In Utero Pediatrics

Research & Practice

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Foreword

With the advances in medical science and technology over time, problems encountered in clinical practice are also evolving. In this context, the traditional classification of medicine into four disciplines (i.e., internal medicine, surgery, obstetrics and gynecology, and pediatrics) may no longer be applicable. Currently, two trends have emerged in medicine—the further subdivision of specialties and the integration of individual disciplines.

In the former, internal medicine and surgery are further divided, with each organ and system or even a distinct disease serving as a subspecialty. For example, the internal medicine can be classified into multiple specialties such as respiratory medicine, gastroenterology, cardiology, neurology, and diabetes, while the general surgery can be further divided into breast surgery, gastrointestinal surgery, orthopedics, hand and foot surgery, etc.

As for the discipline integration, a case in point is fetal medicine, which is not only one of the three subspecialties in obstetrics, but also the result of multi-technology and inter-discipline integration. Cooperation among different specialties is essential for an accurate diagnosis and treatment of a fetal disorder, including, but not limited to, obstetrics, imaging, clinical genetics, clinical laboratory, molecular genetics laboratory, pediatrics, and pediatric surgery.

Given the above trends along with the opportunity of national key clinical specialty evaluation in China, I herein propose to divide obstetrics into three subspecialties, including general obstetrics, maternal medicine, and fetal medicine. The obstetric subspecialties reflect the concept of discipline subdivision, while fetal medicine represents the trend of discipline integration.

The core concept of fetal medicine is "fetus as patient." As the sections targeting the elderly, female, and children are termed as departments of geriatrics, obstetrics and gynecology, and pediatrics, respectively, the one focusing on fetus should be designated as "department of fetal medicine."

Like other clinical departments, it is necessary to integrate various technologies in the diagnosis, treatment, and operation for the fetus. But they are more challenging due to the fact that the fetus is very fragile with small organ size, and the diagnosis, treatment, and operation have to be performed *in utero* with the obstruction of the mother's belly. This requires not only state-of-the-art technologies but also the consideration of ethical issues.

Two types of doctors are engaged in fetal medicine in Europe and North America: obstetricians who have received subspecialty training in maternal-fetal medicine/fetal medicine, and pediatric surgeons who are specialized in

intrauterine fetal and neonatal surgeries. Given the diversity of fetal diseases and the limitation of available technologies, few fetal diseases can be treated and operated *in utero*; on the other hand, most of these disorders are diagnosed *in utero* at early stage and managed at certain developmental stages after birth, requiring the involvement of pediatricians and pediatric surgeons. Hence, the fetal medicine subspecialists must cooperate with the specialists and subspecialists in pediatrics and pediatric surgery, since an abnormality may be noted in any organ and system of the fetus.

Meanwhile, an increasing number of pediatricians and pediatric surgeons are participating in the intrauterine diagnosis and treatment of the fetus as they become increasingly interested in fetal and neonatal diseases. Therefore, the responsibility of fetal medicine specialists is extended to pediatrics and pediatric surgery after birth, while the responsibility of pediatricians and pediatric surgeons is extended to prenatal stage accordingly. In line with the principle of "fetus as patient," the subspecialists in fetal medicine should work with radiologists, clinical geneticists, pediatricians, and pediatric surgeons as a team during the diagnosis and treatment of fetal diseases.

From the perspective of the specialists in obstetrics, maternal-fetal medicine, and fetal medicine, this discipline should be defined as "fetal medicine"; from the pediatricians' viewpoint, it should be termed as "*in utero* pediatrics." I am very glad to find that pediatricians are paying more attention to fetal diseases. In addition, it is also encouraging to see that Professor Kun Sun has proposed the concept of "*in utero* pediatrics" and led the compilation of this monograph. Regardless of whether it is from an obstetric or a pediatric lens, the fundamental principle is the same: "fetus as patient."

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Overview of *In Utero* Pediatrics

1

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1.1 Background of the Development of *In Utero* Pediatrics

In utero pediatrics was originated from the interdisciplinary research and practice of obstetrics, fetal medicine, and other disciplines. As a subspecialty of pediatrics, it was born on the basis of fetal medicine, perinatal medicine, and pediatrics.

In 1967, German Professor Erich Saling first proposed the concept of "perinatal medicine" to strengthen the maternal and perinatal care during the perinatal period, which is a science to study the development of embryos, the physiology and pathology of fetuses, and the diagnosis and prevention of neonatal and maternal diseases [1]. Classical obstetrics focuses on maternal safety during pregnancy and delivery, and the main task is to reduce maternal mortality, while perinatal medicine not only focuses on maternal mortality but also on fetuses and newborns and emphasizes the importance to reduce perinatal mortality. With the development of prenatal imaging, interventional prenatal diagnosis technology, molecular genetic technology and related medical instruments, and the deepening understanding of the pathophysiology of fetal diseases, a new concept of "fetus as a patient" emerged in the 1980s. This concept focuses on comprehensive prenatal screening and diagnosis of fetal diseases, as well as intrauterine intervention for certain congenital abnormalities clearly diagnosed in the womb which means the advent of the era of fetal medicine [2].

Originated from the concept of "fetus as a patient," fetal medicine involves obstetrics, pediatrics, clinical genetics, surgery, and other fields. It focuses on all diseases that may affect the fetus, as well as the diagnosis and treatment of these diseases [3]. In recent years, many studies have found that exposure to adverse factors during pregnancy will have a profound impact on the long-term health of offspring, i.e., the fetal origin of adult diseases (DOHaD theory). Research on chronic noncommunicable diseases shows that "1000 days in the early stages of life" (270 days of pregnancy + 730 days from birth to 2 years of age) is the best intervention window to prevent chronic diseases [4, 5]. However, it is difficult for obstetrics-led fetal medicine to achieve sequential follow-up treatment from the fetal period to infancy and even to the child and adolescent period. Furthermore, at present perinatology and neonatology are still separated, and this is not conducive to the treatment of diseases of intrauterine origin during the neonatal period. In addition, the decline in fertility in recent years is a major challenge faced by many countries, highlighting the necessity for cooperation between pediatricians and obstetricians to ensure the

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newborn survival, improve the prognosis of children with congenital defects, and improve population quality. In view of this, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine has advanced the pediatric diagnosis and treatment window to encourage pediatricians to participate in disease diagnosis and treatment during the prenatal period, to emphasize the continuity and consistency of health management in the early stages of life. In 2021, the team first proposed the concept of "*in utero* pediatrics" in Lancet Global Health [6]. In December of the same year, at the 16th Asian Society for Pediatric Research, the team took the lead in setting up the *In Utero* Pediatrics Forum and reported the Xinhua practice model of *in utero* pediatrics system.

1.2 Concept and Scope of *In Utero* Pediatrics

In utero pediatrics is a discipline that focuses on the study and practice of pediatric diseases originated *in utero* during the first 1000 days of life from a fertilized egg to the first 2 years of life and, subsequently, to childhood and adolescence. It enables the sick children to become healthy adults with full potential. *In utero* pediatrics is the extension of traditional pediatrics to pregnancy and interacts with practice of obstetrics and fetal medicine. It moves the attention and research focus of pediatricians forward to the embryonic period for a consistent management of child diseases. Moreover, *in utero* pediatrics gradually derives into many disciplinary branches, such as *in utero* pediatric internal medicine/surgery/diagnostics/imaging/pathology based on the major organ systems of children and focusing on diseases of intrauterine origin.

As an emerging interdisciplinary clinical diagnosis and treatment model, the intrauterine diagnosis and treatment system involves the integration of various disciplines related to the existing pediatric and obstetrics systems, such as diagnosis, treatment, prevention, and even engineering and basic science. Pediatricians need to be more proficient in prenatal care, and multidisciplinary collaboration is required, including but not limited to maternal and fetal medicine specialists, pediatric surgeons, neonatologists, geneticists, pediatricians, anesthesiologists, radiologists, and possibly other specialists. In 2018, Xinhua Hospital completed the first single-center intrauterine interventional treatment for severe fetal aortic valve stenosis in Asia, improving the intrauterine diagnosis and treatment system and comprehensive life span management model for pediatric congenital heart disease, providing a good example for the discipline construction and promotion of *in utero* pediatrics.

In recent years, technological developments and breakthroughs in fields such as prenatal diagnosis, intrauterine treatment, and perinatal management have provided solid technical support for the construction of the intrauterine diagnosis and treatment system and comprehensive life span management. This book will discuss the development and specific application of *in utero* pediatrics from different systems, including the circulatory system, nervous system, digestive system, respiratory system, urinary system, endocrine system, locomotor system, and reproductive system, and explain the profound knowledge in a simple way, from the conclusive description of basic knowledge to case presentations, such as intervention and sequential treatment of intrauterine cardiovascular structural abnormalities, treatment of fetal cerebral hemorrhage, treatment of congenital megacolon, etc. At the same time, it also integrates imaging and molecular genetic diagnosis, etc. to comprehensively and concisely enable readers to understand *in utero* pediatrics, new developments, and cutting-edge hotspots and development trends in *in utero* pediatrics.

1.3 Future Development of *In Utero* Pediatrics

In the future, we will continue to promote multidisciplinary cooperation to standardize the indications and procedures for the diagnosis and treatment of fetal diseases. Early diagnosis and early prevention of *in utero* pediatric diseases will

be achieved by combination with advanced methods including medical genetics and medical imaging. We will carry out researches on the mechanism of developmental diseases and the follow-up study on the long-term prognosis of intrauterine pediatric diseases, and establish cohorts of intrauterine pediatric diseases. The exploration of emerging techniques involving intrauterine fetal screening, diagnosis, and intervention will be the major project. The main task in the future includes the development of new techniques and methods for intrauterine and delivery room treatment, in order to perform intrauterine intervention, sequential surgery, and hybrid procedure for congenital heart disease, complex congenital malformations and other diseases. Our goal is to achieve comprehensive leadership in intrauterine pediatric treatment technology and become a leading intrauterine pediatric discipline in China and first-class in Asia. The specialties including pediatric medicine and pediatric surgery will be promoted with the development of *in utero* pediatrics, so as to comprehensively improve the discipline level of pediatrics.

The construction of a discipline is inseparable from the professional and technical personnel training. We hereby call on colleagues to work together to promote the training of specialists in the intrauterine diagnosis and treatment system and the development of the textbook and to promote the standardization and systematic development of the discipline.

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2 The Early Life Plan Program

Qian Chen and Jun Zhang

2.1 DOHaD Theory and 1000 Days in Early Life

Noncommunicable diseases (NCDs), including type 2 diabetes mellitus, cardiovascular disease, obesity, and even tumors, are a serious and growing global health burden. There is clear evidence that a series of adverse early exposures may make individuals more vulnerable to NCDs throughout life and that these risk factors can be passed on to future generations, thereby perpetuating the disease cycle. This concept is called "developmental programming" and is the basis for the framework of developmental origins of health and disease (DOHaD) [1].

2.1.1 DOHaD

In the 1990s, UK Professor David Barker studied the cardiovascular mortality of men born in Hertfordshire, UK, at the beginning of the century, and found that deaths due to ischemic heart disease were more common in men who had had underweight at birth and at 1 year of age. On this basis, Professor Barker proposed the hypothesis of "fetal origin of adult diseases," arguing that malnutrition in the second and third trimesters of pregnancy might lead to unbalanced fetal development, which would lead to coronary heart disease in adulthood [2]. Subsequent cohort studies from several countries suggested that lower birth weight was associated with a higher risk of ischemic heart disease, diabetes mellitus or impaired glucose tolerance, hemorrhagic stroke, and hypertension in children and adulthood. Furthermore, more and more epidemiological studies have confirmed that, in addition to nutrition during pregnancy, environmental factors (physicochemical environments, social environments) in the early stages of human life (including early embryo, fetus, and infancy) increase the risk of certain diseases during childhood and even in adulthood [3]. Therefore, the concept of "fetal origin of adult disease" gradually transitioned to "developmental origins of health and disease (DOHaD)."

The DOHaD concept has attracted the attention and interest of a wide range of epidemiologists, and numerous studies have been carried out based on this theory. The International Developmental Origins of Health and Disease Society (International DOHaD Society) was established in 2003 to advocate the start of healthy life worldwide by supporting initiatives, programs, and policies that promote education and research on the early origins of health and disease. In 2008, under the leadership of Professor Duan Tao from the First Maternity and Infant Health Hospital Affiliated to Tongji University and Professor Yang Huixia from Peking

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University First Hospital, the "DOHaD China" was established in Shanghai.

Currently, there are five major theoretical foundations of DOHaD, including developmental plasticity, adaptive response, thrifty genotype, thrifty phenotype, and environment-gene interaction [4–7]. The core point of these theories is that during development, the influence of environmental factors can make the body produce corresponding adjustments and changes to better adapt to the adverse intrauterine environment, and the changes may lead to transgenerational inheritance through epigenetic changes.

2.1.2 1000 Days in Early Life

In the first 1000 days of life, from the beginning of a zygote to 2 years of age, individuals have basically completed the early life development programming. Exposure to any adverse factors at this critical stage may affect the developmental plasticity of fetuses, infants, and early childhood, bring about changes in phenotypic characteristics, resulting in continuous and permanent structural and functional changes, which ultimately lead to the occurrence of a series of childhood and adulthood diseases. Such diseases whose causes can be traced back to early life are called developmental diseases.

The intrauterine stage from the beginning of fertilization to the birth of the fetus is a critical period for the development of tissues and organs, which is most susceptible to adverse exposure factors inside and outside the uterus and causes morphological abnormalities and is known as the "highly sensitive period for teratogenesis." The development windows of various tissues and organs are different; neural tissue develops on days 15–25 after fertilization, the heart develops on days 20–40 after fertilization, limbs develop on days 24–46 after fertilization, and lips develop on days 42–56 after fertilization. Studies have demonstrated that various environmental changes *in utero*, including exogenous substances, maternal psychology and behavior, and environment, etc., can lead to changes in gene expression levels through epigenetic changes in non-gene sequences (such as DNA methylation, chromatin conformational changes, etc.), followed by changes in protein synthesis and function, thereby affecting intrauterine fetal development and continuing this effect into adults or even the next generation.

The first 2 years after birth, including infancy and early childhood, are the most rapid growth and development periods in a person's life, during which weight increases rapidly and the nervous system develops rapidly. On this basis, the psychology of the infants also tremendously changes under the influence of the external environment. Feeding progresses from feeding, weaning, supplementary food addition, and transition to general food; movement progresses from lying down and inability to move freely to touch, fiddle with objects at will with hands and stand on both legs at will, and learn to walk independently and stand on one foot; and transition from complete lack of language and inability to speak to the ability to the simplest communication with language, etc. All of this marks the baby's first steps from a natural, biological individual to a social entity. On the basis of genetic biology, they form the socialized humanity-sociality and gradually adapt to human social life. The nurturing environment, nutrition, and behavior of families during infancy and early childhood have a huge impact on the health of children. Premature infants complete catch-up growth between the ages of 1 and 2. From intrauterine malnutrition to extrauterine overnutrition, blind feeding causes an increased chance of premature infants becoming obese.

In the past 30 years, with the rapid development of economy and the improvement of people's lives, the living environment and lifestyle of children have changed markedly. Correspondingly, the spectrum of children's diseases has also changed significantly. Maternal mortality and under-five mortality are important demographic indicators that comprehensively reflect the population level, health care status, and quality of life of a country or region. As of May 2013, the statistics released by WHO show that China has basically achieved the United Nations Millennium Development Goals.

However, because of the high base value of child mortality in China, even if it drops to two-thirds of the original value, its absolute number is still very high, and the under-five mortality is as high as 15%. Neonatal deaths account for 43% of deaths in children under 5 years of age, of which 75% occur within the first week after birth and 33% occur on the day of birth; complications of premature infants, birth asphyxia, and other complications and birth defects related to labor account for 16%, 15%, and 13% of the causes of death, respectively. Therefore, reducing perinatal mortality is the key to reducing under-five mortality. With the implementation of the "two-child" policy in China, the number of elderly parturients is increasing, and the cesarean section rate is increasing year by year. At the same time, assisted reproductive technology is widely used, environmental factors have worsened, and other adverse exposure in early life is increasing, making the situation of adverse birth outcomes in the perinatal period such as premature labor, birth defects, low birth weight, and fetal macrosomia still severe; on the other hand, chronic noncommunicable diseases have gradually become the leading diseases in the adult period of urban residents in China. Therefore, taking 1000 days of life as the key time point to explore the impact of early life exposure on human developmental diseases and to establish an early intervention model is currently an important strategic task in China. It is of great significance in improving population quality, safeguarding the health of the masses, promoting social and economic development, and building a harmonious society.

2.2 Birth Cohorts

Long-term prospective cohort studies are of great value for more specific understanding of the disease process and its etiology. In the mid-twentieth century, longitudinal cohort studies focused on adults, such as the Framingham Study and the Nurses' Health Study, which collected the necessary data to help identify causal relationships between risk factors and disease outcomes. These studies lay the foundation for effective chronic disease prevention and treatment programs. Following these successful adult cohort studies, birth cohort studies were carried out in many countries in the late twentieth century to assess the risk of harmful chemicals in the air, water, and food and other environmental factors to children. These birth cohort studies usually recruit participants during pregnancy and continue to follow children until puberty or later. These studies generally investigated hundreds to thousands of pairs of mothers and infants at the time of pregnancy or birth and then followed these children regularly for several years.

2.2.1 International Birth Cohorts

2.2.1.1 International Birth Cohort Consortium

Some studies have investigated specific environmental exposures, while others have investigated the environment more broadly, including behavioral and social factors. For example, the Faroese birth cohort study investigates the exposure of mothers and children to environmental pollutants in their diet, and tests are being carried out to assess neurobehavioral development. The Avon Longitudinal Study of Parents and Children (ALSPAC) is a long-term health research project designed to investigate the effects of environmental and genetic factors on health and development in over 14,000 pregnant mothers. The Mothers and Children's Environmental Health (MOCEH) study of South Korea is a prospective hospital-based and community-based cohort study of 1500 women and collects information on environmental exposures (chemical, biological, nutritional, physical, and psychosocial) in pregnant women and children under 5 years of age. Similarly, the French EDEN cohort recruited 2000 children in 2 regions to study pre- and postnatal determinants of child health and development, including specific environmental exposures. However, even these largest birth cohort studies are underpowered to investigate rare childhood diseases and outcomes, such as childhood cancer. To increase the sample size, researchers are working with some older birth

cohort studies to pool data. Their efforts were hampered because old studies did not use the agreed definitions of disease outcomes, time periods of measurement, or methods for measuring biomarkers and chemical contaminants. This makes it very difficult to perform pooled data analysis.

Researchers involved in large-scale twenty-first-century birth cohort studies are trying to reach an agreement on how to evaluate disease outcomes, measure biomarkers, and assess environmental exposures. Researchers from the Japan Environment and Children's Study (JECS) (Japan) and other birth cohort studies discussed possible research coordination at the JECS International Linkage Symposium (Tokyo 2011). The Ministry of Environment of Japan recommended that, in order to further carry out international cooperation, it would be better to establish a working group to identify a list of core elements for inclusion in birth cohort studies. These core elements might include disease outcome measures, biomarkers, and exposure measurements. The Ministry of Environment of Japan expressed interest in providing financial support to coordinate such efforts. Therefore, it was recommended to establish an International Birth Cohort Group on Environment and Child Health to discuss and exchange information on ongoing and upcoming large-scale birth cohort studies. New large-scale studies on the impact of the environment on children's health and development that were planned or conducted in France, Shanghai (China), the United States, and Germany expressed interest in participating. In 2011, experts from these five countries formed the International Birth Cohort Group on Environment and Child Health (hereafter referred to as the ECH group). The common primary goal of these studies is to better understand the broad range of environmental and social factors affecting children's health and well-being. The environment is broadly defined in these studies, including the investigation of chemical, biological, physical, and socioeconomic factors affecting children's growth, development, and health. The ECH group exchange information by meetings and strive to harmonize processes and procedures to create an opportunity to compare methods and results at any time and conduct a comprehensive analysis of results in the future. To this end, harmonization of certain infant health outcomes, biomarkers, environmental measures, and measures of socioeconomic and immigration status has begun.

The ECH group currently includes the Japan Environment and Children's Study, the French ELFE Cohort, the Shanghai Birth Cohort (SBC), and the Danish National Birth Cohort (DNBC) [8].

2.2.1.2 Birth Cohort Consortium of Asia (BiCCA)

Asia is home to half of the world's children, and Asia is the fastest industrializing region in the world. Environmental threats to the health of Asian children are diverse and include typical infectious disease hazards (i.e., pneumonia, dysentery, measles, acquired immunodeficiency syndrome, and tuberculosis). Furthermore, the prevalence of environment-related disorders, such as allergic disorders, attention-deficit hyperactivity disorder (ADHD), and autism, are increasing dramatically. As industrial development proceeds, epidemic transformation of countries in the region, rapid urbanization, unsustainable consumption, and increased industrial disposal of electronic waste, children are facing rapidly increasing new health threats due to exposure to toxic chemicals. In order to address these issues, local and national research efforts must focus on environmental risks. The International Conference on Environmental Threats to the Health of Children was held in Bangkok in 2002 and took the first step to increase awareness on environmental health hazards affecting children in the region. Several actions have been proposed to reduce environmental hazards, including the removal of lead from gasoline, the Clean Water Project in India, the Mercury Pollution Reduction project, the establishment of International Network, and the Anti-Smoking Campaign. However, new challenges continue to emerge. In 2008, hundreds of thousands of infants and young children in China, Hong Kong, and Taiwan developed renal insufficiency after exposure to melamine intentionally added to milk and dairy products. In 2011, plasti-

cizers such as di(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DiNP) were found to be illegally added as emulsion stabilizer in food and beverages in Taiwan. These issues have raised new concerns about the importance of food safety. Another cross-border threat in the Asia-Pacific region is outdoor air pollution. Smoke and sand storms in China and Southeast Asia are not regional threats. More importantly, we are facing the challenges of global climate change and extreme weather. The issue has become a prominent goal of policy reform and public health efforts.

In response to these new threats, Asian governments have taken action since the 1970s to protect children from environmental health threats, and over the past decade, they have developed new methods to assess and manage toxic chemicals. In 2009, the Ministry of Environment, Republic of Korea, in collaboration with the World Health Organization, held the Third International Conference on Child Health and Environment in Busan, Korea. In July 2010, the Korean government hosted the Second Regional Forum on Environment and Health in Southeast and East Asian Countries in Jeju, Korea, which was jointly organized by the UN Environment Regional Office for Asia and the Pacific and the WHO Regional Offices for the Western Pacific and Southeast Asia. A number of relevant meetings further committed to promoting recognition, assessment, and consideration of the impact of harmful environments on children's health and development. Children's environmental health is an issue of global and regional importance. Environmental hazards, lifestyle, and genetic predisposition vary by region and race. Thus, a single cohort or study cannot explore the full picture of this issue. Population size is even a threshold for studying how genes interact or coexist with the environment. Furthermore, replication or validation is essential to interpret the results of genomics techniques such as epigenomics and metabolomics.

The two main goals of BiCCA are (1) to facilitate knowledge exchange and collaboration among teams and researchers and (2) to explore the future needs of children's environmental health research. The BiCCA agenda was first introduced at the Prenatal Programming and Toxicity III (PPTOXIII) held in Paris, France, on May 14–15, 2012. Subsequently, several symposiums/seminars, workshops, and PI meetings were held to raise awareness of the Asian birth cohort and build capacity related to conducting up-to-date research techniques such as exposure measurement and genetic and epigenetic analyses. These activities also identified the criteria for joining BiCCA, completed the first stage checklist, prepared presentation documents, and planned future directions for collaboration.

2.2.2 Shanghai Birth Cohort

With the unprecedented development of China's economy in recent years, the whole country is undergoing great changes. Urbanization has been occurring on a large scale all the time. However, environmental pollution, as a side effect of rapid economic growth, is a prominent problem in many parts in China and may pose a threat to the health of the population. Furthermore, as a developing country, China continues to be affected by traditional pollutants such as lead and mercury. Emerging pollutants, such as endocrine disruptors, are currently being produced in large quantities locally and are widely used in daily life. There is evidence that human exposure to both old and new pollutants is high in China. Of particular concern is how environmental pollutants affect vulnerable populations, such as pregnant women, fetuses, and young children. Early life exposure to a variety of environmental pollutants is associated with reproductive failure, adverse pregnancy outcomes, physical and mental developmental disorders in children, and even adult diseases in future generations. For example, perfluoroalkyl substances (PFAS), a group of environmental endocrine disruptors commonly used in household products, have been found to be associated with dyslipidemia, asthma, renal function, and younger age of menarche in children. The evidence further suggests that the incidence of birth defects, childhood asthma, and precocious puberty is rising in China.

Equally important is overnutrition in urban settings, coupled with less physical activity, where the prevalence of obesity is rising rapidly, particularly among children. For example, the prevalence of overweight and obesity among children aged 7–18 in China increased from less than 2% in 1985 to 19.4% in 2014. Childhood obesity has important effects on long-term health, such as hypertension and type 2 diabetes mellitus. Shanghai, one of China's largest and most developed cities, has undergone rapid changes over the past 30 years. The prevalence of childhood asthma is the highest in the country (7.6%). More and more children are being diagnosed with attention deficit hyperactivity disorder (ADHD). The combined prevalence of overweight and obesity is 49.1% in boys aged 8–15 years and 30.8% in girls. Furthermore, Shanghai residents have higher PFAS levels than those in other parts of China. Therefore, Shanghai is an interesting and important place to study the effects of early exposure to environmental and behavioral factors on child health and even adult health, a key aspect of developmental origins of health and disease (DOHaD) theory.

Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, designed the Shanghai Birth Cohort (SBC) to study the effects of genetic, environmental, and behavioral factors on fertility, pregnancy outcomes, child growth and development, and disease risk 10. The researchers collected information on most of the world's birth cohorts, visited several international cohorts, and invited experts from around the world to participate in the study design. SBC is a member of the Environment and Child Health International Birth Cohort Group (ECHIBCG) led by the World Health Organization and the Birth Cohort Consortium of Asia (BiCCA).

SBC includes a prepregnancy cohort and a pregnancy cohort. From 2013 to 2015, 1180 couples who visited two prepregnancy care clinics in Shanghai to receive care were recruited. The couples were contacted by well-trained research assistants in the waiting room. They met the following conditions: (1) 20 years of age or older (legal age for marriage in China); (2) at least one of the couple was a registered resident of Shanghai; (3) they had recently planned to become pregnant; (4) neither of them had been diagnosed with fertility problems; (5) they planned to seek prenatal care once they became pregnant and to deliver at the SBC-participating hospital; (6) families intended to stay in the catchment area for at least 2 years; and (7) they were willing to sign a consent form and be followed for at least 2 years. Couples who attempted to conceive spontaneously for more than 12 months but were still not pregnant or seeking reproductive assistance were not included in the cohort.

In the meantime, in six SBC-participating hospitals, women who came for an appointment for prenatal care were contacted for recruitment. They were informed about the project and the procedures involved. These six hospitals were located in four districts, two districts in urban areas, one district in suburban areas, and one district in semirural areas. The following couples were eligible for the pregnancy cohort: (1) 20 years of age or older; (2) at least one of the couple was a Shanghai-registered resident; (3) planned to seek prenatal care and to deliver at the SBC-participating hospital; (4) families intended to stay in the catchment area for at least 2 years; and (5) were willing to sign a consent form and be followed for at least 2 years.

In the prepregnancy cohort, women were contacted by phone every 2 months after enrollment to confirm their pregnancy for 12 months. If a woman became pregnant, she would continue to be followed in the first, second, and third trimesters. If a woman was not pregnant at the end of the 12-month follow-up period, she would no longer be followed. If she later became pregnant by any means, she would be followed up again. In the pregnancy cohort, pregnant women were enrolled at the time of prenatal care during the first trimester. The second follow-up was performed at 24–28 weeks of pregnancy, and an oral glucose tolerance test was performed. The third follow-up was performed during routine prenatal care at 32–36 weeks of pregnancy. After delivery, medical records were reviewed and abstracted by

the researchers. As part of routine clinical care, the mother and baby were required to return to the delivery hospital for routine postnatal examinations 42 days after birth. Subsequently, when the children were 6 months, 12 months, 24 months, 4 years old, and 7 years old, they were contacted to return to the hospital for follow-up. Up to now, the follow-up of all 4-year-old children has been completed, and more than 1000 7-year-old children have been followed up.

2.2.3 Early Life Plan (ELP) Program

The ELP program is led by the Ministry of Education-Shanghai Key Laboratory of Children's Environmental Health, in collaboration with the Department of Obstetrics and Gynecology, the Department of Child Health, the Department of Pediatric Internal Medicine, the Department of Pediatric Surgery, and various disciplines of adults in Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. ELP focuses on the environmental and genetic issues of developmental diseases, covering the three major sectors of basic, clinical, and public health research. Using clinical epidemiology, developmental and behavioral pediatrics, molecular neurobiology, cell biology techniques, molecular epidemiology, and epigenetics as the main research means, we conduct in-depth research on environmental factors affecting children's health, and reveal the current status, rules, and related factors affecting the health of children and adults of different environmental factors. The ELP platform will form a clinical integration platform by means of hospital information system (HIS), including electronic medical record database, medical laboratory database, medical imaging database and biobank platform, clinical study center, and central laboratory. Based on this, if it is possible to integrate with other hospitals in the alliance in the future to achieve regional clinical big data, this platform can better serve medical quality supervision, commercial health insurance actuarial calculation, personalized medical care, health insurance supervision, national medical reform monitoring, etc. The establishment of a clinical big data system is of great significance to the transformation of diagnosis and treatment models. The application of clinical early warning and recommendations is an important aspect of future development. The application in the early warning can determine the risk of disease and prompt it based on some vital signs of patients, and clinical researchers can discover the association between exposure and outcomes through disease maps.

2.2.3.1 Observation Indicators of the Study

The primary observation indicators are as follows:

- Pregnancy outcomes
- Children's growth and development information, including height, weight, head circumference, and neurodevelopmental assessment data

The secondary observation indicators are as follows:

- Influence of pregnancy complications and comorbidities on mother and child
- According to the follow-up cohort of children with different diseases and the research direction of clinicians, relevant observation indicators are collected, such as the diagnosis, treatment, and outcome of diseases

2.2.3.2 Study Design

ELP is a multicenter prospective observational study, including cohort studies and case-controlled studies. The duration of the project is initially set at 5 years. It is planned to include 15,000 pregnant women in Xinhua Hospital, and after their delivery in Xinhua Hospital, about 3000 newborns will be included each year. Among them, 15% of the children with premature labor, intrauterine growth retardation, macrosomia and birth defects, or their mothers with gestational diabetes mellitus and preeclampsia will be selected for active follow-up; meanwhile, 5% of the normal children will be selected as the control group. In addition, for the study disease

of interest to the clinicians, the follow-up population and number of children to be followed-up will be determined based on the incidence. At the later stage of the project, other hospitals will participate in this study. The number of patients enrolled in the subcenter will be determined according to the annual delivery volume. The follow-up population and proportion are the same as those in Xinhua Hospital.

All pregnant women participating in the study will be followed up through the ELP APP. Children enrolled in the case-controlled study will be followed up or underwent a routine physical examination at 42 days, 6 months, 1 year, and 2 years of age after their birth. All follow-up work will be performed by establishing structured medical history, follow-up records, children's questionnaire survey, APP questionnaire push, HIS data, and collection of pathological specimens and remaining blood samples to ensure the accuracy and comprehensiveness of information and sample collection.

2.2.3.3 Inclusion Criteria

Inclusion criteria for pregnant women are as follows:

1. Pregnant woman that is registered at the hospital
2. Pregnant woman that has signed the informed consent form
3. Pregnant woman that is willing to comply with the study schedule and requirements, including follow-up of her newborn(s)

Inclusion criteria for newborns are as follows:

1. Newborn that is with no premature labor, no intrauterine growth retardation, no macrosomia, and no birth defects and whose mothers have no pregnancy complications during pregnancy.
2. Newborn with premature labor, intrauterine growth retardation, macrosomia, and birth defects.
3. Newborn whose mother has pregnancy complications such as gestational diabetes mellitus or preeclampsia.
4. Please refer to the corresponding study protocol for the inclusion criteria of newborns undergoing special disease study.

Exclusion criteria are as follows:

- Pregnant woman unwilling to sign informed consent form.

2.2.3.4 Study Schedule

Perinatal-obstetric part: Pregnant women enrolled are required to undergo examinations consistent with the routine prenatal examination procedures and do not need to undergo additional examinations unless they have complications that require additional examination and treatment. Study data will be collected from the first trimester (8 weeks after registration) until 42 days after delivery, including the first prenatal examination and blood glucose screening at the second trimester, third trimester, delivery, and 42 days after delivery. During the study, if a volunteer requests to withdraw from the study, the reason for withdrawal should be recorded.

The follow-up schedule for the pregnant women enrolled is as follows:

Screening

- Volunteer informed consent
- To check inclusion/exclusion criteria

Follow-up 1 (the first trimester)

- Blood collection on an empty stomach
- To collect demographic data, medical history, medication history, etc.
- Physical examination
- Laboratory tests/diagnostic tests
- To retain the remaining blood sample after the tests
- To complete the Questionnaire for Pregnant Women

Follow-up 2 (the second trimester)

- Glucose challenge test (GCT)
- For those who do not need GCT, to determine fasting blood glucose
- To retain the remaining blood sample after the tests

Follow-up 3 (the third trimester)

- Blood collection on an empty stomach
- To retain the remaining blood sample after the tests

Follow-up 4 (delivery period)

- To confirm pregnant women participate the study
- To retain umbilical cord blood
- To record relevant data in HIS

Follow-up 5 (42 days after delivery)

- Physical examination
- To complete the Follow-up Form on 42 Days after Delivery

Pediatric part: After the pregnant women enrolled give birth, 20% of the newborns (children patients and healthy children) will be selected for follow-up questionnaire survey or routine physical examination at 42 days, 6 months, 1 year old, and 2 years old after birth. The routine physical examinations include measurements of height, weight, and head circumference, as well as neurodevelopmental assessments. If the guardian of an enrolled child requests withdrawal from the study during this period, the reason for withdrawal should be recorded.

The follow-up schedule for the children enrolled is as follows:

Screening

All newborns delivered in Xinhua Hospital automatically will enter the general population for follow-up, and those who meet the inclusion criteria of normal control and disease follow-up cohort will enter the disease follow-up cohort.

Follow-up 1 (Day 42)

- To verify the newborns enrolled
- To measure height, weight, and head circumference
- To assess neurodevelopment
- To complete Follow-up Questionnaire for 42-days-old Children
- To record relevant data in HIS

Follow-up 2 (6 months ± 1 month)

- To measure height, weight, and head circumference
- To assess neurodevelopment
- To complete Follow-up Questionnaire for 6-months-old Children
- To record relevant data in HIS

Follow-up 3 (1-year-old ± 1 month)

- To measure height, weight, and head circumference
- To assess neurodevelopment
- To complete the Follow-up Questionnaire for 1-year-old Children
- To record relevant data in HIS

Last follow-up (2-years-old ± 1 month)

- To measure height, weight, and head circumference
- To assess neurodevelopment
- To complete the Follow-up Questionnaire for 2-year-old Children
- To collect blood samples
- To collect urine samples
- To record relevant data in HIS

2.3 Achievements of Cohort Studies

Up to now, the follow-up of all 4-year-old children has been completed, and more than 1000 7-year-old children have been followed up in SBC. In SBC, the physicochemical and social environment exposure status of children is

assessed through questionnaires and laboratory tests. Combined with asthma, obesity, and neurodevelopment of children during follow-up, the effects of these early exposure factors on children's health are discussed. At the same time, a large biobank of SBC is constructed, and a series of biological samples are collected, including more than 340,000 samples from 5398 families of mothers, fathers, and children, which provides a valuable and rich resource for studying the pollutant exposure of pregnant women, men, and children in Shanghai and its effects on reproduction, pregnancy and child health, and an outbound logistics management system and information management system for the collection, transportation, warehousing, sub-packaging, and preservation of biological samples is established.

Since the implementation of the ELP Program, in addition to Xinhua Hospital, three hospitals have joined in the alliance: Linyi Women and Children's Hospital, Jiaxing Maternity and Child Health Care Hospital, and Longyan People Hospital. Up to now, Xinhua Hospital has recruited more than 12,000 pregnant women, including more than 8000 pregnant women who have delivered and more than 1200 children; the three alliance hospitals have also recruited more than 13,000 pregnant women, including more than 9000 pregnant women who have delivered and 1400 children. Follow-up of children is also ongoing, and follow-up of some children at the age of 4 years has begun.

In addition, we have carried out a series of studies based on birth cohorts to investigate the effects of early life factors on children's health.

2.3.1 Child Early Growth and Development

2.3.1.1 Study on the Early Growth and Development Trajectory of Chinese Children

The first 1000 days of life (from pregnancy to 2 years of age) is a period of rapid growth and development which is easily affected by nutrition and the environment. The determination of the normal growth pattern of children is essential in growth evaluation and can provide information for timely intervention. At present, both the World Health Organization (WHO) growth standards and Chinese growth standards are currently used in China. The WHO growth standards for children aged 0–24 months use the longitudinal data of children in six countries from 1997 to 2003, while the current domestic standards are established based on a large cross-sectional study with stratified random sampling of children in nine cities in China from May to October 2005, so the growth standards need to be updated.

Based on the recent six birth cohorts in China, we included 4251 infants (2174 boys and 2077 girls) born from 2015 and established a new *Z*-score growth curve. After comparison, we found that the growth and development curves in this study were higher than the two standards currently used in China. The new growth curve represents the growth pattern of healthy Chinese infants assessed longitudinally from 0 to 24 months of age and provides the latest reference for monitoring the children early growth and development in China [9].

2.3.1.2 Study on Early Growth and Development of SGA Infants

Small-for-gestational-age (SGA) infant is defined as newborns whose birth weight is below the tenth percentile of the average weight of the same gestational age, or below 2 standard deviations of the average weight of the same gestational age. Previous studies have shown that insufficient weight gain during pregnancy is associated with SGA, and insufficient weight gain will increase the risk of developmental retardation in SGA children. However, the long-term relationship between weight gain during pregnancy and catch-up weight gain in SGA children remains unknown. Based on a multicenter prospective cohort study, 56,990 pregnant women were recruited from prenatal examination and children were followed up to 7 years of age. Studies have found that SGA children whose mothers were underweight before pregnancy or who gained

insufficient weight during pregnancy had poor body weight catch-up growth in preschool years [10].

Studies have shown that newborns with intrauterine growth restriction have an increased risk of chronic diseases in adulthood, such as cardiovascular disease, type 2 diabetes mellitus, and adult metabolic syndrome. These risks may be exacerbated when intrauterine growth restriction is combined with rapid catch-up growth after birth. Therefore, it is necessary to construct early prediction models of growth trajectories that are highly associated with childhood overweight/obesity. On the combination of the two prospective cohort studies, the SBC and the United States Cooperative Perinatal Project (CPP), it is found that the change in the BMI percentile after birth was completed around the age of 1, and high-risk growth trajectories closely related to overweight/obesity at the age of 7 have been identified. Children with this trajectory had a BMI percentile around 85% after 1 year of age. Using a prediction model containing four metabolites (tyrosine, glycine, octanoylcarnitine, and stearoylcarnitine), combined with gender, birth weight, and prepregnancy BMI, it was able to predict children at high risk after birth. In the validation data set, the area under the subject operating characteristic curve of the model was 0.869, the sensitivity was 83.3%, and the specificity was 81.1% [11].

2.3.1.3 Study on Mechanisms Related to Early Growth and Development

Fetuin-A is a liver-derived glycoprotein associated with insulin resistance, metabolic syndrome, and bone metabolism. At the same time, both insulin resistance and bone growth are associated with fetal growth, suggesting that fetuin-A may be associated with abnormal fetal growth. Studies have reported that maternal circulating fetuin-A levels are negatively correlated with fetal growth, but there is still little evidence on whether cord blood fetuin-A is related to fetal growth. We conducted a nested case-controlled study in Shanghai birth cohort to assess whether fetal globulin-A was associated with abnormal fetal growth. The results showed that fetuin-A concentrations were higher in cord blood of both SGA and LGA infants. The results indicated that fetuin-A might be associated with fetal overgrowth, and this association was independent of fetal growth factor [12].

Gestational diabetes mellitus (GDM) is a disease characterized by elevated insulin resistance and impaired glucose tolerance in the second and third trimesters of pregnancy, and it has been found that GDM is associated with elevated maternal circulating fetuin-A levels. At the same time, GDM usually induces macrosomia, which may be mainly due to increased glucose transfer from mother to fetus. It is unclear whether GDM can alter the association between cord blood fetuin-A and fetal growth. We assessed the relationship between cord blood fetuin-A levels and GDM and fetal growth based on a nested case-controlled study including 153 pregnant mothers with GDM and 153 matched pairs with normal blood glucose. It was found that maternal GDM was not associated with cord blood fetuin-A levels. In addition, fetuin-A was negatively correlated with both birth weight and length of fetuses with GDM, and no association was found in mothers with normal blood glucose [13].

2.3.2 Childhood Obesity

Childhood overweight and obesity are one of the most serious challenges to child health worldwide. Since 1980, the global prevalence of childhood obesity and overweight has increased by 50%. Childhood obesity is associated with adverse cardiometabolic outcomes, such as type 2 diabetes mellitus, fatty liver disease, dyslipidemia, and hypertension.

Previous studies have shown that caesarean section may increase the risk of childhood obesity, but it is still uncertain whether this association is causal or an effect of medical indications for caesarean section. To assess the association between cesarean section, especially cesarean section without medical indications, and the risk of overweight and obesity in school-aged children, we conducted a large population-based sur-

vey in Shanghai. A retrospective survey of delivery methods and the measurement of height, weight, and waist circumference of 13,724 children showed that cesarean section was associated with an increased risk of overweight and obesity, manifested by increased BMI and abdominal obesity, and this effect was not related to cesarean section indications [14].

New evidence suggests that disturbances in intestinal microbial diversity may affect host energy metabolism and lead to obesity. It is found in animal models that changes in the microbiota due to early exposure to antibiotics, even for a short term, may be sufficient to trigger long-lasting metabolic consequences. Randomized controlled trials in human have also shown that weight gain also occurs with antibiotic exposure. Epidemiological studies on early childhood exposure to antibiotics and weight gain have mainly focused on postnatal antibiotic use. Most studies have shown that antibiotic use in infancy is associated with an increased risk of childhood obesity, but the effect of prenatal antibiotic use on the risk of obesity in offspring is not consistent. We assessed the association between maternal antibiotic use during pregnancy and obesity in children aged 4–7 years on the basis of a prospective cohort study. It was found that repeated use of antibiotics during pregnancy was associated with obesity in 7-year-old children, and the risk of obesity increased with the increase in the number of antibiotic uses, with the strongest association particularly with recurrent use of antibiotics in the second trimester [15].

2.3.3 Allergic Diseases in Children

2.3.3.1 Pregnancy Factors and Childhood Asthma

Aspirin is one of the three classic drugs in the history of medicine, and it is still the most widely used antipyretic, analgesic, and anti-inflammatory drugs in the world. Low-dose aspirin can be used to prevent recurrent miscarriage and preeclampsia associated with antiphospholipid syndrome, usually throughout pregnancy. However, aspirin may lead to asthma and allergic diseases by blocking cyclooxygenase-1 to increase leukotriene synthesis. In order to investigate whether intrauterine exposure to aspirin at different stages of fetal life is associated with childhood asthma, we used the prospective cohort data from the United States to record maternal aspirin use before and during pregnancy and to follow children up to 7 years of age. A total of 19,928 only children without maternal asthma history were included. After analysis, it was found that aspirin use during pregnancy was associated with an increased risk of childhood asthma, and the effect of aspirin use in the second and third trimesters was significant. In addition, aspirin use in the third trimester for more than 2 days can increase the risk of childhood asthma [16].

Furthermore, the World Health Organization recommends folic acid supplementation during pregnancy to prevent neural tube defects. However, methyl groups provided by folic acid can induce epigenetic changes by altering the methylation-sensitive state, thereby enhancing the expression of Th2 cytokines during fetal development, which may alter the inflammatory response and increase the risk of asthma in offspring. However, there were no consistent evidence in the previous epidemiological evidence, and the underlying biological mechanisms are unclear. We conducted a hospital-based case-controlled study in Shanghai, including 548 childhood asthma patients and 816 normal children, and retrospectively investigated the folic acid supplementation of mothers before and during pregnancy. It was found that perinatal folic acid supplementation was associated with an increased risk of childhood asthma, and the risk was related to the time of initiation of folic acid supplementation. We further found that the risk of childhood asthma was most obvious when perinatal folic acid supplementation was continued for more than 6 months [17].

The incidence of childhood asthma has been increasing over the past 30 years. A number of factors, both genetic and environmental, are associated with the development of childhood asthma. However, no single factor can explain this sub-

stantial increase well. Previous evidence suggests that maternal antibiotic use during pregnancy may increase the risk of childhood asthma, but epidemiological studies remain limited and the results are inconsistent. In addition, most studies did not include the first trimester as the window of exposure. To assess the relationship between antibiotic exposure before and during pregnancy and risk of early childhood asthma, we conducted a prospective survey of 39,907 mother-child pairs. The results showed that maternal use of penicillin or chloramphenicol was associated with an increased risk of asthma in children at 7 years of age, and this effect was more significant when penicillin or chloramphenicol was used in the first trimester of pregnancy [18].

2.3.3.2 Perinatal Factors and Childhood Asthma

Cesarean section can save lives of pregnant women and fetuses, which is considered to be an important development of modern medicine. With the progress of social economy and the improvement of medical level, the cesarean section rate in China has been rising rapidly and maintained at a high level. At the same time, the incidence of childhood asthma in China is also increasing year by year. Previous studies have suggested that cesarean section may increase the risk of asthma and allergic diseases in children, but the potential confounding effect of medical indications for cesarean section cannot be ruled out. In order to assess the relationship between cesarean section (CS) itself (without indication) and the risk of childhood asthma and allergic rhinitis, a cluster random sampling was conducted in 26 elementary schools in Shanghai, China, in 2014, and a total of 12,639 children were included in the analysis. The results showed that CS without medical indications was associated with an increased risk of childhood asthma and allergic rhinitis, and CS with fetal complications was associated with an increased risk of childhood allergic rhinitis. Combined with early infant feeding practices, it was found that breastfeeding could reduce these risks. Our asthma case study confirmed this association [19, 20].

2.3.4 Child Neurodevelopment

DOHaD theory believes that the uterine environment can affect the lifelong health trajectory of the offspring. In this process, the placenta provides oxygen and nutrition, acts as a neuroendocrine organ, and plays a key role in early life planning. It also mediates a complex series of maternal-fetal interactions, such as the integration of nutrients and prestress signals into chromatin changes. Thus, damage to the placental microenvironment affects these processes and disrupts fetal brain development. A number of studies have linked the morphological and histopathological features of placental abnormalities to a wide range of adverse neurodevelopmental outcomes. We prospectively collected information on placental pathology and assessed neurodevelopment in children at the age of 8 months, 4 years, and 7 years. It was found that placental inflammatory pathology reduced motor and psychological scores at 8 months and was associated with low IQ at 4 years of age; however at 7 years of age, this association was weakened. Mediation analysis showed that this effect had nothing to do with gestational age and was a direct effect of placental inflammatory pathology [21].

Thyroid hormones (T4 and T3) are essential for growth and neurodevelopment of fetuses. From the second trimester of pregnancy, the fetal hypothalamic-pituitary-thyroid axis gradually functions. Thyroid peroxidase antibodies (TPOAb) are thyroid autoantibodies, and neonatal TPOAb is mainly derived from the mother. Positive TPOAb in cord blood is associated with a higher risk of autoimmune thyroiditis in children and adolescents. The TSH level in cord blood elevates in neonate who has experienced intrapartum stress, including induced labor, long labor, and vaginal delivery. However, association between maternal factors (including pregnancy-induced hypertension, preeclampsia, gestational diabetes mellitus (GDM)) as well as birth outcomes and neonatal thyroid hormones were not consistent. Therefore, it is necessary to pay attention to thyroid hormones in cord blood of children in China and to explore potential perinatal-related factors. Nine hundred and

twenty-two mother-infant pairs in Shanghai prospective birth cohort from 2012 to 2013 were included, and the concentrations of FT3, FT4, TSH, and TPOAb in neonatal cord serum were measured. It was found that FT3 in neonatal cord serum was higher and TSH was lower with cesarean delivery compared with vaginal delivery. In addition, the older the maternal age at delivery, the lower the FT3 level. Further studies are needed to understand whether this association may mediate the adverse effects of advanced maternal age on early neurodevelopment in the offspring [22]. Subsequently, we further explored the association of thyroid hormones in cord blood with neurodevelopment at 2 years of age. It was found that boys in the lowest FT4 group had a 5-point decrease in the communication domain, a 3.25-point decrease in the fine motor domain, and a 3.84-point decrease in the personal social domain compared with infants with intermediate thyroid hormone levels; boys in the highest FT3 group had a 4.46-point increase in the personal social domain; however, these associations were not observed in girls. No association was found between TSH in cord serum and neurodevelopment at 2 years of age [23].

Vitamin D deficiency in pregnant women has been identified as a significant public health problem. During pregnancy, the fetus obtains vitamin D from the mother entirely through the placenta. More and more evidences suggest that vitamin D deficiency may also contribute to offspring obesity by regulating inflammation, lipogenesis, and adipocyte secretion. Some longitudinal studies in Europe have found that maternal vitamin D deficiency during pregnancy is associated with greater fat mass in the offspring during childhood. At the same time, animal studies have also shown that reduced vitamin D concentrations during pregnancy can affect neuronal differentiation and brain development, thereby increasing the risk of abnormal behavior in offspring. In humans, however, the evidence remains limited and inconsistent. To explore the association between maternal vitamin D and vitamin D in cord blood during pregnancy, in a Shanghai prospective cohort study, we examined vitamin D levels in maternal in the first, second, and third trimesters and in cord blood, respectively. It was found that neonatal vitamin D levels in cord blood were positively correlated with maternal serum vitamin D levels during pregnancy, with the strongest correlation in the third trimester [24]. Further combined with the growth in infancy, obesity, and neurodevelopment at 2 years of age, the analysis revealed that vitamin D concentration in cord blood has nothing to do with the growth and neurodevelopment in infancy, suggesting that the role of vitamin D in the early origin of obesity needs to be reconsidered [25].

2.3.5 Child Cardiovascular Health

Hypertension in pregnancy, including preeclampsia, are major complications affecting 5–10% of all pregnancies and are associated with maternal morbidity and mortality worldwide. Preeclampsia and gestational hypertension in pregnant women are important factors affecting perinatal health, including perinatal death, premature labor, and intrauterine growth restriction. In addition, many epidemiological and experimental studies have shown that the offspring of women with gestational hypertension have an increased risk of developing cardiovascular disease in later life. Several studies have shown that maternal preeclampsia may have a strong impact on the blood pressure (BP) of their children. Maternal preeclampsia may affect the future cardiovascular health of offspring through adverse intrauterine environment, which may lead to a variety of pathophysiological factors, such as angiogenic imbalance, immune response, and inflammation. Therefore, intervention for preeclampsia during pregnancy may be a way to affect blood pressure in the offspring.

For decades, low-dose aspirin has been widely recommended for the secondary prevention of cardiovascular diseases in adults, as well as for the treatment of gestational hypertension and obstetric antiphospholipid syndrome. It is found in a series of randomized trials and multiple meta-analyses that the use of low-dose aspirin during pregnancy can reduce the risk of

preeclampsia and other placenta-mediated complications, including intrauterine growth restriction and premature labor. Although the biological mechanism of these benefits is unknown, aspirin is thought to promote placental implantation and growth. Some studies have further shown that early prophylactic aspirin initiation is associated with better effect. Aspirin given to pregnant women can cross the placenta into the fetal circulation. In addition to the benefits for placental development, it is unclear whether aspirin also affects fetal vascular development.

Based on a prospective cohort study, women were divided into high, intermediate, and low risk according to their medical history, and the time of aspirin exposure was divided into 4 weeks before the last menstruation, the first trimester, the second trimester, and the third trimester of pregnancy. The analysis found that aspirin use during pregnancy could reduce the risk of maternal hypertension, and early aspirin use in women at high risk was associated with a lower incidence of preeclampsia/eclampsia. At the same time, the protective effect of aspirin against term preeclampsia/eclampsia and gestational hypertension might continue into the third trimester of pregnancy [26]. The children were continuously followed up to 7 years of age, and it was found that aspirin use during pregnancy could reduce the risk of elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP) at 7 years of age, with a mean decrease in SBP of 0.62 mmHg, and the earlier aspirin use during pregnancy, the lower the risk of hypertension in children [27].

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3 *In Utero* Pediatrics in Maternal-Fetal Medicine

Lei Wang, Xing-Wei, Hong Zhu, and Lu-Ming Sun

3.1 Development of Maternal-Fetal Medicine

Since the 1980s, with the popularization of the concept of "the fetus as a person and the sick fetus as a patient" and the rapid development of prenatal imaging, molecular genetic diagnosis, and *in utero* treatment technologies, fetal medicine has emerged as a branch of obstetrics and an important research component of maternal-fetal medicine. In the era of maternal-fetal medicine, the target of prenatal screening and diagnosis include not only fetal genetic diseases and structural abnormalities but also complex twins/multiple pregnancy, maternal-fetal alloimmunization, maternal vascular malperfusion (MVM), viral infection, and all other diseases that may affect the health of the fetus. The purpose of prenatal screening and diagnosis is no longer to detect serious birth defects and terminate pregnancy, but to use various techniques to provide accurate prenatal diagnosis, genetic counseling, close monitoring for the high-risk fetus, possible fetal therapies, etc. so as to minimize perinatal mortality and improve the quality of life (QoL) after birth while ensuring maternal safety.

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3.2 Role of Maternal-Fetal Medicine Specialists in Diagnosis and Treatment of Fetal Structural Abnormalities

As the leader of multidisciplinary consultation, maternal-fetal medicine specialists are versatile medical professionals who have received maternal-fetal medicine subspecialty training and need to have many professional skills such as prenatal imaging diagnosis, invasive prenatal sampling and genetic counseling, fetal therapy, *in utero* monitoring and delivery of the affected fetus, and management of high-risk pregnancy. In the multidisciplinary consultation, the main roles of fetal medicine specialists include the following:

1. To ensure maternal safety: the mother is an important participant, decision-maker, and recipient for *in utero* diagnosis, and treatment of fetal diseases, sometimes even the patient (e.g., mothers suffer from genetic diseases with mild phenotype), the mother must be placed in the "primary" position in the diagnosis and treatment of fetal diseases. As the

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guardian of maternal safety, fetal medicine specialists should take the safety and health of the mother as the primary prerequisites for all *in utero* diagnosis and treatment.
2. To make an accurate diagnosis for fetal diseases: use evaluation methods such as prenatal imaging and molecular genetics to exclude potential concomitant structural or genetic abnormalities and provide disease-oriented multidisciplinary consultation after precise prenatal diagnosis.
3. To perform prenatal monitoring and perinatal management: depending on the severity of fetal structural and developmental abnormalities, fetal medical specialists give individualized prenatal monitoring and necessary *in utero* intervention and decide the timing, mode, and location of delivery.

3.2.1 Prenatal Screening for Fetal Structural Abnormalities

Ultrasonography is the most commonly used noninvasive and reproducible examination method in screening for fetal structural abnormalities. The prenatal ultrasound screening is mainly to assess fetal growth and development and detect severe structural abnormalities. It is essential in reducing birth defects and improving the quality of birth population. The screening is usually divided into the first trimester (11 to 13 + 6 weeks of gestation) and the second trimester (20 to 24 + 6 weeks of gestation). The accuracy of prenatal ultrasound screening is affected by many factors including gestational week, fetal position, amniotic fluid volume, obscure of observation site caused by the thick abdominal wall fat of pregnant women, and the gradual development and changes of fetal organs (some fetal malformations are formed or manifested in the second and third trimesters). All these factors may cause false screening results. In addition, there may be artifacts in ultrasound images. Therefore, prenatal ultrasound screening cannot detect all fetal malformations.

Ultrasound screening in the first trimester should be performed from 11 to 13 + 6 weeks of pregnancy. It includes confirmation of embryo/fetal viability, accurate assessment of gestational age, determination of the number of fetuses, identification of amnioticity and chorionicity in multiple pregnancies, comprehensive assessment of fetal anatomy in the first trimester, screening for aneuploidy, evaluation of the uterus and adnexa, and measurement of fetal crown-rump length (CRL) and nuchal translucency (NT) thickness. About 40–66% of fetal abnormalities are identified in the first trimester. Transvaginal ultrasonography shows specific structures with greater clarity. A combination of transabdominal and transvaginal ultrasonography improves the detection rate of structural abnormalities. The detection of severe abnormalities in the first trimester provides families with earlier prenatal diagnosis and pregnancy options, but ultrasonography in the first trimester should not replace the routine structural screening in the second trimester because some organs are not mature in the first trimester and not all fetal structural abnormalities are visible in the first trimester. When fetal structural abnormalities are identified or suspected in ultrasonography during the first trimester, a referral should be made in time for further assessment and counseling [1].

Ultrasound screening for fetal structural abnormalities in the second trimester has become a standard procedure for fetal anatomical assessment. ISUOG recommends that the second trimester ultrasound screening should be provided for all pregnant women at 18–24 weeks of gestation. In this period, the fetal anatomical structure has been formed, the fetal size and amniotic fluid are moderate, the influence of bone echo is small, the image is clear, and most severe fetal malformations are manifested. Routine ultrasonography in the second trimester assesses cardiac impulse, number of fetuses (and chorionic and amniotic membranes in the case of multiple pregnancy), gestational age/fetal size, basic fetal anatomy, placental appearance and location, and amniotic fluid volume [2].

In addition to screening for abnormal fetal anatomy and assessment of the number and size of fetuses, placenta, amniotic fluid, and length of the cervix, ultrasonography during the second

trimester can also screen for soft markers of aneuploidy, such as thickened nuchal soft tissue, absent or hypoplasia of nasal bone, and aberrant right subclavian artery [3].

The sequence and content of fetal structure screening in the second trimester are as follows:

Head: intact skull and normal head shape, septum pellucidum, choroid plexus, cerebral falx, thalamus, lateral ventricles, cerebellum, cerebellomedullary cistern, and nuchal soft tissue
Face: visible bilateral orbits and eyeballs, normal facial profile of the midline sagittal section, normal nasal bone, and intact upper lip
Neck: no mass
Chest: normal shape/size of chest cavity and lung without diaphragmatic hernia
Heart: normal four-chamber cardiac view, normal heart position (left atrium and left ventricle are located on the left side), normal size and position of aortic and pulmonary artery outflow tract, normal left ventricular outflow tract, and normal three-vessel view or three vessel and trachea view
Abdomen: normal position of stomach bubble (located on the left side), normal intestinal canal (no dilation or echo enhancement), gallbladder on the right side, visible bilateral kidneys (no renal pelvis dilatation), normal bladder, and normal insertion site of umbilical cord in abdominal wall
Bone: no spinal defect or mass (transverse section and sagittal section), visible bilateral upper arms and hands, visible bilateral lower limbs and feet, and normal joint positions
Placenta: position of the placenta, and relationship between placenta and cervix, no mass
Umbilical cord: three vessels and normal umbilical cord insertion site
External genital organs: normal male or female external genital organs
Cervix: normal length of cervix

Although fetal structural screening in the second trimester systematically screens fetal anatomy, not all fetal structural abnormalities can be ruled out. The incidence of major fetal congenital structural abnormalities in all pregnancies is about 2–3% [4]. Most identifiable structural abnormalities can be detected with two-dimensional ultrasound, but some conditions such as facial clefts, spinal abnormalities, and brain midline structural abnormalities can be further evaluated by three-dimensional ultrasound [5, 6]. The detection rate of fetal structural abnormalities by ultrasound before 24 weeks is between 16% and 56% [7–9]. The detection rate of serious and fatal abnormalities (84%) is high [8, 10]. The screening sensitivity varies with the organ system involved, the detection rate of lung and central nervous system abnormalities (82%) is high, and the detection rate of cardiac abnormalities (13%) is low. The false positive rate of screening is 0.6–5.3%, with hydronephrosis and pleural effusion resolved during pregnancy being the most common [11, 12]. Although ultrasound screening in the second trimester can detect most fetal structural abnormalities, there is still a possibility of missed diagnosis. As fetal growth and development is a continuous process, not all abnormalities are manifested in the second trimester, and this should be fully informed in the fetal structural screening.

3.2.2 Prenatal Diagnosis of Fetal Structural Abnormalities

If fetal structural abnormalities are found during ultrasound screening in the first and second trimesters, the pregnant women should be referred to a level III diagnosis and treatment institution or a regional fetal medical center for further assessment. The assessment is to collect information such as family medical history and obstetric history, more detailed fetal anatomical examination findings under ultrasound or MRI to identify the type (such as isolated or non-isolated) and severity of structural abnormalities, provide genetic counseling to discuss the available genetic testings and the advantages and disadvantages of different options, provide multidisciplinary consultation to discuss the prognosis of fetal structural abnormalities, and possible *in utero* treatment and postnatal treatment regimen for selection.

Imaging diagnosis includes diagnostic ultrasound and MRI. Diagnostic ultrasonography is to provide further targeted, systematic, and comprehensive ultrasonography for high-risk fetuses detected by prenatal ultrasound screening and to provide a basis for prenatal consultation and establishment of diagnosis and treatment regimen. With the introduction of real-time ultrasound and the continuous improvement in the accuracy of scanning images, most of the fetal structural abnormalities can be detected in prenatal ultrasound screening in the first and second trimesters, and the extent and nature of the lesions can also be evaluated effectively, which makes ultrasonography the most commonly used method for prenatal diagnosis of fetal structural abnormalities. MRI has become an effective supplementary method for prenatal ultrasound diagnosis due to its high soft tissue contrast, high resolution, multidirectional imaging capability, and large imaging field of view. Especially for the pregnant women with concomitant oligohydramnios, poor fetal position, or excessive obesity, it is ideal to compare the fetal structural examination with ultrasound findings. Fetal abnormalities that can be assessed by MRI include central nervous system (CNS) abnormalities, fetal head and neck tumors, thoracic masses, digestive system disorders, congenital heart disease, urinary system disorders, and adrenal neuroblastoma. Especially for the assessment of CNS abnormalities, the diagnostic accuracy of prenatal ultrasound is 68%, while that of MRI is 93% [13]. Before *in utero* surgery, both the examination methods is also helpful to accurately evaluate the *in utero* condition of the fetus. For example, in the evaluation of congenital diaphragmatic hernia, LHR and O/E LHR results by ultrasound and FLV and O/E FLV results by MRI in combination with the specific gestational age, herniated contents, and location can provide an important reference for the selection of suitable population for diaphragmatic hernia treatment and comprehensive judgment of the condition.

Invasive sampling techniques in prenatal diagnosis Invasive prenatal sampling techniques include amniocentesis, chorionic villus sampling, percutaneous umbilical cord blood sampling, and fetal tissue biopsy, among which amniocentesis and chorionic villus sampling are the most commonly used. At present, amniocentesis is the most widely used and relatively safe sampling technique, which is usually performed after 15 weeks of gestation. Fetal cells, DNA, or metabolites in amniotic fluid can be used to diagnose genetic or infectious diseases. The procedure-related complications such as fetal loss (0.1–1%), fetal damage, hemorrhage, chorioamnionitis, and amniotic fluid leakage are rare. Chorionic villus sampling (CVS) is a sampling method for genetic diagnosis of fetus in the first trimester (after 10 weeks of gestation). There are two sampling paths, i.e., transabdominal and transcervical paths. Complications associated with CVS procedure are also rare. The fetal loss rate in the transabdominal CVS performed by experienced physicians is similar to amniocentesis in the second trimester. Confined placental mosaicism occurs in approximately 1% of CVS cases, resulting in ambiguous genetic test results that require amniocentesis for further examination.

Selection of prenatal genetic tests for fetal structural abnormalities. By conventional karyotyping analysis, the detection rates of abnormal karyotypes in fetal structural abnormalities in the first and second trimesters were 49% and 17%, respectively. With the development of prenatal genetic testing technologies, chromosomal microarray analysis (CMA) is used to detect the DNA copy number imbalances, especially the changes in the submicroscopic structure of chromosomes that cannot be identified by conventional karyotyping. In 2012, some scholars explored the value of CMA in the detection of prenatal fetal abnormalities and found that CMA could increase the detection rate by 6–7% compared with conventional karyotyping [14], and the additional detection rate by CMA in isolated renal and cardiac abnormalities was 15.0% and 10.6%, respectively. Therefore, the guidelines recommend CMA as the first-line test for prenatal genetic assessment of fetal structural abnormalities [15]. However, CMA has limitations, including the inability to detect most of

monogenic diseases. In recent years, with the application of exome sequencing (ES) in prenatal diagnosis of fetal diseases, the detection rate of fetal structural abnormalities by ES was analyzed, and it was 6.2–80% due to the detection indications (e.g., structural abnormalities in a single system or multiple systems) and the differences in data interpretation by different laboratories [16–23]. The detection rates of fetal cardiac structural abnormalities, urinary system abnormalities, and central nervous system abnormalities are 10%, 12.3%, and 44%, respectively. Therefore, current guidelines recommend that when CMA or karyotype is normal, ES can be provided to fetuses with structural abnormalities [24]. Whole genome sequencing (WGS) has a short detection cycle; in addition to the detection of copy number variations with a resolution higher than CMA, it can also detect protein coding variations that may be missed by ES. With the reduction of costs and the deepening of noncoding DNA research, WGS shows significant advantages over ES. Therefore, WGS may become the major tool for prenatal genetic assessment of fetal structural abnormalities in the future.

3.2.3 Principles and Classification of *In Utero* Treatment of Fetal Structural Abnormalities

The purpose of *in utero* treatment is to prevent irreversible fetal damage or death caused by further deterioration of the disease, provide opportunities for postnatal diagnosis and treatment, and improve prognosis. The diseases of fetal structural abnormalities that need *in utero* intervention are very limited. Before initiation of *in utero* treatment, it is necessary to make accurate diagnosis and stage of fetal diseases and follow (1) the favorable principle, the treatment can only be implemented when the benefits outweigh the risks; (2) the necessary principle, the surgeon must fully evaluate whether *in utero* treatment is necessary; (3) the effective principle, definite evidence must be available to demonstrate that *in utero* treatment is effective for fetal diseases; (4) the principle of impartial consulting, to inform the current situation of *in utero* treatment at home and abroad and the risks and benefits to the mother and the baby without tendency; (5) the voluntary principle, the treatment must be implemented with the informed consent of pregnant women and their families; and (6) the ethical principle, the treatment must be subject to ethical discussion.

Classification of *in utero* treatment: It can be divided into drug therapy and surgical treatment according to the treatment methods, and into *in utero* treatment for fetus and *in utero* treatment for fetal appendages according to the surgical site. Based on the level of evidence in EMB, it can be divided into (1) *in utero* treatment supported by randomized controlled clinical studies, such as fetoscopic laser coagulation of placental anastomoses for twin-to-twin transfusion syndrome (TTTS), open surgery for fetal meningomyelocele, and fetal endoscopic tracheal occlusion for severe congenital diaphragmatic hernia; (2) *in utero* interventions with high awareness supported by rich clinical experience, but there is still a lack of multicenter randomized controlled clinical studies, such as fetal cardiac interventional for severe pulmonary/aortic valve stenosis or atresia; and (3) *in utero* treatment techniques that are still in the clinical exploration stage due to a few number of cases, such as *in utero* intervention for amniotic band syndrome.

3.2.4 Pregnancy and Delivery Management of Fetal Structural Abnormalities

The frequency of *in utero* follow-up, timing, and mode of termination of pregnancy for fetal structural abnormalities should be based on the nature and severity of the structural abnormality, the possibility of *in utero* progression, and the intervention options available before and after birth. *In utero* monitoring schemes and perinatal plans should be developed through multidisciplinary collaboration with pediatric teams, including neonatology and pediatric subspecialties.

***In utero* monitoring**: For the majority of fetal structural abnormalities that can maintain stable *in utero*, ultrasonography can be performed every 4 weeks to assess fetal growth, amniotic fluid volume, and Doppler blood flow changes. If there are manifestations such as fetal growth restriction or amniotic fluid abnormalities, the frequency of monitoring should be increased to every 2 weeks. For diseases that are likely to progress/deteriorate *in utero*, the frequency of *in utero* monitoring needs to be individualized. For example, in the case of a large congenital pulmonary airway malformation mass with the high risk of edema, the frequency of follow-up should be increased to once a week or even 2–3 times a week.

Delivery planning: For most fetal structural abnormalities that are hemodynamically stable *in utero*, delivery can be performed in institutions with appropriate care for the mother and newborn. For ductus-dependent congenital heart disease (such as hypoplastic left heart syndrome, interruption of aortic arch, and transposition of the great arteries), fetal tumors with hemodynamic instability and congenital diaphragmatic hernia that may require extracorporeal membrane oxygenation (ECMO), delivery should be scheduled in tertiary institutions with neonatal intensive care unit (NICU) and pediatric subspecialties. If the institution does not meet these conditions, transfer shall be arranged before delivery [25].

Timing and mode: For the majority of fetal structural abnormalities and under stable maternal or fetal condition, induced labor or planned cesarean section before 39 weeks is not recommended due to the risk of delivering an early-term newborn (before 39 weeks) [26]. There is no evidence that cesarean section can improve pregnancy outcome in fetuses with structural abnormalities, and the final delivery method should be based on obstetric indications [27]. For fetal structural abnormalities that may increase the risk of dystocia, rupture, infection, and bleeding during vaginal delivery, such as large omphalocele, giant sacrococcygeal teratoma, and severe hydrocephalus, the indications for cesarean section can be relaxed appropriately.

3.2.5 Care for "M" Cannot Be Ignored

The initial purpose of maternal-fetal medicine is to solve fetal and/or maternal problems equally and to combine the treatment of high-risk pregnancy with the diagnosis and treatment of fetal complications. However, due to obesity, diabetes mellitus, hypertension, increased cesarean section rate, and the widespread application of assisted reproductive technology in eldgravida and women of childbearing age with chronic diseases, the incidence of high-risk pregnancy continues to rise.

It is important to emphasize that chronic comorbidities and complications of pregnancy have a significant impact on the fetus at all stages of pregnancy and that fetal diseases cannot be completely separated from the mother, and that both pregnancy and abortion, *in utero* monitoring and intervention, timing and mode of delivery, and postnatal treatment, all depend on maternal participation. Maternal-fetal medicine is to provide diagnosis and treatment of fetal diseases under the premise of fully taking care of the mother. Its basic point of view is to ensure the fundamental safety of the mother. Maintaining the safety of the mother is the premise of all *in utero* diagnosis and treatment. Therefore, paying attention to "M" care in maternal-fetal medicine is essential for optimizing obstetric management and improving pregnancy outcome.

3.3 New Research Field

With the rapid development of prenatal diagnosis technology, the target of fetal-free DNA detection in maternal plasma has been gradually changed from the screening for aneuploidy to the screening for chromosomal structural abnormalities, genomic diseases, and even monogenic diseases. With the deepening of research, cffDNA or specific gene cfRNA may also be used as a molecular marker for noninvasive prenatal detection of pregnancy complications such as preeclampsia, premature labor, and fetal growth restriction. Artificial intelligence is also gradually

applied to the monitoring and management of maternal complications of pregnancy such as diabetes mellitus and premature labor, monitoring of *in utero* fetal conditions, fetal structure, and growth assessment. Future research areas in *in-utero* therapies include how to improve perinatal outcomes by use of accurate diagnosis on the new molecular genetic diagnostic technology platform and to explore indicators that can sensitively predict fetal functional diseases and long-term poor prognosis. In addition, embryonic stem cells and gene therapy also bring important treatment opportunities for some fetal and neonatal disorders, such as *in utero* treatment of hemophilia and skeletal system abnormalities.

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4 Effects of Maternal Environmental Exposure on Early Life Growth and Development

Cui-Ping Wang and Ying Tian

4.1 Overview

Environment is the basis of survival, reproduction, and continuous development for humans on the Earth. However, with the rapid development of industrialization and urbanization, significant amount of wastes is discharged into the environment during human production and daily-life activities, polluting the air, water, soil, and other natural environment. Pollutants released into the environment, especially persistent organics and heavy metals, circulate and accumulate in nature, posing serious threats to human health.

Pregnant women experience dramatic changes during pregnancy in terms of vascular physiology, metabolism, reproductive organs, endocrine activity, and immune system, some of which may increase the risk of fetal exposure to certain environmental pollutants. For example, lead enters the body and predominantly accumulates in bones, but can be mobilized during pregnancy in response to calcium release, increasing the risk of lead exposure in the fetus. Since fetal period is a critical stage of growth and development, characterized by active cell differentiation and proliferation, immature organs and systems (such as the incomplete blood-brain barrier), and lower detoxification enzyme activity than that of adults, fetuses are particularly susceptible to environmental pollutions. Excess xenobiotics may alter the developmental programming of the fetus, affecting the growth and development both *in utero* and after birth. This impact may even persist into the adulthood and pass down to the offspring for several generations.

With the development of Developmental Origins of Health and Disease (DOHaD) theory, an increasing number of researchers have been paying attention to the effects of maternal exposure to adverse environmental factors on the growth and development of the offspring. Advanced scientific technologies allow us to explore the underlying biological mechanisms, which is essential to achieve the goals of preventing and controlling of environmental pollution and improving the quality of population. In this section, we will discuss the effects of maternal exposure to common environmental pollutants (such as endocrine-disrupting chemicals, heavy metals, and air pollutants) on the growth and development of the offspring and the underlying mechanisms associated with these effects.

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4.2 Effects of Maternal Exposure to Environmental Endocrine-Disrupting Chemicals on the Growth and Development in Early Life

Environmental endocrine-disrupting chemicals (EDCs) refer to a class of chemicals widely distributed in the environment, which may interfere or inhibit the synthesis, secretion, transport, conjugation, reaction, and metabolism of hormones in the endocrine system as they mimic the physiological and biochemical effects of natural hormones, resulting in a variety of health damage. There are various EDCs, such as bioaccumulative perfluoroalkyl substances, polybrominated diphenyl ethers, organochlorine pesticides, heavy metals, degradable phenols (e.g., bisphenol A, triclosan, and parabens), phthalate esters, organophosphorus and pyrethroid pesticides, etc. Growing evidence suggest that EDCs may enter the fetus through the placenta following maternal exposure and interfere fetal growth and development programming and increase the risk of growth and development abnormalities, neurodevelopmental disorders, and metabolic diseases in the offspring.

4.2.1 Perfluoroalkyl Substances

Perfluoroalkyl substances (PFAS) are an emerging group of synthetic-persistent organic pollutants in which all hydrogen atoms linked to carbon atoms are substituted by fluorine atoms. PFAS exhibit hydrophobic and oleophobic properties with high-temperature resistance and high surface activity. They were originally used in waterproof and stain-resistant layers, and now have been widely used in the products of various industrial and household consumables such as materials for nonstick cookers, food packaging materials, waterproof and oil-proof coatings, furniture, carpets, and foam-extinguishing agents. However, the stable physical and chemical properties of PFAS also make them difficult to undergo degradation in the environment and organisms, which renders them the properties of obvious environmental persistence, long-distance migration, and bioaccumulation. As such, PFAS are extensively distributed in a variety of environmental media (e.g., different water body, sediment, household dust, atmospheric particles), as well as organisms such as aquatic/terrestrial organisms and humans [1, 2].

4.2.1.1 Population Exposure Level of Perfluoroalkyl Substances

According to EPA report, more than 9000 PFAS have been produced to date, with perfluoroalkyl carboxylic acids (e.g., perfluorooctanoic acid (PFOA)) and perfluoroalkyl sulfonic acids (e.g., perfluorooctane sulfonic acid (PFOS)) as the most representative species. Previous studies have demonstrated that exposure to long-chain PFAS, such as PFOA and PFOS, can cause dysfunction of multiple organs, and significantly increase the risk of adverse health outcomes such as liver and kidney dysfunction, obesity, cardiovascular disease, glucose and lipid metabolism abnormalities, thyroid dysfunction, and neurological and reproductive abnormalities in humans [3, 4]. At present, several countries have restricted the production and use of PFOA and PFOS [5]. In contrast, given the needs of promoting industrial and national economic development, China has not issued the corresponding laws and regulations to control PFAS production. It was in 2017 that PFOA was first included in China's list of chemicals with high pollution and environmental risk for management and control. At present, long-chain PFAS such as PFOA and PFOS are still being produced and used in China at a large scale. The Shanghai Birth Cohort Study conducted since 2013 revealed that the blood concentration of PFAS, especially the concentration of PFOA in women of childbearing age in Shanghai (19.97 ng/mL), was significantly higher than the values obtained from other regions both in China and abroad (Colorado, USA: 1.1 ng/mL; Canada: 1.7 ng/mL; Aarhus, Denmark: 2.0 ng/mL; and Korea: 2.7 ng/mL) [6].

4.2.1.2 Effects of Maternal Exposure to Perfluoroalkyl Substances on the Growth and Development in Early Life

Unlike other persistent organic pollutants that accumulate in fat, PFAS preferentially bind to proteins due to their hydrophobic and oleophobic properties and are mainly enriched in the blood, liver, muscle, and other organs [7]. Toxicology studies have shown that PFAS exposure can cause a series of health damages to the endocrine system, nervous system, immune system, and cardiovascular system [8]. However, studies evaluating the effect of prenatal PFAS exposure on early life growth and development are still limited.

PFAS can cross the placental barrier for maternal-fetal transmission. Fetuses are exposed to PFAS *in utero*, which may cause developmental toxicity and embryotoxicity, inducing spontaneous abortion, preterm birth, fetal death, ectopic pregnancy, birth defects, low birth weight, neonatal asphyxia, and other adverse pregnancy outcomes [9]. For example, Steenland et al. found that a 1-ng/mL increase in maternal PFOA in the first trimester was associated with a change in birth weight of −3.3 g (−9.6 g, 3.0 g), and a 1-ng/mL increase in maternal PFOA in the second and third trimesters was associated with a decrease in birth weight of −17.8 g (−25 g, −10.6 g). However, no significant correlation between maternal exposure to PFAS and birth weight was observed in the Shanghai Birth Cohort Study, whereas a significant negative correlation between the exposure to PFAS (PFOS, PFNA, PFDA, PFUA, and PFDoA) during the first trimester and body length at birth was found in this cohort, which was statistically significant only in female fetuses [10]. In addition, the results of the Danish National Birth Cohort Study showed that maternal exposure to PFAS (PFOA, PFHpS, and PFAS) was significantly associated with an increased risk of miscarriage, which was more pronounced in multiparous women. Meanwhile, maternal exposure to PFAS (PFOA and PFOS) was also significantly associated with an increased risk of preterm birth in this cohort study [11]. However, in a prospective nested case-controlled study in Shanxi Province and the Shanghai Birth Cohort Study, there were no significant correlation between maternal exposure to PFAS and preterm birth [12].

PFAS in pregnant women can cross the placental barrier and fetal blood-brain barrier and impair nervous system development in fetus. Animal studies have shown that intrauterine exposure to low doses of PFAS induced irreversible neurotoxic effects in offspring that persist into adulthood. Several prospective cohort studies have also demonstrated that maternal exposure to PFAS may affect the neurobehavioral development of the offspring. In the Shanghai Birth Cohort Study, Luo et al. noted that maternal exposure to PFAS during the first trimester significantly affected the neurobehavioral development in 2-year-old toddlers, as evidenced by the negative correlation between the maternal exposure to PFOS, PFNA, PFDeA, and PFUnDA and cognitive scores; the negative correlation between maternal exposure to PFNA, PFDeA, PFUnDA, and PFHxS and the language scores; and the negative correlation between maternal exposure to PFNA and PFUnDA and motor function scores in this population [13]. Similar findings have also been observed in other studies. For example, the Shanghai Minhang Birth Cohort Study demonstrated that exposure to PFAS (PFOS, PFSA, PFDA, and PFNA) during pregnancy was associated with increased risk of neurobehavioral (fine motor and problem-solving domains) development problems/delays in 6-month-old female infants and increased risk of neurobehavioral (personal-social skills) development delays in 4-year-old children [14]. The Danish National Birth Cohort Study demonstrated that exposure to PFAS (PFNA) during pregnancy was significantly associated with increased neurobehavioral development problems (externalizing behavioral difficulties) in children aged 7 and 11 years old [15]. At present, the mechanism by which PFAS effects on neurobehavioral development is not clear. Thyroid hormones (THs) play an important role in the temporal and spatial regulation of certain processes including neural cell proliferation,

migration, differentiation, and myelination as well as synapse formation during fetal nervous system development. Animal studies have shown that PFAS (PFOS) exposure during pregnancy was associated with decreased serum thyroxine (T4) and 3,5,3′-triiodothyronine (T3) in pregnant mice and also the total T4 in pups, which may impair the neural development of the pups. Population studies have demonstrated decreased free and total T4 levels with an elevated TSH level in serum obtained from mothers exposed to PFAS during pregnancy, while the levels of total T3 and total T4 in the umbilical cord blood decreased, suggesting that PFAS exposure affects maternal and fetal thyroid hormone homeostasis with subsequent effects on fetal neurobehavioral development. On the other hand, brain-derived neurotrophic factor (BDNF) is widely distributed in the central nervous system (CNS) and plays an important role in the survival, differentiation, growth, and development of neurons during the CNS development process. The Shanghai Birth Cohort Study showed that the PFAS (PFHxS) concentration in maternal blood during the first trimester of pregnancy was negatively correlated with the BDNF concentration in cord blood. It is speculated that PFAS may affect the neurodevelopment of the offspring by reducing the BDNF concentration in cord blood [16]. In addition, in vitro studies revealed that PFOS had direct effect on neurobehavioral development through changing the neurotransmitter types of the neurons by promoting the differentiation of PC12 cells into the acetylcholine phenotype subsequent to the depletion of dopamine phenotype [17].

Prenatal PFAS exposure can interfere with the growth and developmental programming, which will not only affect the short-term growth and development of the fetus but also cause irreversible changes in the structures and functions of the body, resulting in profound and even lifelong effects on human health [18]. The length-for-age Z score (LAZ), the weight-for-length Z score (WFL), and the BMI-for-age Z score (BAZ) are commonly used to evaluate the physical development of infants and toddlers. The follow-up results of the Shanghai Birth Cohort Study showed that prenatal PFAS exposure could adversely affect the physical development trajectory of the offspring after birth. Specifically, an increased concentration of PFHpA during pregnancy was negatively correlated with the LAZ of infants aged 0 to 1 year old; the PFBS concentration was negatively correlated with the WFL and BAZ; and the PFDoA concentration was positively correlated with the WFL and BAZ. In addition, the cohort study also found a positive association between PFBS exposure during pregnancy and the development of obesity in girls aged 5 and 7 years old. Maternal exposure to PFAS significantly increased the risk of overweight and obesity in the offspring [19–21].

4.2.2 Phenols

Bisphenol A (BPA) and nonylphenol are typical EDCs, which have endocrine disrupting effect as they are structurally similar to natural estrogen. As one of the most widely produced and used phenolic EDCs, BPA is used to make polycarbonate plastics and epoxies in a wide range of commodities, including food containers, sports equipment, medical and dental equipment, spectacle lens, and electronics. Human exposure to BPA is primarily derived from the diet. BPA is prone to hydrolysis and can be released from products such as food cans, beverage bottles, and baby bottles followed by entering the body via food. In addition, BPA in heat-sensitive paper or cosmetics is taken up into the body through skin absorption, while BPA in air and dust enters the body through breathing.

4.2.2.1 Population Exposure Level of Environmental Phenolic Endocrine-Disrupting Chemicals

Bisphenol A originated from the environment can accumulate in the human body through biomagnification. Multiple studies have shown that BPA was detected in human fluids (urine, blood, amniotic fluid, milk, etc.) and tissues. For example, the median concentration of BPA in the urine samples obtained from pregnant women was 0.48 μg/L in the Laizhou Bay Birth Cohort Study. In a study

conducted in Tianjin by Zhang et al., the blood and urine samples from 10 children, 50 adults (27 males), and 40 females (30 pregnant women) along with the blood samples from 30 fetuses were analyzed for BPA. The results showed that BPA was detected in 46% of all the blood samples, with a mean concentration of 0.19 μg/L; the maximum blood concentration of BPA (2.60 μg/L) was detected in children, and to a lesser extent in pregnant women (0.60 μg/L) [22].

4.2.2.2 Effects of Maternal Exposure to Environmental Phenolic Endocrine-Disrupting Chemicals on the Growth and Development in Early Life

Bisphenol A in pregnant women is delivered to their fetuses through placental transfer, which may affect the growth and development of the fetus and further contribute to adverse pregnancy outcomes. For example, a previous study indicated that exposure to BPA significantly increased the risk of recurrent pregnancy loss, which may be related to BPA exposure-induced oxidative stress and immune imbalance [23]. Huang et al. and Cantonwine et al. found that urinary BPA concentration was positively correlated with the occurrence of premature delivery. Huo et al. noted that elevated urinary BPA concentration in pregnant women was associated with an increased risk of low-birth-weight infants, and the association was more pronounced in female infants than that in male infants [24].

Animal studies have reported that BPA exposure in early life could induce neurobehavioral development problems in offspring, including hyperactivity, aggression, anxiety-like behavior, and decline in memory and learning abilities [25]. Similar findings have been obtained in the epidemiological studies. For example, Roen and Perera et al. demonstrated that BPA exposure during pregnancy was associated with neurobehavioral abnormalities in the male offspring, including the increase in emotional and aggressive behaviors and symptoms of anxiety and depression [26]. Braun et al. demonstrated a negative correlation between prenatal BPA exposure and executive function scores in the male offspring at 3 years of age, characterized by decrease in working memory and increase in internalizing and somatization behaviors [27]. The biological mechanisms underlying the effects of BPA exposure during pregnancy on the neurodevelopment in offspring have not been fully understood. Bisphenol A has previously been shown to act as an antagonist of thyroid receptors, leading to fetal hypothyroidism and retarded brain development by disrupting thyroid hormone signaling. Meanwhile, maternal exposure to BPA can adversely affect the neurobehavioral development in offspring by disrupting the synthesis, transport, and release of neurotransmitters (dopamine, serotonin, norepinephrine, and glutamate).

Maternal exposure to BPA may have long-term effects on the physical development in offspring. Studies have shown that BPA exposure during pregnancy may promote the progression of obesity by disrupting the metabolism of lipid and glucose in offspring, and ultimately induce the onset of diabetes [28]. In addition, the Shanghai Birth Cohort Study revealed that BPA exposure during pregnancy was associated with increased systolic blood pressure and diastolic blood pressure in 2-year-old female offspring and increased blood glucose in male offspring of the same age, suggesting that maternal exposure to BPA may increase cardiometabolic risk in offspring [29].

4.2.3 Effects of Maternal Exposure to Polybrominated Diphenyl Ethers on Early Life Growth and Development

Polybrominated diphenyl ethers (PBDEs) are a class of EDCs that contain bromine atoms and have excellent flame retardancy, which have been widely used in electronic equipment, furniture, and industrial products (such as plastics and textiles) since the 1960s. Compounds in this class are characterized by high stability, high lipid solubility, and long-distance migration, which can be distributed into air, soil, water, and other environmental media for a long time and enter the human body through the food chain and other possible pathways.

4.2.3.1 Population Exposure Level of Polybrominated Diphenyl Ethers

At present, PBDEs have been completely banned in Europe, Canada, and the United States. In 2014, some of the PBDEs (tetrabromodiphenyl ether, pentabromodiphenyl ether, hexabromodiphenyl ether, and heptabromodiphenyl ether) in electronic products were banned by the China's Measures for the Control of Pollution from Electronic Information Products, but the use of PBDEs in other products was not prohibited. However, as PBDEs exhibit high stability and lipid solubility, humans would be exposed to PBDEs for a long time through food chain transmission and the biomagnification. The waters in the East China Sea and the Pearl River Basin have been heavily polluted by PBDEs, with a level of 12.7–7361.0 ng/g on average. At present, PBDEs have been detected in human tissues, including the liver, fat tissues, blood, and milk. In the south bank of Laizhou Bay in Bohai Sea, one of the largest production areas of brominated flame retardants in China, the median serum PBDE concentration in pregnant women was 22.91 ng/g lipid, which was higher than that of Guangzhou (4.40 ng/g lipid). In cities with more concentrated e-waste industry, such as the Lu Qiao District of Taizhou (30.38 ng/g lipid) and Wenling (123.70 ng/g lipid), the corresponding PBDE concentrations were higher than those in Hong Kong (5.25 ng/g lipid) and Tianjin (3.99 ng/g lipid) [30].

4.2.3.2 Effects of Maternal Exposure to Polybrominated Diphenyl Ethers on the Growth and Development in Early Life

Previous studies have shown that exposure to PBDEs during pregnancy may result in PBDEs entering the fetus through the blood-brain barrier and placental barrier, and affecting the growth and development of the fetus. Epidemiological studies have demonstrated that PBDE exposure during pregnancy can significantly increase the risk of adverse pregnancy outcomes, such as preterm birth and low birth weight [31]. The results of the Laizhou Bay Birth Cohort Study showed that maternal exposure to PBDEs (BDE-28 and BDE-100) was negatively correlated with the birth length and birth weight in offspring [30].

Animal studies indicated that during the critical period of brain development, exposure to PBDE congeners at a similar level to humans caused irreversible damage to the brain function of the animals, which were manifested as abnormal locomotor activity and a decline in learning and memory abilities. Similar findings have been observed in epidemiological studies. The results of the Laizhou Bay Birth Cohort Study showed that exposure to PBDEs (BDE-99) during pregnancy was significantly associated with poor neurobehavioral development (language and social-emotional responses) in toddlers at 2 years old [32]. The mechanisms underlying the effects of PBDE exposure on neurobehavioral development in the offspring have not been fully understood. Thyroid is one of the target organs for PBDEs, and some of the PBDE congeners can alter or mimic the effects of thyroid hormones. Maternal exposure to PBDEs, regardless of at low or high levels, can lead to thyroid hormone imbalance [33]. In addition, PBDEs also affect the gene expression of BDNF by influencing the methylation of this gene, and thus impair the neurobehavioral development of the offspring [34].

In addition to neurodevelopmental toxicity, maternal exposure to PBDEs may also affect the physical development of the offspring. The Laizhou Bay Birth Cohort Study showed that exposure to PBDEs in the maternal blood during the third trimester of pregnancy may affect the physical development of 8-year-old children, characterized by increase in body weight, body height, and waist circumference. With stratification by gender, it was found that the PBDE concentration was positively correlated with body height and waist circumference of boys, and the association was not statistically significant in girls, suggesting a gender difference in the effect of maternal PBDEs on the offspring's growth and development. The underlying mechanisms for the gender differences remain unclear. As a class of known EDCs, PBDEs may disturb the sexual-hormone-secreting system by acting on the steroid receptors and thus affect the growth and

development of the offspring, while the sex dimorphism in the distribution of sex steroids and their receptors in the central nervous system and lipid-rich tissues may account for the gender difference in PBDE exposure effects.

4.2.4 Effects of Maternal Exposure to Phthalates on the Growth and Development in Early Life

Phthalates (PAEs) are the most widely used plasticizers in hundreds of products such as toys, food-packaging materials, medical blood bags and latex tubes, vinyl flooring and wallpaper, detergents, lubricating oils, and personal care products such as nail polish, hair sprays, soaps, and shampoos. Since PAEs bind to the polymer matrix via weak hydrogen bonds or van der Waals force without formation of strong covalent bonds, they are easily released and migrated into the surrounding environments. Phthalates can interfere with the endocrine system and induce adverse health outcomes, as their chemical structures are similar to that of natural estrogen.

4.2.4.1 Population Exposure Level of Phthalates

Foods and drinking water are the most important routes of exposure to PAEs. The Hygienic Standard for Use of Additives for Food Containers and Packaging Materials (GB9685-2008) specifies the maximal residual amount of diethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), and dibutyl phthalate (DBP) in food and food additives, which are 1.5 mg/kg, 9.0 mg/kg, and 0.3 mg/kg, respectively. The phthalates that entered the body are rapidly hydrated and metabolized, with the metabolites primarily excreted in urine. Therefore, the concentration of phthalate metabolites in urine is often used as a measure of phthalate exposure. In a survey conducted in Wuhan, China, the levels of DEHP metabolites in urine samples of pregnant women in the first, second, and third trimesters of pregnancy were 100.2 ng/mL, 84.1 ng/mL, and 104.2 ng/mL, respectively, which were much higher than the maternal exposure level in Japan (13.2 ng/mL) [35, 36].

4.2.4.2 Effects of Maternal Exposure to Phthalates on the Growth and Development in Early Life

Previous studies have shown that phthalates and their metabolites were detected in biological samples such as placental tissue, amniotic fluid, umbilical cord blood, and meconium, indicating that PAEs could cross the placental barrier and exert direct impacts on the fetus following maternal exposure. Phthalates have antiestrogenic and/or anti-androgen activities. Epidemiological studies have shown that exposure to PAEs could interfere with the endocrine processes of multiple sexual hormones in humans including estrogen, testosterone, progesterone, follicle-stimulating hormone, and luteinizing hormone, which are essential for the maintenance of normal pregnancy [37]. In addition, maternal exposure to PAEs can also induce inflammatory response and oxidative stress, alter genome-wide DNA methylation in the placenta and angiogenesis, and increase the risks of adverse pregnancy outcomes such as preterm birth and low birth weight [38].

Animal studies have shown that maternal exposure to PAEs (DEHP) could affect neuronal migration and neurite outgrowth in the offspring, and therefore induce neurobehavioral abnormalities, as evidenced by a decline in environmental exploration ability [39]. The effects of maternal exposure to PAEs on the neurobehavioral development of the offspring have also been observed in epidemiological studies. For example, a cohort study in Korea showed that maternal exposure to PAEs was associated with decreased mental development index (MDI) and psychomotor development index (PDI) in 6-month-old infants [40]. The results from a Norwegian cohort study showed that maternal exposure to PAEs (DEHP) was significantly associated with an increased risk of attention deficit hyperactivity disorder (ADHD) in 3-year-old children [41]. Based on the results from animal studies, PAEs could display developmental toxicity by decreasing the expression of T3, T4, FT3, and FT4 [42].

In addition, animal studies have also shown that maternal exposure to PAEs increased the risk of obesity and metabolic syndrome in the offspring. Significant positive associations between maternal exposure to PAEs and offspring obesity were observed in the US and Korean cohort studies [43, 44]. However, some studies suggest that PAEs may disturb the normal growth of children, rather than induce obesity, Lee et al. observed a significant negative association between the prenatal exposure to DEHP and the BMI Z score of the offspring in the meta-analysis, while no significant association between the prenatal exposure to DEHP and the body fat percentage of the offspring was observed [45]. The mechanism underlying the effects of maternal exposure to PAEs on the metabolism of the offspring requires further investigation.

In summary, a series of animal studies and epidemiological investigations have demonstrated that environmental EDCs including perfluoroalkyl substances, phenols, polybrominated diphenyl ethers, and phthalates could cross the placental barrier and enter the fetus following maternal exposure, interfere with normal fetal growth and development programming, and increase the risk of adverse pregnancy outcomes, neurodevelopmental abnormalities, and physical development abnormalities in the offspring. At present, China is still a leading country in the global production and application of perfluoroalkyl substances, phenols, polybrominated diphenyl ethers, phthalates, and other environmental EDCs. Therefore, determining the population exposure level of such pollutants and the corresponding health risks can promote the pollutant management in China.

4.3 Effects of Maternal Exposure to Heavy Metals on the Growth and Development in Early Life

Heavy metals generally refer to metals and metalloids with a density greater than 4.5 g/cm^3 (e.g., lead and mercury, arsenic). In recent years, due to the rapid industrialization and continuous mining, smelting, and processing of various minerals, a large number of heavy metals have been discharged into the soil, rivers, lakes, and oceans. Heavy metals in the environment cannot be degraded, and they enter animals and plants through water, soil, and other pathways and accumulate in the human body through food chain amplification, endangering public health. Studies have shown that heavy metals can cross the placental barrier and blood-brain barrier, accumulate in fetal tissues, and exert adverse effects on fetal growth and development.

4.3.1 Lead

Lead exposure is mainly attributed to the recycling of electronic waste and lead-acid batteries, environmental pollution resulting from poor regulation of lead mining and smelting, traditional therapies containing lead, food containers coated with lead-containing ceramic glazes, lead-containing pipelines and other plumping fittings, and lead-containing coatings. Lead compounds can enter the human body through the digestive tract, respiratory tract, and skin. Following the absorption of exogenous lead into the blood, it is partly excreted through the kidney and digestive tract. A small fraction of the lead remaining in the body accumulates in the brain and liver, while approximately 95% is accumulated in the bone with few or no clinical symptoms. There is increased demand for calcium during pregnancy due to the growth and development of the fetus, and the lead existed in the maternal bone will be released into the blood subsequent to the calcium mobilization in the bone, resulting in increased blood level of lead in pregnant women. In that case, the fetus is not only exposed to the lead that enters the maternal body from environment during pregnancy (exogenous lead) but also to the lead that originally accumulates in the bones of the maternal body (endogenous lead).

4.3.1.1 Population Exposure Level of Lead

The Shanghai Birth Cohort Study showed that the geometric mean blood concentration of lead in the pregnant women in Shanghai was 1.47 μg/

dL, which was lower than that in the Norwegian study (2.5 μg/dL) and Laizhou Bay Birth Cohort Study (3.2 μg/dL) and similar to that of pregnant women in South Africa (1.4 μg/dL). Approximately 20% of the pregnant women enrolled in this study had a blood lead concentration of >2 μg/dL. The lead concentration in the neonatal cord blood was 1.34 μg/dL, and about 17% of these newborns had concentrations exceeding 2 μg/dL [46].

4.3.1.2 Effects of Maternal Exposure to Lead on the Growth and Development in Early Life

Lead can be transferred from mother to fetus through the placenta, which may cause direct damage to the placental tissue, and consequently have an adverse effect on fetal growth and development. Epidemiological studies have shown that exposure to lead could give rise to adverse pregnancy outcomes such as miscarriage, stillbirth, malformation, preterm birth, and low birth weight. In the Laizhou Bay Birth Cohort Study, the mean lead concentrations in maternal blood and neonatal cord blood were 3.20 μg/dL and 2.52 μg/dL, respectively. Even at such low levels of lead exposure, significant negative associations of maternal lead level with birth weight and birth length were still observed [47].

The effects of maternal exposure to lead on neurodevelopmental toxicity are mainly generated through three biological pathways. First, studies have shown that lead exposure elicited various pathological responses such as oxidative stress, lipid oxidation, mitochondrial damage, and excitotoxicity in the neurons, which eventually contribute to neuronal necrosis or apoptosis. Second, lead exposure could affect cell signaling by interfering with calcium metabolism. Under normal physiological conditions, protein kinase, adenosine cyclophosphate, and phosphodiesterase are activated in response to calmodulin activation by calcium ions, among which protein kinase C is involved in the differentiation, proliferation, and long-term potentiation of neurons. Following excessive lead exposure, calcium ions are replaced by lead with the resulting loss of function as the second messenger, which will ultimately have an adverse effect on the normal physiological activity of neurons. Finally, lead exposure may also contribute to changes in neurotransmitters and their receptors, such as affecting the synthesis, release, and storage of acetylcholine, dopamine, and amino acid neurotransmitters. For example, the cholinergic system can affect learning and memory functions through its regulatory effect on hippocampal synaptic transmission, while lead exposure damages the muscarinic receptors and causes disorders in hippocampal synaptic transmission, resulting in lead-induced memory dysfunction [48]. In 2010, the US Centers for Disease Control and Prevention recommended that the blood lead levels in pregnant women should be less than 10 μg/dL to mitigate neurodevelopmental toxicity to infants. However, even if the blood lead concentration is lower than this cut-off value, there are still adverse effects on the cognitive and motor development in the offspring. Therefore, the American Academy of Pediatrics recommends that all children with blood lead concentrations above 5 μg/dL should receive appropriate management [49]. However, although the mean blood concentration of lead in pregnant women from the Shanghai Birth Cohort Study was 1.47 μg/dL, which was well below the above cut-off value for management, this study also demonstrated that low-level maternal lead exposure significantly affected neurobehavioral development of 2-year-old children indicated by decreased scores in social-emotional skills [46]. Therefore, the monitoring and management of lead exposure should be further strengthened, and the use of lead in production and daily life should be strictly restricted, so as to protect the population from the harm of lead exposure.

4.3.2 Mercury

The main sources of anthropogenic mercury emission include mining, the combustion of coal and oil, and the emission of industrial waste gases, waste liquids, and waste residues that contain mercury. Mercury is a liquid metal at room temperature, displaying the characteristics of

migration, persistence, bioaccumulation, and biological toxicity. There are three forms of mercury in the environment, including the organic mercury (predominantly methylmercury), inorganic mercury, and metallic mercury. Methylmercury and inorganic mercury are readily absorbed, which enter the human body directly or indirectly through mercury-polluted water, mercury-polluted atmosphere, and mercury-polluted soil as well as mercury-enriched food chain (such as shellfish or marine fish). In 2017, methylmercury is classified as Group 2B carcinogens by the International Agency for Research on Cancer (IARC) under the World Health Organization (WHO). Metallic mercury is liquid at room temperature and primarily enters the blood circulation through the respiratory tract in the form of mercury vapor at high temperatures.

4.3.2.1 Population Exposure Level of Mercury

In the Laizhou Bay Birth Cohort Study, the median concentrations of mercury in maternal blood and neonatal cord blood were 0.84 μg/L and 1.46 μg/L, respectively, which were lower than those measured in Zhoushan, Zhejiang Province (5.6 μg/L in cord blood), and Wuchuan, Guizhou Province (21.5–33.9 μg/g in hair samples), in China, and similar to those measured in the National Health and Nutrition Examination Survey (NHANES) in the United States [50, 51].

4.3.2.2 Effects of Maternal Exposure to Mercury on the Growth and Development in Early Life

Extensive animal studies and epidemiological studies have shown that mercury has significant embryonic developmental toxicity and neurotoxicity. Mercury accumulates in vivo after entering the fetus through the placental barrier, interfering with or damaging the normal cell behavior, affecting the development and differentiation of embryonic cells, and inducing adverse birth outcomes such as preterm birth, growth retardation and low birth weight, malformation, and even embryo death [52, 53].

Mercury is distributed to various organs and tissues after entering the human body and mainly causing damages to the nervous system. Studies have shown that mercury compounds have strong affinity to sulfydryl group, and they can conjugate with sulfydryl-containing compounds to form mercury-thiolate complex and enter the human body. Several key enzymes in the body (e.g., ATPase, cytochrome oxidase, and lactate dehydrogenase) contain sulfydryl groups. When mercury compounds enter the human body, they can conjugate with the sulfydryl groups of these enzymes leading to cell apoptosis. In addition, mercury compounds stimulate the efflux of Ca^{2+} from mitochondria and inhibit the uptake of Ca^{2+} by the mitochondria, resulting in an elevated Ca^{2+} concentration, intracellular calcium overload, and enhanced calpain activity, which in turn leads to the disturbance of mitochondrial oxidative phosphorylation, the decrease in mitochondrial membrane potential, and the activation of phospholipases and proteases in cytoplasm, resulting in irreversible damage to the neurons. A case-controlled study of a neural tube defect surveillance program conducted in Texas, USA, also showed that maternal exposure to mercury significantly increased the risk of neural tube malformations in the fetus [54]. Animal studies have revealed that maternal exposure to methylmercury induced significant behavioral changes and memory impairment in the offspring in a dose-dependent manner. Long-term maternal exposure to low-dose (below the safe level) methylmercury could significantly alter the offspring's motor performance and coordination [55]. The Laizhou Bay Birth Cohort Study showed that maternal exposure to low levels of mercury was associated with significant increase in the neurodevelopmental scores (adaptive and social scores) in the offspring. In fact, maternal exposure to mercury mainly comes from dietary sources, especially from fish and seafood, and fish is rich in nutrients (such as unsaturated fatty acids and selenium) that are beneficial to the neurodevelopment of the offspring. In this study, there was a significant positive correlation between fish food intake during pregnancy and the neurobehavioral development scores of the offspring, and the positive

correlation between maternal mercury exposure and the neurobehavioral development of the offspring could be attributed to the effect of numerous nutrients in the fish [51]. Indeed, in the Mothers and Children's Environmental Health Birth Cohort Study with high mercury exposure in South Korean, maternal mercury exposure significantly reduced neurobehavioral development scores in children aged 3 years old, and this effect was more pronounced in those pregnant women with low-folate intake during pregnancy [50].

4.3.3 Arsenic

Arsenic, an element widely distributed in nature, is listed as one of the top ten chemical elements endangering public health by the World Health Organization. It is classified as "Group 1" human carcinogens by the International Agency for Research on Cancer (IARC). Arsenic pollution primarily arises from industrial wastewater from mining, metallurgy, chemical industry, chemical pharmacy, pesticide production, textile, glass, tanning, and other sectors. The human body is exposed to inorganic arsenic mainly through intake of arsenic-contaminated groundwater and foods. Arsenic exists in organisms predominantly in trivalent and pentavalent forms.

4.3.3.1 Population Exposure Level of Arsenic

In the Laizhou Bay Birth Cohort Study, the median arsenic concentrations in the maternal blood and cord blood were 8.05 μg/L and 6.03 μg/L, respectively [56]. In a study conducted in Wuhan, the median concentrations of inorganic arsenic in urine samples of pregnant women during the first, second, and third trimesters of pregnancy were 2.45 μg/L, 2.23 μg/L, and 2.00 μg/L, respectively; the median concentrations of dimethylarsenic acid were 8.81 μg/L, 7.55 μg/L, and 6.82 μg/L, respectively; the median concentrations of monomethylarsenic acid were 1.04 μg/L, 0.87 μg/L, and 0.75 μg/L, respectively; and the median concentrations of total arsenic were 13.08 μg/L, 11.59 μg/L, and 10.36 μg/L, respectively, which were all higher than those measured in the US NHANES study (total arsenic, 8.3 μg/L; dimethylarsenic acid, 3.6 μg/L) [57, 58].

4.3.3.2 Effects of Maternal Exposure to Arsenic on the Growth and Development in Early Life

Arsenic can enter the embryo by crossing the maternal-placental barrier and cause embryotoxicity. Animal studies have shown that maternal arsenic exposure was associated with low birth weight, malformations, and death in fetuses. In epidemiological studies, a significant negative correlation between maternal arsenic exposure and neonatal birth weight and birth length was observed, and maternal arsenic exposure could increase the risk of small-for-gestational-age infants [59].

In addition, arsenic could go through the blood-brain barrier and accumulates in the brain tissue. Studies in rats have shown that maternal exposure to high-dose arsenic was associated with oxidative stress, epigenetic changes, mitochondrial dysfunction, thinning of postsynaptic density, widening of synaptic cleft, and neuronal cell apoptosis in the nervous system of fetuses [60]. A study conducted by Wang et al. in Shanghai revealed that maternal arsenic exposure significantly reduced the neonatal behavioral neurological assessment (NBNA) scores of the newborns [61]. Similarly, in the US Health Outcomes and Measures of the Environment (HOME) study, maternal exposure to arsenic was associated with significant reductions in neurodevelopmental scores in children at 3 and 5 years of age, as indicated by reductions in mental development index and full-scale intelligence quotient [62].

In conclusion, maternal exposure to heavy metals (lead, mercury, and arsenic) could significantly increase the risk of adverse pregnancy outcomes and have long-term adverse effects on the growth and development, especially the neurodevelopment in the offspring. Thus, it is important to strengthen health education to the public with regard to the hazards of heavy metal exposure and improve the public awareness of health protection among pregnant women, aiming to reduce maternal exposure to environmental heavy met-

als, thereby protecting and promoting the healthy development of the offspring.

4.4 Effects of Maternal Exposure to Air Pollutants on the Growth and Development in Early Life

Air pollution is one of the major environmental pollution factors in China. Air pollutants are generally divided into particulate pollutants and gaseous pollutants. Atmospheric particulate matters have solid and liquid forms. The common solid atmospheric particulate matters include carbon black dust, combustion particle, soil dust, and coal dust, while the liquid atmospheric particulate matters mainly includes raindrops, fog, and sulfuric acid mist. Gaseous pollutants consist of gases and vapors. Nitrogen-containing compounds (nitric oxide, nitrogen dioxide, ammonia, etc.), sulfur-containing compounds (sulfur dioxide, sulfur trioxide, hydrogen sulfide, etc.), and carbon-containing oxides (carbon monoxide, formaldehyde, etc.) are the common gaseous pollutants. Due to the increase in the respiratory rate and tidal volume of pregnant women, the health hazards caused by air pollution exposure in this population are much higher compared to the non-pregnant population. Therefore, it is of great significance to explore the effects of maternal exposure to air pollutants on the growth and development in the offspring as well as the underlying mechanism.

4.4.1 Particulate Matters

Particulate matters refer to solid or liquid particles dispersed in the air. The dust from coal combustion, automobile exhaust, industrial exhaust gas, and ground dust are the primary sources of ambient particulate matters. Indoor particulate matters mainly come from kitchen cooking, smoking, indoor decoration, secondary dust, etc. The chemical composition of atmospheric particulate matters is quite complex, including inorganic water-soluble ions, carbonaceous components, heavy metals, and organic substances, which are toxic and harmful to human health. The particle size ranges from several nanometers to tens of microns. Particulate matters with smaller particle size have larger specific surface area and are more likely to adsorb toxic and harmful substances. Particulate matters can be deposited in mouth, nose, throat, trachea, bronchus and alveoli, and other sites in our body. Atmospheric particulate matters at nanometer level can cross the blood-gas barrier and the placental barrier, thus directly interfering with the growth and development of the fetus *in utero*.

4.4.1.1 Population Exposure Level of Particulate Matters

In the Shanghai Birth Cohort Study, the mean exposure level of fine particulate matters ($PM_{2.5}$, with aerodynamic equivalent diameter of $\leq$2.5 μm) in pregnant women was 49.3 μg/m^3 during pregnancy, which exceeded the secondary standard (35 μg/m^3) of China's National Annual Mean Ambient Air Quality Standards [63]. In recent years, China has taken a series of measures in atmospheric environment protection and has achieved positive results. According to the "China Ecological Environment State Bulletin in 2021" issued by the Ministry of Environmental Protection, the mean concentration of $PM_{2.5}$ in the atmosphere in 2021 was 30 μg/m^3, which was much higher than the primary standard of China's National Annual Mean Ambient Air Quality Standards (15 μg/m^3), indicating that the air pollution control remains a great challenge in China, and it is necessary to continue to strengthen the control of air pollutants and the management of air hygiene quality.

4.4.1.2 Effects of Maternal Exposure to Particulate Matters on the Growth and Development in Early Life

The biological mechanisms underlying the effects of maternal exposure to atmospheric particulate matters on the fetal growth and development include the increase in oxidative stress and inflammatory response, coagulation dysfunction

and endothelial dysfunction, neuroendocrine disorder, DNA damage, and mitochondrial dysfunction. These mechanisms may act alone or in combination to affect the structure and function of the placenta as well as the growth and development of the fetus. For example, atmospheric particulate matters can increase maternal blood pressure during pregnancy by causing autonomic nervous dysfunction, oxidative stress, and endothelial dysfunction, which may further impair the structure and function of placental spiral artery, decrease uteroplacental perfusion, and reduce the delivery efficiency of oxygen and nutrients of placenta, displaying adverse effect on the normal growth and development of the fetus [64]. The Shanghai Birth Cohort Study showed that maternal exposure to $PM_{2.5}$ significantly increased the risk of adverse pregnancy outcomes, such as low birth weight and preterm birth [63].

The effects of prenatal exposure to PM2.5 on birth outcomes has been studied extensively, but studies of postnatal outcomes are less common. A prospective cohort study conducted in the United States demonstrated that maternal exposure to $PM_{2.5}$ was significantly associated with the occurrence of overweight and obesity (OWOB) in children aged 2–9 years [65]. A birth cohort study conducted by Zhou et al. in Beijing also showed that maternal exposure to $PM_{2.5}$ was positively correlated with WFL and BAZ of infants aged 1 year old and was associated with an increased risk of overweight and obesity in these children [66].

In addition, studies in rats have shown that maternal exposure to $PM_{2.5}$ induced mitochondrial dysfunction and ultrastructural changes characterized by apoptosis in the cerebral cortex and hippocampus neurons of the offspring, resulting in cognitive and social behavior changes, anxiety, and depression-like emotions in the offspring [67]. The Shanghai Birth Cohort Study revealed that maternal exposure to $PM_{2.5}$ during the third trimester and the whole pregnancy was negatively correlated with the neurobehavioral development scores in children aged 1 year old, which were mainly indicated by the decrease in the scores of gross motor, problem-solving, and personal-social ability. Similarly, two birth cohort studies conducted in Spain and Mexico City revealed a significant association between maternal exposure to $PM_{2.5}$ and the decline in the cognitive function in the offspring [68, 69]. $PM_{2.5}$ is a complex mixture consisted of various chemical components. In the Shanghai Birth Cohort Study, black carbon [BC], mineral dust, organic matter, ammonium, nitrate, and sulfate might play important roles in the neurodevelopmental toxicity of maternal $PM_{2.5}$ exposure [70].

4.4.2 Nitrogen Oxides

Nitrogen oxides in the atmosphere mainly come from the burning of fossil fuels and plants, as well as the conversion of nitrogen-containing compounds in farmland, soil, and animal waste. Indoor nitrogen oxides are primarily generated from heating and fuel combustion during cooking, indoor smoking, and the nitrogen oxides penetrated from outdoor atmosphere. Nitrogen oxides (NOx) in the atmosphere generally include nitric oxide (NO) and nitrogen dioxide (NO_2). As NO is not stable, it will be converted to NO_2 immediately through oxidization. Therefore, NOx is generally referred to as NO_2 for simplicity, which is also one of the important measures for air quality.

4.4.2.1 Population Exposure Level of Nitrogen Oxides

In a cohort study conducted in Shanghai, the maternal exposure level to NO_2 was 48.2 μg/m^3 in 2014–2015, which was higher than the annual mean concentration specified in ambient air quality standards of China's National Annual Mean Ambient Air Quality Standards (40 μg/m^3). The study demonstrated that maternal exposure to NO_2 was significantly associated with an

increased risk of preterm birth [71]. With the implementation of the Action Plan on Air Pollution Prevention and Control, the air pollution has been effectively controlled in China. According to the "China Ecological Environment State Bulletin in 2021" issued by the Ministry of Environmental Protection, the mean concentration of NO_2 in 168 prefecture-level cities was 28 μg/m^3, which was lower than the China's National Annual Mean Ambient Air Quality Standards.

4.4.2.2 Effects of Maternal Exposure to Nitrogen Oxides on the Growth and Development in Early Life

Nitrogen dioxide is a gaseous pollutant with poor water solubility, which has mild irritation to the respiratory tract. Short-term exposure to high concentrations of NO_2 can lead to respiratory tract inflammation, thickening and narrowing of bronchial endothelium, which may be accompanied by pathological changes such as interstitial edema, arterial wall thickening, endothelial cell necrosis, and tissue fibrosis. Among them, systemic inflammation caused by respiratory tract inflammation can induce adverse pregnancy outcomes such as preterm birth, stillbirth, low birth weight, and intrauterine growth restriction [72].

Epidemiological studies have indicated that maternal exposure to NO_2 may impair the neurobehavioral development of the offspring. For example, a cohort study conducted in Shanghai showed that maternal exposure to NO_2 was significantly associated with the decrease in gross motor scores, fine motor scores, and social behavior scores in infants aged 24–36 months [73]. The Spanish INMA cohort study demonstrated that maternal exposure to NO_2 was significantly associated with decreases in cognitive and language scores in the male children aged 4–6 years old [74].

In addition to neurodevelopmental toxicity, epidemiological studies have shown that maternal exposure to NO_2 could also affect the physical development of the offspring. In a multicenter prospective cohort study conducted in Wuhan, maternal exposure to NO_2 significantly reduced the fetal biparietal diameter, abdominal circumference, body weight, femur length, humerus length, and head circumference [75].

4.4.3 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed and relocated in the environment as a result of the incomplete combustion of organic matter, such as coal, petroleum, tobacco, etc. Many PAHs and their epoxides are highly toxic, mutagenic, and/or carcinogenic to humans.

4.4.3.1 Population Exposure Level of Polycyclic Aromatic Hydrocarbons

In a birth cohort study conducted in Taiyuan, China, the levels of eight PAH metabolites (2-OHNap, 1-OHNap, 3-OHFlu, 2-OHFlu, 2-OHPhe, 9-OHPhe, 1-OHPhe, and 1-OHPyr) in the urine samples of pregnant women were determined. The geometric mean concentration of 2-OHPhe (0.09 ng/mL) was similar to that measured in a Puerto Rico study (0.11 ng/mL), lower than that measured in a Czech study (0.16 ng/mL). The geometric mean concentration of 1-OHPyr (0.07 ng/mL) was lower than that measured in a New York study (0.15 ng/mL) [76].

4.4.3.2 Effects of Maternal Exposure to Polycyclic Aromatic Hydrocarbons on the Growth and Development in Early Life

Benzo[a]pyrene (BaP) is a typical and representative compound of PAHs with stable chemical properties. The potent teratogenicity and carcinogenicity of BaP have been well-established in large number of studies. The carcinogenicity of BaP is mainly driven by the carcinogenic activity of its final metabolite dihydrodiol epoxy benzo[a]pyrene (BPDE). BaP could cross the placenta and exert a direct adverse effect on the fetus. BaP can be transferred to 7,8-epoxybenzo[a]pyrene by cytochrome P450, and then transferred to 7,8-dihydroxybenzo[a]pyrene by epoxide hydrolase, which is further oxidized by CYP1A1 to 7,8-dihydroxy-9,10-epoxybenzo[a]pyrene (i.e.,

BPDE). The active metabolite BPDE can covalently conjugate with the biopolymers such as DNA, RNA, and protein in the fetus to form adducts, which cause alterations in the structure and function of biopolymers, triggering the activation of signaling pathways (e.g., DNA damage repair and cell cycle regulation) as well as abnormal expression of relevant target genes, and leading to adverse pregnancy outcomes such as fetal malformation or death.

There are increasing evidence in China and abroad about the neurodevelopmental toxicity of PAHs. PAHs and their metabolites are highly lipophilic and can distribute into the central nervous system after entering the body and crossing the blood-brain barrier and are widely distributed in the hippocampus, striatum, brain stem, and other brain tissues. Toxicological studies have shown that exposure to PAHs in pregnant rats resulted in impairment in the spatial learning and memory ability, cognitive ability, and behavioral function. With regard to epidemiological studies, the Taiyuan Birth Cohort Study showed that maternal exposure to PAHs significantly reduce the neurobehavioral development scores in children aged 2 and 3 years old [76, 77]. Epidemiological studies conducted in Poland and the United States have also noted adverse effects of maternal exposure to PAHs on the neurobehavioral development in the offspring [78, 79].

4.4.4 Tobacco Smoke

Exposure to environmental tobacco smoke (ETS), also known as passive smoking, refers to the nonsmokers inhaling the smoke exhaled by smokers and the smoke generated by spontaneous combustion of the cigarette butts at a frequency of 1 day (more than 15 min a day) out of a week, which is also referred to as "involuntary smoking" and "second-hand smoke."

4.4.4.1 Population Exposure Level of Tobacco Smoke

According to several domestic studies, the rate of pregnant women exposed to ETS ranges from 38% to 75%, and the indoor environment, such as home and work environment, is the primary place where inhalation of second-hand smoke occurs [80].

4.4.4.2 Effects of Maternal Exposure to Tobacco Smoke on the Growth and Development in Early Life

Epidemiological studies have shown that women who are exposed to environmental tobacco smoke are at a 1.25 times greater risk of delivering a newborn with birth defects than those without such exposures. After pregnant women are exposed to environmental tobacco smoke, harmful substances in tobacco such as particulate matter (tar), nicotine acid, CO, hydrogen cyanide, phenols, benzene, anthracene, and other components may enter fetal blood circulation by crossing the placental barrier and exerts a direct toxic effect on the fetus. In addition, nicotine, CO, and other substances can cause vasoconstriction, resulting in slowed blood flow and reduced blood oxygen concentration, which may further lead to insufficiency in fetal nutrition and oxygen and significantly increase the risk of fetal death, stillbirth, preterm birth, low birth weight, birth defects, and other adverse birth outcomes.

Maternal exposure to tobacco smoke is associated with significant damages to the cognitive ability of the offspring in rodents, characterized by the decline of spatial memory and learning ability. In the early stage of brain development, the cholinergic system is involved in multiple critical processes, such as axon growth, the survival, proliferation, and differentiation of cells along with neurogenesis. It is considered as a marker of growth and developmental signals. Studies have shown that prenatal exposure to tobacco smoke had adverse effect on the levels of nicotinic acetylcholine receptors and neurotransmitters in the central nervous system of the offspring. A study conducted in Guizhou Province showed that maternal exposure to tobacco smoke was significantly associated with decrease in the scores of cognitive and language functions in 2-year-old toddlers [81]. Similarly, based on the birth cohort studies conducted in Poland and South African, adverse effects of maternal expo-

sure to tobacco smoke on neurobehavioral development (associated with decreased language and motor function scores) were observed in toddlers aged 6 months, 1 year, and 2 years old [82, 83].

Air pollution is one of the major environmental pollutants in China. During the past decade, China has made great progress in the prevention and control of air pollution. However, as human beings cannot live without the atmospheric environment, how to effectively prevent and control the health hazards caused by air pollution during pregnancy should be highlighted in future studies.

4.5 Prospects

Although we have made great progress in exploring the effects of maternal exposure to environmental pollution on the growth and development of the offspring, researches on the comprehensive evaluation strategy for the health risks associated with the environmental pollution remain in the early stage. More attention should be paid to the following aspects in future studies.

Many countries around the world have established long-term and systematic monitoring programs to detect the environmental biohazardous substances at population level. For example, since 1999, the US Centers for Disease Control and Prevention has conducted the National Health and Nutrition Examination Survey (NHANES), which monitors the levels of approximately 265 environmental pollutants in the human body, including metals, pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers, volatile organic compounds, tobacco smoke, metabolites of polycyclic aromatic hydrocarbons, perfluoroalkyl substances, phthalates, and their metabolites. However, there are currently ~30,000 chemicals commonly used in production and daily life, any of which may be released into the environment during the processing or usage, and only less than 1% of them have been evaluated in detail for toxicity and health-related risks. Most of the basic and epidemiological studies conducted to date are limited to the investigation of exposures to traditional environmental pollutants. As a result, the effects of maternal exposure to novel pollutants on the growth and development of the offspring have not been fully understood. More importantly, humans are often exposed to multiple pollutants at the same time, and previous studies have only considered the association between individual pollutant or a single class of pollutants and the health outcomes. The characteristics of mixed exposure to various pollutants and the corresponding mixed exposure effects have not been taken into adequate consideration. Studies have revealed that simultaneous exposure to multiple chemicals can have additive or synergistic effects on health, especially for the same adverse health outcomes, and that an analysis of one chemical at a time may underestimate its potential health effects in the presence of other chemicals. Therefore, future studies should focus on the effects of combined exposure to multiple environmental pollutants during pregnancy on the growth and development of the offspring.

The state of human health is determined by the combination of environmental and genetic factors. The completed genome-wide association studies (GWASs) for chronic diseases demonstrated that most genetic alterations could account for 10% of the genetic variability in these diseases. In view of the genetic variability, the susceptibility to the adverse effects of toxic chemicals may vary among different individuals. For example, glutathione S-transferase M1 (GSTM1), the enzyme responsible for catalyzing phase II metabolic reactions, plays an important role in the catabolism of exogenous carcinogens. A study demonstrated that higher maternal blood levels of organochlorine insecticides were associated with an increased risk of idiopathic premature delivery in pregnant women with a GSTM1 deletion polymorphism, as they lacked the activity of enzymes responsible for detoxification [84]. Incorporating gene-environment interactions in risk assessment may help to identify and protect the vulnerable groups.

The Human Genome Project, omics big data, biological detection technology, artificial intelligence, and big data analysis technology have provided technical support for the precision

medicine. These technologies bring an increasing amount of biomedical data, including mass data on genomics, transcriptomics, proteomics, and metabolomics, as well as adequate data on clinical manifestations, pathological findings, biochemical parameters, and immunological parameters. There are three urgent problems to be addressed in the future: (1) how to correlate the clinical phenotypes with the omics big data; (2) how to uncover the underlying mechanism and therapeutic targets involved in the effects of maternal exposure to environmental pollution on the health; and (3) how to assess the health damages caused by maternal exposure to environmental pollution and provide treatment and prevention services to the target population in a precise manner. Meanwhile, all sectors of the society, including the government decision-making departments, scientific researchers, clinicians, and community workers, must cooperate closely with each other to translate the scientific research findings into practical policies and strategic actions. These are essential for the contributions to the sustainable development of mankind.

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5 Effects of Maternal Internal Environment on Early Life Growth and Development

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5.1 Overview

The first 1000 days of life, defined as the period between early embryogenesis and 2 years old, is the critical window for the growth and development of an infant. According to the well-known theory of DOHaD (Developmental Origins of Health and Disease), adverse factors such as malnutrition and poor environment in early life (including early embryo, fetus, and infancy phases) will increase the risk of obesity, diabetes, cardiovascular disease, and other chronic diseases in adulthood. The concept of "life course health management" is no longer limited to disease prevention and treatment in adulthood but also focuses on the gestation period, including gametogenesis, early embryonic development, organogenesis, and fetal growth. Fetus is conceived *in utero* throughout the pregnancy. There will be physiological and pathological changes in maternal nutritional metabolism, endocrine, neurological, and immune pathways during pregnancy, and these changes interact with and are influenced by each other. Challenges posed by the adverse external environment and/or adverse maternal environment (e.g., high maternal blood glucose/lipid levels, maternal thyroid disease, and gestational hypertension) during embryonic development may alter the growth trajectory of the offspring, resulting in growth and development restriction together with various organ and system dysplasia, which in turn cause a series of short-term and long-term diseases in the offspring. As for life cycle health management, efforts should be made to move the "window of opportunity" forward into the pregnancy (prenatal period) and provide appropriate interventions to significantly reduce the risk of chronic metabolic diseases in adulthood.

Huang et al. based on their study results and the findings from other teams demonstrated that adult metabolic diseases, including cardiovascular disease, diabetes, obesity, and tumors, could be attributed not only to the adverse intrauterine environment during the prenatal period but also to the adverse maternal or paternal environment during the gametogenesis (i.e., development of ovum and sperm), such as cardiovascular disease, diabetes, obesity, and unhealthy lifestyle (e.g., smoking). On this basis, Huang et al. proposed the hypothesis of gamete, embryo, and fetal origin of adult diseases in 2013. Extensive studies have shown that early life starting from spermatogenesis and oogenesis to the development of embryo and fetus *in utero* plays a crucial role in the onset of adult cardiovascular diseases after birth. Hyperglycemia, hypertension, or hyperlipidemia can impair the development of the ovum or sperm, and therefore significantly increase the risk of adult-onset chronic diseases in the offspring.

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The intrauterine environment with maternal hyperglycemia induced by insulin resistance during pregnancy has certain impacts on the offspring, causing life cycle adverse outcomes including obesity, metabolic syndrome, cardiovascular diseases, and gestational diabetes mellitus. The hyperglycemic intrauterine environment may alter the epigenetic modification (DNA methylation) in the offspring, which poses a higher risk of diabetes to the offspring. Decreased maternal sensitivity to insulin associated with a hyperglycemic environment may cause metabolic disorders of varying severity and excessive weight gain during pregnancy. The insulin resistance in the muscles, fat tissues, and liver in combination with excessive nutrition obtained from the diet contributes to elevated levels of glucose, amino acid (AA), free fatty acid (FFA), and several inflammatory factors. These substances can cross the placental barrier and enter into the fetus, which have adverse effects on various functions of the fetus, including the insulin secretion function of β-cells, differentiation of stem cells, mitochondrial function, and appetite regulation. Excessive glucose and AA may cause fetal hyperinsulinemia, while excessive FFA may lead to increased blood triglyceride (TG) and the development of fatty liver in the offspring. Pregnant women experience changes in microbiome composition and maintain a rapid weight gain after delivery, while overfeeding of the baby further contributes to development of metabolic diseases such as childhood obesity. After the fetus grows up and gives birth to his or her children, this vicious cycle will pass down from one generation to another.

As the largest endocrine organ in the body, the thyroid is responsible for promoting metabolism and maintaining normal growth and development. The special physiological changes that occur during pregnancy will cause fluctuations in thyroid hormones level; therefore, pregnant women are susceptible to pregnancy-related thyroid diseases such as gestational hypothyroidism, hypothyroxinemia, and transient thyrotoxicosis. Recently, an increasing number of studies have shown that maternal thyroid hormone level during pregnancy are associated with the risk of several pregnancy complications and are closely related to fetal growth and development as well as nervous system development. The normal development of the fetus *in utero* depends on the adequate level of maternal thyroid hormones during pregnancy, especially during the first trimester (up to 20 weeks of gestation). As the fetal thyroid is still immature, it relies largely on the maternal thyroid hormone levels to provide essential hormones to maintain its normal growth and development. Therefore, the fetus will be affected in the case of maternal thyroid dysfunction.

In addition, as gestational hypertension is a common complication in obstetrics, there are many theories on the occurrence and development of this disease. Gestational hypertension will eventually affect the blood supply and oxygen supply to the placenta with subsequent placental dysfunction, which leads to insufficient fetal nutrition supply and intrauterine growth restriction, along with the long-term risk of metabolic diseases and cardiovascular diseases in the offspring. Polycystic ovary syndrome is the most common endocrine disease affecting young women, which may interfere with their normal ovulation and lead to infertility. As these women become pregnant after all attempts, the placenta is exposed to an environment characterized by hyperandrogenemia and insulin resistance during the first trimester of pregnancy, which has adverse effects on the development of the placenta and fetus, and further leads to placental abnormalities and pregnancy complications.

5.2 Effects of Maternal Internal Environment on Early Life Growth and Development

5.2.1 Effects of Gestational Hyperglycemia on Early Life Growth and Development

Gestational hyperglycemia is defined as different types of abnormal glucose metabolism during pregnancy, including pregestational diabetes mellitus (PGDM), prediabetes, and gestational

diabetes mellitus (GDM) [1]. Risk factors associated with gestational hyperglycemia include age [2], race [3], overweight and obesity (especially severe obesity) [4], intrauterine environment (high or low birth weight) [5], insulin resistance, and family history of diabetes [6]. Gestational hyperglycemia not only increases the long-term risk of obesity, type 2 diabetes, cardiovascular diseases, cognitive function impairment, and nonalcoholic liver disease in the offspring, leading to the intergenerational vicious cycle of obesity and diabetes that affect the health of the entire population [7, 8], but also causes adverse pregnancy outcomes such as macrosomia, cesarean delivery, premature delivery, and preeclampsia [9]. The placenta plays a crucial role in maintaining fetal development and growth. Changes in maternal or fetal circulation may lead to alterations in placental structure and function, which have potential effects on fetal growth and development. In view of the adverse effects of gestational hyperglycemia on maternal and children's health, timely diagnosis and early intervention/treatment, including lifestyle intervention (diet and exercise) and insulin therapy, are of great importance. However, due to the persistence of insulin resistance, these interventions and treatments have limited efficacy. As such, there is an urgent need to develop safe, effective, and feasible treatments.

5.2.1.1 Short-Term Effects of Gestational Hyperglycemia on the Offspring

Several retrospective and prospective studies have revealed that gestational hyperglycemia is associated with adverse maternal and offspring outcomes. The risk of maternal, fetal, and neonatal complications increases with the increase in maternal blood glucose levels [10, 11]. Short-term complications include preeclampsia, polyhydramnios, cesarean delivery, shoulder dystocia, birth canal laceration, fetal overgrowth (also known as macrosomia), neonatal hypoglycemia, jaundice, and perinatal death [12–14]. Pregnant women with gestational hyperglycemia usually have risk factors associated with adverse outcomes, including maternal overweight, advanced age, reduced physical activity, or minority group. For this reason, there has been a long-standing debate on whether the adverse outcomes associated with gestational hyperglycemia are caused by maternal hyperglycemia itself or other factors [15]. Subsequently, the results from a large multinational landmark study (i.e., the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study) confirmed that maternal hyperglycemia as an independent factor for preeclampsia, preterm delivery, cesarean section, large for gestational age (LGA), shoulder dystocia, neonatal hypoglycemia, hyperbilirubinemia, and admission to the neonatal special care unit in a hierarchical linear manner without clear cut-off points [16]. Among women with gestational hyperglycemia diagnosed using the "International Association of Diabetes and Pregnancy Study Groups" (IADPSG) criteria, fasting glucose levels obtained from the oral glucose tolerance test (OGTT) showed a stronger association with adverse outcomes compared to the postprandial glucose levels at 1 h and 2 h. Two large randomized controlled clinical trials have demonstrated that the treatment of gestational hyperglycemia is effective in reducing or preventing short-term maternal and fetal complications, specifically reducing the incidence of LGA to the expected normal range while reducing the incidence of preeclampsia by approximately 50% [17, 18].

5.2.1.2 Long-Term Effects of Gestational Hyperglycemia on the Offspring

1. Increased Risk of Diabetes in Offspring

   Studies in animal models of gestational hyperglycemia indicated that the offspring derived from dams with gestational hyperglycemia were at increased risk of hyperglycemia, diabetes, obesity, cardiovascular diseases, and structural changes in the hypothalamus during pregnancy; however, these could be prevented if the maternal blood glucose was within the normal range during pregnancy [19, 20]. This has also been confirmed by a clinical study conducted on the offspring of pregnant women with different types of diabetes. In this study, increased risks

of diabetes and obesity were observed in the offspring of pregnant women with diabetes [7, 8, 21]. A Danish study showed that 21% of the offspring (aged 18–27 years old) of pregnant women with gestational hyperglycemia had prediabetes or diabetes, representing an eightfold increased risk compared to the background population [22]. In addition, the risks of overweight and metabolic syndrome were even higher (twofold and fourfold, respectively) [23], with subsequent decreases in insulin sensitivity and insulin secretion [24]. In a study conducted on approximately 100,000 pregnant women, infants born to mothers with gestational hyperglycemia had increased risks of higher fasting blood glucose levels, insulin resistance, obesity, and cardiovascular disease [25]. The HAPO-FUS study also demonstrated that although maternal obesity is a significant risk factor for offspring obesity, gestational hyperglycemia remained to be a significant risk factor following adjusted for maternal BMI [26–28].

2. Effects on the Cognitive Function of the Offspring

   Although several results have been reported with regard to the effect of gestational hyperglycemia on the cognitive function of the offspring, there was no conclusive evidence that maternal gestational hyperglycemia may act as an independent factor in the pathogenesis of cognitive impairment [21, 29]. Studies have shown that the offspring of pregnant women who were diagnosed with gestational hyperglycemia during the first trimester had an increased risk of autism spectrum disorder [30] and the offspring of pregnant women with gestational hyperglycemia requiring medical treatments were at an increased risk of attention-deficit/hyperactivity disorder (ADHD) [31]. In contrast, animal studies have found that treatment of pregnant hyperglycemia did not appear to improve the long-term outcomes for the offspring [32, 33]. However, the duration of the follow-up studies in postpartum women with gestational hyperglycemia is relatively short (4–10 years), and the long-term outcomes remain to be investigated. In conclusion, gestational hyperglycemia is part of an intergenerational vicious cycle. The offspring of pregnant women with gestational hyperglycemia are more likely to develop gestational hyperglycemia during their pregnancy, and there are currently no effective interventions to interrupt or mitigate this cycle.

3. Hepatic Steatosis in the Offspring

   A study on rat models with induced severe hyperglycemia during pregnancy showed that under the same dietary conditions, a higher percentage of the offspring delivered by diabetic rats with normal weight developed hepatic steatosis compared with the rats in the control group. A small-scale human study of 25 newborns revealed that prepregnancy body mass index was more strongly associated with neonatal liver fat content than maternal hyperglycemia. This finding was supported by a recent study in the EPOCH cohort, which also demonstrated that maternal obesity, rather than maternal hyperglycemia, was associated with higher liver fat content in children and adolescents.

5.2.1.3 Changes in Placental Function in Women with Gestational Hyperglycemia

The placenta serves as a connection between maternal and fetal circulation, and hence plays a pivotal role in transporting maternal nutrients to the fetus so as to maintain fetal growth and development. Changes in maternal or fetal circulation may lead to alterations in placental structure and function, which have potential effects on fetal growth and development.

The placenta is directly exposed to the maternal environment, meanwhile providing oxygen, macronutrients, and micronutrients to the fetus. The interaction between these factors and fetal genotype, including the epigenotype, determines the fetal phenotype [34]. The placenta allocates the nutrients from the maternal circulation to meet its own needs while maintaining fetal growth, thereby regulating the effects of maternal metabolic disorders on the fetus. These metabolic alternations include, first of all, hyperglycemia,

which is both a predictor and a main therapeutic target of gestational hyperglycemia.

5.2.1.4 Gestational Hyperglycemia and Metabolic Disorders

Metabolic disorders include abnormal concentrations of fatty acids and amino acids in the maternal circulation.

1. Placental Glucose Transport

   There are numerous transporters in the placental, allowing for an adequate supply of glucose, lipids, and amino acids in the context of normal maternal metabolism. However, it does not protect the fetus from glucose oversupply in the presence of gestational hyperglycemia [35]. Transplacental glucose transfer is saturated only if the difference in glucose concentrations between the maternal and fetal circulation is ≥25 mmol/L [36]. Therefore, the glucose concentration gradient between maternal and fetal circulation is the most important determinant of maternal glucose transferred to the fetus. This gradient depends on maternal and fetal glucose levels, and the latter is affected by fetal insulin levels. Fetal hyperinsulinemia promotes glucose uptake into peripheral tissues, yielding a steeper concentration gradient. Under this circumstance, the fetus continues to absorb ("steal") glucose from the maternal circulation (i.e., the so-called fetal glucose steal phenomenon) [37], resulting in more maternal glucose transferred to fetal circulation.
2. Placental Fatty Acid Transport

   The placental transferring system transports fatty acids at a much lower efficiency than that for glucose, with only ~3% of the maternal fatty acids access to the fetal circulation [38, 39]. The fetus uses glucose as the precursor to synthesize its nonessential fatty acids; therefore, only 20% of the fatty acids in neonatal fat are derived from maternal origins. Docosahexaenoic acid is an essential component for the development of the fetal brain and retina. However, gestational hyperglycemia is associated with reduced expression of NLS1 (encoded by MFSD2A) by approximately 30% in the placenta [40], which reduces the ability of the placenta to transport docosahexaenoic acid, thus having an adverse effect on the fetal intelligence and vision development. In the third trimester of pregnancy, only about 9–10% of the placental surface is involved in nutrient transport [41], and this proportion remains unchanged in the presence of gestational hyperglycemia. The vast majority of nutrients absorbed from the placental surface enter the metabolic pool of the placenta, thereby maintaining the placental function [35]. In general, the placenta does not actively increase the number of nutrients in fetal circulation by the end of delivery in pregnant women with gestational hyperglycemia, and therefore does not directly contribute to excessive fat accumulation in fetuses.
3. Adaptation of Placenta to Intrauterine Blood Glucose Concentration

   The placenta of pregnant women with gestational hyperglycemia needs to make a series of changes to protect the placenta itself and the fetus, including placental hypervascularization. Hyperinsulinemia in pregnant women with gestational hyperglycemia stimulates aerobic metabolism in the fetus, and there is an increase in placental capillaries in response to the increased fetal oxygen demand [42]. In pregnant women with gestational hyperglycemia, hypoxia, hyperinsulinemia, and changes in the levels of angiogenic factors in the fetal circulation stimulate placental angiogenesis [43, 44]. Although these regulatory signals are generated in fetus, some of the regulatory signals may also come from placental trophoblasts and macrophages [45, 46]. In pregnant women with gestational diabetes, the number and function of these cells may also be altered, including changes in molecules secreted by these cells, which contribute to the regulation of placental vascularization. Overall, there are multiple signals contributing to the hypervascularization of the placenta in women with gestational diabetes.

   In pregnant women with gestational hyperglycemia, the placenta removes cholesterol from fetoplacental circulation to prevent the

formation of pre-atherosclerotic lesions, which may lead to inadequate blood flow. The placenta appears to have evolved to be capable of buffering the stress in the intrauterine environment, thus adapting itself to changes in this environment [47]. However, in the event of extreme changes in the maternal environment, such as untreated gestational hyperglycemia or gestational hyperglycemia complicated with obesity, the buffering capacity of the placenta may be insufficient to prevent the pathological changes in fetus [48]. Some evidence suggests that placental adaptive responses are more pronounced in female fetuses. During the second half of pregnancy, the organogenesis in fetus has completed, and the placenta (as a fetal tissue) is controlled primarily by the fetus. Compared with the first trimester of pregnancy, the placenta is less susceptible to the maternal environment during this period [49]. For example, during the first 10–12 weeks of pregnancy, the placenta has poor antioxidant defenses (e.g., low levels of the antioxidant enzyme catalase) [49], resulting in the placenta being particularly sensitive to oxidative and metabolic stress, especially in women with hyperglycemia, obesity, and/or gestational hyperglycemia [50, 51]. However, whether the development of gestational hyperglycemia in late pregnancy will affect the placenta or the growth and development trajectory of fetus still needs further investigation.

5.2.2 Effects of Thyroid Hormones on Offspring During Pregnancy

5.2.2.1 Overview

Thyroid hormones are one of the most important endocrine hormones in humans, regulating the metabolism of almost all tissues and organs of the body. Pregnancy is often associated with physiological changes in hormones and metabolism. Thyroid dysfunction is one of the common endocrine diseases during pregnancy, with an incidence greater than 15%. If no effective intervention is given promptly, it will increase the risk of adverse pregnancy outcomes, endangering the long-term and short-term health of pregnant women and their newborns. Hypothyroidism is a common type of thyroid disease, which is often associated with fatigue, chills, constipation, and even hypomnesia. In women of childbearing age, hypothyroidism will affect the secretion of sex hormones, resulting in abnormal menstruation intensity, prolonged menstrual periods, and even amenorrhea and infertility. If maternal hypothyroidism occurs in the first trimester of pregnancy during which the fetal thyroid has not yet formed, the thyroid hormones needed for fetal growth and development are completely dependent on the maternal supply. Any maternal thyroid dysfunction will have adverse effects on fetal growth and development. Studies have shown that even mild abnormalities in thyroid hormones level during pregnancy may increase the risk of miscarriage, premature delivery, gestational hypertension, preeclampsia, low birth weight, and impaired mental development in childhood [52–55]. In view of the adverse effects of abnormal thyroid hormones level during pregnancy on the health of the pregnant woman and their offspring, prompt diagnosis and early intervention/treatment are of great importance. However, due to the limited reports on the effectiveness of aggressive interventions, there is an urgent need for safe, effective, and feasible treatments and relevant high-quality clinical evidence.

5.2.2.2 Intrauterine Thyroid Hormone

1. Maternal-Fetal Transfer of Thyroxine

   Fetal brain development during the first half of pregnancy relies on maternal thyroid hormones (THs). Although thyroid hormones are lipid-soluble compounds, the local action of thyroxine in placental tissue is still affected by the differential expression of proteins such as thyroxine receptor, deiodinase (DIO), thyroid transporters (e.g., monocarboxylate transporter family, L-type amino acid transporter, and organic anion transporting polypeptide). An analysis with the plasma membrane of the microvilli on the surface of syncytiotrophoblasts isolated from human term placental tis-

sue showed that the uptake of saturable tetraiodothyronine (T4) was mediated primarily (67%) by L-type amino acid transporters and monocarboxylate transporter 10, while the uptake of saturable triiodothyronine (T3) was mediated predominately (87%) by monocarboxylate transporter 8 and monocarboxylate transporter 10 [56]. At the placenta level, maternal free thyroxine (FT4) is the major thyroid hormone transported to the fetus. Maternally derived T4 is converted to T3 by DIO2 or to inactive rT3 by DIO3. Thyroid hormone receptor alpha is mainly expressed on the stromal cells, and to a lesser extent on trophoblast cells, while thyroid hormone receptor beta is primarily expressed on trophoblast cells and epithelial cells. DIO3, the predominant type of deiodinase expressed in placental tissue, plays an important role in regulating the T4 level in fetal circulation [57]. With the increase in gestational weeks, the expression of most thyroid transporters is upregulated [58], while DIO2 and DIO3 expression are downregulated, indicating that the local regulation of DIO is more pronounced during the first trimester, while thyroid hormones play an important and direct role in placental development during the third trimester.

2. Effects of Thyroid Hormones on Placental Function

Thyroid hormones are responsible for the regulation of placental development. The optimal concentration of maternal thyroxine can promote the normal development of the placenta by regulating the secretion of key cytokines and angiogenic growth factors in human decidual cells and prevent fetal immune rejection. Thyroid hormones play a critical role in maintaining the balance of inflammatory response in the first trimester of pregnancy. An in vitro study conducted by Oki et al. reported the effect of maternal thyroid dysfunction on placental development [59], suggesting that triiodothyronine (T3) could increase the expression of matrix metalloproteinases and integrins in extravillous cytotrophoblasts (EVTs) in early pregnancy, with subsequent enhancement of EVT invasion. Invasive trophoblasts are involved in placentation, hormone secretion, decidual vascularization, lymph angiogenesis, and spiral artery remodeling in the maternal uterus [60]. Precise coordination of the vascular remodeling process *in utero* is essential for the success of pregnancy, as it allows for the normal delivery of nutrients to the fetus and protects the fetus from exposure to the deleterious effects of reactive oxygen species [61]. Abnormally low levels of maternal thyroid hormones may affect fetal-placental development; impair decidualization, vascularization, and development of the placenta; increase apoptosis; and decrease trophoblast proliferation and stromal trophoblast invasion, thereby affecting uterine spiral artery remodeling [62, 63]. High levels of thyroid hormones may attenuate the pro-inflammatory activities of monocytes and macrophages, while low levels of thyroid hormones are associated with enhanced phagocytosis, increased ROS levels, and high expression of pro-inflammatory molecules, including macrophage inflammatory protein-1 alpha and interleukin-1 beta. In addition, thyroid hormones may also impact the activity of natural killer cells and cell-mediated immune responses [64]. Inflammatory cytokines are important components of the placental immune response, which may affect trophoblast invasion of the decidua through exerting effects on extracellular matrix remodeling and the vascular area of the uterine stroma-decidua [65]. Silva et al. noted the changes in glycogen cells and trophoblast giant cells in the placenta of hypothyroid rats, with a significant decrease in the gene and/or protein expression of interleukin-10, Nos2, interferon-γ, macrophage migration inhibitory factor, matrix metalloproteinase-2, and matrix metalloproteinase-9 in the placenta and placental leptin. These findings suggested that hypothyroidism affected maternal immune function by impairing the development of an anti-inflammatory environment at the maternal-fetal interface and also had an impact on intrauterine trophoblast migration [66].

3. Development of Fetal Thyroid

The development of fetal thyroid function involves a complex process, and the fetal thyroid plays a crucial role in controlling fetal metabolic rate, cardiac output, and brain development. The fetal thyroid starts to develop from 5 to 6 weeks of gestation and cannot synthesize endogenous thyroid hormones until 13 weeks of gestation. By 18 weeks of gestation, the fetal thyroid begins to produce large amounts of thyroid hormones and perform its key endocrine functions [67, 68]. Human thyroid development follows a precise time-dependent gene expression programming, with its terminal differentiation consisting of a colloid formation phase (7–11 weeks of gestation) and a follicular growth phase (from 12 weeks of gestation onwards) [69]. To date, little is known about the initiation of functional maturation of the human fetal thyroid during the follicular phase of growth, and there were only some reports on specific gene expression patterns in human thyroid samples [70] or the transcriptome of the fetal thyroid during early pregnancy [71]. Recent single-cell RNA sequencing of the thyroids obtained from juvenile and adult zebrafish revealed thyroid heterogeneity during development, highlighting the potential of single-cell molecular profiling in the understanding of thyroid maturation [72]. Nevertheless, the dynamics of the cellular composition during fetal thyroid maturation in mammals (especially in humans) remains unclear. Genome-wide transcriptome studies of human thyroid development, particularly at the single-cell level, are in urgent need for understanding human thyroid development, identifying potential early diagnostic markers, and exploring treatments for thyroid diseases.

5.2.2.3 Classification and Diagnosis of Gestational Thyroid Dysfunction

According to the criteria stipulated by the National Academy of Clinical Biochemistry, a 95% confidence interval is selected to establish the pregnancy-specific reference range (i.e., the 2.5th percentile is the lower limit, and the 97.5th percentile is the upper limit). Gestational hypothyroidism is characterized by a group of clinical syndromes caused by insufficient synthesis, secretion, or biological effect of thyroid hormones due to various reasons. The diagnostic criteria for this disease are a TSH level above the upper limit of the pregnancy-specific reference range and an FT4 level below the lower limit of the pregnancy-specific reference range. Gestational subclinical hypothyroidism (SCH) is defined as a serum TSH level higher than the upper limit of the pregnancy-specific reference range and a FT4 level within the pregnancy-specific reference range. Isolated maternal hypothyroxinemia (IMH) refers to the condition when a pregnant woman has normal serum TSH level, but her FT4 level falls below the lower limit of the pregnancy-specific reference range. Gestational transient thyrotoxicosis is characterized by a TSH level below the lower limit of the reference range (or 0.1 mU/L) with normal or elevated FT4 or FT3 level in the first trimester, excluding Graves' disease and other hyperthyroidism. Gestational subclinical hyperthyroidism is diagnosed based on the TSH level below the lower limit of the reference range (or <0.1 mU/L in the first trimester) with normal FT4 or FT3 levels.

5.2.2.4 Factors Affecting Intrauterine Thyroid Hormone Levels

There is an increased maternal demand for thyroid hormones as well as changes in hormone levels such as human chorionic gonadotropin (hCG) during pregnancy, which will affect thyroid function, while the increase in serum thyroglobulin level can also cause corresponding changes in the secretion of thyroid hormones. Based on the analysis of iodine nutritional status and thyroid function of 1154 pregnant women within 8 weeks of pregnancy, Chinese researchers discovered that excessive iodine intake in the first trimester could lead to a significant decrease in the level of maternal serum FT4. More than adequate or excessive iodine intake in the first trimester was associated with an increase in the

prevalence of gestational subclinical hypothyroidism and isolated maternal hypothyroxinemia in pregnant women [73]. Our preliminary cohort study found that only 8.4–24.8% of the thyroid diseases that occurred during the first trimester could persist into the third trimester if left untreated [74]. These results suggested that the majority of gestational thyroid dysfunction diagnosed in the first trimester is transient, most likely due to the failure of the thyroid gland to meet the increased demand for thyroid hormones in the first trimester [75]. This increased demand can be explained by fetal consumption of thyroid hormones, the increase in thyroxine-binding globulin, and the inactivation of thyroid hormones by DIO3. In addition, after evaluating the thyroid morphology, fetal thyroid endocrine function as well as the protein and transcript expression during fetal development in the absence and presence of altered maternal lifestyle, we found that maternal exposure to smoke and overweight/obesity during the critical second trimester were associated with disrupted fetal thyroid morphology and neonatal thyroid function in a gender-specific manner [76]. Furthermore, iron deficiency in the first trimester [77], environmental endocrine-disrupting chemicals (such as PM2.5 [78], perfluoroalkyl, and polyfluoroalkyl substances [79]), drugs, and positive maternal thyroid antibodies [80] could also lead to thyroid dysfunction in pregnant women.

5.2.2.5 Adverse Pregnancy Outcomes Associated with Maternal Thyroid Dysfunction

1. Maternal Thyroid Hormone Levels and Premature Delivery

   Thyroid-stimulating hormones, thyroxine and triiodothyronine, are essential for the normal growth and development of the fetus. Their bioavailability in the uterus depends on the development of the hypothalamic-pituitary-thyroid axis in the fetus along with the abundance of thyroid hormone transporters and deiodinase that may affect the level of bioactive hormones in the tissue. The T4 and T3 concentrations in the fetus are also influenced by gestational age, nutritional and endocrine conditions *in utero*, and placental permeability of maternal thyroid hormones, which varies with placental morphology in different species. Thyroid hormones also promote terminal differentiation of fetal tissues during several weeks before delivery and play a pivotal role in mediating the prepartum maturational effect of the glucocorticoids that ensure neonatal viability. Thyroid hormones act directly through the anabolic effect on fetal metabolism and stimulation of fetal oxygen consumption; in addition, they also exert indirect effects by controlling the bioavailability and effectiveness of other hormones and growth factors that influence fetal development, such as catecholamines and insulin-like growth factors (IGFs). Fetal thyroid hormones are involved in regulating tissue proliferation and differentiation, allowing for the activation of physiological processes necessary for survival after birth, such as gas exchange in the lung, thermogenesis, hepatic glucose production, and cardiac adaptation [81]. The oxidative phosphorylation capacity of muscle increases primarily after birth, and fetal thyroxine deficiency inhibits oxidative phosphorylation, preventing prenatal upregulation of mitochondrial density and cyclic electron transport proteins in fetal skeletal muscles. This temporal difference between prenatal maturation and upregulation of neonatal mitochondrial oxidative capacity may protect the newborn from birth-related oxidative stress while ensuring the energy supply to the newborn, which has potential implications for neonatal viability and adult metabolic health [82].
2. Effects of Maternal Thyroid Hormone Level on Fetal Growth *In Utero*

   Birth weight is a key predictor of childhood obesity and subsequent metabolic diseases in adulthood. Studies conducted both in China and abroad have shown that lower FT4 concentrations and IMH may lead to higher mean birth weight in newborns [54, 83]. Studies have also demonstrated that even mild changes in thyroid hormones can have a significant impact on lipid metabo-

lism by regulating several key genes in lipid synthesis, stimulating lipid transport, and altering enzyme activity to enhance lipid hydrolysis. Maternal hyperglycemia and insulin resistance with subsequent neonatal hyperinsulinemia are considered to play a significant role in neonatal overgrowth; however, recent studies have shown that other energy sources, such as lipids and amino acids, may be the underlying factors in the overgrowth of newborns born to nondiabetic mothers [84]. In addition to acting as energy substrates for fetal lipogenesis and fetal growth, some fatty acids can induce the secretion of placental insulin-like growth factor-1 (IGF-1), suggesting a potential pathophysiological association between maternal plasma lipids and high fetal birth weight [85]. Knight et al. found that gestational IMH was associated with adverse maternal lipid levels [86]. Based on a study conducted in mice, the lipid dysregulation in the offspring of dams with gestational hypothyroidism may be more pronounced than those of dams with hypothyroidism before pregnancy [87]. Lower serum FT4 concentrations or higher FT3/FT4 ratios in the first trimester are associated with an increased risk of gestational diabetes, with approximately 69% of the association being mediated by specific lipids (i.e., PC (O-36:1), PE (P-38:6), and DG 18:0/18:1) [88]. Therefore, maternal thyroxine level may increase the risk of gestational diabetes, macrosomia, and large for gestational age through regulating maternal lipid metabolism.

3. Effects of Maternal Thyroid Hormone Level on Fetal Intelligence Development

Maternal thyroid hormones during the first trimester of pregnancy are essential for fetal brain development, particularly the development of cerebral cortex responsible for language, hearing, and intelligence [89, 90]. Studies have shown that during fetal brain development, thyroid hormones can regulate the growth of neuronal axons and dendrites, and thus participate in synapse formation, myelination, and differentiation of specific neuronal population [91–93]. The development of the human brain can be divided into three stages. Stage I is the first 12 weeks of gestation, during which the neural development of the brain and brain stem as well as the neuronal migration are observed. At this stage, the fetus's thyroid gland is immature, so the thyroid hormones needed for brain development are completely dependent on the supply from the maternal supply. Stage II refers to the period from the third trimester of pregnancy to birth, characterized by axons elongation, active synapse formation, and important neuron maturation. Since the fetal thyroid becomes mature during this stage, brain development is influenced by the maternal thyroid hormone level, and to a lesser extent, by the fetal thyroid hormones level. Stage III is 2–3 years after birth, during which the formation of glial cells and myelin sheath as well as the proliferation and differentiation of cerebellar cells till the maturation of the brain is completed. Brain development at this stage is entirely dependent on the thyroid hormones produced by infants [92]. Single-cell sequencing has demonstrated the co-expression of organic anion transporting polypeptide-1 (SLCO1C1) and DIO2 in lateral radial glia (the universal stem cells of the cerebral cortex), indicating the close cooperation of the T4 transporters OATP1C1 and DIO2 in local T3 synthesis, thus underscoring the potentially important role of brain-derived T3 in neural development [94]. Recent studies have also demonstrated an "inverted U-shaped" relationship between maternal free thyroid hormones level in the first trimester of pregnancy and the IQ values and brain development in the offspring [90, 95]. Currently, there have been no clinical reports on the association between elevated TSH levels (with free thyroid hormones concentration in the normal range) and impaired neurocognitive development in offspring. Therefore, compared with TSH, the rapid recovery of FT4 is more important to obtain a favorable outcome.

5.2.2.6 Intervention and Treatment of Gestational Thyroid Dysfunction

Levothyroxine (LT4) is recommended for the treatment of hypothyroidism newly diagnosed during pregnancy. The recommended dose is 2.0–2.4 μg/kg/day based on TSH value, which should be adjusted according to the thyroid function after 2 weeks in order to achieve the goal as soon as possible. The TSH level should be controlled at the lower limit of the reference range (or 0.1–2.5 mU/L) throughout the course of pregnancy [96]. The treatments, the goal of TSH control before and during pregnancy, and the frequency of monitoring for subclinical hypothyroidism are the same as those for hypothyroidism. Treatment options are based on the serum TSH level and TPOAb positivity. There is no consensus about whether LT4 is an appropriate treatment for patients with IMH. According to the 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease during Pregnancy, there is no sufficient evidence from interventional clinical studies to support the use of LT4 for the treatment of women with IMH [97]. However, the 2014 European Thyroid Association Guidelines for the Management of Subclinical Hypothyroidism in Pregnancy and in Children recommend that LT4 should be administered in the first trimester of pregnancy, but not in the second and third trimesters of pregnancy [98]. The strength of recommendations in both guidelines is weak, which is mainly due to the fact that there are no compelling proofs from evidence-based medicine to support the treatment benefits in IMH patients. The most recent Chinese guideline recommends LT4 treatment in early pregnancy [96], and no treatment in mid-and late pregnancy is suggested in both 2018 [99] and 2020 Chinese guidelines [96].

As the fetus is particularly susceptible to the potential teratogenic effects of the antithyroid drugs (ATDs) during the first 6–10 weeks of pregnancy, propylthiouracil (PTU) is considered as the preferred option, and methimazole is a second-line treatment during the first 10 weeks of pregnancy. Thyroid function should be monitored every 1–2 weeks in the first trimester of pregnancy and every 2–4 weeks in the second and third trimesters of pregnancy, with serum FT4 or total thyroxine (TT4) level being the preferable indicators. The dose of ATDs should be adjusted according to the results of thyroid function monitoring. The recommended dose is the minimum dose at which FT4 can be controlled at or slightly higher than the upper limit of the normal range. If serum TRAb is negative in the first trimester, it is not necessary to retest during pregnancy or postpartum. If serum TRAb level is elevated in the first trimester, it should be thoroughly monitored during 18–22 weeks of pregnancy and in the third trimester. For women with TRAb above three times of the upper limit of the reference range in the second and third trimesters of pregnancy, fetal heart rate should be monitored, and an ultrasound examination is required to evaluate the volume and the growth/development of fetal thyroid. Meanwhile, neonatal thyroid function should be closely monitored postpartum. These allow for early identification of hyperthyroidism or hypothyroidism in fetuses and newborns. Treatment with ATDs is generally not recommended for gestational transient thyrotoxicosis.

5.2.2.7 Clinical Cases: Pregnancy Outcome and Neonatal Follow-Up Results from a Case of Severe Gestational Hypothyroidism

The 33-year-old pregnant woman, G2P1, previously delivered a healthy full-term baby girl in 2012. The patient was diagnosed with pituitary prolactinoma (head MRI: pituitary tumor 13*20 mm, PRL: 62.2 ng/mL), Sicca syndrome (antinuclear antibody 1: 320, anti-SSA antibody ++, anti-SSB antibody ±, anti-Ro-52 antibody ++), and hypothyroidism (TSH > 100 μIU/mL, FT3: 2.58 pmol/L, FT4: 5.52 pmol/L) in another hospital from 2017 to 2018. The patient was treated with bromocriptine and chloroquine; oral levothyroxine was given for the treatment of hypothyroidism, with a starting dose of 25 μg qd, which was gradually increased to 100 μg qd. During the routine follow-up of thyroid function,

a decreasing trend in TSH was noted, and FT4 gradually increased to the normal range. In December 2019, a retest of thyroid function performed in another hospital demonstrated the following results: TSH: 23.19 μIU/mL, FT3: 34.05 pmol/L, and FT4: 9.18 pmol/L. After that, levothyroxine supplements were discontinued by the patient, and thyroid function was not followed up during this period. In August 2020, the patient presented to another hospital due to "amenorrhea for 64 days with cold intolerance, cold limbs, and memory deterioration," and the blood test results were shown as follows: TSH > 490 mIU/L, FT3: 2.09 pmol/L, FT4: 0.62 pmol/L, and PRL: 20.84 ng/mL. The ultrasound result revealed early intrauterine pregnancy, with the embryo of 9.1 mm and a visible fetal heart. According to these findings, the gestational week was estimated to be 7 weeks, and the corrected expected date of delivery (EDD) was March 29, 2021. The patient was referred to our hospital, and given oral levothyroxine at the starting dose of 50 μg qd, which was up-titrated to 175 μg qd + 200 μg biw, followed by a maintenance treatment at this dose. From 9+4 weeks of gestation, prednisone acetate tablets 15 mg qd and α-calcitriol were added for the treatment of Sicca syndrome; meanwhile, low-molecular-weight heparin at 4100 IU qd and aspirin 75 mg qd were given for anticoagulation. Aspirin was discontinued at 37 weeks of pregnancy, and low-molecular-weight heparin was administered until delivery. At 10 weeks of gestation, FT4 increased to normal level (FT4: 9.8 pmol/L, TSH: 96.28 mIU/L, TPOAb: 35.7 mIU/L, TgAb: 364.2 mIU/L). At 18+1 weeks of gestation, TSH decreased to the lower half of the normal range (TSH: 0.76 mIU/L, FT4: 14.8 pmol/L, TPOAb: 24.5 mIU/L, TgAb: 150 mIU/L). Thyroid function was well controlled during pregnancy, TSH fluctuated between 0.65 and 0.98 mIU/L, and FT4 fluctuated between 12.5 and 14.8 pmol/L. On March 17, 2021, when the patient was in her 38+2 gestational weeks, she was admitted to the emergency department due to "irregular lower abdominal pain with vaginal bleeding for 3 h." The results of obstetrical examination were listed as follows: uterine height: 37 cm, abdominal circumference: 99 cm, regular and weak uterine contraction, cervix dilated to 2 cm, head presentation, resting at −2, and spontaneous rupture of fetal membrane for 2 h, with small amount of transparent amniotic fluid. Therefore, the patient was admitted to our hospital for "38+2 weeks of gestation, G2P1, in labor, pregnancy complicated with hypothyroidism, pregnancy complicated with Sicca syndrome, and premature rupture of membranes." Finally, the patient successfully delivered a baby girl via the vagina. The labor process was smooth. The newborn weighed 3335 g, with a body length of 49 cm and an Apgar score of 10. The amniotic fluid was clear, with normal placenta and umbilical cord. After delivery, the newborn was placed in the same room with her mother and was breastfed. The newborn's general condition was good and responded well.

Two weeks postpartum, the dose of levothyroxine was adjusted to 50 μg qd based on the results of thyroid function tests (TSH: 1.24 mIU/L, FT3: 3.0 pmol/L, FT4: 12.7 pmol/L). The general condition of the newborn was good during the follow-up, and the neonatal behavioral neurological assessment (NBNA) score was 36 (the full score was 40, and score ≥35 is considered as normal).

Six months after delivery, the infant's growth and development appeared normal (body weight: 8.7 kg, body height: 71.3 cm, head circumference: 42 cm). There were no abnormalities in body temperature, sleep, feeding, and defecation, and the physical examination revealed normal results. There were no findings in hearing and vision screening, and the neurocognitive development was assessed according to age using the Mental Development Screening Test (DST), with the results of a developmental quotient (DQ) of 93 (DQ ≥ 85 is considered as normal).

In this case, although the patient was complicated with severe hypothyroidism, following timely and active treatments, FT4 increased to the normal range at week 10 of gestation, and TSH decreased to the normal range at the 18th gestational week. This case suggests that even pregnant women with severe hypothyroidism in early pregnancy can achieve favorable maternal

and infant outcomes as long as they receive active treatments in a timely manner. This case not only provides great confidence to other pregnant women who are worried about their pregnancy due to overt hypothyroidism but also provides new insights to clinicians: pregnant women with overt hypothyroidism can continue their pregnancy and deliver healthy babies based on their own wishes. However, although no abnormalities have been observed in the neonatal thyroid function tests and neurological/intelligence tests, the first 2–3 years after birth is another critical period of neurodevelopment, and further follow-up is still required. In addition, for complex obstetric diseases, co-management based on multidisciplinary cooperation should be strengthened. Although the patient in this case had severe hypothyroidism before pregnancy (TSH up to 490 mIU/L, FT4 as low as 0.62 pmol/L), a normal outcome of neurological/intellectual development was observed in the fetus following timely and active intervention with LT4. This result also confirms that early intervention with LT4 supplements is the key to achieving a satisfying perinatal outcome for pregnant women with overt hypothyroidism during pregnancy.

5.2.3 Short-Term and Long-Term Effects of Gestational Hypertensive Disorders on the Fetus

5.2.3.1 Pathophysiological Mechanism of Hypertensive Disorders in Pregnancy

During normal pregnancy, the placental trophoblasts differentiate into villous trophoblasts and extravillous trophoblasts (EVTs). The EVTs invade the endometrial stroma up to one third of the inner myometrium, and they can also enter the lumen of the uterine spiral arteries to gradually replace the vascular smooth muscle cells and the vascular endothelial cells on the vascular wall. Adequate remodeling of the uterine spiral arteries allows for the enlargement of the vascular diameter, and the arteries transform from high resistance and low-flow-volume vessels to low resistance and high-volume vessels, which increases the placental blood flow to meet the needs of fetal growth and development. However, in the case of preeclampsia, the invasive ability of EVTs is impaired, resulting in the shallow implantation of the placenta and inadequate uterine spiral artery remodeling. In such cases, only the spiral arteries in the decidual layer are remodeled, and the diameter of the arteries may be 50% of the normal size, leading to increased vascular resistance and decreased blood flow [100].

Placental formation requires extensive endometrial angiogenesis to support the establishment of a suitable vascular network to provide oxygen and nutrients to the fetus. The developing placenta produces various pro-angiogenic and anti-angiogenic factors, and the balance between these factors is essential for normal placental development. Local hypoperfusion caused by the failure in uterine artery remodeling may also lead to the release of various factors, such as inflammatory cytokines and antiangiogenic factors, resulting in a systemic inflammatory response, imbalance between pro-angiogenic factors and antiangiogenic factors, the decline of placental function, and decrease in placental perfusion [101].

The fetus is regarded as a semi-allograft to the mother. During a normal pregnancy, the maternal immune cells on the maternal-fetal interface show a low level of reactivity to placental trophoblasts, which contributes to an adequate maternal-fetal immune tolerance. As the EVTs invade the spiral arteries, they come into contact with the decidual natural killer cells (dNKs) and the NK cells ($CD56^+CD16^+$) and T cells in maternal blood. HLA-C and HLA-G expressed by EVTs can serve as the ligands of killer cell immunoglobulin-like receptors (KIR) on NK cells. If the expression of HLA-G is reduced or blocked, EVTs will inevitably be killed by cytotoxic NK cells. In order to avoid being killed by the NK cells, EVTs invade the spiral arteries at a shallow layer, and the spiral artery lumen becomes narrower.

In summary, gestational hypertension and preeclampsia are driven by multiple mechanisms and pathways. Firstly, deficient remodeling of the

uterine spiral arteries leads to placental ischemia and hypoxia, which releases a variety of placental factors. These molecules enter the maternal blood circulation, with subsequent activation of systemic inflammatory response and vascular endothelial injury, resulting in systemic small vessel spasm, vascular endothelial injury, and reduced blood perfusion of maternal organs and placenta. Taken together, these changes have an adverse effect on fetal growth and development [102, 103], such as intrauterine growth restriction and fetal distress or even premature placental abruption secondary to rupture of placental bed arteries.

5.2.3.2 Effects of Gestational Hypertension and Preeclampsia on the Fetus

1. Intrauterine Growth Restriction

   The process of normal fetal growth consists of three consecutive yet slightly overlapping phases. The first phase is characterized by cell proliferation, which covers the first 16 weeks of pregnancy. The second phase is defined as the 16–32 weeks of pregnancy, during which cell proliferation occurs simultaneously with cell enlargement, involving an increase in both cell number and cell size. The third phase is the period from week 32 of gestation to full term, which is characterized by cell enlargement, with a rapid increase in cell size. According to this pattern, fetal growth restriction can be classified as symmetrical or asymmetrical types.

   Various factors may lead to systemic small vessel spasms and vascular endothelial injury in patients with gestational hypertension and preeclampsia, resulting in decreased placental function and uteroplacental perfusion and eventually causing fetal ischemia and hypoxia. In order to adapt to this adverse intrauterine environment, the fetal systemic blood flow is redistributed, preferentially supplying the vital organs (such as the brain and heart) while reducing the blood supply to non-vital organs (such as the abdominal organs, lungs, skin, and kidney). These changes lead to intrauterine growth restriction of the fetus, which is mainly manifested as an asymmetrical type [104]. The fetus and placenta of preeclampsia patients are smaller, with reduced organ weight and decreased cell size compared to those of healthy mothers; however, the weight of vital organs such as the heart and brain is close to a normal level, and there is no decrease in cell numbers. Compared with the fetuses born to healthy mothers, those born to patients with preeclampsia have 5% lower birth weight, on average, a difference that is more pronounced in pregnant women with early-onset preeclampsia. A prospective study based on a Chinese population [105] indicated that earlier onset of gestational hypertensive disorders was associated with a higher risk of impaired growth and development of the fetus. These results suggest that longer duration of gestational hypertensive disorders may have greater effects on fetal growth and development.

2. Intrauterine Fetal Distress and Fetal Death

   Oxygen is delivered from mothers to their fetuses through uteroplacental circulation, while the carbon dioxide produced by the fetuses is released into the maternal circulation. In pregnant women with insufficient placental function due to gestational hypertension and preeclampsia, disorders may be observed in this oxygen supply and carbon dioxide release process, and long-term chronic ischemia and hypoxia may also lead to fetal intrauterine growth restriction.

   Intrauterine fetal distress is mainly manifested as hypoxemia or even metabolic acidosis in severe cases. Blood gas parameters of the umbilical artery can be measured after delivery to assess the degree of neonatal ischemia and hypoxia, but the umbilical artery blood can only be collected after delivery. The flow velocity waveform measurements of umbilical artery blood by ultrasound are helpful to judge placental blood perfusion during pregnancy. End-diastolic blood flow is the most important indicators for umbilical blood flow monitoring, which increases gradually with the increase of gestational age, along with a decrease in the resistance index (RI).

During normal pregnancy, the diastolic blood flow cannot be detected until 16 weeks of gestation. The abnormal umbilical artery spectrum is characterized by an increase in S/D, PI, and RI, and the diastolic blood flow is not visible or even reversed. If the umbilical artery S/D is >3 after 32 gestational weeks or the umbilical artery diastolic flow is absent or reversed after 16 gestational weeks, it can be considered as placental function impairment. The absence of umbilical artery diastolic flow indicates that more than 75% of the placental vascular bed has necrotized, and the probability of fetal hypoxemia and acidosis is 85% and 50%, respectively. In recent years, an increasing number of studies have shown that the early diastolic notch of the uterine artery is an important indicator to evaluate fetal distress. Some studies have demonstrated that the abnormal changes in uterine artery blood flow have already occurred in patients with gestational hypertensive disorders when the indicators of umbilical artery blood flow are still within normal ranges.

Severe intrauterine hypoxia may cause irreversible damage to the fetus, even fetal death. Studies have shown that gestational hypertension is significantly associated with fetal death in developing countries, while placental dysfunction and premature placental abruption are the main causes of fetal death in pregnant women with gestational hypertension.

3. Oligohydramnios

During normal pregnancy, the production and absorption of amniotic fluid maintain a dynamic equilibrium. The secretion and absorption of amniotic fluid mainly depend on the amniotic epithelial cells through 20 weeks of gestation. After this period, fetal urine becomes the main source of amniotic fluid, which is absorbed by fetal swallowing. Due to the low placental perfusion in patients with gestational hypertension and preeclampsia, fetal blood is redistributed to ensure the blood perfusion of vital organs, among which the kidney is the most severely affected organ. As such, there is a decrease in fetal urine production, resulting in oligohydramnios. In addition, oligohydramnios is an important signal of fetal intrauterine anoxia; besides, oligohydramnios can also lead to umbilical cord compression, which in turn exacerbates fetal anoxia. Studies have shown that compared with normal pregnancies, neonates born to mothers with oligohydramnios have a sevenfold increased risk of mild neonatal asphyxia and a 13-fold increased risk of perinatal mortality [106].

4. Premature Placental Abruption

Preeclampsia is an independent risk factor for premature placental abruption. The risk of premature placental abruption in pregnant women with preeclampsia has been reported to be 2–4 times higher than that in women with normal pregnancies. The incidence of premature placental abruption in pregnant women with early-onset preeclampsia was as high as 4.1–22.9%. This can be explained by the generalized vasospasm and sclerosis in pregnant women with preeclampsia; additionally, vasospasm and sclerosis may also occur to the spiral arteries in the basal decidua, which leads to the ischemic necrosis with subsequent rupture and hemorrhage of distal capillaries. The blood enters the space between placenta and basal decidua, followed by hematoma formation, and ultimately causing the separation of placenta from the uterine wall.

In the event of severe premature placental abruption, the placental villi and decidua at the abrupted areas release a large amount of tissue thromboplastin, which in turn activates the coagulation system and leads to disseminated intravascular coagulation (DIC). Progression of DIC can activate the fibrinolytic system and cause secondary hyperfibrinolysis, which eventually induces severe coagulation disorders and life-threatening conditions. Based on the reports from China, the incidence of DIC in patients with premature placental abruption was approximately 1–3.7%, and about 1% of the pregnant women with placental abruption died. The perinatal mortality was as high as 12%, which was

20-fold higher than that of normal pregnant women [107].

5. Iatrogenic Premature Delivery

Gestational hypertensive disorders are one of the important causes of preterm delivery, particularly iatrogenic premature delivery. Gestational hypertensive disorders are not regarded as an absolute indication for cesarean section, and the timing of pregnancy termination should be determined on the basis of gestational weeks, disease severity, and fetal conditions. According to the 2020 Chinese guidelines for diagnosis and treatment of hypertensive disorders complicating pregnancy [108], pregnant women with gestational hypertension and preeclampsia classified as non-severe are expected to persist to 37 weeks of gestation. For pregnant women with severe gestational hypertension and preeclampsia, termination of pregnancy is recommended for those who are less than 26 weeks of gestation and are still critically ill after treatment. For pregnant women at 26–28 weeks of gestation, termination of pregnancy should be decided based on maternal and fetal conditions in combination with the diagnosis and treatment capabilities of the hospital. For pregnant women at 28–34 weeks of gestation with a worsening condition despite active treatment, and for those beyond 34 weeks of gestation who have severe maternal and fetal complications or life-threatening conditions, termination of pregnancy should be considered. The risk of premature termination increases with the increasing severity of the disease. Although timely termination of pregnancy may avoid the occurrence of some adverse outcomes, given the immature development of various organs and systems in premature infants, there may be a variety of complications, such as neonatal respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis [109, 110]. Among these, neonatal respiratory distress syndrome is the main cause of death in premature infants, and preterm birth is also the primary cause of perinatal death [110]. There are many factors affecting the prognosis of premature infants, among which gestational age and birth weight are of great importance. Smaller gestational age and lower birth weight are associated with poor prognosis. Data from the United States in 2013 showed that the mortality rate of preterm infants with birthweights of 1000–1250 g and 2000–2500 g was 61.7‰ and 9.9‰, respectively. The mortality rate of preterm infants with less than 28 weeks of gestation was 374.7‰, while the mortality rate of those with 28–31 weeks of gestation was 35.7‰.

5.2.3.3 Long-Term Effects of Gestational Hypertension and Preeclampsia on the Offspring

1. Effects on Nervous System Development in the Offspring

Maternal hypertension during pregnancy is the main factor affecting the development of the central nervous system in the fetus. With worsening hypertension, the proportion of neonates with abnormal central nervous system increases accordingly, and the incidence of neonatal hypoxic-ischemic brain injury increases significantly, which has a direct impact on the survival and long-term prognosis of newborns. The types of brain injury include neonatal hypoxic-ischemic encephalopathy, intracranial hemorrhage, and periventricular leukomalacia. Studies have shown that brain injuries in term infants are mainly hypoxic-ischemic encephalopathy and intracranial hemorrhage, while brain injuries in premature infants are primarily periventricular white matter lesions and intraventricular hemorrhage. Due to premature separation from the maternal environment, the cerebrovascular regulation mechanism and collateral vessels in preterm infants have not been fully developed. Since the periventricular white matter is located in the terminal area of the cerebral artery blood supply, periventricular ischemia is likely to cause periventricular leukomalacia [111].

The offspring of patients with gestational hypertensive disorders are predisposed to vari-

ous types of neurodevelopmental abnormalities. Studies conducted in China have revealed that the incidence of severe neurological sequelae (e.g., cerebral palsy and mental retardation) in the offspring of pregnant women with gestational hypertensive disorders was 1.5%, while 15.2% of these children had mild neurodevelopmental abnormalities. In addition, the follow-up results showed that 3.68% of the children with neurodevelopmental abnormalities recovered after active intervention and rehabilitation treatment, which also suggested that early intervention has a positive effect in reducing neurological sequelae.

The effect of gestational hypertension on perinatal infants depends on its severity. Among pregnant women with severe preeclampsia, there is a notable reduction in uteroplacental blood perfusion, and the placenta cannot maintain normal function, which has a significant effect on infants. The time to onset of severe preeclampsia has great effects on neonatal prognosis. Studies have demonstrated that the incidence of severe brain injury in neonates born to mothers with early-onset severe preeclampsia is significantly higher than those born to mothers with late-onset severe preeclampsia. The reasons underlying this are described as follows: (1) The incidence of premature delivery in mothers with early-onset severe preeclampsia is extremely high. Studies have shown that 86% of the neonates whose mothers have early-onset severe preeclampsia are preterm infants, and preterm birth is the main reason for the high incidence of neonatal brain injury. (2) A subset of pregnant women whose early-onset severe preeclampsia is controlled after treatment continue their pregnancy to full term or close to full term; however, the prolonged exposure to the adverse intrauterine environment has a significant effect on fetal development, leading to an increased incidence of *in utero* brain injury [112].

2. Effects on the Incidence of Chronic Diseases in the Offspring During Adulthood

The "Developmental Origins of Health and Disease (DOHaD)" hypothesis proposed by David Barker revealed an association between fetal malnutrition *in utero* and the development of certain adult diseases [113]. According to this theory, an adverse intrauterine environment resulting from decreased placental function may permanently alter the structure and function of the biological feedback system, and there will be an increased susceptibility to disease in the future [110]. Based on the DOHaD hypothesis, subsequent studies have demonstrated that even in neonates of preeclamptic pregnant women with a birth weight of more than 2500 g, hypertension may still develop in childhood and adolescence, with the increase in systolic blood pressure as the most pronounced manifestation [114, 115], and these neonates are at increased risk of stroke in their later lives. In addition, studies have also noted changes in physical development, sensorimotor reflexes, body mass index, neuroanatomy, cognitive function, and hormones in the offspring of preeclamptic pregnant women [116, 117].

5.2.4 Effects of Intrauterine Hyperandrogenism on the Offspring of Pregnant Women with Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disease in women of childbearing age, characterized by hyperandrogenemia, ovulatory dysfunction, and ovarian polycystic morphology, which may lead to fertility impairment. In addition to the adverse effects on reproductive function, PCOS is also associated with multiple comorbidities such as metabolic disorders, insulin resistance, obesity, diabetes, and cardiovascular diseases. Hyperandrogenemia is one of the key features of PCOS and also contributes to the aggravation of reproductive symptoms and metabolic syndrome, among which hyperandrogenism is the most heritable phenotypic trait [118, 119]. Increasing evidence suggests that the hyperandrogenic intrauterine environment in women with PCOS

during pregnancy is the potential cause of the adverse effects on their fetuses and the development of PCOS features in the offspring [120, 121].

5.2.4.1 Origins of Intrauterine Hyperandrogenism in PCOS

There are five types of androgens identified in women: dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T), and dihydrotestosterone (DHT). These androgens are ranked in decreasing order of serum concentrations in women as follows: DHEAS > DHEA > A4 > T > DHT, and also in increasing order of biological activities as follows: DHEAS < DHEA < A4 < T < DHT [122]. DHEAS, DHEA, and A4 are the major androgen precursors, whereas T and DHT are potent androgens that may induce biological effects by direct binding to the androgen receptors (ARs).

Hyperandrogenemia is an important manifestation of PCOS, and increasing evidence has shown that women with PCOS may have a hyperandrogenic intrauterine environment during pregnancy [123]. There are three potential origins of hyperandrogenism in women with PCOS: (1) Maternal-fetal transmission: Several studies have shown that the levels of A4, T, and DHEAS in peripheral blood of women with PCOS were significantly increased [124, 125], while the levels of T and A4 in cord blood obtained from the offspring of women with PCOS were increased [126, 127]. These results indicated that the excessive androgens in the circulation of women with PCOS during pregnancy were probably transferred to the fetus through the placenta, which exposed the fetus to a hyperandrogenic intrauterine environment. (2) Changes in placental structure and enzyme activity in women with PCOS: Changes have been observed in the activity of two steroidogenic enzymes in the placenta of women with PCOS. Specifically, there was an enhanced activity of 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD-1) and a reduced activity of CYP450 aromatase, which promoted the production of androgens in women with PCOS during pregnancy [128]; this may be a potential origin of the hyperandrogenic intrauterine environment. (3) Androgen secreted by the fetal ovary and adrenal gland: The fetal ovary is capable of synthesizing androgens in the second trimester of pregnancy. Moreover, fetal adrenal mainly involves in secreting DHEA *in utero*, which can be converted into androstenedione, testosterone, and estradiol by the placenta [129]; this may serve as another origin of intrauterine hyperandrogenism.

5.2.4.2 Intrauterine Hyperandrogenism in the Pathogenesis and Development of PCOS

A large number of clinical cohort studies and animal studies have shown that PCOS is a complex disease that is mediated by multiple factors, including genetic, epigenetic, and maternal-fetal environmental factors. Although recent genome-wide association studies (GWAS) have identified several genetic susceptibility loci in patients with PCOS, these loci account for less than 10% of the heritability of the disease [130]. Therefore, other factors contributing to the susceptibility to PCOS should be investigated, including intrauterine environment and epigenetic factors. It is generally believed that intrauterine exposure to hyperandrogenism as an environmental factor may cause epigenetic modifications in fetal genes, mainly those involved in the regulation of ovarian steroidogenesis, follicular development, gonadotropin release, and insulin resistance.

In animal studies, the prenatally androgenized (PNA) animal models were frequently used [131] to investigate the effects of exposure to a hyperandrogenic intrauterine environment on the offspring following injection of testosterone or DHT at different stages of pregnancy [132]. Excessive androgens could eventually lead to the inhibition of placental formation and fetal growth restriction through different mechanisms [133, 134]. Some studies have found that the female offspring of pregnant rats exposed to a hyperandrogenic intrauterine environment during pregnancy showed changes in the methylation of certain genes in ovarian tissue during adulthood, which led to hyperandrogenemia and PCOS-related

phenotypes in the offspring [135, 136]. Recent studies have shown that autophagosomes were significantly increased in ovarian granulosa cells of the PNA mouse model. Excessive androgens (DHT) in vitro could lead to hypomethylation of the promoter regions of *Map3k1* (encoding MEKK1) and *Map1lc3a* (encoding LC3II) in granulosa cells, which was associated with upregulation of *Map3k1* and *Map1lc3a* mRNA expression, and directly led to increased autophagy. It has also been reported that changes in autophagy might be driven by the activation of the MAPK/p53 signaling pathway, suggesting that epigenetic imbalance may trigger a cascade of downstream reactions through activation of the MAPK signaling pathway, which plays an important role in follicular maldevelopment, excessive autophagy, and metabolic disorders in PCOS [137].

5.2.4.3 Effects of Intrauterine Hyperandrogenism in Women with PCOS on Adverse Pregnancy Outcomes and the Offspring

5.2.4.3.1 Effects of Intrauterine Hyperandrogenism in Women with PCOS on Adverse Pregnancy Outcomes

Women with PCOS are considered to be at high risk of adverse pregnancy complications such as preeclampsia, gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR), and premature delivery [138, 139]. The placenta of a pregnant woman with PCOS is exposed to an environment characterized by hyperandrogenemia and insulin resistance (IR) during the first trimester of pregnancy, which has adverse effects on the development of placenta and fetus, and even leads to placental abnormalities and pregnancy complications in some cases.

1. Preeclampsia

   Normally, placental steroidogenic enzymes convert DHEA to A4, which is further converted to T. Placental aromatase is responsible for the conversion of placental androgens to non-androgenic metabolites, including estrone and estradiol [140]. However, there is a reduced expression of mRNA and proteins of placental aromatase in women with preeclampsia, which inhibits the metabolism of DHEA and T to estrogen metabolites, and produces a shift in the equilibrium between estrogens and androgens to the latter [141]. Studies have shown that in pregnant women who eventually develop preeclampsia, maternal androgen levels have increased in the second trimester, along with elevated levels of androstenedione, testosterone, and free testosterone at 17 and 33 weeks of gestation; on the contrary, the androgen precursor DHEA-S was only elevated at 17 weeks of gestation [142]. Based on these findings, Shao et al. demonstrated that testosterone downregulated the mRNA expression of aromatase in human trophoblasts via the miR-22-mediated mechanism [143]. Therefore, hyperandrogenemia associated with PCOS can be considered as an early risk biomarker for preeclampsia and may be involved in the pathogenesis of preeclampsia.
2. GDM

   Pregnant women with GDM have lower levels of sex hormone-binding globulin (SHBG) in the first trimester of pregnancy [144], and there is a significant negative correlation between the SHBG level in the first trimester and the subsequent development of GDM [145]. In addition, the expression of androgen receptor (AR) was significantly increased, while the protein expression of aromatase was decreased in the placenta of pregnant women with GDM compared with those of the control group [146]. Since women with PCOS have altered placental steroidogenesis [147], persistent insulin resistance (IR), and altered hormonal profiles including high androgen levels and low SHBG levels, PCOS is considered as a risk factor for GDM.
3. IUGR

   Recent studies have revealed a gender-dependent association between maternal androgen levels and offspring BMI and body weight. Maternal hyperandrogenism is only associated with lower BMI at birth in female

neonates, suggesting that maternal androgens may have differential effects on the programming of intrauterine growth depending on fetal gender [148]. In the sheep model of maternal hyperandrogenemia, it has been demonstrated that prenatal hyperandrogenemia could lead to reduced birth weight and height in both male and female neonates [149]. This may be attributed to the decreased availability of IGFs in response to an increase in insulin-like growth factor-binding proteins (IGFBPs), leading to intrauterine growth retardation. In addition to the effect of IGFs on fetal growth, placental dysfunction is a major contributor to fetal growth restriction [150, 151]. In a rat model of maternal hyperandrogenemia, fetal growth restriction induced by a high level of maternal androgens is associated with decreased placental amino acid transport activity [152]. Given that pregnant women with PCOS have placental alterations and changes in the IGFs and insulin pathways, it is important to evaluate the risk of IUGR and other potential effects on the offspring of these patients.

4. Premature Delivery

PCOS women with hyperandrogenism are at high risk of preterm delivery. Hyperandrogenism is considered as a risk factor in these women, but not the only one. Androgens are involved in the physiological processes of pregnancy establishment, maintenance, and delivery. DHEA-S and DHT as well as AR signaling pathways act on cervical remodeling, including cervical ripening, in preparation for delivery. In addition, studies also showed that AR could affect uterine muscle growth and proliferation, which were essential for pregnancy progression, and it also acted on uterine muscle contractions involved in parturition. The downregulation of AR expression in the myometrium was associated with preterm labor in humans. Although exposure to low concentrations of androgens did not affect myometrial contractility, uterine muscle relaxation was observed following exposure to high levels of androgens in animal models [153]. Studies have shown that antiandrogen pretreatment before pregnancy could reduce the risk of premature delivery [154], suggesting that hyperandrogenism may be a risk factor for premature delivery. Therefore, androgen-related pathways may be altered in PCOS women with hyperandrogenism, leading to adverse pregnancy outcomes and an increased risk of premature delivery.

5.2.4.3.2 Effects of Intrauterine Hyperandrogenism on the Offspring of PCOS Women

PCOS has been generally recognized as a disease with familial aggregation tendencies. Moreover, emerging evidence suggests that the etiology of PCOS is more likely to have a strong environmental component rather than a genetic basis. The offspring exposed to the hyperandrogenic intrauterine environment in the setting of PCOS showed manifestations similar to PCOS phenotypes [155, 156], involving the reproductive system, metabolic system, cardiovascular system, nervous system, etc.

Effects of Intrauterine Hyperandrogenism on the Reproductive System of the Offspring

1. Reproductive System Malformation

It has been reported that prenatal androgen exposure may lead to increased anogenital distance, abnormal nipple development, and abnormal reproductive tract morphology in female rats [157]. Studies in rodents have shown that maternal hyperandrogenism could block steroid feedback mechanisms, impair follicular recruitment, and cause reproductive system malformations in female offspring [158–160]. Prenatal exposure to hyperandrogenism during intrauterine development in women was associated with impacts on steroid target tissues, reproductive changes in adolescence and adulthood [161], irregular or absent estrous cycles, abnormal follicular development, and low responsiveness of the uterus to hormones, thereby impairing the fertility [162, 163].

2. Follicular Dysgenesis

The hyperandrogenic intrauterine environment may affect the development of ovarian

follicles in the offspring, resulting in increased recruitment of primordial follicles and decreased ovarian reserve. In the studies using a prenatally androgenized rat model of PCOS, downregulation of folliclestatin gene expression and upregulation of steroidogenesis pathway gene expression (cytochrome P450-17 (CYP17), GATA-binding protein (GATA6), and steroidogenic acute regulatory protein (StAR)) were noted in ovarian follicular theca cells in adulthood [164, 165]. It was also found in a prenatally androgenized rat model of PCOS that the expression of follicle-stimulating hormone receptor (FSHR) and activin receptor (actR) genes in ovarian granulosa cells (GCs) were decreased, which might be one of the mechanisms underlying the effects of fetal androgens exposure *in utero* on the folliculogenesis and ovulation dysfunction in the adulthood [166]. Studies have shown that maternal hyperandrogenism, especially in the third trimester of pregnancy, could cause impaired follicular development and disturbance in sex hormone synthesis in the female offspring. These impairments may be partially due to the reduced expression of FOXL2 and CYP19A1 in ovarian granulosa cells [167]. Recent studies also demonstrated that adult rats prenatally exposed to hyperandrogenism were at risk of marked pathological changes, such as disturbances in the cell cycle of uterine tissue, dysregulated cell death and survival pathways, proliferative uterus, anovulation, and mating failure [168].

Effects of Intrauterine Hyperandrogenism on the Metabolic System of the Offspring

1. Risk of Obesity in the Offspring

   A prospective cohort study conducted by Finnbogadóttir et al. showed that there was no difference in the birth weight and other indicators between the offspring of PCOS mothers and non-PCOS mothers, but the BMI at 3 years of age was significantly higher in the offspring of PCOS mothers compared with the offspring of non-PCOS mothers [169]. However, in another large prospective cohort study [170], there was no significant difference in the body weight, body height, and body mass index (BMI) at 3 years old between the children born to PCOS mothers and non-PCOS mothers. A recent multivariate linear regression analysis showed that the BMI of PCOS patients before pregnancy was positively associated with the BMI of the offspring at the age of 6–8 years [171]. It was also shown in a rat model that significant weight gain in adulthood was observed in the offspring of rats exposed to hyperandrogenism during the fetal period [172]. These studies suggest that maternal PCOS during pregnancy may be a significant weight gain effect as the PCOS offspring age increases.
2. Endocrine Profiles in the Offspring

   Maternal PCOS status not only changes the intrauterine environment and leads to endocrine abnormalities in the offspring during the fetal period but also continues to impair the endocrine health of the offspring in various growth and development stages after birth. Exposure to a hyperandrogenic intrauterine environment may lead to increased visceral adipocyte volume, insulin resistance, increased fasting blood glucose, and impaired glucose tolerance in the female rat offspring, which has a direct and negative effect on the pancreatic organ of the offspring. As a result, there were increased pancreatic β cells in the offspring, contributing to long-term changes in glucose and lipid metabolism [173, 174]. Recent studies conducted with murine models demonstrated that female F1–F3 offspring developed metabolic phenotypes similar to those found in women with PCOS. The transgenerational transmission of metabolic dysfunction in androgens was demonstrated, as evidenced by increased fat mass, larger adipocytes, and disturbed lipogenesis [175]. A DHEA-induced model was established in which female rats were exposed to this androgen on a daily basis from the prepubertal stage (Day 27) through the pubertal stage (Day 46) after sexual maturity; the study showed that the rats in the DHEA group had impaired estrous cycle and ovarian morphology, along with impaired glucose tolerance and abnormal lipid metabolism [176].

Effects of Intrauterine Hyperandrogenism on the Cardiovascular System of the Offspring

High levels of androgens in the circulation of PCOS women may increase the risk of cardiovascular diseases in the offspring. Prenatal exposure to androgens leads to the development of hypertension in the offspring and alters their cardiac structure and function in adulthood [177–179]. Studies have shown that the offspring of PCOS mothers presented a series of cardiovascular abnormalities in early childhood (2.5–4 years old), including a significant decrease in diastolic blood pressure, increased aortic pressure, and increased left ventricular diameter, and carotid intima thickening was also observed in childhood (6–8 years old) [180]. Animal studies have demonstrated that exposure to intrauterine hyperandrogenism during the first trimester of pregnancy could inhibit insulin-like growth factor 1 (IGF-1) and cause IUGR in both female and male offspring, along with the inhibition of cardiomyocyte proliferation and maturation in the offspring. Exposure to intrauterine hyperandrogenism will not only lead to the development of PCOS and cardiovascular diseases in female offspring but also associated with complications of IUGR and cardiovascular diseases in male offspring [181]. Hou et al. [182] found that prenatal exposure to hyperandrogenism induced cardiac hypertrophy in adult female rats by upregulating the expression of PKCδ, a member of the protein kinase C family in cardiomyocytes. Recent studies in mice showed that exposure to DHT in the uterine of dams with or without obesity increased the risk of cardiac hypertrophy in adult female offspring and that remodeling of cardiac hypertrophy was associated with impaired expression of genes related to hypertrophy, fibrosis, calcium, and redox signaling, as well as dysregulation of androgen-related genes. In addition, increased expression of transcription factors and calmodulin gene *Slc8a2*, which were involved in the cardiac hypertrophy remodeling, was observed in the hearts of neonates from the PNA group on the first day of birth. Overall, in women with PCOS, maternal hyperandrogenism may increase the risk of cardiac dysfunction in the offspring [183].

Effects of Intrauterine Hyperandrogenism on the Nervous System of the Offspring

In several studies registered in the Swedish National Registry, prenatal androgens exposure has been proposed to have a potential causal relationship with the development of neuropsychiatric disorders in the offspring of PCOS women [184–186]. Studies have shown that the daughters of women with PCOS had an increased risk of attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), while their sons were at lower risks [184]. However, some other studies have found that boys born to women with PCOS were more likely to develop ADHD-related behaviors [187]. Recent studies revealed that prenatal exposure to androgens may lead to permanent reprogramming of the fetal nervous system. In addition, children born to women with PCOS had a higher risk of childhood anxiety and an increased risk of psychiatric and mild neurodevelopmental disorders [188, 189].

Exposure to androgens during the first and second trimesters of pregnancy led to the reprogramming of behaviors in adolescent and adult female offspring. Some studies have demonstrated that female monkeys exposed to androgens exhibited male-typical infant vocalization, decreased close social interactions with their mothers and interests in the infant, and reduced participation in female-typical sexual interactions with males [190]. A recent report of intrauterine hyperandrogenism in rodent models noted the anxiety state in female offspring exposed to hyperandrogenism *in utero* accompanied by upregulation of amygdala gene expression, which was likely due to the activation of the central noradrenergic system and the hypothalamic-pituitary-adrenal axis [191]. A recent study showed that in a mouse model of PCOS, maternal intrauterine hyperandrogenism led to the transgenerational transmission of anxiety-like behaviors to the female offspring (F1 and F3), but not to the male F1, F2, and F3 offspring. This study further demonstrated that the first generation of the male offspring exposed to maternal hyperandrogenism and obesity transmitted the anxiety-like behaviors to subsequent

male offspring (mF3), although they themselves seemed unaffected. These behavioral changes noted in F3 offspring (female and male) were accompanied by the altered expression of the genes in the amygdala, suggesting that elevated maternal androgen levels in mothers with PCOS and obesity may pose a risk of transgenerational transmission of anxiety to their offspring, an effect that can be mediated through epigenetic reprogramming [192].

5.2.4.4 Prevention and Intervention of PCOS

PCOS not only affects the perinatal outcomes of the offspring but also has a persistent impact on the health status of the offspring from fetal period through the various stages of postnatal growth and development. Therefore, active lifestyle management in patients with PCOS (including continuous and effective diet, exercise, and behavior interventions) has important implications for the prevention and control of PCOS. Women with PCOS should start to improve their hyperandrogenic conditions before pregnancy to avoid intrauterine hyperandrogenism. This is of great significance for the early prevention of PCOS or related phenotypes in their offspring. In addition, the offspring of PCOS should be monitored at an early stage, and effective interventions should be administered as appropriate.

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Fetal Growth and Its Trajectory

6

Yi-Wen Wang and Yong-Jun Zhang

6.1 Large for Gestational Age and Macrosomia

6.1.1 Epidemiology

Large for gestational age (LGA) is defined as birth weight (BW) above the 90th percentile of weight for infants of the same gestational age. However, it has also been suggested that LGA should be defined as the 97th percentile of birth weight higher than the weight of the same gestational age (2 standard deviations above the average), because the latter can more accurately represent the infants at the highest risk of perinatal complications and death [1, 2]. According to the US reference standard for single live birth infants, the 90th percentile of birth weight is 4000 g, and the 97th percentile is 4400 g for infants at the gestational age of 40 weeks [3]. LGA infants, especially at full-term or post-term, are at an increased risk of perinatal complications and long-term metabolic syndrome.

Diagnosis of macrosomia can be confirmed when the birth weight exceeds a specific threshold regardless of gestational age. The previous threshold was 4000 g. The American College of Obstetricians and Gynecologists (ACOG) recommended 4500 g be used as the threshold for the diagnosis of macrosomia as the incidence of complications increased significantly above this threshold.

The incidence of macrosomia in developed countries has increased from 5%–20% to the current 15%–25% in the past 20 to 30 years, mainly due to the increase in maternal obesity and diabetes [4]. Although there are few data on changes in the prevalence of macrosomia in developing countries, a study in China pointed out that the prevalence of macrosomia increased from 6.0% in 1994 to 7.8% in 2005 [5].

Although the mechanism of fetal weight gain and fetal growth is not completely clear, the cause of fetal overgrowth may be the increase in nutrients delivered to the fetus,

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which is associated with genetic factors, in utero environmental factors and the interaction between the two.

1. Genetic factors: There are ethnic differences in the occurrence of LGA. A study in the United States included all full-term singleton live births born between 1995 and 1997, with significantly more White, American Indian, or Samoan mothers among LGA infants [2]. Another study found that Latino babies had a higher risk of developing macrosomia than African-American babies (19% vs. 50%) [6]. LGA also has familial characteristics, and mothers large for gestational age at birth are more likely to have LGA delivery than mothers appropriate for gestational age at birth. In addition, macrosomia may be a characteristic phenotype of certain genetic syndromes [2], namely, LGA due to early overgrowth, including Beckwith-Wiedemann syndrome, Simpson-Golabi-Behmel syndrome, Sotos syndrome, Weaver syndrome, and Berardinelli lipodystrophy.
2. In utero environmental factors: Mothers with diabetes, obesity, or excessive weight gain during pregnancy are more likely to have LGA delivery [7]. Macrosomia is common in infants of diabetic mothers (IDMs), especially when the mother's diabetes is not well controlled. Thereby, too many nutrients are delivered to the fetus, resulting in hyperglycemia and hyperinsulinemia in the infant, and accelerating the growth. Disproportionate growth with an increased ponderal index is sometimes present in macrosomia with diabetic mothers, who have higher head-chest ratio and head-shoulder ratio, higher body fat content, and thicker upper limb skin folds than macrosomia with nondiabetic mothers. This disproportionate macrosomia greatly increases the risk of birth trauma, especially shoulder dystocia. In addition, the risk of LGA delivery increases linearly with the magnitude of maternal weight gain during pregnancy. In addition, excessive maternal weight gain during pregnancy can also lead to macrosomia. Among women with normal BMI before pregnancy, the risk of LGA delivery with gain more than 15.9 kg during pregnancy is almost 2.5 times higher compared to those with weight gain of 11.3–15.9 kg [8]. Since overweight and obese mothers tend to have more weight gain during pregnancy, they are at the highest risk of delivering LGA. Even if overweight and obese mothers gain less weight than women with normal BMI, they still have a higher risk of LGA delivery. This correlation was not associated with an increased prevalence of gestational diabetes among obese mothers.
3. Epigenetic factors: Limited research data suggest that epigenetic changes in the placenta may accelerate fetal growth [9–12].
4. Other factors: Other factors associated with LGA include multipara, advanced maternal age, post-term pregnancy, male infants, history of LGA delivery, and a mother's birth weight of more than 4000 g.

6.1.2 Prenatal Diagnosis

Two-dimensional ultrasonography is the standard method for diagnosing macrosomia and LGA. Hadlock formula includes the measurements of abdominal circumference (AC), head circumference (HC), and femur length (FL), which can provide useful information.

6.1.2.1 Ultrasonography

Estimated fetal weight: Ultrasonography usually involves the measurement of multiple biometric parameters, and these parameters are included in the formula to generate the estimated fetal weight (EFW). The most commonly used parameters in combination are biparietal diameter (BPD), HC, AC, and FL. The most commonly used formula is the Hadlock formula [13, 14], modified Shepard's formula [15], and the Warsof formula [16]. These formulas are included in the component of most ultrasonic ultrasound devices:

Hadlock Formula

$$\log_{10} \mathrm{BW} = 1.3598 + 0.051(\mathrm{AC}) + 0.1844(\mathrm{FL}) - 0.0037(\mathrm{AC} \times \mathrm{FL}), \text{or.}$$

$$\log_{10} \mathrm{BW} = 1.4787 + 0.001837(\mathrm{BPD})^2 + 0.0458(\mathrm{AC}) + 0.158(\mathrm{FL}) - 0.003343(\mathrm{AC} \times \mathrm{FL})$$

Shepard Formula

$$\log_{10} \mathrm{BW} = -1.7492 + 0.166(\mathrm{BPD}) + 0.046(\mathrm{AC}) - \left[2.646(\mathrm{AC} \times \mathrm{BPD})/100\right]$$

Abdominal circumference: AC is the most important parameter for assessing the risk of macrosomia [17, 18]: Macrosomia can be predicted by AC of 35–38 cm alone [19]. AC is measured on a specific plane through the liver because changes in liver size often reflect abnormalities in fetal growth and development [20]. AC measurements obtained with two-dimensional measurements or ellipse evaluation are both accurate. If AC is >90th percentile or 2–3 weeks advanced for the specific gestational age, it may be an early indication of macrosomia that has appeared or is about to appear, even if the EFW is normal. When ultrasound assessment shows that the AC value is high, the fetus should be re-assessed after 3–4 weeks, especially for the fetus with diabetic mother. The occurrence of macrosomia can usually be predicted after two consecutive ultrasound examinations that indicate the AC value is high. If the AC value remained <90th percentile, then increasing the frequency of ultrasound examinations does not improve the predictive accuracy [21]. Continuous dynamic assessment of AC growth rate from 21 to 22 gestational weeks can also help predict macrosomia [22].

6.1.2.2 Non-ultrasonic Method

Physical examination: In clinical practice, fetal weight can be estimated by simple palpation of the abdomen of the pregnant woman (e.g., Leopold maneuvers) and/or measurement of the uterus fundal height (distance from above the pubic symphysis to the highest point of the uterine fundus). On examination, the pregnant woman needs to be in the supine position and empty the bladder. Although symphysis-fundal height measurement combined with Leopold maneuvers is inexpensive and simple to learn, relevant prospective studies have shown that the sensitivity of this method to predict macrosomia is only 10%–43%, and the positive predictive accuracy is only 28%–53% [23]. Therefore, for the general obstetric population, the ability to diagnose macrosomia prenatally by this clinical method is limited, but it is still with diagnostic value in populations at high risk.

MRI and two-dimensional ultrasound: Theoretically, MRI should be a better technique for assessing macrosomia because its advantage on assessing adipose tissue is better than ultrasound [24]. A few studies have evaluated the estimation of EFW by MRI and found that this method performs better than two-dimensional ultrasound based on the measurement of total fetal body volume. A meta-analysis that included studies comparing MRI with two-dimensional ultrasound for prediction of birth weight >4000 g or >90th percentile found that EFW estimated by MRI was more sensitive than two-dimensional ultrasound (93% vs. 56%), but EFW estimated by MRI was not significantly more sensitive than AC >35 cm measured by two-dimensional ultrasound (93% vs. 80%) [25]. Another prospective study included more than 2000 pregnant women and compared EFW measured by MRI and two-dimensional ultrasound at 36 weeks of gestation, respectively. For infants with birth weight ≥95th percentile, the detection rate of MRI was 80% (positive and negative predictive values were 42% and 99%, respectively), and the detection rate of ultrasound was 59.1% (positive and negative predictive values were 35.4% and 98%, respectively) [26].

Novel biomarkers: Previous studies have shown that the expression levels of several RNAs (MicroRNAs, miRNAs) in the placenta of macrosomia are significantly increased [27]. miRNAs may be associated with placental growth and development and may affect fetal growth and body shape. Other biomarkers that are expected to help predict macrosomia include maternal glucose-related markers, for example, glucose and 1,5-anhydrosorbitol, and hormones currently thought to be involved in placental nutrient transport, for example, adiponectin and insulin-like growth factor-1 [28, 29].

6.1.3 Complications and Potential Long-Term Effects

6.1.3.1 Complications

Macrosomia and LGA not only significantly increase the incidence of maternal complications, such as postpartum hemorrhage and emergency cesarean section, but also greatly increase the risk of their own various adverse outcomes [4, 30]. Common adverse outcomes include the following:

Birth trauma: Shoulder dystocia and birth trauma are more common for macrosomia, including brachial plexus injury and clavicle fracture [2, 31, 32]. For LGA infants, the incidence of birth trauma is higher in vaginal delivery compared to cesarean section. In a large case-series study, LGA infants (with birth weight of 4500–5000 g) were three times more likely to have birth trauma during vaginal delivery compared to cesarean section (9.3% vs. 2.6%) [31].

Respiratory distress: LGA infants have a higher probability of respiratory distress than AGA [2, 33]. This is mainly due to higher risk of neonatal respiratory distress syndrome (RDS), also known as hyaline membrane disease, in LGA, especially in IDMs, because of the increased incidence of preterm birth. The higher incidence of cesarean section in LGA infants appears to increase the risk of transient tachypnea of newborns. In addition, meconium aspiration is a common respiratory complication of LGA and may be associated with an increased risk of perinatal respiratory depression.

Hypoglycemia: Hypoglycemia is more common in LGA infants due to the interruption of placental glucose supply after birth. Data from 1997–2002 Dutch National Perinatal Registry showed an incidence of hypoglycemia of 19% and 15% in LGAs of all LGAs and nondiabetic mothers, respectively [34]. Meanwhile, 0.3% and 0.2% of the children in the above population developed seizures due to hypoglycemia, respectively. Another large case-series study of 887 German LGA newborns (birth weight >90th percentile) monitored blood glucose in the early postnatal period and found that 16% LGA infants had hypoglycemia (blood glucose level <40 mg/dL) within the first 24 h after birth [35].

Polycythemia: Polycythemia is more common in LGA infants with both diabetic and nondiabetic mothers compared to AGA [36]. It is speculated that the pathogenesis of polycythemia is that the increased oxidative demand due to hyperglycemia and hyperinsulinemia leads to the relative hypoxia of the fetus, which in turn leads to the increase of erythropoietin production, and eventually leads to polycythemia.

Perinatal asphyxia: Macrosomia is at a higher risk of perinatal asphyxia, especially in IDMs. The indirect evidence for an increased risk of perinatal asphyxia in LGA infants is that a lower Apgar score is more common in LGA infants compared to AGA infants [1, 2, 31, 37]. The related factors are increased in utero oxygen utilization due to fetal hyperglycemia and hyperinsulinemia (particularly for IDMs), and delivery complications associated with shoulder dystocia.

6.1.3.2 Potential Long-Term Effects

In the long run, macrosomia is more likely to be complicated by obesity in childhood, adolescence, and adulthood [38], as well as metabolic syndrome and cardiovascular disease in adulthood [39, 40].

6.1.4 Neonatal Management

The neonatal management of LGA includes screening and treatment of macrosomia-related complications, identifying the cause of overgrowth as far as possible, and routine neonatal

care. Before delivery, the need for neonatal resuscitation should be assessed based on gestational age, expected birth weight, presence of congenital abnormalities or complications in labor, mode of delivery (e.g., cesarean section), and maternal history. Routine neonatal care immediately after birth includes drying the skin, removing airway secretions, and keeping warm, as well as rapid assessment of the infant's clinical status based on the degree of respiratory effort, muscle tension, heart rate, and examination for the presence of major congenital abnormalities or genetic syndromes. The need for further intervention is determined based on the results of these initial assessments. If additional resuscitation is not required, the infant should be given to its mother in the delivery room for mother-to-child skin contact, and breastfeeding should be initiated. LGA infants should be fed as soon as possible after delivery to avoid hypoglycemia. Further evaluation after transfer from the delivery room includes a comprehensive examination to determine the presence of any potential genetic syndrome, birth trauma (e.g., perinatal respiratory depression, brachial plexus injury, or clavicle fracture), or congenital defect. Laboratory screening for hypoglycemia and polycythemia should be performed within a few hours after birth. If there are no important complications requiring further intervention, routine neonatal care is provided.

6.1.5 Research Findings

Our research group has previously conducted a series of studies on LGA. One study included 3316 full-term singleton live births in LGA infants who were classified as no catch-down (BMI still above the 85th percentile at age 7), small catch-down (BMI between the 60th and 85th percentiles at age 7), and high catch-down (BMI above the 30th–55th percentiles at age 7) according to the degree of decline in postnatal growth trajectory. Compared with term-appropriate-for-gestational-age infants, term LGA babies without catch-down growth had increased risks of obesity and hypertension at 7 years of age. Those with high catch-down growth had higher risks of growth restriction and low intelligence quotient (IQ). Nevertheless, infants with small catch-down growth had lower risks of obesity, growth restriction, low IQ, and hypertension at 7 years of age. The study results suggested that term LGA infants with small catch-down growth had no increased risks of adverse outcomes [41]. It was also found that children with high-risk trajectories (i.e., BMI above the 85th percentile at 1–4 years of age) were significantly correlated with subsequent overweight/obesity. We established a predictive model based on the four metabolites (tyrosine, glycine, octenoylcarnitine, and stearoylcarnitine) at birth combined with gender, birth weight, and maternal prepregnancy BMI, which can identify high-risk children who may be overweight/obese in the future early after birth [42]. Another study found that taking aspirin in the first and second trimester of pregnancy can reduce the risk of high systolic blood pressure in children at the age of 7 by 11% and 7%, and reduce the risk of high diastolic blood pressure by 29% and 13%, suggesting that taking aspirin during pregnancy may have long-term beneficial effect on childhood blood pressure, especially on diastolic blood pressure [43].

6.2 Fetal Growth Restriction and Small for Gestational Age

6.2.1 Epidemiology

A fetus that has not achieved its full intrauterine growth potential due to genetic or environmental factors is defined as fetal growth restriction (FGR), also known as intrauterine growth restriction (IUGR), which increases the risk of serious complications and death compared to infants with normal intrauterine growth. Several terms have been used to describe infants whose birth weight (BW) is lower than that of the same gestational age, including small for gestational age (SGA) and fetal (in utero) growth restriction. Although many SGA infants have FGR, many of them are healthy infants. FGR can also manifest as appropriate for gestational age (AGA) or even LGA. Thus, these two terms are not synonyms.

Fetal growth restriction: FGR/IUGR is often defined as the estimated fetal weight <tenth percentile. In clinical practice, most FGR infants are found because their birth weight is lower than SGA of the tenth percentile of the weight of infants of the same gestational age (GA). Moderate FGR is defined as birth weight in the third–tenth percentile, and severe FGR was defined as birth weight <third percentile. FGR infants can be divided into the following: Symmetrical FGR: the body, head circumference, and body length are affected proportionally, and the function of all organs is reduced to varying degrees, accounting for 20%–30% of FGR cases. Symmetrical FGR usually begins in early pregnancy and is often caused by intrinsic factors such as chromosomal abnormalities or congenital infections, as well as reduced nutrient supply during early development [44]. Nonsymmetrical FGR: It accounts for 70%–80% of FGR cases. The growth restriction of the children is disproportionate, with normal head circumference and slightly shorter body length, and the body weight is most affected. Therefore, a normal-sized head appears relatively large compared to the size of the body and limbs. Abnormal growth begins in the late of second trimester or third trimester of pregnancy, when reduced fetal nutrients reduce fat and glycogen reserves, but allow the brain to continue to grow [44].

Ponderal index: Birth weight parameters are not sensitive measures for FGR. Ponderal index (PI) is a useful tool for detecting FGR, especially in infants with nonsymmetrical FGR [45]. PI refers to the ratio of body weight to body length and is expressed as PI = [body weight (g)*100]/[length (cm)]^3. Under normal growth, the PI gradually increases between 30 and 37 weeks of gestational age and then remains constant. Adipose tissue and skeletal muscle are the main components of body weight, and reduced growth of both leads to smaller PI. PI <tenth percentile indicates fetal malnutrition; PI <third percentile indicates severe emaciation [46]. Other body proportion ratios, such as the ratio of head circumference to body weight, body length, or abdominal circumference, or the ratio of femur length to abdominal circumference, are also used in the detection of FGR.

Small for gestational age: SGA is usually defined as birth weight below the tenth percentile for gestational age weight [47]. However, this definition cannot distinguish between physically normal small SGA infants and growth-restricted and small infants. Constitutionally small infants are defined as normal infants with only birth weight less than the tenth percentile due to constitutional factors. Constitutional factors include maternal height, weight, ethnicity, and parity. These small-sized infants are not at increased risk for perinatal death and complications [47]. Another definition of SGA is that the birth weight and/or length are more than 2 standard deviations (SD) below the average of infants for the corresponding gestational age (i.e., <2.3rd percentile) [48, 49].

The incidence of FGR varies among different populations, with the smaller the gestational age, the higher the incidence. In developed countries, about 10% of full-term infants are SGA, while in resource-limited countries, this proportion is 20% [44, 50, 51]. In 2012, a study by the Child Health Epidemiology Reference Group (CHERG) reported that 19.3% of live births were SGA in low- and middle-income countries. The study was based on 14 birth cohorts and the birth weight criteria of International Fetal and Newborn Growth Consortium for the 21st Century, INTERGROWTH-21st [50]. The incidence of SGA in preterm infants is quite different reported in the literatures. The Neonatal Research Network Database of National Institute of Child Health and Human Development (NICHHD) showed that 22% of 4438 infants with birth weights of 500–1500 g were classified as SGAs [52]. A larger study included 20,000 very low birth weight (VLBW, birth weight <1500 g) infants with gestational age of 25–30 weeks, and found that the incidence of SGA was only 9% [53].

Nutrient supply is impaired in fetuses with FGR. In order to increase the chance of survival, the fetus will respond as follows: reducing the size of its overall type, preserving the growth of the brain, accelerating the maturation of the

lungs, and increasing the production of red blood cells [54]. The fetus redistributes blood flow from less important organs to the heart, brain, placenta, and adrenal glands. The total body fat, bone mineral content, and lean body mass were reduced, resulting in infants with severe FGR presenting a thin face [55]. The contents of nitrogen and protein are low due to the reduced muscle mass [44]. In addition, fetal plasma glucose and insulin concentration are low, resulting in reduced glycogen content in the liver and skeletal muscle [44].

FGR can be caused by fetal, placental, and maternal factors. However, no underlying cause is found in at least 40% of FGR infants. Among infants with underlying causes, about 1/3 of FGR is caused by genetic diseases, and 2/3 of them are associated with in utero environment [56].

6.2.2 Prenatal Diagnosis

6.2.2.1 Screening Test

Selective measurement of the symphysis-fundal height: The distance from the upper edge of the pubic symphysis to the top of the fundus is measured with a measuring tape. This simple and inexpensive technique is widely used for prenatal screening for FGR and can also be used to detect other diseases that lead to inconsistent fetal size/gestational age. If this measurement is found to be inconsistent with the expected value for the corresponding gestational age, FGR should be suspected initially. A variety of methods have been used to define this inconsistency: The most commonly used standard is that the uterine height (cm) is at least 3 less than the expected value of the corresponding gestational age (week), for example, the uterine height is 32 cm at 36 weeks of gestation [57]. Another definition method is that the uterine height of the gestational age is lower than the 3rd or 10th percentile: The INTERGROWTH-21st International Program has published standards for the determination of the symphysis-fundal height that can be printed (symphysis-fundal height measurement standards), and the 3rd, 10th, 50th, 90th, and 97th percentiles of uterine height are determined based on eight well-nourished urban healthy female populations [58]. In clinical practice, screening for FGR by measuring uterine height remains controversial. Factors that may affect sensitivity include maternal BMI, bladder volume, parity, and ethnicity [59–62].

Ultrasound screening: Routine ultrasound screening is another method to screen FGR. There is no consensus on the timing and number of screenings. Typically, if two screening tests are scheduled after fetal anatomy at 18–22 weeks of gestation, they are scheduled at approximately 32 and 36 weeks of gestation [63]. If only one screening test is performed, it is scheduled between 32 and 36 weeks of gestation, and the predictive power close to 36 weeks is higher [64].

6.2.2.2 Diagnosis

Ultrasound EFW <tenth percentile for gestational age or abdominal circumference <tenth percentile for gestational age is the optimal test result for the diagnosis of FGR.

6.2.2.3 Biological Measures

Abdominal circumference: When fetal growth is impaired, fetal abdominal circumference is smaller than expected due to depletion of abdominal adipose tissue and decreased liver size due to glycogen depletion. Most studies have reported that reduced abdominal circumference is the most sensitive single biological measure of FGR [65–70]. Smaller abdominal circumference is also associated with FGR complications: Biochemical markers of hypoxia and acidemia are more likely to occur when abdominal circumference is less than the fifth percentile for gestational age [71].

Ratio of biological measures: Head circumference/abdominal circumference ratio and femur length/abdominal circumference ratio have been used to identify FGR, and these ratios have the best sensitivity for predicting nonsymmetrical FGR. FGR associated with uteroplacental insufficiency is often asymmetrical, while FGR associated with other etiologies is often symmetrical, so the ratio of fetal biological measures predicts the former better than the latter.

Head circumference/abdominal circumference ratio: The head circumference/abdominal circum-

ference ratio decreases linearly throughout pregnancy; if the ratio is more than 2 standard deviations (SD) above the mean of the same gestational age, it is considered abnormal. A prospective study using the head circumference/abdominal circumference ratio to detect asymmetrical FGR due to uteroplacental insufficiency showed that 79% of fetuses had a normal ratio, and none of them had FGR at birth; the remaining 21% of fetuses had an abnormal ratio, and all were correctly diagnosed as FGR [72]. In the population of FGR due to various causes, the sensitivity, specificity, positive predictive value, and negative predictive value of abnormal head circumference/abdominal circumference ratio are 36%, 90%, 67%, and 72%, respectively [73]. Not all the fetuses with an increased head circumference/abdominal circumference ratio are asymmetrical FGR. Macrocephaly due to enlargement of any part of the head (brain, cerebrospinal fluid, blood, or bone) or increased intracranial pressure can also cause an increased head circumference/abdominal circumference ratio, so it should be excluded.

Femur length/abdominal circumference ratio: FGR can be predicted using the fetal biological measurement parameter femur length/abdominal circumference ratio, which correlates with both weight and length, and is independent of gestational age in fetuses with normal growth in the second half of pregnancy. It is reported that the sensitivity of femur length/abdominal circumference ratio >23.5% to identify asymmetrical FGR is 56%–64%, and the specificity is 74%–90% [74, 75], but symmetrical FGR cannot be detected. In children with FGR, the sensitivity, specificity, positive predictive value, and negative predictive value of the 90th percentile of the femur length/abdominal circumference ratio are 30%, 91%, 14%, and 96%, respectively [76–78].

Amniotic fluid volume: Oligohydramnios is one of the complications of FGR. When the fetus presents with oligohydramnios and EFW <third percentile, it is highly predictive of poor outcome [79]. A certain percentage (15%–80%) of FGR fetuses do not have low amniotic fluid volume. Although oligohydramnios is usually insensitive in predicting FGR [80, 81], FGR may be the most common cause of oligohydramnios if there is no rupture of membranes, congenital genitourinary abnormalities, or post-term pregnancy.

6.2.2.4 Protein Biomarkers

It is one of the research directions to find sensitive, specific, and noninvasive biomarkers to predict FGR. The biochemical analysis of maternal peripheral blood in a large number of studies have suggested that β-human chorionic gonadotropin (β-HCG), pregnancy-associated plasma protein-a (PAPP-A), placental growth factor (PIGF), and soluble fms-like tyrosine kinase-1 (sFlt-1) are important serum biochemical markers that can be used to predict placental dysfunction in early pregnancy.

β-human chorionic gonadotropin (β-HCG): It is a glycoprotein secreted by the trophoblastic cells of the placenta which increases rapidly in early pregnancy over time after embryo implantation. Studies have demonstrated that maternal free β-hCG levels below the fifth percentile in the first trimester are associated with the incidence of SGA, with a sensitivity of 6%–34% and a specificity of 90%–96% for detecting SGA [82]. However, other studies [83, 84] have not showed the correlation between low β-hCG and SGA. Therefore, the clinical use of low beta-hCG in early pregnancy to predict the increased risk of FGR in pregnancy is controversial.

PAPP-A is a placenta-derived protein that binds to insulin-like growth factor and is associated with placental function and fetal growth. In a prospective cohort study (n = 1792) [85], uterine artery pulsatility index (PI) combined with PAPP-A was used for the early diagnosis of FGR. The area under the curve, sensitivity, and specificity for this model were 0.788 (95% CI: 0.735, 0.842), 0.816, and 0.758, respectively.

Placental insulin-like growth factor (PIGF): It is secreted by the placenta and classified as an angiogenic factor because of its similarity to vascular endothelial growth factor (VEGF), which promotes vascular health through receptor-mediated signaling of vascular endothelial cells [86]. PIGF in the first trimester is significantly lower in the blood of pregnant women delivering SGA infants [87–89]. However, the univariate prediction model constructed using PIGF, similar to PAPP-A, performs

poorly, with only sensitivity of 27% and specificity of 90% [90]. In a large prospective study [91] (n = 3348), SGA infants without preeclampsia showed a 60% increase in the incidence of low PIGF (<280 pg/mL) at 22 to 26 weeks of gestation compared to non-FGR infants. If combined with uterine artery Doppler abnormalities in the second trimester, the risk will increase to 2.7-fold. In studies of fetuses with FGR diagnosed by ultrasound, PIGF less than the fifth percentile in early pregnancy can diagnose severe placental pathology with the sensitivity of 98% and the specificity of 75% [92]. A recent study [93] has shown that a combination of all markers, including maternal factors, mean arterial blood pressure (MAP), umbilical artery pulsatility index (UtA-PI), PIGF, and PAPP-A predicted delivery before 37 weeks of gestation in 48.6% of SGA newborns (AUC 0.795) and before 32 weeks of gestation in 59.1% of SGA newborns (AUC 0.8257), regardless of PE. The combined prediction model based on maternal characteristics, MAP, UtA-PI, PAPP-a, and PIGF at 11–14 weeks of gestation had the sensitivity of 67.2% and the specificity of 82.7% for screening FGR [94]. In conclusion, PIGF is closely associated with FGR infants and uteroplacental dysfunction and has potential in FGR screening if combined with other diagnostic biomarkers or models.

Soluble fms-like tyrosine kinase to placental insulin-like growth factor ratio (sFlt-1/PIGF): It has been widely accepted and used as a marker for the diagnosis and prediction of FGR in preeclampsia. sFlt-1/PIGF ratio >33 is shown to be a threshold for predicting FGR with the sensitivity and specificity of 0.63 (95% CI: 0.54–0.71) and 0.84 (95% CI: 0.83–0.85), respectively, and the AUC of 0.8345 [95]. When Doppler assessment is not feasible, sFlt-1/PIGF can help to grade the severity of early-onset FGR and guide the clinical management.

6.2.3 Complications and Potential Long-Term Effects

6.2.3.1 Complications

FGR infants have a high risk of perinatal complications [96].

Preterm birth: FGR infants are at risk of preterm birth. Sometimes early induction of labor is required because the harm to the fetus remaining in utero may be greater than that due to premature delivery. Compared to preterm infants appropriate for gestational age as controls, preterm infants with FGR have a higher risk of preterm birth-related death and complications, such as necrotizing enterocolitis, respiratory distress syndrome, bronchopulmonary dysplasia, and retinopathy of prematurity [53, 97–100].

Perinatal asphyxia: Infants with severe FGR may have difficulties in transition during delivery due to additional hypoxic stress during the uterus contraction. This is especially true for FGR fetuses due to placental lesions. Impaired placental function may lead to hypoxia and metabolic acidosis and increase the risk of multiple organ dysfunction, such as ischemic cardiac failure, neonatal hypoxic-ischemic encephalopathy, persistent pulmonary hypertension, meconium aspiration, and renal and acute gastrointestinal damage.

Impaired thermoregulation: FGR infants are at higher risk of hypothermia compared to AGA infants as control [101]. Causes of hypothermia include increased calorie loss in FGR infants due to decreased subcutaneous fat and decreased heat production due to factors such as too little nutrient stores and consumption of catecholamines (essential substances for heat generation from brown fat) due to in utero stress [44]. FGR infants should be in an environment with appropriate temperature, such as an incubator, to prevent the occurrence of hypothermia.

Hypoglycemia: Hypoglycemia is common in FGR infants. Therefore, glucose levels must be monitored [101–103]. Growth restriction may lead to decreased protein, glycogen, and fat stores; the higher the severity of growth restriction, the higher the risk of hypoglycemia. Since low insulin concentration in utero may lead to decreased glycogen storage and glycogen synthesis, infants begin to have a tendency to develop hypoglycemia in utero. After birth, poor coordination of insulin counter-regulatory hormones and peripheral insensitivity to these hormones can contribute to hypoglycemia [104].

Hypoglycemia usually occurs within 10 h after birth.

Polycythemia and hyperviscosity: Polycythemia and hyperviscosity occur more frequently in FGR, and the risk increases with the severity of growth restriction [102]. It may be associated with the increased secretion of erythropoietin due to fetal hypoxia [105].

Impaired immune function: Cellular immune function may be affected in FGR infants during the neonatal period and even throughout childhood. A cross-sectional study showed that peripheral T lymphocytes and B lymphocytes were reduced at birth in pediatric patients; the number of T lymphocytes basically returned to normal in later childhood, but their proliferative capacity remained low [106]. The ability of the skin to produce delayed-type hypersensitivity to phytohemagglutinin is also reduced in FGR infants from birth to childhood.

Hypocalcemia: FGR infants born preterm or born with asphyxia are at risk of early hypocalcemia, which increases with the severity of growth restriction, mainly during the first 2–3 days after birth [102, 107].

6.2.3.2 Potential Long-Term Effects

Physical development: SGA infants may present with multiple growth patterns after birth, depending on the etiology and severity of growth restriction. Infants with mild-to-moderate SGA may experience growth acceleration in the first 6–12 months after birth, completing catch-up growth [108, 109]. A study showed that 87% of 3650 full-term infants with birth length less than 2 standard deviations (SD) reached normal height at 1 year of age [109]. However, a report of data from a national survey in the United States showed that the weight of SGA infants appeared to catch up with AGA infants in the first 6 months after birth, but in terms of height, SGA babies still have a gap of about 0.75 standard deviations from AGA babies by 47 months [110]. Another prospective study of FGR infants whose mothers had severe early-onset hypertensive disorders during pregnancy followed up to 12 years of age found that the height and weight of these children were comparable to those of control children of the same age [111]. On the contrary, compared to AGA infants, severely affected SGA infants are usually lighter in weight and shorter in height throughout childhood and adolescence. Adolescents with birth weight below the third percentile had a lower average height than AGA controls at the age of 17 (169 cm vs. 175 cm for boys and 159 cm vs. 163 cm for girls) [112] and were also more likely to have a height lower than the tenth percentile by puberty (OR for boys and girls was 4.13 and 3.32, respectively).

Neurodevelopment: SGA infants are more likely to experience neurodevelopmental impairment (NDI), including cognitive and motor disorders [113–117]. Some data show that SGA (especially severe SGA) has lower intelligence and cognitive test scores in adolescents and early adulthood, is more likely to have learning difficulties, and is at greater risk of cerebral palsy [118–122].

Chronic diseases in adults: FGR may be a contributing factor to chronic diseases in adults, including coronary heart disease, hyperlipidemia, hypertension, and chronic kidney disease (CKD) (Barker hypothesis). Coronary artery disease: Adults with FGR may be more likely to develop ischemic heart disease and related diseases. The correlation between FGR and coronary and vascular disease in adults is based on the hypothesis (fetal origins of adult disease or Barker hypothesis) that fetal undernutrition predisposes vascular disease during adulthood, which includes stroke, hypertension, hypercholesterolemia, and diabetes (Barker hypothesis) [123–125]. A cohort study included 6425 SGA or preterm infants born in four major Swedish delivery units from 1925 to 1949 as subjects, and the correlation between SGA and adult ischemic heart disease was well demonstrated and elaborated [126]. Studies of follow-up in 1987–2002 found that SGA infants were more likely to have ischemic heart disease in adulthood (adjusted HR 1.64, 95% CI: 1.23–2.18) compared to age-/sex-matched AGA controls born >35 weeks, and the correlation was independent of gestational age. Other studies have demonstrated thickening of the aortic wall (a marker of early atherosclerosis) [127, 128] as well as increased aortic stiffness [129] on ultra-

sound in FGR infants compared to infants with normal intrauterine growth. In addition, an autopsy study of children aged 1–13 years confirmed birth weight was negatively correlated with the extent and severity of aortic lesions [130]. Although these findings suggest that fetal factors can increase the risk of cardiovascular disease later in life, long-term longitudinal studies are still required to understand the clinical significance of these changes and whether they will accelerate atherosclerosis [131]. However, the hypothesis (fetal origins of adult disease) is not generally accepted [132–135]. A smaller case cohort study showed that 50-year-old adults born full-term with a birth weight below the tenth percentile (defined as FGR) had no significant difference in health quality outcomes compared to the controls with a birth weight above the tenth percentile [132].

Chronic kidney disease (*CKD*): Data show that SGA is at risk of CKD, including end-stage renal disease (ESRD) [136]. For example, a large population study in Norway analyzed volunteers born 1967–2004 and found that SGA patients were more likely to develop ESRD than AGA after adjusting for confounding variables such as congenital malformations, multiple labor, maternal age, and preeclampsia (RR 1.5, 95% CI: 1.2–1.9) [137]. Another systematic review also confirmed the relationship between low birth weight and CKD [138].

6.2.4 Neonatal Management

1. *Initial management*: The initial management of FGR neonates is supportive and focusing on the prevention or management of related complications. Infants with no definite cause can be further assessed after their condition is stable. If the fetus is known to have severe growth restriction, the delivery should be planned in a perinatal center with experienced pediatric health care providers. For the severely affected cases, the pediatric delivery team should be prepared to manage the following complications: perinatal asphyxia and neonatal encephalopathy, meconium aspiration, hypoglycemia, pulmonary hypertension, and hypoxia. Due to impaired thermoregulation in FGR infants, infants should be wiped dry and placed in a radiant warmer immediately after delivery to avoid heat loss. Immediate resuscitation, including clearance of meconium in the airway as needed, should be performed. Many infants should be admitted to the neonatal intensive care unit (NICU) because they require higher levels of treatment and monitoring than the usual measures in the normal nursery. The management of full-term FGR infants in the general neonatal nursery includes the following: (1) Physical examination: Physical examination can reveal any abnormalities that may alter the normal course of the newborn, or medical problems that should be managed, such as malformations, birth trauma, jaundice, or cardiopulmonary disease. These include accurate measurement of body length, weight, and head circumference and accurate assessment of gestational age. (2) Thermoregulation: An incubator or radiant warmer rather than an open crib may be required to maintain proper temperature. (3) Blood glucose monitoring: Monitoring for hypoglycemia is initiated within 1–2 h after birth. Blood samples should be collected prior to feeding. In infants with low blood glucose concentrations (less than 40 mg/dL (2.2 mmol/L)), continuous monitoring should be carried out until a more stable feeding is established and blood glucose has returned to normal. (4) Blood calcium monitoring: SGA infants born prematurely or with birth asphyxia are at risk of hypocalcemia. Ionized calcium concentrations should be monitored 12 h after birth, and adequate calcium intake should be provided. (5) Monitoring for polycythemia: If the infant has symptoms or signs that may be due to polycythemia, such as cyanosis, tachycardia, feeding difficulties, and vomiting, hematocrit or hemoglobin should be tested. (6) Nutrition: Enteral feeding should be started early and in appropriate amounts based on the infant's weight [139, 140]. The optimal caloric intake amount of FGR infants is not clear [54]. The

goal of management should be to provide adequate nutrition to achieve postnatal growth similar to that of normal fetuses of the same gestational age or infants of the same corrected gestational age. Breastfeeding is preferred because it can meet most nutritional needs and has short-term and long-term advantages over formula milk. Infants unable to receive enteral feeding require parenteral nutrition under closer monitoring.

2. *Further assessment*: Once the condition is stable, children whose underlying etiology is still unclear should be further assessed to determine the cause of FGR. However, in a large proportion of cases, the underlying cause has not been identified. If possible, the management of children should be guided by the treatment of underlying causes and monitoring of long-term complications (e.g., abnormal growth and poor neurodevelopmental outcomes). A detailed assessment of the maternal history and pregnancy history may reveal the causes of the growth disturbance. Pathological examination of the placenta for evidence of infarction or infection may be helpful. A comprehensive physical examination should be performed to identify features that may indicate an underlying chromosomal abnormality or syndrome. In some cases, genetic counseling as well as chromosomal testing may be helpful. Prenatal exposure to drugs or poisons (e.g., alcohol) should be considered. Diagnosis can be made based on assessment and diagnostic tests. Congenital infection may cause FGR (e.g., cytomegalovirus infection) even if the clinical signs are not obvious. Congenital infection can be assessed by serological tests or urine tests.

6.2.5 Research Findings

A series of studies on SGA were conducted previously. A study included mothers and singleton neonates delivered at Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine from September 2013 to December 2016. They were divided into four groups based on the percentile of different birth weights of offspring: mild SGA group (SGA5th to 10th), moderate SGA group (SGA3rd to 5th), severe SGA group (< SGA3rd), and normal control group (10th to 90th). The study showed that different SGA groups have varied high-risk factors. Maternal low body mass index (BMI) and gestational hypertension are risk factors for moderate SGA, and assisted reproduction is a risk factor for severe SGA [141]. Another study has found that the optimal growth trajectory for full-term SGA infants may be fast catch-up growth to about the 30th percentile in the first several months, with modest catch-up growth thereafter, to be around the 50th percentile by 7 years old [142].

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Circulatory System

7

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7.1 Overview

The early embryonic development of the heart and blood vessels is a very complex process. The abnormal development of cardiac system at the embryonic stage will lead to congenital cardiac and vascular malformations. Thus, knowledge of the embryonic development of the circulatory system is of great importance in understanding the pathological changes and clinical diagnosis of congenital cardiovascular malformations. At present, the common screening and diagnosis methods for *in utero* congenital heart diseases include fetal cardiac ultrasound and MRI. Most congenital heart diseases can be diagnosed in fetal period.

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7.1.1 Normal Development of *In Utero* Cardiovascular System Structure

7.1.1.1 Development of the Heart

The embryonic development of the heart undergoes stages such as heart tube formation, looping, and septation of the atria, ventricles, atrioventricular canal, and conotruncus [1]. At the beginning of the third week of human embryonic development, the mesoderm develops from the ectoderm, while the heart mainly develops from the mesoderm. The heart precursor cells located at both sides of the primitive streak migrate and form the cardiogenic plate in the prechordal region. The rapid development of the somite region leads to the ventral bending of the outer edge of the embryo. The closing up and fusion of the left and right heart tubes to form a straight primitive heart tube on day 20 of the embryonic stage, and on day 21, looping of the heart tube begin. Pulsation of the heart tube begins to appear on days 22–26.

Septation inside the heart occurs between day 26 and day 37. The atrial separation starts from the appearance of septum primum in the middle of the dorsal wall of the primitive atrium, followed by the formation and closure of foramen primum, the appearance of septum secundum, and the formation of foramen secundum until the fusion of septum primum and septum secundum after birth, that is, the closure of foramen ovale, which is the completion of atrial septation [1].

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The endocardial cushions of atrioventricular canal, the right dorsal conotruncal ridge, and the ventricular wall are all involved in the formation of atrioventricular valve. Conotruncus is involved in the formation of outflow tract and supraventricular crest after septation, migration, and absorption. The interventricular ridge develops and bulges, forming the interventricular septum with the trabeculae and conotruncal septum at the base of the inflow tract, separating the primitive ventricles into the left and right ventricles. After the primitive ventricles are separated, the left and right ventricles continue to enlarge and develop. In addition to the outward extension of the ventricular chamber, the maturation of the muscles in the inner wall of the ventricle also expands the ventricular chamber, while the inner wall of the ventricle presents uneven trabecularization.

7.1.1.2 Development of Blood Vessels

At the beginning of the embryonic stage, the human aorta is the branchial-type artery, including aortic sac, aortic arch, and paired dorsal aortas. After the formation of the primitive heart tube, the aortic sac begins to connect to the first pair of aortas. With the successive appearance of intersegmental artery, ventral aorta, dorsal aorta, and aortic arch, the aortic sac begins to separate into the aorta and the pulmonary artery. After the six pairs of aortic arches undergo evolution of appearance, disappearance, discontinuation, and displacement, they eventually form the asymmetric mature arterial arches and the arterial system [1].

On day 30 of the embryonic development, pulmonary capillaries begin to appear and surround the pharynx and developing trachea. The proximal end of left pulmonary artery and right pulmonary artery are derived from the proximal end of left and right sixth pair of aortic arches. During weeks 20–32, bronchial vessels appear and form anastomoses with pulmonary arteriole [1, 2]. The capillary plexus on the surface of the lung bud develops into the pulmonary venous plexus, eventually into the pulmonary veins.

In the early stages of embryonic heart development, the myocardium draws nutrients directly from cardiac chambers. Sinus venosus-derived vessels develop into the first coronary vascular population, which is located on the outer myocardial wall [3–5]. Endocardium-derived vessels develop into the second coronary vascular population, which is mainly located in the core of the heart, including the interventricular septum and the inner myocardial wall of the postnatal heart, and eventually connects to sinus venosus-derived vessels. Sinus venosus-derived vessels and endocardium-derived vessels merge to form complementary and overlapping regions of coronary vessels.

7.1.1.3 Characteristics of Fetal Circulation

The fetal circulation accomplishes gas exchange through the placenta, and the systemic and pulmonary circulation is "open" to each other and is connected to each other. The fetal circulation consists of two main pathways; one is the "via sinister" from the placenta to the upper part of the body, with higher blood oxygen concentration; the other is the "via dexter" from the superior vena cava to the placenta, with lower blood oxygen concentration. During fetal period, right-to-left communication exists between the foramen ovale site and the ductus arteriosus. Under physiological conditions, the pressure of the left and right ventricles is close; the overall blood oxygen level of fetal circulation is lower than that after birth. The right ventricle of the fetus is the main part of the heart to pump blood, with the output accounting for 60–70% of the total.

7.1.1.4 Structural Abnormalities of *In Utero* Cardiovascular System

Development abnormalities of *in utero* cardiovascular structure is the pathological basis of fetal congenital heart disease after birth; thus, exploring abnormal embryonic development is an important topic of congenital heart disease. The abnormal development of endocardial cushions can cause various degrees of atrioventricular septal defects, atrioventricular valve malformations, etc. Atrioventricular septal defect (AVSD) accounts for about 4% of all congenital heart diseases. About 40% of children with Down's syndrome have congenital heart diseases, of which 40% are

with atrioventricular septal defects [6]. In addition, the abnormal development of conotruncus is also associated with a variety of congenital heart malformations. Abnormal conotruncus can be divided into incomplete septal development, uneven segmentation or conical displacement, poor spiral, and poor alignment, ultimately leading to the tetralogy of Fallot, transposition of great arteries, ventricular septal defect, double outlet of right ventricle, aortopulmonary septal defect, etc. However, the mechanism of embryonic development in specific disease is still unclear.

There are a wide variety of congenital heart diseases, and the pathological anatomy of each disease is complex and quite different. To clarify the diagnostic nomenclature, sequential segmental diagnosis is used. Segmental diagnosis includes atrial position, ventricular position, atrioventricular connection, large artery position, ventricular large artery connection and cardiac position, apical orientation, thoracic and abdominal organ position, and combined cardiac and noncardiac malformations. Segmental diagnosis approach is not only essential but also should be used as the basis for the diagnosis of congenital heart disease.

7.1.2 Development and Conduction Abnormalities of *In Utero* Cardiovascular Conduction System

7.1.2.1 Embryonic Development of *In Utero* Cardiac Conduction System

The cardiac conduction system is composed of special myocardial cells located in the myocardium that can produce and conduct impulses, including sinoatrial node, internodal bundle, atrioventricular node, atrioventricular bundle, left and right bundle branches, and Purkinje fibers. The embryonic development of the cardiac conductive tissues described above begins at the primitive heart tube stage of the heart.

The primitive heart tube has formed from about 3.5 days to the third week of the embryonic stage. The primitive heart tube consists of five parts: truncus arteriosus, bulbus cordis, primitive ventricle, primitive atrium, and sinus venosus. Each two adjacent parts are slightly narrowed to form a ring, which is called the sinoatrial ring, atrioventricular ring, bulbo-ventricular ring, and truncobulbar ring, respectively. Early studies of the human embryonic heart have confirmed the existence of annular constrictive rings between the segments of the straight primitive heart tube, i.e., between the primitive venous sinus, the primitive atrium, the primitive heart bulb and the primitive arterial trunk. This tissue ring has properties of autorhythmicity and conduction; as the heart tube twists, these rings bend in and approach each other in the heart, forming a conductive bundle of the heart.

The sinoatrial node is thickened tissue at the junction of the superior vena cava and the venous sinus from the region of the sinoatrial ring. By week 8 of embryonic stage, sinoatrial ring cells at the junction of the superior vena cava and atrial appendage accumulate around a developing artery; by week 10 of embryonic stage, sinoatrial nodes are formed anteriorly and laterally at the junction of the superior vena cava and atrial appendage. There is a special conduction pathway between the sinoatrial node and the atrioventricular node, i.e., the internodal bundles are present. At weeks 5–6 of the embryonic stage, the atrioventricular node begins to form; at about week 10 of the embryonic stage, the developed and shaped atrioventricular node can be identified. But at the same time, there are still a vast of muscle bridges spanning across the developing septal annulus. In the primitive embryo, the ventricles and atria are connected to each other. During development, the connective tissue of the endocardium and epicardium wedges at the atrioventricular junction and separates the atria and ventricles to form the atrioventricular ring, but a continuous bundle of muscle fibers remains. This continuous bundle arises from the back of the right atrium, crosses the fibrous tissue to the top of the muscular part of the interventricular septum, and is the primitive His bundle. For the embryonic heart at week 6, the bundle branch can be seen descending along both sides of the ventricular septum, eventually forming branches and entering the trabecular fovea; at

week 18, the left bundle branch has formed a fan-shaped structure, and the main trunk of the left bundle branch is located at the left lateral side of the interventricular septum, starting from the joint level formed by the aortic ring, extending 1–2 cm before the branch; the right bundle branch begins to branch when it extends from the septum to the middle zone, and during this path, the initial segment of the right bundle branch and the terminal segment before the branch are both subendocardial and superficially located, while the middle segment is deeply hidden in the muscularis and deeply located.

7.1.2.2 Development Abnormalities of *In Utero* Cardiac Conduction System

Development abnormalities of cardiac conduction system can lead to *in utero* abnormal cardiac conduction in the fetus and postnatal population, which is one of the important causes of arrhythmia and sudden death. These include developmental disorders of the cardiac conduction system and structural abnormalities of the cardiac conduction system.

Developmental disorders of cardiac conduction system: For people who have developed to the adult stage, and the tissue structure of cardiac conduction system has not yet reached the normal mature structure for the development degree, which is called developmental disorders of cardiac conduction system. Pathomorphological manifestations show seven types: (1) Abnormal proportional distribution of node and bundle cells and interstitium of conduction system. It often presents with excessive stroma, significantly reduced cell number (sparse in severe cases), or focal increase in local stroma. (2) Displacement of atrioventricular node and atrioventricular bundle tissue. There are two types of displacement: total or partial tissue displacement. (3) Undersized. If the size of a certain part of the structure is less than 1/2 of the normal range, it can be considered undersized. (4) Abnormal neurodevelopment. These include a decrease in the number of synaptic vesicles in nerves or nerve terminals distributed in the sinoatrial node. (5) Atrioventricular nodularization of atrioventricular bundle. It manifests as the atrioventricular node is not developed or absent, and a structure simulating the atrioventricular node appears at the bifurcation of the atrioventricular bundle. (6) Fetal atrioventricular node. It refers to the heart still retains the pattern of the atrioventricular node during fetal period in adulthood, that is, the atrioventricular node is located in the central fibrous body, and part of the nodal tissue extends into the central fibrous body, and is distributed in islands of varying sizes, with nuclear pyknosis and cytoplasmic vacuolation of some nodal cells in the island. (7) Absence of a certain part in cardiac conduction system.

Structural abnormalities of the cardiac conduction system: The spontaneous rhythm of the normal human heart is caused by the depolarization wave generated by its primary pacemaker-sinoatrial node, and through the conduction channel, that is, the sinoatrial node-internodal bundle-atrioventricular node-atrioventricular bundle-left and bundle-right branches, to transmit the impulse to the atria and ventricles and to activate various parts of the myocardium. If there is another abnormal conduction outside the normal conduction pathway, it is called accessory conduction bundle. Five types are known at present: (1) Atrioventricular accessory pathway (also known as accessory atrial-ventricular bundle, accessory atrioventricular muscle bundle, Kent bundle). This accessory bundle is composed of common myocardial tissue and does not pass through the atrioventricular node, atrioventricular bundle, but directly connects the atrial muscle and ventricular muscle. (2) Atrial-bundle accessory pathway (atrium-His bundle), which is an accessory bundle connecting the atrium to the atrioventricular bundle or its branch parts. (3) Atrioventricular internodal pathway (James fiber), which is an accessory bundle connecting the sinoatrial node and the inferior part of the atrioventricular node or atrioventricular bundle. (4) Nodoventricular accessory pathway (node-ventricular accessory bundle, Mahaim fiber), which connects from the atrioventricular junction to the interventricular septum. (5) Bundle-ventricular accessory pathway (atrioventricular bundle-ventricular accessory bundle, Mahaim

fiber), often composed of the common myocardium, connects the atrioventricular bundle or bundle branch to the ventricular myocardium.

7.1.3 Screening and Diagnosis of *In Utero* Cardiovascular Disease

7.1.3.1 Screening and Diagnosis of *In Utero* Cardiovascular Structural Abnormalities

Congenital heart disease ranks first among the 23 types of congenital birth defects, which seriously affects the life and quality of life of newborns. Therefore, prenatal examination and early diagnosis are important. At present, the commonly used prenatal imaging examination items for congenital heart disease in clinical practice include fetal cardiac ultrasound, MRI, etc. Among them, fetal cardiac ultrasound is the most commonly used, the most convenient, noninvasive, and efficient method. Most complex congenital heart diseases can be detected by screening during the fetal period. In addition, fetal cardiac ultrasound also involves the monitoring of fetal hemodynamic function. Therefore, fetal cardiac ultrasound not only plays a key role in the diagnosis and interventional treatment of congenital heart disease but also plays a vital role in the diagnosis and treatment of fetal arrhythmia and cardiac insufficiency. Current studies have demonstrated that diagnostic ultrasound has little or no effect on tissues, but the ultrasound energy conducted during the examination is not completely harmless. For example, ultrasound can cause thermal effect and cavitation effect in the inspected body [7]. Therefore, the indications of fetal cardiac ultrasound should be strictly grasped [8], and the examination time should be controlled [9]. If the mother suffers from congenital cardiovascular malformations, exposure to cardiovascular teratogenic factors in early pregnancy, or has other metabolic diseases, the screening of fetal congenital heart disease should be emphasized. If the fetus has risk factors such as abnormal obstetrical ultrasonography, extracardiac malformation, and chromosomal abnormalities, they are also important indications for screening.

7.2 Clinical Practice

7.2.1 *In Utero* Intervention and Sequential Treatment of Structural Abnormalities of Cardiovascular System

7.2.1.1 Aortic Stenosis

Aortic stenosis (AS) is a congenital cardiovascular structural abnormality, which is aortic valve stenosis and left ventricular outflow tract obstruction due to congenital dysplasia of the aortic valve due to various causes, and may be complicated with hypoplastic left heart syndrome (HLHS) with the progression of the disease. Percutaneous *in utero* fetal aortic valvuloplasty (FPV) is currently the main *in utero* interventional treatment for severe aortic stenosis during fetal period [1, 2]. Although the incidence of fetal aortic stenosis is lower in China compared to Europe and the United States, it usually develops progressively during fetal period. Severe stenosis will affect the remodeling of the heart and eventually develop into HLHS, with a high neonatal mortality rate after birth.

(Case) A pregnant woman at 24–5 weeks of gestation visited the Diagnosis and Treatment Center for *In Utero* Pediatric Diseases of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. At the initial visit, the fetal echocardiography showed cardiothoracic ratio of 29%, atrial level right-to-left shunt, left ventricular short-axis meridian of 6.9 mm, right/left ventricular short-axis ratio of 1.33, aortic annulus diameter of 3.8 mm, peak velocity across aortic valve of 2.54 m/s, and pressure gradient (PG) of 25 mmHg, without aortic valve and mitral valve regurgitation (MR), suggesting aortic valve stenosis and left ventricular shrinkage, with cardiac function and structure not significantly involved (Fig. 7.1). The pregnant woman was recommended to be followed.

We closely monitored the growth and development of the fetus and disease changes. The pregnant women was followed up for fetal echocardiography at 30 weeks of gestation. The disease has progressed: The left ventricular short-axis meridian was significantly widened, the aortic flow velocity increased than before, the

flow velocity increased to 4.8 m/s, the aortic valve pressure gradient (PG) gradually increased from 25 to 90 mmHg, the left and right ventricles were gradually disproportional, and pericardial effusion and mitral regurgitation occurred at the same time (Fig. 7.2), suggesting that fetal aortic valve stenosis was worsened.

Therefore, Xinhua Hospital organized a multidisciplinary consultation expert team (pediatric cardiology, pediatric cardiac surgery, obstetrics, neonatology, imaging, etc.) to conduct accurate, effective, and comprehensive consultation before delivery, so that the pregnant woman and her families can fully understand the disease and the possible benefits and risks of *in utero* intervention and elucidate the purpose of *in utero* cardiac intervention. With the informed consent of the patient and her families, the physical and mental risks to the pregnant woman during the perioperative period were assessed, and fetal aortic valvuloplasty (FAV) was performed at 31 weeks of gestation.

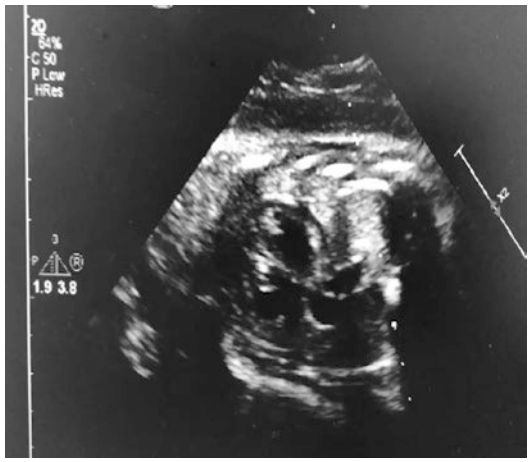

Fig. 7.1 Fetal echocardiography at 24 + 5 weeks of gestation: left ventricular shrinkage

During the operation, the pregnant woman was placed in the supine position, and the fetal position was assessed by preoperative ultrasound. When the fetal spine was located posteriorly, ultrasound was used to determine that the puncture needle was parallel to the direction of the left ventricular outflow tract. The umbilical vessels were punctured under the guidance of ultrasound, the fetus was anesthetized, the fetal movement was significantly reduced after withdrawal of the puncture needle, and then the fetal ventricular puncture point was determined. Thereafter, the sheathed puncture needle was further guided into the fetal left ventricle under continuous ultrasound monitoring, pulsatile blood flow was observed after removing the inner needle, and a preset guidewire 3.0–4.0-mm coronary dilatation balloon was immediately placed. The balloon was delivered into the ascending aorta under the guidance of ultrasound and adjusted to be placed at the aortic valve. The balloon dilatation was performed for two to four times under an appropriate pressure, during when fetal heart rate was monitored. After the completion of dilatation, increased blood flow passing through the aortic valve was observed (Fig. 7.3).

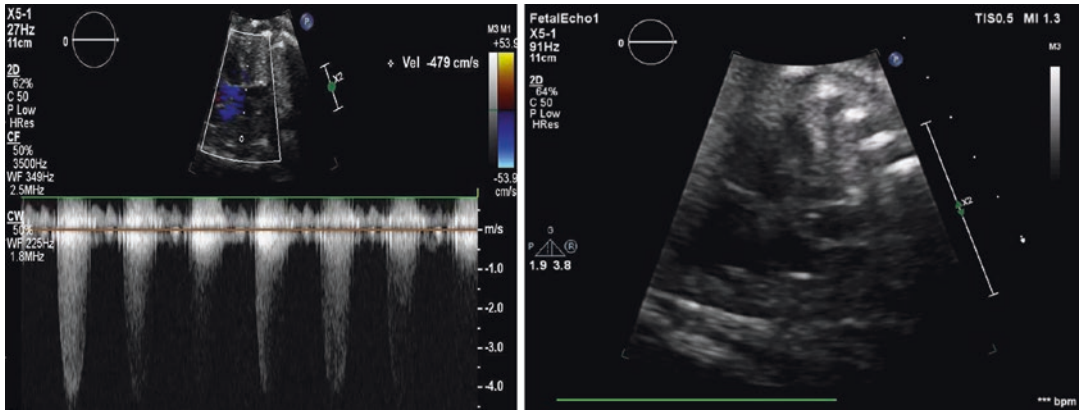

Fig. 7.2 Fetal echocardiography at 31 weeks of gestation: the aortic flow velocity increased to 4.8 m/s in the left figure, and fetal pericardial effusion was observed in the pericardial cavity indicated by the arrow in the right figure

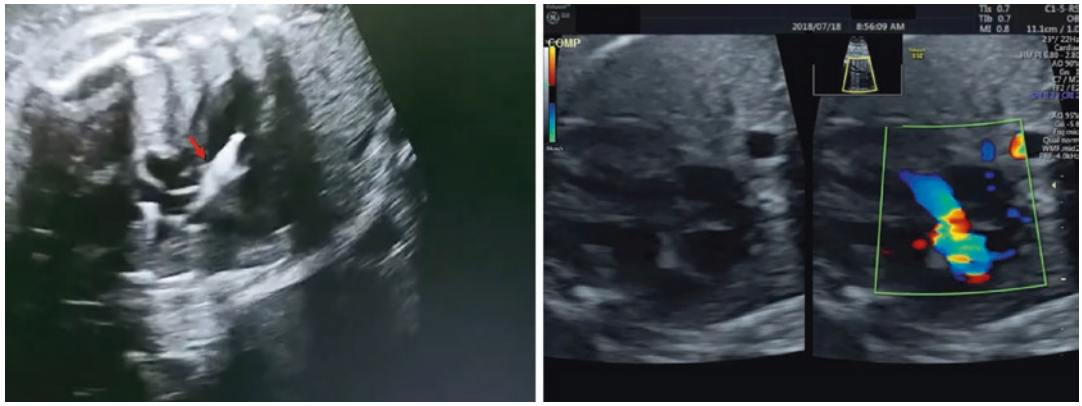

Fig. 7.3 Balloon dilatation of fetal aortic valve: the red arrow in the left figure indicated the dilatation balloon, and the blood flow passing through the dilated aortic valve was observed in the right figure

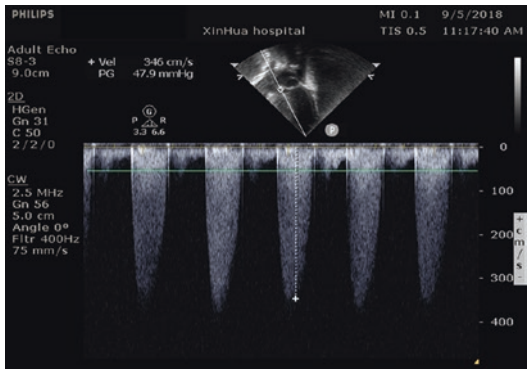

Fig. 7.4 Neonatal echocardiography: aortic flow velocity decreased to 3.4 m/s and pressure gradient of 48 mmHg

Postoperative fetal echocardiography revealed that the left ventricular short-axis meridian was significantly improved, the aortic valve flow rate decreased, the peak pressure gradient was significantly reduced, the mitral regurgitation disappeared, and the fetal heart function was significantly improved. After delivery, the newborn had an Apgar score of 10–10–10 points and a birth weight of 3.9 kg. By re-examination of echocardiography, the newborn was diagnosed to have aortic stenosis, left ventricular wall hypertrophy, poor systolic activity of left ventricular wall, patent ductus arteriosus, aortic blood flow velocity decreased to 3.4 m/s, and pressure gradient (PG) of 48 mmHg (Fig. 7.4).

The postnatal circulation was stable, and the patient was discharged from the hospital after 1 week of hospitalization. The patient underwent corrective surgery for aortic stenosis 2 months after birth. The operation was successful, and the biventricular circulation was established. During follow-up to date, the patient has developed well, and the daily activities and movements are not affected.

7.2.1.2 Pulmonary Atresia with Intact Ventricular Septum

Pulmonary atresia with intact ventricular septum (PA/IVS) is a rare cyanotic congenital structural abnormality of the cardiovascular system, which accounts for about 0.42–0.81 in 10,000 newborns. The main pathological manifestations are complete pulmonary atresia, two independent ventricles, intact ventricular septum, and open tricuspid valve, accompanied by various degrees of right ventricular dysplasia and the possibility of coronary artery dysplasia. At present, the main *in utero* interventional therapy for PA/IVS is fetal pulmonary valvuloplasty (FPV) [3, 4].

(Case) A pregnant woman was diagnosed with PA/IVS at 23 + 5 weeks of gestation. Fetal echocardiography at 29 weeks of gestation showed cardiothoracic ratio of 43%, atrial level right-to-left shunt, right ventricular short-axis meridian of 11.7 mm, right/left ventricular short-axis ratio of 0.6, pulmonary artery membranous atresia, visible valvular structure, tricuspid annulus diameter of 11.3 mm, bicuspid valve ratio of 0.93, severe tricuspid valve regurgitation (TR), and other car-

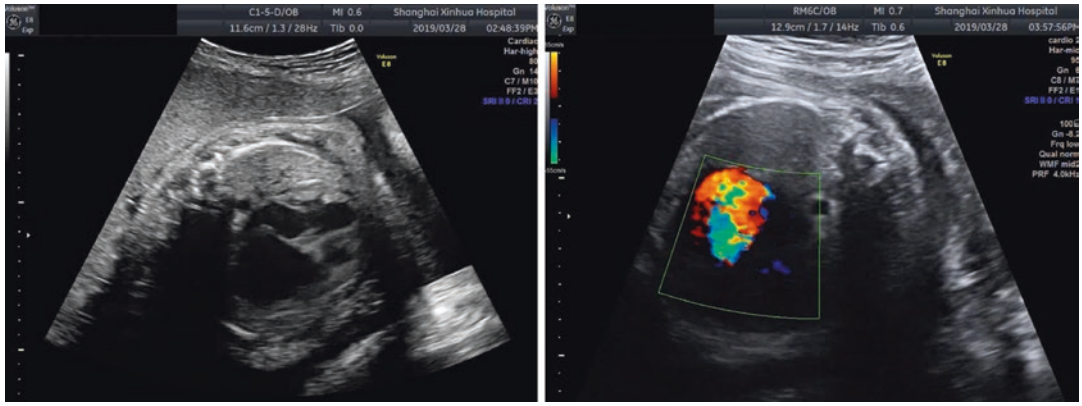

Fig. 7.5 Fetal echocardiography: enlarged right atrium in the left figure and severe tricuspid regurgitation in the right figure

diac structures normal (Fig. 7.5), suggesting that the fetus had PA/IVS, tricuspid regurgitation, and right ventricular dysplasia.

Considering that the fetus has right heart dysplasia, it was recommended that the pregnant woman be hospitalized. Therefore, we organized a multidisciplinary diagnosis and treatment team, including pediatric cardiology, pediatric cardiac surgery, obstetrics, neonatology, and imaging departments; conducted a comprehensive and systematic management for the pregnant woman; and developed an integrated sequential diagnosis and treatment plan from *in utero* treatment to postnatal follow-up and intervention. The pregnant woman and her families were adequately informed of the surgical methods and their related risks and benefits, and the informed consent was obtained. After considering the situation of the pregnant woman and the fetus, it was decided to perform *in utero* fetal pulmonary valvuloplasty (FPV) for the pregnant woman at 29 + 4 weeks of gestation.

During the operation, the pregnant woman was placed in the supine position, and the fetal position was assessed by preoperative ultrasound. Since the right ventricular outflow tract was not parallel to the inflow tract, it was not easy for the puncture needle to reach the pulmonary valve through the narrow and twisted right ventricular outflow tract. Thus, when the fetal spine was located at 5–7 o'clock, ultrasound was used to determine that the puncture needle was parallel to the direction of right ventricular outflow tract. After the direction of the puncture needle was confirmed, the umbilical vessels were punctured under the guidance of ultrasound, anesthetics were injected to anesthetize the fetus, decreased fetal movements were confirmed by ultrasound after withdrawal of the puncture needle, and then the ventricular puncture point was determined. Under continuous ultrasound monitoring, the sheathed puncture needle was guided into the right ventricle of the fetus, and a coronary dilatation balloon was placed immediately after pulsatile blood flow was observed by removing the inner needle, and the guidewire was delivered into the main pulmonary artery through the right ventricular outflow tract under the guidance of ultrasound. The balloon was adjusted to place at the pulmonary valve, balloon dilatation was performed for 2–4 times under an appropriate pressure, and fetal heart rate was also monitored. After the completion of dilatation, the puncture needle and balloon dilatation system were withdrawn, and the forward blood flow of the pulmonary artery was monitored under ultrasound, indicating the atretic pulmonary artery was successfully opened during surgery (Fig. 7.6).

After operation, the pregnant woman and fetus were closely followed up to monitor the fetal growth and development, pulmonary arterial blood flow, and related cardiac development of structure and function. Fetal echocardiography at 35 + 3 weeks of gestation showed pulmonary artery open, pulmonary arterial blood flow veloc-

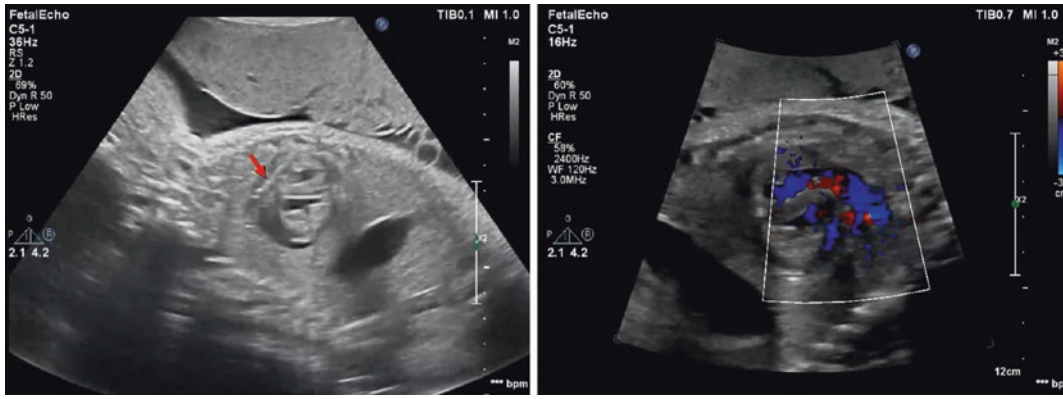

Fig. 7.6 Balloon dilation of fetal pulmonary valve: the red arrow in the left figure refers to the puncture needle; and for dilated pulmonary valve blood flow, see the right figure

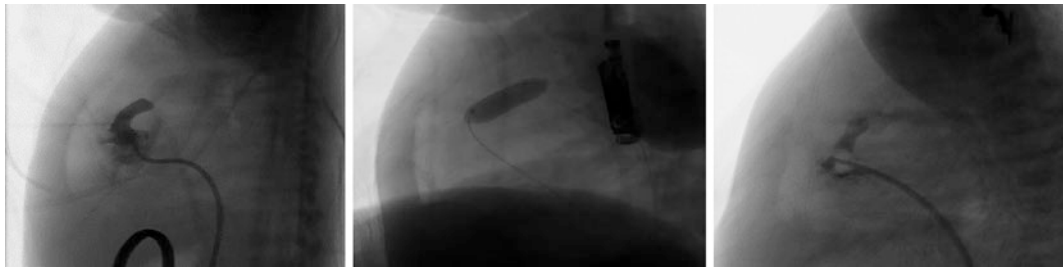

Fig. 7.7 Percutaneous balloon pulmonary valvuloplasty: the left figure showed significant pulmonary artery stenosis by preoperative angiography, the middle figure showed balloon dilatation of the stenosis, and the right figure showed significantly increased postoperative pulmonary artery forward blood flow

ity 1.11 m/s, mild pulmonary regurgitation, improved tricuspid regurgitation, and regurgitation velocity of 3.73 m/s. The fetus was delivered by cesarean section at 39 + 3 weeks of gestation with a birth weight of 4258 g and a neonatal APGAR score of 9–10–10.

Percutaneous balloon pulmonary valvuloplasty (PBPV) was performed at the age of 2 days and also 1 year and 1 month after birth due to echocardiographic findings of pulmonary artery stenosis (Fig. 7.7).

Follow-up echocardiography at 3 years of age showed decreased pulmonary artery blood flow velocity, mild pulmonary regurgitation, mild tricuspid regurgitation, and decreased flow velocity of 2.97 m/s, which was significantly improved. The child patient has been followed up to date, with normal growth and development and good cardiac function.

7.2.1.3 Total Anomalous Pulmonary Venous Connection

Total anomalous pulmonary venous connection (TAPVC) is a congenital anomaly of the pulmonary veins. Due to the ectopic connection of pulmonary veins, the pulmonary veins do not normally merge into the left atrium, but directly or indirectly affluxing into the right atrium through the body vein. It can be divided into supracardiac, cardiac, infracardiac, and mixed based on the site of connection to the heart according to Darling.

(Case) At 23 + 4 weeks of gestation, the pregnant woman underwent fetal echocardiography in the *in utero* Pediatric Diagnosis and Treatment Center of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. The results showed slightly enlarged right atrium and right ventricle, confluence of four pulmonary

veins into vertical veins running downward, entering the hepatic venous system, horizontal right-to-left shunt at foramen ovale, and open right-to-left shunt of ductus arteriosus. The remaining cardiac structures showed no significant abnormalities, suggesting total anomalous pulmonary venous connection (TAPVC) (infracardiac) in the fetus. Fetal echocardiography was followed up after 8 weeks, and the ultrasound findings were consistent and showed no significant improvement (Fig. 7.8).

Since it is infracardiac, as the most serious type of total anomalous pulmonary venous connection, the incidence of obstruction is high, easily leading to pulmonary congestion and pulmonary edema, which can develop into severe heart failure shortly after birth and resulting to death. After a comprehensive assessment of fetal growth and development, through the multidisciplinary consultation of obstetrics, neonatology, and pediatric heart center, it was recommended that the pregnant mother deliver in the Obstetrics Department of Xinhua Hospital. The child underwent complete anomalous pulmonary venous connection and patent ductus arteriosus correction after birth.

During the operation, after routine disinfection and draping, a median sternal incision was made under anesthesia, and the ascending aorta and right atrial appendage were cannulated, respectively, to establish extracorporeal circulation. The patent arterial duct was dissociated, sutured, and cut. The bypass was cooled, the ascending aorta was blocked, the root was injected with myocardial protective fluid, and the heart was stopped. Under deep hypothermia and low flow, the right atrioventricular groove was dissociated, two pulmonary veins on the right side were observed, and one pulmonary vein on the left side converged with the vertical vein to pass through the diaphragm. The vertical vein was cut by horizontal suture from the diaphragm, and the anterior wall of the vertical vein and common vein was dissected and anastomosed side-to-side with the incision of the posterior wall of the left atrium. After intracardiac exhaust, the heart automatically resumes beating, and the rhythm was sinus rhythm. Postoperative transesophageal ultrasound revealed a pulmonary vein anastomosis of 15 m/s. The extracorporeal circulation was stopped. After the blood pressure was stable, the superior and inferior venous cannulas and ascending aortic cannulas were removed, respectively, and protamine was intravenously infused to neutralize the effect of heparin. Mediastinal drainage tube was placed in the child. After careful examination of each incision and no obvious bleeding point was found, the chest closure was delayed, the skin edge was covered with latex membrane, and the child was returned to the ICU with endotracheal intubation.

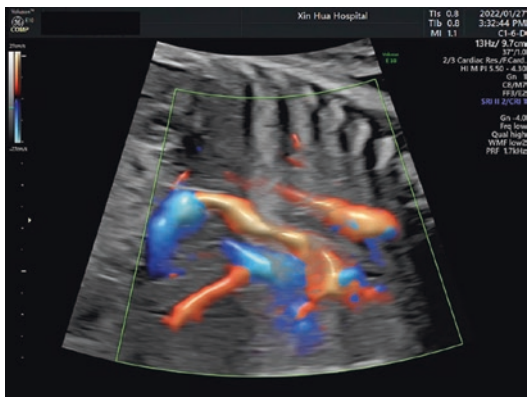

Fig. 7.8 Fetal echocardiography at 31 + 4 weeks of gestation: after the pulmonary veins converge, the vertical veins descend into the hepatic venous system

Three months after the operation, the echocardiography showed that the inner diameter of pulmonary vein-left atrium anastomosis was 4.3 mm, the flow velocity was 2.23 m/s, the flow velocity of left pulmonary vein was 0.64 m/s, and the flow velocity of right pulmonary vein was 0.8 m/s; the atrial orifice was 3.2 mm, with left-to-right shunt; the right atrium and ventricle were slightly enlarged, with acceptable systolic activity of left ventricular wall (LVEF 77%), acceptable open activity of atrioventricular valve, tricuspid regurgitation at regurgitation flow rate of 2.3 m/s, and without evident pericardial effusion (Fig. 7.9).

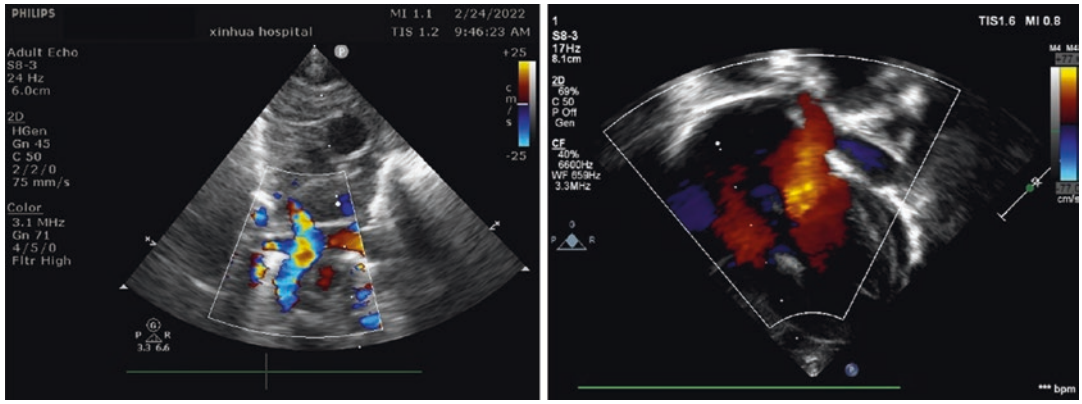

Fig. 7.9 Echocardiography 3 months after the operation: the four-chamber cardiac view in the left figure shows the pulmonary vein-left atrium anastomosis; the suprasternal fossa view in the right figure shows the pulmonary vein confluence

The results suggested that the flow velocity of pulmonary vein anastomosis was slightly increased after operation, and the atrial orifice was left to right shunt. Balloon dilation of the pulmonary veins was further performed. During follow-up at 1 month after operation, the child had no complaints of discomfort, with regular rhythm, strong heart sounds, insignificant murmur, and good general activity.

Congenital structural malformations of the cardiovascular system are currently the most prevalent disease among birth defects [5]. Through early screening, diagnosis, and intervention for such structural malformations, the survival rate of the fetus, postnatal quality of life, and the outcome of the disease can be effectively improved. The sound intervention of structural abnormalities of the cardiovascular system and the integrated sequential diagnosis and treatment system are essential to achieve this goal, which allows for the timely referral of fetal cardiac anomalies detected during maternal screening for accurate and effective prenatal diagnosis and evaluation. *In utero* intervention of the fetal heart can effectively reduce the severity of fetal abnormalities and reduce the birth of infants with severe birth defects.

7.2.2 *In Utero* Intervention and Sequential Treatment of Conduction Abnormalities of Cardiovascular System

7.2.2.1 Case 1 of *In Utero* Intervention and Sequential Treatment of Fetal Tachyarrhythmia

A 28-year-old mother was diagnosed with "fetal supraventricular tachyarrhythmia" at 33 weeks of gestation, with the ventricular rate of 218 beats/min and the CVPS score of 10 points. After maternal cardiac ultrasound, electrocardiogram, and other examinations, it was confirmed that there was no contraindication for prenatal intervention of transplacental transfer of digoxin. Thus, the mother was given oral digoxin (0.25 mg/dose, twice daily), and the plasma concentration of digoxin fluctuated steadily within 1.0–2.0 ng/mL for 5 days of medication, and the fetus achieved *in utero* conversion to sinus rhythm at 33 + 6 weeks of gestation, and digoxin maintenance therapy was continued. One week later (35 weeks of gestation), digoxin was reduced to 0.125 mg/dose, twice daily, and the fetus developed recurrent tachyarrhythmia on the night of dose reduction. Thus, the pregnant woman was given increased digoxin dose (0.25 mg/dose, bid), and the fetus converted to

sinus rhythm successfully again after 1 day of treatment. Then the pregnant woman continued to maintain oral digoxin (0.25 mg/dose, bid) until delivery. The affected fetus was delivered via vagina at 42 weeks of gestation, with a birth weight of 3330 g. Neonatal ECG showed sinus rhythm. During 8.5 years of follow-up, physical and neurological development were normal, ECG showed sinus rhythm, and echocardiography showed normal systolic and diastolic function.

7.2.2.2 Case 2 of *In Utero* Intervention and Sequential Treatment of Fetal Tachyarrhythmia

A 28-year-old mother was diagnosed with "fetal supraventricular tachyarrhythmia" at 25 + 3 weeks of gestation, with the ventricular rate of 222 beats/min and the CVPS score of 9 points (minus 1 point for valve regurgitation), without fetal edema. After maternal cardiac ultrasound, ECG and other examinations, it was confirmed that there was no contraindication for prenatal intervention of transplacental transfer of digoxin. Thus, the mother was given oral digoxin (0.25 mg/dose, twice daily). After treatment for 5 days, the plasma concentration of digoxin fluctuated within 1.0–2.0 ng/mL. After 7 days of treatment, the affected fetus still did not convert to sinus rhythm. Sotalol (80 mg/dose, twice a day, gradually increased to 160 mg/dose, twice a day) was added for several weeks, but the affected fetus still did not convert to sinus rhythm. The decision to continue treatment postpartum was made after assessment by a maternal-fetal medicine specialist. During this period, fetal movement was good, and multiple re-examinations of fetal cardiac echocardiography did not indicate fetal edema or heart failure. After evaluation by the maternal-fetal medicine expert team and full communication with the family members, it was agreed that the gestational age of the affected fetus was less than 35 weeks, the left ventricular function was normal, and there were indications for continued prenatal intervention. Therefore, digoxin combined with sotalol was continued until full term, and the fetal cardiopulmonary development was relatively intact. After evaluation by the maternal-fetal medicine expert, it was decided to continue postpartum treatment. The affected fetus was delivered by cesarean section at 37 + 3 weeks, with a birth weight of 3150 g. "Neonatal atrial flutter" was diagnosed by neonatal electrocardiogram. Antiarrhythmic treatment with digoxin combined with propafenone, propranolol, and amiodarone was successively given for more than 20 days, but all ineffective, and finally conversion was achieved using neonatal electrical cardioversion. During 3.3 years of follow-up, physical and neurological development were normal, ECG showed sinus rhythm, and echocardiography showed normal systolic and diastolic function.

7.2.2.3 Case 3 of *In Utero* Intervention and Sequential Treatment of Fetal Tachyarrhythmia

A 27-year-old mother was diagnosed with "fetal atrial flutter" at 32 + 5 weeks of gestation, with the atrial rate of 441 beats/min and the ventricular rate of 250 beats/min, showing a 2:1 downward conduction, the CVPS score of 9 points (minus 1 point for valve regurgitation), without fetal edema. After maternal cardiac ultrasound, ECG, and other examinations, it was confirmed that there was no contraindication to prenatal intervention for transplacental transfer of digoxin. Thus, the mother was given oral digoxin (0.25 mg/dose, twice daily). After treatment for 5 days, the plasma concentration of digoxin fluctuated within 1.0–2.0 ng/mL. After 7 days of treatment, the affected fetus still did not convert to sinus rhythm. Oral sotalol (80 mg/dose, twice daily) was added, and the affected fetus converted to sinus rhythm after 1 day of treatment, and then digoxin combined with sotalol was continued and gradually reduced and stopped. The affected fetus was delivered via vagina at 39 weeks of gestation, with a birth weight of 3530 g. Neonatal ECG showed sinus rhythm. During 2.8 years of follow-up, physical and neurological development were normal, ECG showed sinus rhythm, and echocardiography showed normal systolic and diastolic function.

7.2.2.4 Case 4 of *In Utero* Intervention and Sequential Treatment of Fetal Tachyarrhythmia

A 27-year-old mother was diagnosed with "fetal atrial flutter" at 31 + 6 weeks of gestation, with the atrial rate of 451 beats/min and the ventricular rate of 242 beats/min, showing a 2:1 downward conduction, the CVPS score of 8 points (minus 1 point each for pericardial effusion and valve regurgitation), without fetal edema. After maternal cardiac ultrasound, ECG, and other examinations, it was confirmed that there was no contraindication to prenatal intervention for transplacental transfer of digoxin. Thus, the mother was given oral digoxin (0.25 mg/dose, twice daily) during 31 + 6 weeks of gestation. After treatment for 5 days, the plasma concentration of digoxin fluctuated within 1.0–2.0 ng/mL, and the fetus achieved control of ventricular rate (atrial flutter was still maintained, ventricular rate <180 beats/min). Oral digoxin was maintained to control ventricular rate, but fetal atrial flutter recurred after 12 days. Obstetric ultrasound of the mother revealed progressive increase in amniotic fluid, accompanied with threatened preterm labor. The baby was born prematurely at 34 + 6 weeks of gestation, with a birth weight of 2900 g. Paroxysmal atrial tachycardia was diagnosed by birth electrocardiogram, and sinus rhythm conversion was successful after oral maintenance therapy with intravenous bolus of cedilanid followed by digoxin. During 8.4 years of follow-up, physical and neurological development were normal, ECG showed sinus rhythm, and echocardiography showed normal systolic and diastolic function.

7.2.2.5 Case 1 of *In Utero* Intervention and Sequential Treatment of Fetal Immune-Related First-Degree Atrioventricular Block

A 32-year-old female patient was diagnosed with "undifferentiated connective tissue disease" before pregnancy. She was treated with long-term oral "hydroxychloroquine (200 mg, bid)" (without drug discontinuation during pregnancy). Her previous two artificial insemination attempts failed, and it was the third artificial insemination. The patient underwent routine obstetrical examination at an outside hospital at 20 weeks of gestation and found that the AV interval of twins was prolonged (not specified). Interval prolongation was not improved after treatments with gamma globulin twice (400 mg/kg/day). She visited our hospital at 24 + 2 weeks of gestation, and examination of autoantibodies revealed positive anti-SSA. Fetal echocardiography revealed that the Doppler value of the tissue at the junction of the right ventricular free wall and tricuspid valve suggesting the AV interval of twin 1 was 148 ms and that of twin 2 was 150 ms, without cardiac structural abnormalities, endocardial thickening, and fetal edema. Fetal cardiac ultrasound at 25 weeks of gestation revealed the AV interval of twin 1 was 170 ms and that of twin 2 was 160 ms. The diagnosis of "immune-related fetal first-degree atrioventricular block" was considered. The mother was given dexamethasone 4.5 mg/day orally, and fetal echocardiography was performed weekly to monitor AV interval. The results suggested that the AV intervals of the twins were gradually decreased, but the femoral length and biparietal diameter length showed growth delay. The oral dose of dexamethasone was gradually reduced to 3.0 mg/day. Unfortunately, fetal echocardiography at 29 weeks of gestation revealed AV interval prolongation again, accompanied by endocardial thickening. The dose of dexamethasone was again increased to 3.75 mg/day, and at 34 weeks of gestation, the AV intervals of the twins decreased to the normal range, and the dexamethasone was gradually reduced to 1.125 mg/day for maintenance without further recurrence; at 35 + 4 weeks of gestation, the twins were delivered by cesarean section due to premature rupture of membranes. Birth weights of the twins were 2220 g (0.3th) and 1980 g (≤0.1th) and body length 47 cm (2.8th). Electrocardiogram showed the AV interval of newborn 1 was 118 ms and that of newborn 2 was 120 ms; serological tests showed that the two newborns were positive for anti-SSA antibodies. Blood routine and liver function were normal, without rash manifestation. The newborns were followed up and

observed. At 4 months of age, the serum anti-SSA antibodies became negative. After a follow-up of 46 months, the 2 children were physically and mentally comparable to their peers; no abnormalities were found in the cardiac ultrasound and electrocardiogram.

7.2.2.6 Case 1 of *In Utero* Intervention and Sequential Treatment of Fetal Immune-Related Second-Degree Atrioventricular Block

One affected fetus was diagnosed with type II second-degree atrioventricular block at 25 weeks of gestation, with the CVPS score of 10 points, the atrium rate of 131 beats/min, and the ventricle rate of 86 beats/min. The pregnant mother was diagnosed with Sjogren syndrome during pregnancy and treated with dexamethasone 4.5 mg/dose/day + HCQ 200 mg/dose (twice a day). After 2 weeks of treatment, the fetus converted to sinus rhythm; dexamethasone was gradually reduced and stopped. The affected fetus was born at 38 weeks of gestation, with the birth weight of 3730 g and the birth length of 48 cm. Electrocardiogram revealed sinus rhythm. At present, the child is 2 years and 10 months of age, with normal growth and development indicators and no neurological dysplasia. Prenatal genetic examination revealed no abnormalities.

7.2.2.7 Case 2 of *In Utero* Intervention and Sequential Treatment of Fetal Second-Degree Atrioventricular Block

A fetus was diagnosed with type II second-degree atrioventricular block at 21 weeks of gestation, with the CVPS score of 10, the atrial rate of 136 beats/min, and the ventricular rate of 66 beats/min. The pregnant mother's autoantibodies suggested positive anti-SSA and anti-SSB antibodies and was treated with dexamethasone 4.5 mg/dose/day + HCQ 200 mg/dose (twice a day). The fetus progressed to third-degree AVB *in utero* and was born at 37 weeks of gestation, with the birth weight of 2450 g and the birth length of 46 cm. Electrocardiogram after birth revealed third-degree AVB, QT prolongation, recurrent torsades de pointes, and Adams-Stokes syndrome after birth. Genetic testing was completed, and long QT syndrome (type 2) was confirmed. No recurrence occurred after the placement of an epicardial permanent pacemaker and oral metoprolol treatment.

7.2.3 *In Utero* Origin and Early Management of Cardiovascular Health

The theory of "developmental origins of health and disease" states that exposure to adverse factors in early life may increase the risk of chronic diseases in adulthood by changing epigenetic characteristics [6]. As an important field in the prevention and treatment of adult chronic diseases, cardiovascular diseases have attracted the attention of many scholars at home and abroad. Taking hypertension, the most common cardiovascular disease, as an example, massive epidemiological evidence has shown that adverse exposures such as nutrition deficiency and developmental disorders in early life increase the prevalence of hypertension in children and adults. There is a "trajectory phenomenon" of hypertension, that is, children with higher blood pressure in childhood have an increased risk of hypertension in adulthood [7, 8]. The management of early cardiovascular health in children, especially blood pressure health, may be an effective measure to reduce the risk of cardiovascular disease in adulthood.

(Case) To explore the origin of developmental diseases and establish an early intervention model, Xinhua Hospital, in conjunction with four other tertiary hospitals in Shanghai, has established the "Early Life Plan" program, integrating research and intervention since 2016 [9]. As a large prospective cohort of developmental diseases, it focuses on the critical period of developmental plasticity in early life "the first 1000 days of life from the fertilized egg to 2 years of age," aiming to discover the effect of adverse exposures in early life on the growth and development as well as the occurrence and development of diseases in adulthood and to pro-

vide a scientific basis for the prevention and control strategies for developmental diseases. It is an important scientific research and transformation platform for the practice of *in utero* pediatrics in Xinhua Hospital.

Early studies by the team have found that fetuses that have experienced adverse exposure *in utero* experienced structural and functional changes in the cardiovascular system early in life. Among them, excessive gestational weight gain [10], maternal gestational diabetes [11], and excessive birth weight [12] are all associated with increased blood pressure and cardiovascular structural changes in early childhood. Excessive gestational weight gain and excessive birth weight will increase the risk of left ventricular hypertrophy in offspring. The offspring of mothers with gestational diabetes, especially boys, have higher blood pressure levels during childhood. In addition, adverse growth patterns in early life [13], overweight, and elevated blood pressure in children [14] may all lead to changes in cardiac structure and function. Body mass index in children is more closely related to changes in cardiac structure, while elevated blood pressure affects changes in cardiac function more. It should be noted that these changes in cardiovascular structure and function have been detected as early as the age of 4 years, which suggests that for preschool children, especially those who have experienced adverse exposure *in utero*, routine blood pressure test and cardiovascular health management are necessary. The American College of Cardiology proposes that routine blood pressure test in children should start at the age of 3 years [15]. Our center has performed routine blood pressure monitoring and cardiac color ultrasonography to follow-up children since the age of 4. For the children with elevated blood pressure, blood pressure retest and 24-h ambulatory blood pressure examination at different time points are performed, and etiological screening and lifestyle intervention are performed for the children with hypertension. At present, nearly 100 children with hypertension are regularly followed up, and most of these children's blood pressure is well controlled.

7.3 Research Progress

7.3.1 Research Progress on Structural Abnormalities of Cardiovascular System

7.3.1.1 Fetal Factors

Fetal-related pathogenic factors of congenital heart disease are divided into three major factors: simple genetic factors, simple environmental factors, and genetic-environmental interaction factors. Among them, simple genetic factors mainly include chromosomal abnormalities and monogenic genetic defects. Chromosomal abnormalities are divided into chromosomal aneuploidy abnormalities and chromosomal copy number variations (CNV). Cardiac malformations due to chromosomal abnormalities account for 30% of the total number of patients with congenital heart disease. The most common chromosomal aneuploidy abnormalities, such as trisomy 21, trisomy 18, and trisomy 13, may be associated with congenital heart disease in 50%, almost 100%, and 80% cases, respectively. CNV-related syndromes, including 22q11.2 deletion/duplication syndrome, Noonan syndrome, and Holt-Oram syndrome, are usually associated with congenital heart diseases such as atrial septal defect, ventricular septal defect, atrioventricular septal defect, and the tetralogy of Fallot. With the development of prenatal diagnosis and detection technology, chromosome karyotyping and chromosomal microarrays (CMA) can achieve the detection of chromosomal aneuploidy abnormalities, chromosomal microdeletions, and microduplications associated with fetal congenital heart diseases [10]. Monogenic genetic defects are also one of the main genetic pathogenic factors of congenital heart disease. Studies have shown that more than 10% of congenital heart diseases are caused by de novo mutations. Various studies have confirmed that genes related to congenital heart disease include NKX2.5, TBX5, GATA4, and ZIC3. NKX2.5 gene mutation may lead to atrioventricular septal defect, the tetralogy of Fallot, while GATA4 gene mutation may lead to the tetralogy of Fallot, double-outlet right ventricle, etc. Pollard et al. published on paper

the role of protein interactome of transcription factors GATA4 and TBX5 in congenital heart disease in cell, confirming that the interaction between transcription factors and other cofactors can be disrupted, thereby impairing transcription synergy and leading to cardiac malformations and revealing the potential mechanism by which deleterious mutations in transcription factors leading to congenital heart disease [11]. In recent years, Professor Kun Sun's group from Xinhua Hospital has published studies on SOX7 gene mutation that may lead to atrioventricular septal defect in cell death and disease [12] and molecular genetics and genomics [13], verifying the regulatory effect of SOX7 gene on Wnt signaling pathway and its possible interaction with GATA4, revealing the important role of SOX7 in cardiac development and the occurrence of congenital heart diseases.

Over the past decade, the development of whole-exome sequencing (WES) has greatly updated our understanding of the genetic causes of congenital heart diseases, but there are still more than 50% of congenital heart diseases whose genetic factors are still unclear. Studies have shown that the occurrence of congenital heart disease may not be caused by a single detrimental mutation, but by a combination of multiple genes [14]. For example, in the mouse model with Zic3 (+/−), Nodal (+/−) compound heterozygote, the penetrance of congenital heart disease has increased. Studies have reported that patients with congenital heart disease may have cellular populations with different genetic backgrounds, that is, genetic mosaicism, which is also the reason why the pathogenesis of many cases cannot be explained by gene mutations. In the heart tissue of patients with congenital heart disease, there is also the phenomenon of allele-specific expression. In addition, some databases of noncoding DNA regions sequencing have been established recently, suggesting new areas for future research on the genetic etiology of congenital heart disease.

According to the theory of early life programming and reprogramming, fetal development is a programming process in which genetic information and environmental factors interact. Recently, more studies suggest that genes and environmental factors can interact and regulate the development and differentiation of myocardium in an epigenetic manner, leading to the occurrence of congenital heart disease [15]. For instance, overmethylation of CpG island in the promoter of the SCO2 gene may be associated with the development of the tetralogy of Fallot and ventricular septal defect; CpG methylation at APOA5 and PCSK9 gene loci is associated with the development of aortic stenosis; aberrant methylation of DOK7 and NOS3 genes is associated with the pathogenesis of atrioventricular septal defect and aortic coarctation [16]. The pathogenesis of congenital heart disease is complex. To explore the etiology of congenital heart disease through epigenetic regulation is also a new research direction in the future.

7.3.1.2 Maternal Factors

Despite the great efforts in genetics, researchers have been able to provide genetic explanations for only a small proportion of cases. This makes researchers no longer focus on the role of genes in the pathogenesis of congenital heart disease. Maternal health is closely related to the growth and development of the fetus. Hence, more studies have explored the influence of maternal factors on the development of the cardiovascular system of the offspring.

Diabetes is a disorder of glucose metabolism. It is now estimated that 425 million people worldwide suffered from diabetes, and this number is expected to rise to 0.629 billion by 2045. The offspring of mothers with diabetes (types I and II) have an approximately threefold increased risk of congenital heart disease of any type, while gestational diabetes increases the risk of congenital heart disease by an approximately 1.5-fold [17]. The prevalence of congenital heart disease in offspring is associated with increased or poorly controlled maternal blood glucose levels, and the measurement of glycosylated hemoglobin strongly suggests that hyperglycemia is the major teratogen [18]. Nevertheless, there is no consensus on how hyperglycemia leads to congenital heart disease. Extensive research on various animal models has generated many different hypoth-

eses, including increased hypoxia and/or oxidative stress [19], activation of polyol [20] or hexosamine pathways [21], increased apoptosis [22], or endoplasmic reticulum stress [23]. Maternal obesity during pregnancy (prenatal body mass index >30) is associated with a number of pregnancy-related adverse outcomes, including an increased risk of congenital heart disease [24]. Obesity is often accompanied by type II diabetes or impaired glucose tolerance, making it difficult to separate the two diseases. Therefore, it is generally assumed that congenital malformations due to obesity and diabetes may share a common etiology. However, it has been shown that maternal weight gain is associated with an increased risk of congenital heart disease after adjusting for glucose tolerance [25]. It is unclear exactly how maternal weight gain alone affects embryonic heart development. In a population study of the Shanghai Birth Cohort, we found that excessive maternal weight gain during pregnancy, especially during the second and third trimesters, was a risk factor for left ventricular eccentric and concentric hypertrophy in offspring [26]. Our findings highlight the importance of maternal weight control during pregnancy.

In addition to metabolic diseases, infection and fever during pregnancy will also increase the risk of congenital heart disease. Rubella infection in pregnant women during the first 10 weeks of pregnancy can cause birth defects in up to 90% of cases, of which about half have heart defects, including stenosis of pulmonary artery branches, patent ductus arteriosus, and ventricular septal defect [27]. Although exactly how maternal viral infection leads to birth defects is unknown, there is evidence that elevated body temperature is a teratogen rather than the virus itself. It is found in an epidemiological study that during weeks 3–8 of gestation, maternal exposure to extreme high temperatures of 3–11 days or more (daily maximum temperature ≥ 95%) increased the risk of congenital heart disease by 50% [28]. Studies in different species have confirmed that maternal hyperpyrexia may lead to a series of embryonic defects, including cardiac defects [29]. Among all the proposed mechanisms, the activation of heat shock response [30] and temperature-activated ion channels in neural crest cells [31] are currently the two most possible mechanisms.

Toxic substances exposed to the pregnant mother during pregnancy can act directly or indirectly on the embryo itself and interfere with the normal embryonic development process. Thalidomide is the most notorious human teratogen, and the fetal malformations it causes also include congenital heart disease. The exact mechanism by which thalidomide causes birth defects has only been identified in recent years. Thalidomide binds to normal cell protein CRBN and enhances its effect. CRBN is a subunit of CRL4CRBNE3 ubiquitin ligase, which targets many proteins for degradation through the ubiquitin pathway, including the transcription factor SALL4, which is necessary for limb and heart development in the embryo [32]. High-dose alcohol consumption (i.e., ≥ 50 g during a single drinking) during pregnancy can lead to fetal alcohol syndrome, and cardiac defects are common in fetal alcohol syndrome, mainly including ventricular septal defect, atrial septal defect, and conotruncal defect. Animal experiments have shown that the time period and amount of alcohol consumed determine the nature and severity of the outcome [33]. The mechanism of alcohol-induced cardiac defects is still unclear and may be related to competitive inhibition of retinoic acid synthesis [34], direct deleterious effects on cardiac neural crest cells [35], or direct effects on DNA methylation, histone modifications, and/or noncoding RNA regulation, which leads to extensive epigenetic changes in embryos [33].

7.3.1.3 Environmental Pollutants

In addition, the exposure of harmful environment in the first trimester is also one of the mechanisms that lead to abnormal structural development of the cardiovascular system in offspring. The major air pollutants produced by industrial emissions, urbanization, and fuel engines include carbon monoxide, nitrogen oxides, sulfur dioxide, and particulate matter. The conclusions drawn from the available population studies are not completely consistent, and this heterogeneity is associated with the classification of congenital heart defects, exposure assessment, and control

of confounding factors used in various studies. In general, maternal exposure to air pollutants is a risk factor of the increased incidence of congenital heart disease. Meanwhile, paternal exposure also plays a key role in the risk of congenital heart disease in offspring. Air pollutants may affect the occurrence of congenital heart disease through DNA methylation modification [36], histone modification [37], and other pathways. However, these pathways still should be confirmed by more definitive evidence.

Maternal heavy metal exposure (including lead, nickel, arsenic, cadmium, and manganese) is associated with the incidence of congenital heart disease. Differences in nickel levels were observed in samples with ventricular septal defect, conotruncal defect, and outflow tract stenosis, suggesting that different exposure levels may pose a risk for specific types of congenital heart disease [38]. There is a strong synergistic interaction between arsenic and cadmium exposure, which is positively correlated with congenital heart disease, emphasizing the importance of studying the extent of environmental exposure and interaction [39].

The offspring of professionals exposed to endocrine disrupting substances are particularly susceptible to congenital heart disease. Many occupations are exposed to endocrine-disrupting substances, such as painters, farmers, metal workers, or carpenters. Certain endocrine-disrupting chemicals, such as phthalates, alkylphenol compounds, pesticides, solvents, and polychlorinated organic compounds, are all related to congenital heart disease [40]. The mechanism of how these occupational factors contribute to the development of congenital heart disease is not fully understood. Many endocrine disrupting chemicals are estrogenic and may cause impaired semen quality [41], impaired oocyte maturation [42], or fetal epigenomic changes [43]. Although biphenols have not been widely associated with congenital heart disease in humans, animal studies have shown that biphenols may also interfere with the cardiogenic process and lead to transgenerational inheritance of congenital heart disease [44]. In addition, we found in population studies that prenatal BPA exposure in pregnant mothers was associated with cardiac metabolic risk factors in offspring [45], emphasizing the importance of *in utero* exposure to environmental contaminants such as BPA in cardiac health later in life.

7.3.2 Research Progress on Conduction Abnormalities of Cardiovascular System

At present, one of the main research directions on fetal cardiovascular conduction abnormalities is the immune injury of fetal cardiac conduction system. Isolated CHB and CAVB are mainly associated with fetal exposure to high-titer anti-SSA/Ro antibodies, which may trigger inflammation, necrosis, and fibrosis of the atrioventricular node in the fetus. Anti-Ro antibodies (SSA) bind apoptotic cardiac cells and block their electrophysiological conduction. The injury process may appear as early as 16–18 weeks of gestation. This immune damage to the fetal heart can last throughout pregnancy, or even early life after birth, resulting in varying degrees of fetal and pediatric cardiac conduction system diseases.

Anti-Ro/SSA and La/SSB antibodies in pregnant women may all be involved in the development of CHB, but Ro52 antibodies in anti-SSA antibodies seem to play a dominant role. Studies have shown that anti-Ro52 antibodies have a direct pathogenic effect on cardiac conduction and calcium homeostasis in vitro and in vivo, which may cause fibrosis and eventual calcification of the atrioventricular node, leading to CAVB. The correlation of maternal anti-Ro52 antibodies with AVB in children is significant with respect to the role of other autoantibodies. Additional studies have shown that although low titers and isolated anti-Ro/SSA 60 kDa antibodies are associated with positive pregnancy outcomes, high titers of anti-Ro/SSA 60 kDa antibodies are closely associated with high probability of fetal CHB.

In addition, scholars have noticed that why not all anti-SSA/Ro antibody-positive pregnan-

cies develop fetal heart block, but the incidence is only 1–2%. Why does first-degree atrioventricular block (AVB) never progress in some cases but rapidly to second-degree and third-degree AVB in others? Observations have shown that different fetuses with positive anti-SSA/Ro antibodies in different pregnant women have different outcomes, suggesting that the expression of fetal genes may determine the severity and progression of the disease. Therefore, researches on gene polymorphisms and related influencing factors are also gradually being carried out.

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The Nervous System

8

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8.1 Introduction

The fetal period is a critical time window for nervous system development. Major developmental events of brain structure and function are completed during this period under the regulation of genetic factors and impact of *in utero* environmental factors. Neurological structural diseases (e.g., encephalocele and hydrocephalus) and neurodevelopmental disorders (e.g., mental retardation and autism spectrum disorders) may occur if any disturbance occurs during this period. Early *in utero* screening, prevention, and intervention for high-risk factors during periconception may improve the disease prognosis to a greater extent.

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8.1.1 Normal Development of *In Utero* Nervous System

8.1.1.1 *In Utero* Neurostructural Development

At 3 weeks of gestational age, the ectoderm is induced by notochord mesoderm to differentiate into neuroectoderm. The neuroderm cells proliferate and thicken to form a slipper-shaped "neural plate." The neural plate expands and grows into the "neural crest" and then closes to form the neural tube, with the caudal end forming the spinal cord, the broader rostral end forming the cerebrum, and the hollow tube forming the ventricular system of the mature brain. At the rostral end of the tubular structure, there are clearly demarcated anterior cerebral vesicles, middle cerebral vesicles, and posterior cerebral vesicles. The anterior cerebral vesicles form the cerebral hemisphere, basal ganglia and thalamus, the middle cerebral vesicles form the midbrain, and the posterior cerebral vesicles form the main part of the brainstem and the cerebellum. The cerebral hemispheres are located at the top of the brainstem, and the cerebellum is located behind the brainstem. The cerebellum is immature at birth and is the finally developed part of the central nervous system. After the anatomical structure of the fetal brain was formed at 3 months of gestational age, brain cells continue to develop and go through six stages: neural tube formation, forebrain development, neuronal proliferation, neuronal migration, organization, and myelination [1, 2].

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Neural tube formation Gestational age of 3–7 weeks is the stage of neurulation or neural tube formation. Under genetic control, the neural tube closes in a specific order, and fetus who fails neural plate fusion may develop anencephaly or spina bifida with meningomyelocele. In addition, there are other factors that may lead to neural tube defects. For example, maternal folic acid deficiency during pregnancy increases the risk of neural tube defects in the fetus [3].

Forebrain development Induced by the anterior mesodermal lobe of the notochord during 2–3 months of gestational age, the forebrain begins to develop, with facial formation, hemispheric, and ventricular division. Thus, if severe disturbances occur during this period, consequent facial development abnormalities often occur. Holoprosencephaly or holoprosencephaly is such a group of complex craniofacial malformations due to disruption of forebrain development.

Neuronal proliferation At 3–4 months of gestational age, neural cells rapidly divide and proliferate, and then migrate to the upper layers of the developing brain. If the critical period of neuronal proliferation is inhibited, microcephaly may occur. Alcohol, radiation, and intrauterine infection during this period may result in appearance characteristics similar to microcephaly.

Neuronal migration Normal central nervous system development relies on extensive cell migration. At 3–5 months of gestational age, neurons migrate rapidly to the cortex and cerebellum. Glial cells play an important role in the migration path. If glial cell function is impaired, migrating neurons may stop and differentiated into ectopic neurons, resulting in epilepsy, mental retardation, and so on.

Organizational process of the nerves From 5 months of gestational age to early childhood, neurons are still undergoing continuous organizational processes, including thickening and lengthening of neuronal axons and dendrites, synaptogenesis, and selective trimming of neuronal synapses. The symptoms of Down's syndrome, fragile X chromosome syndrome, etc. may result from defects in the organizational process of the brain. For example, a significant decrease in the number and surface area of dendritic spines in children with Down's syndrome when compared to typically developing children may partly explain the developmental delay.

Myelination Myelination of nerve fibers is a process in which the fat layer (i.e., myelin sheath) wraps axons and is closely correlated with the rapid proliferation of glial cells. Myelination of nerve fibers produces an insulating effect that allows rapid transmission of nerve impulses, a significant sign of mature conduction function.

8.1.1.2 *In Utero* Brain Function Development

During fetal development, the structures of the central nervous system develop chronologically in the rostral-caudal development sequence. Old structures in phylogenesis develop earlier than those that appear later in evolution, e.g., earlier development is seen in the spinal cord than the brainstem (including the cerebellum), in the subcortical structures than the cortex, in the primary motor area of the cerebral cortex than the primary sensory area, and the connecting cortex is the last region to develop well. In addition, the sensory system also develops in a specific sequence. The vestibule, somatic sensation, taste sense, and smell sense are functional at birth, but the auditory and visual sense are still poorly developed at birth. Although the motor cortex develops earlier than the sensory cortex, the motor behavior develops later than the sensory behavior, which may be related to the immaturity of the cerebellum and motor nerve pathways and late development of the sensory-motor connecting cortex [1, 2].

8.1.2 *In Utero* Etiology of Neurodevelopmental Disorders

Neurodevelopmental disorders (NDDs) are a group of conditions (e.g., attention-deficit hyperactivity disorder, autism spectrum disorder, and

mental retardation) characterized by brain dysfunction such as emotional and cognitive disorders originated from abnormal brain development. Symptoms of abnormal functioning are usually evident in children or adolescents and can persist throughout life [4]. The etiological mechanism of NDDs is still unclear. Current studies suggest that genetic factors and *in utero* exposure to adverse environmental factors are closely correlated with pathogenesis [5, 6]. Therefore, focusing on the exposure window in early life, exploring adverse factors that increase the risk of disease, and taking targeted prevention and control measures become the optimal strategies to reduce the public health costs associated with NNDs.

8.1.2.1 Genetic Factors

The material basis for children's behavioral development is the nervous system, especially the central nervous system. The development and differentiation of neurons and glial cells, which are closely correlated with developmental behavior and the final physiological, biochemical, and behavioral functions, are regulated by embryonic genes during the *in utero* period [7]. In recent years, molecular genetics and genetically modified biotechnology have been ideal tools for further research. It has been found that aggressive behavior in children may be correlated with monoamine oxidase (MAO) A deficiency [8]. Genetic linkage analysis has confirmed that the pedigree genetic defect in children with impulsive aggression is located on the X chromosome with the linked locus at Xp11-21, where the structural gene of MAO is also located, resulting in the mutation of 936C to T in the MAO coding region and the substitution of the glutamine codon by a stop codon. Structural changes in MAO lead to abnormal physiological function in children. The brain structure of MAOA-knockout mice lacking MAOA in the body was significantly changed, and the male mice after weaning showed obvious aggressiveness.

8.1.2.2 *In Utero* Environmental Factors

Not all defects in brain development are genetically determined. Neural synapses are very susceptible to the changes of the *in utero* environment. Changes in the environment may have impacts on the development of the nervous system by affecting synaptic connections, pruning, and even the growth and migration of nerve cells. Studies have suggested that physicochemical environments such as nutrients, toxins, and compounds may also affect growth and development of children [9]. For example, maternal folic acid deficiency during pregnancy is correlated with the absence of the neural tube. The adverse *in utero* environment (e.g., hyperglycemia during pregnancy, maternal immune activation, etc.) can lead to an increased risk of attention-deficit hyperactivity disorder, mental retardation and autism, etc. in the offspring [3, 10, 11].

8.1.3 Common Diseases and Pathogenic Factors of Abnormal Fetal Structural Development

Encephalocele The pathogenesis of encephalocele is still not fully understood. The first relevant theory was based on Vou-Recklinghausen's clinical observations, who pointed out the defect present in the formation of primitive brain neurulation. However, if this theory holds, there should be significant neurostructural deformities and skin defects in all encephaloceles, and all defect sites should protrude (Fig. 8.1) [12].

Research on animal models and human embryos supports another theory that after the neural layer develops, impaired separation of the nervous system from the ectoderm leads to encephalocele. The formation of the mesoderm is generally a gradual, continuous, and segmented process. From the rostral to the caudal direction, it typically starts at the rostral end and ends at the caudal end. Studies on skeletal, neurological, and oropharyngeal morphology suggest that in some types of encephalocele, a dysfunction of the mesoderm affects the formation of the occipital bone, brain, and meninges, which may be the main pathological changes of encephalocele [13, 14]. Defects in nerve organization are minor compared to these changes.

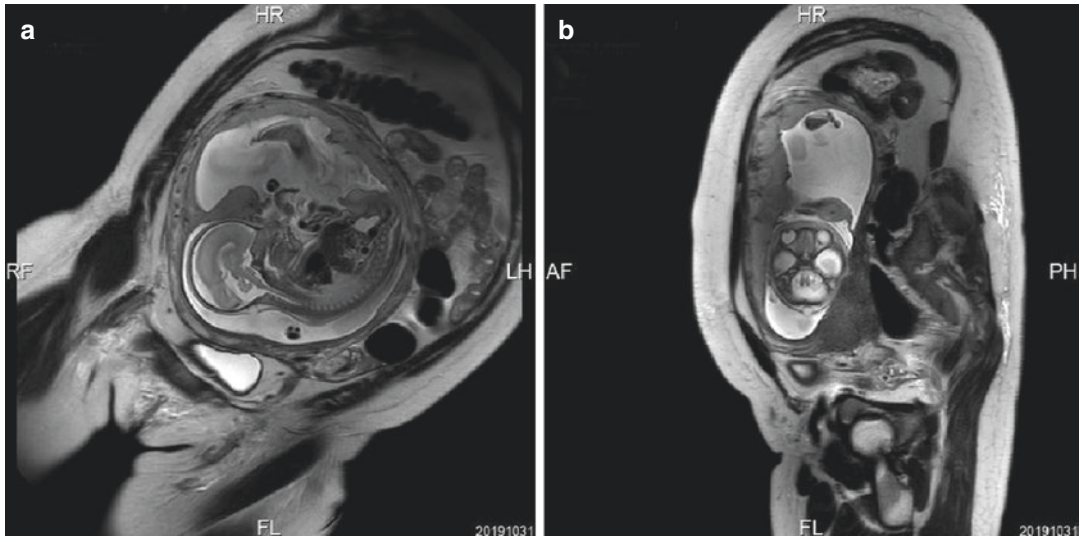

Fig. 8.1 MRI findings of fetal meningocele. Panel (**a**) shows the fetus in sagittal position, at 23 weeks and 6 days of gestation. A subcutaneous cystic lesion of about 6 mm is seen in the posterior occipital region. Panel (**b**) shows the fetus in coronal position

As for the encephalocele in the anterior cranial fossa, the evolutionary blockade hypothesis suggests that the neural tube may not be fully closed at the anterior neural foramen, similar to the pathological changes of spina bifida. It usually occurs around 24 days of gestation in the blind hole area. The theory was established based on a case of anterior parietal encephalocele with agenesis of the corpus callosum. However, this is not the origin of frontal or anterior skull base encephalocele [15]. The two reasons are as follows: firstly, these lesions are not complicated with other neural tube defects; secondly, immunohistochemical morphological analysis of surgically resected and neonatal autopsy tissues using neuron-specific enolase revealed no manifestations of neural tube insufficiency in these brain tissues. Although neonates with brain malformations are exposed to neural placodes containing brain residues, these tissues cannot be stained by neuron-specific enolase, and herniated brain tissue in anterior parietal encephalocele can be stained by neuron-specific enolase despite distorted cellular structures. Therefore, it is anencephaly rather than encephalocele, which is equivalent to spina bifida of the brain.

Another hypothesis suggests that cerebral and meningeal hernias are due to skeletal hypoplasia of the anterior skull base or the persistence of the craniopharyngeal duct. The cause may be excessive extension of the primitive neural tube or increased intracranial pressure during delivery. Unfortunately, this statement is not consistent with the research by embryologists and anatomists who point out that meningeal and neural protrusion has emerged from the beginning and is accompanied by skeletal developmental disorders.

The most widely accepted theory was proposed by Geoffrey St. Hilaire in 1827, who stated that the neural cleft occurred before neural tube closure. As the cleft heals, adhesion occurs between the neuroectoderm and the skin ectoderm, thereby preventing mesoderm from forming the skull. Molecular biology studies have led to new insights into the pathogenesis of encephalocele. Neural tube formation is a process controlled by genes and the proteins they encode. These proteins can be transcription factors, membrane receptors, or ligands. Several genes have been identified to date, such as sonic hedgehog (SHH), which is expressed in chordates and affects cell structure formation on the

ventral side of the spinal cord [16]. It is currently known that bone morphogenetic proteins affect the formation of dorsal structures of the spinal cord. These new findings undoubtedly enhance our understanding of the pathogenesis of encephalocele.

Dandy-Walker Syndrome Dandy-Walker syndrome (DWS) is characterized by the following three characteristics: cystic dilatation of the fourth ventricle, dysplasia of all or part of the cerebellar vermis, and supratentorial hydrocephalus, but hydrocephalus is not a necessary condition for the diagnosis of Dandy-Walker syndrome [17]. In 1887, Sutton et al. first described hydrocephalus complicated with cystic dilatation of the fourth ventricle. In 1914, Dandy reported a 13-month-old girl with severe hydrocephalus and cystic dilatation of the fourth ventricle. He was the first person to correlate these two conditions. Dandy analyzed the origins leading to DWS. After studying the pathology of nine cases, he concluded that the cause of DWS was impaired development, specifically, the *in utero* maldevelopment of the foramen of Magendie and Luschka (median and lateral foramina of the fourth ventricle) or obstruction due to an inflammatory response after birth, or both. In 1941, Sahs reported a case of congenital cerebellar anomaly without hydrocephalus due to the patent foramen of Luschka. In 1954, Brenda proposed a theory that has been accepted so far. He conducted the autopsy study in six cases and proposed that congenital abnormalities of the fourth ventricle were correlated with the pathogenesis of DWS. He speculated that the main abnormality of DWS was the cerebellar fissure with meningocele-like sac at the posterior medullary velum site. In 1959, other researchers carried out studies in mouse with congenital hydrocephalus, which demonstrated that cerebellar vermis dysplasia and fourth ventricle malformation occurred at an earlier embryonic stage than the formation of foramens of Luschka and Magendie. This suggests that the malformation develops earlier than the opening of the foramens of the fourth ventricle. Concomitant facial and cardiovascular abnormalities occur more frequent around 4 weeks of gestation. Since DWS is accompanied by non-CNS abnormalities and genetic disorders, there are still many unknowns about the pathogenic causes [18].

Hydrocephalus Hydrocephalus refers to the state of excessive accumulation of cerebrospinal fluid in the ventricular system and/or arachnoid membrane due to excessive secretion, circulatory obstruction, or malabsorption of cerebrospinal fluid with various causes (Fig. 8.2). It is often accompanied by ventriculomegaly, corresponding decrease in the brain parenchyma, and increased intracranial pressure [19]. On the contrary, a condition where cerebrospinal fluid increases accordingly within the skull due to the decrease of brain parenchyma volume resulting from brain atrophy, loss of local brain tissue, etc. is not hydrocephalus.

The causes of hydrocephalus include excessive secretion of cerebrospinal fluid, circulatory obstruction, malabsorption, or both. Based on the nature of the lesion, it may include congenital dysplasia, inflammation, hemorrhage, tumor, trauma, etc. Hydrocephalus in children is more common due to congenital developmental abnormalities, and hydrocephalus is reported more in adults due to tumor, subarachnoid hemorrhage, and trauma [20].

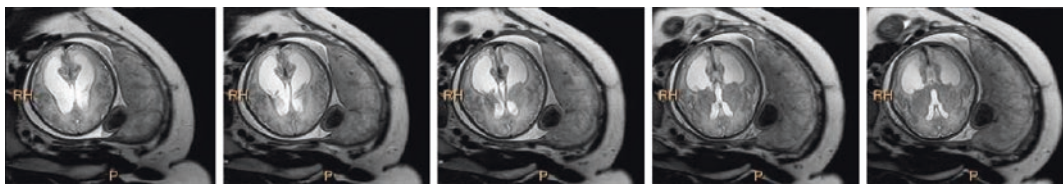

Fig. 8.2 *In utero* hydrocephalus MRI shows that the bilateral and the third ventricles of the fetus are significantly enlarged. Hydrocephalus is considered first

1. Cerebrospinal fluid circulation is obstructed by the ventricular system or the IV ventricular outlet.
   (a) Congenital dysplasia: aqueduct stenosis or occlusion, cerebellar tonsillar herniation malformation (Arnold-Chiari malformation), IV ventricular median foramen, and lateral foramen occlusion (Dandy-Walker syndrome)
   (b) Inflammation: subacute or chronic hydrocephalus due to ventriculitis, intraventricular adhesion, and septation
   (c) Hemorrhage: acute hydrocephalus due to rapid compression or blockage of the interventricular foramen, aqueduct of the midbrain, or outlet of the IV ventricle by blood clots due to intracranial hemorrhage resulting from rupture of malformed vessels, such as rupture due to vascular malformations, and subacute or chronic hydrocephalus due to secondary adhesions at the above sites
   (d) Intracranial space-occupying lesions: hydrocephalus resulting from tumors, parasitosis, cysts, and other compression or blockage of the interventricular foramen, midbrain aqueduct, or IV ventricular outlet
2. Cerebrospinal fluid circulation is obstructed by subarachnoid space.
   (a) Congenital cisternal hypoplasia.
   (b) Meningitis, subarachnoid hemorrhage, meningeal metastases, etc. cause adhesion and blockage of the subarachnoid cavity, resulting in the blockage of cerebrospinal fluid circulation.
3. Cerebrospinal fluid circulation is obstructed by arachnoid granulations or venous return.
   (a) Congenital absence of arachnoid granulations
   (b) Occlusion of arachnoid granulations due to inflammation or hemorrhage
   (c) Increased venous pressure in the superior sagittal sinus
4. Abnormal cerebrospinal fluid.
   (a) Cerebrospinal fluid hypersecretion, e.g., intraventricular choroid plexus papilloma.
   (b) Increased pulsatile cerebrospinal fluid pressure, for example, intraventricular choroid plexus papilloma.
   (c) Changes in the composition of cerebrospinal fluid, e.g., some tumors cause an increase in cerebrospinal fluid protein content and an increase in viscosity, which affects the absorption of cerebrospinal fluid.

Meningomyelocele The spinal cord develops from the ectoderm during the embryonic stage. On the 18th day of the embryonic stage, a neurogenic node forms and develops into a neural groove at the caudal end. On the 21st day, the nerve folds on both sides of the neural groove roll and migrate toward the dorsal midline, eventually fusing to form the neural tube. After formation, the neural tube gradually separates from the surface ectoderm that forms the skin and invades into the embryo body. The rostral end of the neural tube forms the brain vesicle, and the caudal end develops into the spinal cord. In the 11th week of the embryonic stage, the bony spinal canal from the mesoderm heals completely. In the third month of the embryonic stage, the spinal cord extends over the entire spinal canal, and its caudal end ends at the end of the spinal canal. The spinal nerve emerges from the spinal cord rectangularly and runs toward the corresponding intervertebral foramen horizontally. Later, since the spinal canal grows faster than the spinal cord, the caudal end of the spinal cord gradually migrates to the rostral end of the spinal canal, and the angle at which the spinal nerve starts from its spinal cord to entering the intervertebral foramen also gradually changes from a right angle to an acute angle, that is, from a horizontal course to a downward inclination course. As a result, the spinal nerve starts at the T1 spinal cord, and the more it runs toward the caudal end, the more it tilts, and the nerve lengthens accordingly. At full term, the spinal cord has developed to 15–17 cm in length, and the conus medullaris has risen to the level of the lower edge of the L3 vertebral body. After birth, the conus medullaris continues to move toward the rostral end. By the age of

3 years after birth, the caudal end of the conus medullaris is at the lower edge of L1 vertebral body or the upper edge of L2 vertebral body. The dura mater rises less and generally remains at the level of the original corresponding vertebral body, rising from S4 to S5 (embryo) to S3 (adult) [21–23].

Meningocele is caused by abnormal neurulation (Fig. 8.3). The neural groove located in the middle of the neural placode is a remnant of the central canal. The spinal nerve roots emerge from the anterior placode area, with the ventral roots arranged medially and the dorsal roots located laterally. The fusion-deficient dura mater is lateral to the fascia, and functional nerve tissue may be located at the caudal end of the neural placode or on the root of the nerve that emerges from the neural placode.

Most meningomyeloceles (85%) occur at the end of the thoracolumbar region or more distally, 10% at the chest, and the rest at the neck. Almost all children with meningomyelocele are accompanied with Chiari II malformation and a series of pathologic changes affecting the central nervous system. Among them, brainstem defects include medullary kinking, apical cap sharpening, and internal abnormal nuclear. Supratentorial abnormalities include partial or complete corpus callosum agenesis, polymicrogyria, massive interthalamic adhesion, and gray matter heterotopia. The development of the neural mesoderm is also affected, presenting as narrow posterior fossa, short clivus, decreased position of the tentorium cerebelli and sinus confluence, widened notch, enlarged occipital foramen, and parietal invagination [24].

Most children with meningomyelocele (80–90%) are accompanied with hydrocephalus, and treatment is required. Hydrocephalus is caused by obstructive or communicating factors. Syringomyelia occurs in 40–80% of patients with spina bifida, but the cavity usually does not progress. Nervous system degeneration in children with spina bifida may result from hydrocephalus or Chiari II malformation, or result from syringomyelia or tissue adhesion. However, the most common cause of nervous system degeneration is hydrocephalus due to shunt failure.

The terminal filament is a connective tissue-like filament formed by the degeneration of cells at the caudal end of the cone and the spinal pia mater, which attaches to the dorsal side of the first caudal periosteum and fixes the spinal cord. During embryonic development, due to the fineness of the terminal filament, the spinal cord is

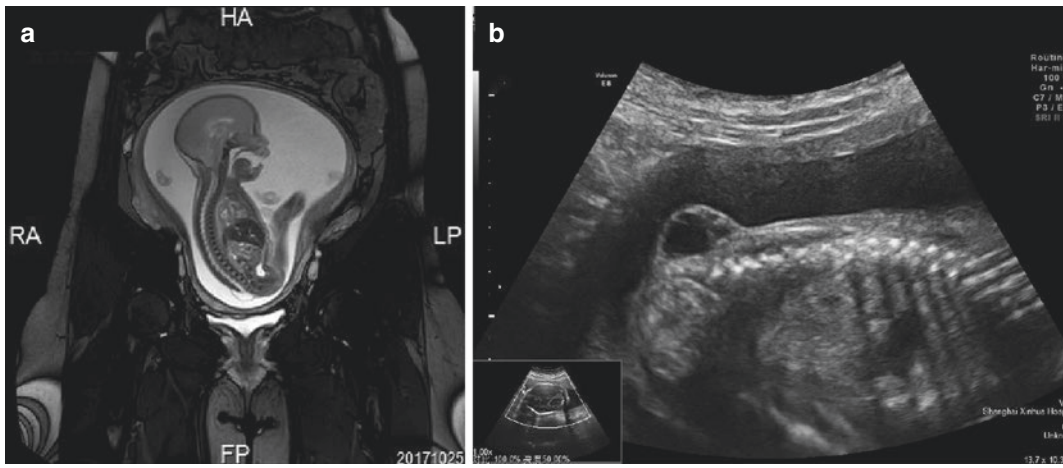

Fig. 8.3 Imaging of *in utero* meningomyelocele. Panel (**a**): *In utero* MRI, 24 weeks of gestation, singleton. Breech fetus, amniotic fluid volume, and the placenta are normal. Cystoid signal with nodular shadow is present on the skin of the sacrum caudal region of the fetus, with a size of 1.8 * 1.0 cm. The lesion is connected with the spinal canal. Panel (**b**): *In utero* ultrasound shows beaded strong echo in the sacrum caudal region, and a cystic mass protruding is seen here, with clear boundary, thin wall, and no echo inside

allowed to slowly rise. If it is pulled for various reasons, it can prevent the spinal cord from rising and pull the spinal cord, resulting in a series of neurological disorders. Furthermore, abnormalities occur during the closing of the neural tube in the embryo, and the ectoderm tissue, which will form the skin, separates prematurely from the ectoderm tissue, which will form the neural tube. The mesoderm tissue at both sides of the neural fold enters the neural tube and evolves into fat, fibrous tissue, smooth muscle, and striated muscle. Incomplete closure of the neural tube may cause spina bifida or cranioschisis. Incomplete closure of the mesoderm alone may cause spina bifida occulta or cranioschisis [25, 26].

8.2 Clinical Practice

8.2.1 Periconceptional Folic Acid Supplementation for Prevention and Treatment of Neural Tube Defects

Neural tube defects (NTDs) are congenital birth defects of the brain, spine, or spinal cord. NTDs occur in developing fetuses within the first month of pregnancy. The most common NTDs are spina bifida and anencephaly. Normally, during the first month of pregnancy, the two sides of the fetal spine are connected together to cover and protect the spinal cord, spinal nerves, and meninges. At this point, the developing brain and spine are named the neural tube. When this structure is not completely closed, NTDs occur. More than 300,000 children worldwide suffer from NTDs each year [27]. Some infants with NTDs are asymptomatic, while most of them experience severe disabilities throughout their lives. Despite medical, surgical treatment, rehabilitation, and training, most of them suffer from complications such as lower extremity paralysis, loss of body sensation, urinary incontinence, refractory skin ulcers, and genitourinary discomfort, which mean lifelong dependence and poor quality of life. Even minor spina bifida often adversely affects daily life due to fatigue, back pain, lumbalgia, and growth retardation. Infants with spina bifida and anencephaly are usually stillborn or die shortly after birth due to the complications of the defect. Healthcare providers and scientists haven't discovered the exact cause of NTDs, but it is generally believed due to a combination of genetic, nutritional, and environmental factors.

Folic acid is the natural form of vitamin B9 and is important for the healthy development of the fetus. Before pregnancy and during the first trimester of pregnancy, low levels of folic acid in the human body may affect the development of congenital diseases. Folic acid is critical in the development of the fetal brain and spinal cord. Folic acid deficiency before and during pregnancy may increase the risk of spina bifida and other NTDs in neonates.

In 1992, the US Public Health Service recommended that all women of childbearing potential to intake at least 400 μg of folic acid every day to prevent NTDs. By 1998 in the United States, folic acid was added to all cereal products labeled as rich in folic acid, such as breakfast cereals. This process is called folic acid fortification. In the United States, folic acid fortification has reduced the incidence of NTDs by 35%. Fortification of folic acid in foods is an effective way to increase folic acid intake in women. Expanding the scope of folic acid fortification in low- and middle-income countries can prevent 150,000–210,000 NTDs every year. Countries such as Chile, South Africa, Canada, and Costa Rica have reduced NTDs due to folic acid fortification. After 3 years of the largest evidence-based medicine study of nearly 250,000 cases, it has been confirmed that folic acid supplementation in northern China can reduce NTDs by 85% [28–31]. On September 5, 2020, the "China Multidisciplinary Expert Consensus on Rational Folic Acid Supplementation" was first released in Beijing, China. It has provided an evidence-based basis and reference for standardized and rational folic acid supplementation. Evidence-based research on folic acid in preventing NTDs was published in *The New England Journal of Medicine*, the *Chinese Medical Journal*, and *The Lancet* was extended to more than 50 countries worldwide. The great task of folic acid fortification worldwide and its positive impact on the

health and well-being of numerous children and families can serve as a great example or model of nutritional intervention or prevention for neurodevelopmental disorders during pregnancy.

8.2.2 *In Utero* Screening and Diagnosis of Down's Syndrome

Down's syndrome, the most common type of chromosomal disorder, is one of the common causes of mental retardation [32]. The disorder is originated from failure of separation in chromosome 21 during meiosis of germ cells due to the influence of certain detrimental factors. Among live infants, the incidence is 1/800–1/600. According to karyotype, it can be divided into three categories: standard, translocation, and mosaicism. The standard type and translocation type are not easily distinguished in terms of phenotype. The clinical manifestations of mosaicism depend on the proportion of normal cells, which can range from near normal to typical manifestations. At birth, the patient may have an obvious unusual face (wide eye distance, small eye fissures, oblique outer canthus), physical retardation, and so on. With the increase of age, manifestations of mental retardation gradually become obvious with moderate-to-low level of intelligence quotient. About half of the children have congenital heart disease, compromised immune function, and increased incidence of leukemia.

The pathogenic etiology is correlated with genetic factors (such as maternal folic acid metabolism-related gene polymorphism) and *in utero* environmental factors (such as advanced maternal age, use of chemical drugs during pregnancy, reflex irradiation, viral infection, etc.). Currently no effective treatments or therapies have been found. Therefore, prenatal screening and diagnosis are effective measures to reduce the incidence. To reduce the deficiencies of invasive prenatal diagnosis such as amniocentesis, fetal ultrasound, and detection and screening of maternal serum biochemical markers (such as alpha-fetoprotein, human chorionic gonadotropin, free estriol, etc.) are usually performed first in clinical practice. In recent years, noninvasive DNA detection technique has been widely used in clinical screening. This technique uses high-throughput sequencing technology to extract cell-free fetal DNA from maternal peripheral blood plasma to detect and analyze chromosomal abnormalities of the fetus. This technique has higher specificity and sensitivity compared to serological screening. If prenatal screening is highly indicative of chromosomal abnormalities, further prenatal diagnostic technique can be carried out. Samples can be obtained through chorion villus sampling, amniocentesis, and umbilical cord puncture, and then fetal genetic disorders can be diagnosed. The commonly used techniques include chromosomal karyotyping, fluorescent in situ hybridization, chromosomal microarray analysis, and gene sequencing technology. With the rapid development of *in utero* screening and diagnostic techniques, the birth rate of children with Down's syndrome has been effectively reduced, and the population quality has been greatly improved.

8.2.3 Management and Sequential Treatment of Fetal Cerebral Hemorrhage During Perinatal Period

8.2.3.1 Case Report

8.2.3.1.1 Medical History

Complaints: At 37 weeks + 6 days of gestation, fetal brain abnormalities were found for 8 days.

History of present illness: The childbearing history of the pregnant women was 1-0-2-1. She gave birth to a child at full term by cesarean section in August 2005. The menstruation was irregular. The menstrual blood volume was moderate without dysmenorrhea. LMP was on September 10, 2015. EDC was on June 17, 2016. The patient had menopause for 1+ month and urine pregnancy test showed β-hcg (+). The patient had mild early pregnancy reaction (nausea and vomiting) at 2 months of pregnancy. The fetal movement occurred at 4+ months of

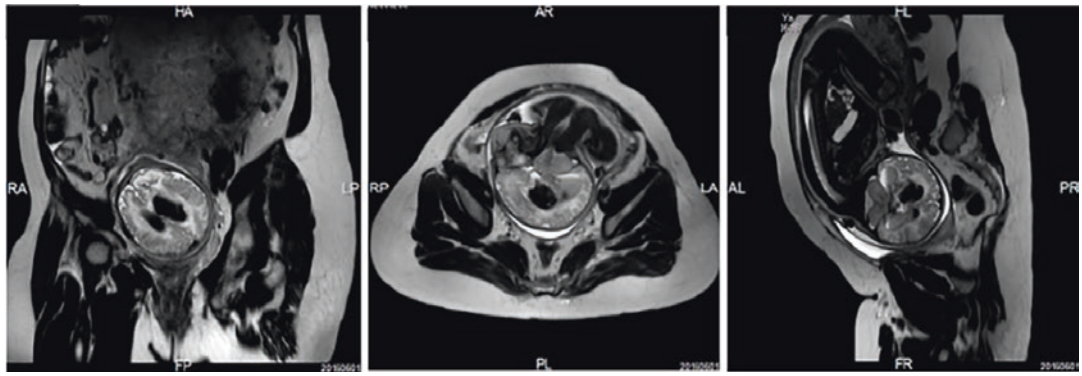

Fig. 8.4 Fetal MRI: bilateral ventricular dilatation with hematocele, abnormal signal from left frontal lobe to basal ganglia, and possible vascular disorder with hemorrhage. (Singleton, cephalic position was seen on each sequential scanning. Amniotic fluid volume and placenta were normal. The fetal craniocerebral ring was intact, the cerebral ventricles were enlarged, low-signal shadow of each sequence was present in the ventricles, liquid-liquid level was also present in some parts, and spotty T2W low-signal shadow was present in the left frontal lobe and basal ganglia)

pregnancy, with normal fetal movement. Regular prenatal examinations were performed at local hospital, with no special abnormality observed. The birth defects test and fetal chromosome examination were normal. On May 26, 2016, ultrasound at local hospital showed 19 mm of the left lateral ventricle of the fetus, 40 * 28 mm enhanced echo at the midline of the brain, and oligohydramnios (AFI 75). Later the patient visited our hospital for treatment. On May 31, 2016, the maximum depth of amniotic fluid pocket was 58 * 62 mm, and S/D of umbilical artery was 1.8 by ultrasound. On June 1, 2016, fetal MRI showed bilateral ventricular dilatation with hematocele, abnormal signal from left frontal lobe to basal ganglia, and possible vascular disorder with hemorrhage (Fig. 8.4). At 37 weeks + 6 days of gestation, she had no abdominal pain or vaginal bleeding and was admitted through the outpatient department.

Conditions of specialist examination: Prenatal examination: abdominal circumference of 109 cm, uterine height of 31 cm. Fetal position of LOA, fetal heart rate of 145 beats/min.

Preliminary diagnosis: 37 weeks + 6 days of gestation, G4P1, fetal ventricular dilatation with hemorrhage, scarred uterus (previous cesarean section), advanced maternal age.

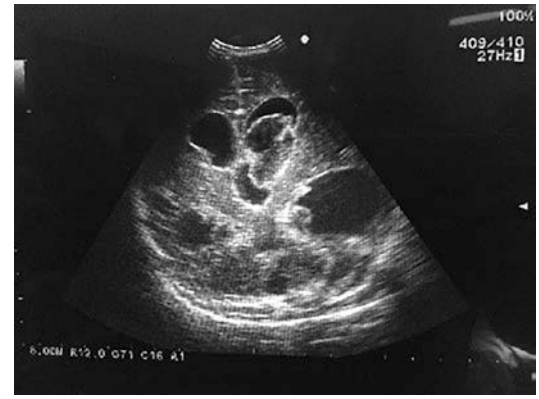

Fig. 8.5 Cranial ultrasonography was performed immediately after birth

8.2.3.1.2 Treatment

At 38 weeks + 3 days of gestation, cesarean section with transverse incision in the lower uterine segment was performed. During the operation, the top of the fetus was observed, with LOT, moderate menstrual blood volume, and clear amniotic fluid. About 800 mL of amniotic fluid was drawn, and the head was easy to hold. The newborn was delivered, with the umbilical cord wrapped around the neck for a circle. The umbilicus was cut and treated away on the table. The Apgar score was 9 points (skin -1 point) in Figs. 8.5 and 8.6.

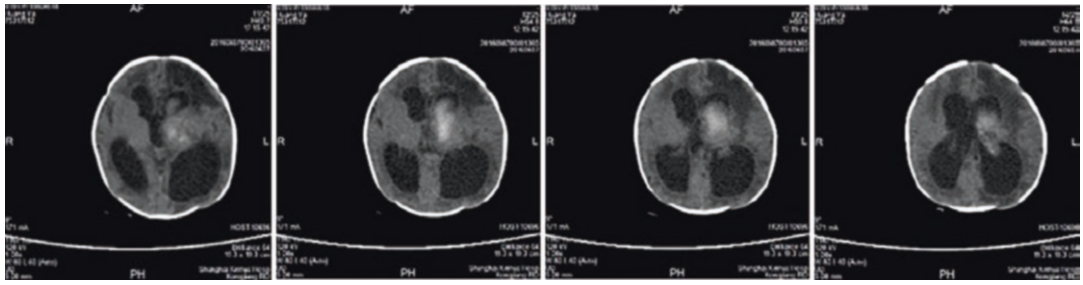

Fig. 8.6 Head CT: left thalamic hemorrhage and rupture into the ventricles, hydrocephalus, and left frontal encephalomalacia, with partial absorption of hematoma correlated with fetal MRI and aggravation of hydrocephalus. (The midline structure was in the middle; the ventricular system was significantly enlarged, especially in the left lateral ventricle. The anterior fontanel and posterior fontanel were enlarged and full. A mass-like slightly high-density shadow was present in the left thalamus, communicating with the left lateral ventricle, and a large low-density shadow was present in the left frontal lobe)

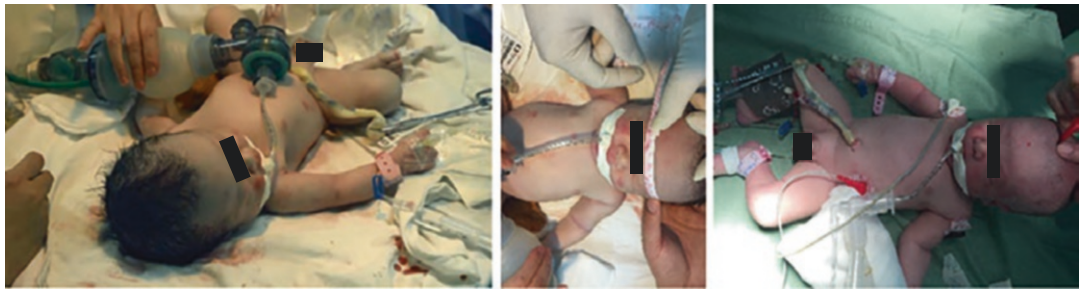

Fig. 8.7 Preoperative preparation for the child patient

The child immediately underwent "ventriculoscopic exploration + intraventricular irrigation + intracerebral hematoma evacuation + Ommaya placement in the left lateral ventricle for external ventricular drainage" under general anesthesia (Figs. 8.7 and 8.8).

Cranial MRI was repeated 1 month after surgery (Fig. 8.9).

8.2.3.2 Case Analysis

Intracranial hemorrhage (ICH) is rare in fetal period, with the detection rate of about 0.5–1/1000. Fetal ICH may occur spontaneously or be correlated with maternal or fetal abnormalities. Various causes such as coagulation dysfunction, trauma, infection, and fetal chromosomal and genetic abnormalities in pregnant women can lead to fetal ICH. Fetal ICH includes hemorrhages that occur in the ventricles, parenchyma, cerebellum, and extracerebral spaces. Among them, germinal matrix-intraventricular hemorrhage (GM-IVH) is

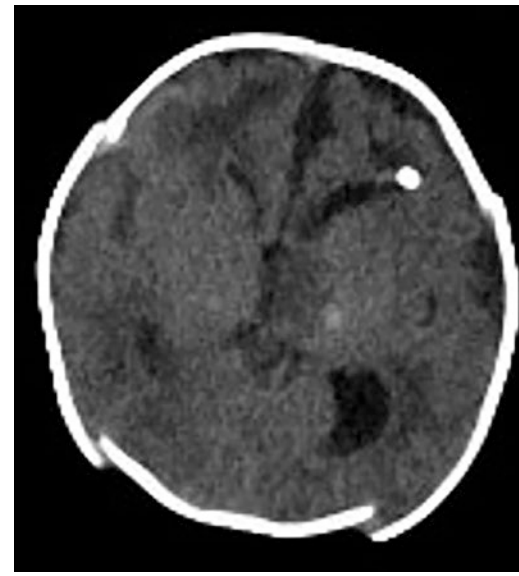

Fig. 8.8 Postoperative head CT: intraventricular hematoma was removed, and hydrocephalus was significantly improved than that before the surgery

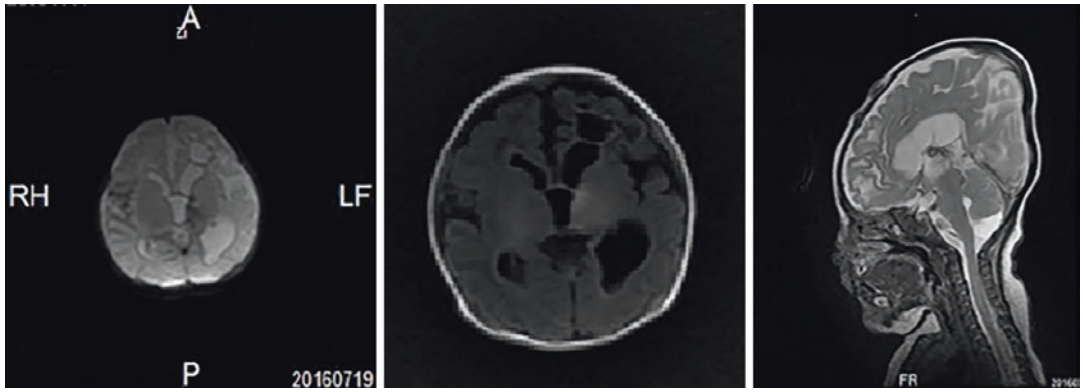

Fig. 8.9 Cranial MRI: after the surgery for "intracranial hematoma," in the absorption of hemorrhage in the left thalamus, the hematocele in the posterior horns of bilateral ventricles was basically absorbed compared to the previous image on June 14, 2016; encephalomalacia in the left frontal lobe communicated with the left lateral ventricle (secondary porencephaly), with periventricular leukomalacia (PVL) at both sides

the most common type. The matrix of subependymal germinal layer is originated from glial cell precursors. Glial cell precursors are mainly gelatinous tissue and lack surrounding supporting structures. It is a capillary network composed of peripheral blood vessels, with weak wall, with head of caudate nucleus most prone to hemorrhage. After hemorrhage, glial cell precursors are destroyed, affecting the subsequent brain development. The fetal germinal layer and subependymal vascular traffic network are formed after 20 weeks of gestation, and the germinal layer matrix is extremely sensitive to hypoxia, infection, etc. After 22 weeks of gestation, when the fetus experiences hypoxia, hypocapnia, circulatory dysfunction, and electrolyte imbalance, the germinal layer matrix is prone to vascular rupture and bleeding. It can break through the ependyma into the lateral ventricle or spread to the surrounding white matter after bleeding, forming intraventricular hemorrhage or periventricular hemorrhage. Fetal intracranial hemorrhage can affect the development of the nervous system. Long-term poor prognosis includes cerebral palsy, motor and cognitive impairment, epilepsy, and microcephaly. The location and severity of hemorrhage are also closely correlated with the occurrence and development of hydrocephalus, which can be complicated by porencephaly.

Since fetal ICH often occurs in the second and third trimesters, it is suggested that ultrasound screening or even fetal MRI is essential in the second and third trimesters. Though the previous ultrasound examination is normal, the possibility of intracranial hemorrhage should also be concerned. Ultrasound is the preferred method for diagnosing fetal ICH. Most fetal ICH is discovered by chance during prenatal ultrasonography, but ultrasound results are easily affected by factors such as cranial artifacts, obesity in pregnant women, etc. Due to the different bleeding periods, it is difficult for ultrasound to differentiate it from other intracranial diseases. The location and ultrasonographic findings of fetal ICH are various and vary over time, with fresh hemorrhage showing hyperechogenicity, partial liquefaction, and absorption 1–2 weeks after hemorrhage showing mixed echogenicity of hyperechogenicity and no echogenicity, and complete liquefaction showing no echogenicity after 1 month of hemorrhage. Fetal MRI can identify abnormalities that are difficult to detect by ultrasound, including the ability to accurately localize and grade intracranial hemorrhage and determine the period of hemorrhage, which is an important supplement to ultrasonography. A multicenter prospective cohort study found that MRI examination can improve the diagnostic accuracy of intracranial malformations in fetuses. Fetuses with suspected cranial abnormalities on ultrasonography should undergo MRI examination to assist clinical decision-

making. Although MRI is superior to ultrasound in the diagnosis of intracranial tumors, subarachnoid hemorrhage, etc., ultrasound can be repeated in real time and can be monitored for many times during pregnancy. Fetal intracranial hemorrhage can be detected early, the bleeding site can be identified and accurately graded, and changes can be dynamically followed up to guide clinical treatment in a timely manner. Therefore, accurate diagnosis of fetal ICH by ultrasound and MRI is essential for prenatal consultation.

Clinically, the treatment regimen for mild fetal ICH is conservative, and the treatment regimen for severe cases in the second trimester is mainly to terminate the pregnancy in a timely manner. Postnatal treatment of fetuses with ICH mainly includes supportive treatment and monitoring of intracranial pressure, early neurological assessment, and development of rehabilitation plans. Children with severe hydrocephalus with intraventricular hemorrhage or even total intraventricular hemorrhage require immediate surgical treatment, including ventriculosubgaleal shunt, ventriculoscopic removal of an intracranial hematoma, or external ventricular drainage, and those with large hematoma can also undergo craniotomy for intracerebral hematoma evacuation. These surgical procedures are effective in reducing intracranial pressure and the incidence of hydrocephalus.

8.3 Research Progress

8.3.1 Prenatal Risk Factors

The hypothesis of developmental origins of health and disease (DOHaD) argues that prenatal development is a particularly vulnerable period of development, during which exposure to adverse environments, such as malnutrition, infection, or stress, can have a long-term or permanent impact on the health trajectory of the offspring—a process known as "development programming." More and more studies have shown that adverse effects of early life environment are also related to long-term consequences of neurological development in future generations, including effects on increased risk of neurodevelopmental disorders. The developing brain may be particularly vulnerable during pregnancy, as the significant developmental trajectory emerges during this period. The adverse *in utero* environment can affect the neurodevelopment of the fetus through direct effects or indirectly through maternal signals and may also lead to the consequences of effects due to preterm birth.

Both maternal malnutrition and overnutrition may have an impact on the neurodevelopment of the fetus. Studies of people with severe *in utero* malnutrition during the Dutch Hunger Winter have shown that the deleterious effects of maternal malnutrition are characteristic. In early 1944, the western part of the Netherlands was occupied by Germany and endured severe food shortages. During that period, the population, including pregnant women, has an estimated daily caloric intake of 400–800 calories over a period of 5–6 months. Long-term follow-up studies of people aged 56–59 years showed that those exposed in early pregnancy performed worse on selective attention tasks, which was correlated with accelerated age-related cognitive decline [33–36]. Maternal obesity is also correlated with impaired neurodevelopment and executive function and adverse neuropsychiatric outcomes in children, including attention-deficiency hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) [37–40]. Gestational diabetes mellitus (GDM) is a disease that develops during pregnancy. Diabetes during pregnancy is associated with a range of fetal effects in various organ systems, including an increased incidence of NTDs. Some effects of maternal obesity on offspring may be mediated by associated increases in inflammation. Higher levels of maternal inflammation during pregnancy are correlated with an increased risk of neurodevelopmental delay in childhood and mediate the effects of adverse prenatal environmental factors on mental retardation in children. This is consistent with the multiple hit hypothesis commonly cited for schizophrenia but may also be correlated with other neurological disorders [41–44]. Maternal immune activation during pregnancy is correlated with an

increased risk of several mental disorders in the offspring during childhood. Researchers used the Danish National Register, a population-based national cohort of people born in Denmark from 1978 to 2015 and followed for 38 years, to study the relationship between maternal autoimmune diseases before delivery and the risk of mental disorders in the offspring. Data analysis was performed from March 01, 2020, to September 30, 2021. According to the Danish National Patient Register, mothers were diagnosed with autoimmune diseases before or during pregnancy. Their main outcome was mental disorders in the offspring, as defined by the diagnosis in hospital. The study included 2,254,234 singleton infants, of whom 2.26% of the mothers had autoimmune diseases before delivery. Participants who had been exposed to autoimmune diseases had an increased risk of overall mental disorders compared to participants who had not been exposed to autoimmune diseases. The offspring with type 1 diabetes and rheumatoid arthritis had an increased risk of overall mental disorders in different age groups. With regard to specific mental disorders, an increased risk of somatic disorders, schizophrenia, obsessive-compulsive disorders, mood disorders, and a range of neurodevelopmental disorders (e.g., childhood autism and ADHD) was observed after exposure to any maternal autoimmune disease [11].

Studies have shown that maternal viral infection during pregnancy may increase the risk of mental disorder in offspring, and the timing of infection is particularly important. For example, a large Danish study found a significant association between maternal hospitalization for influenza in the first trimester of pregnancy and the development of ASD in her offspring. Maternal exposure to measles, rubella, and polio is all correlated with an increased risk of schizophrenia in the offspring. Vertical transmission of viral pathogens to the fetus may be correlated with severe neurodevelopmental consequences. Infection with Zika virus and cytomegalovirus in the first trimester of pregnancy is associated with microcephaly. Bacterial infections during pregnancy are also associated with neural dysplasia in the offspring. The Danish study reported a modest association between bacterial infections in the second month of pregnancy and ASD in offspring. In a large-scale US study, mental disorders in adult descendants of mothers who experienced bacterial infections during pregnancy increased significantly.

In recent years, the effects of mental stress during pregnancy on the offspring have received much attention. The offspring exposed to high levels of prenatal maternal stress or anxiety are at a higher risk of depression, ASD, schizophrenia, and ADHD as well as various emotional and behavioral problems; the timing of stress and the sex of the fetus play an important role in these outcomes. There is also evidence that maternal prenatal depression and socioeconomic status may interact with polygenic scores for major depression, thereby regulating risks. In addition, good maternal mental health during pregnancy was positively correlated with cognitive performance of the offspring at 2 years of age [45–50].

There is also evidence that crisis events during pregnancy have adverse effects on neurodevelopmental outcomes. Offspring born to mothers who became pregnant during the 1998 Quebec ice storm crisis had lower cognitive and language abilities at 5.5 years of age than control infants who became pregnant during the same period. In a US cohort, investigators investigated the relationship between severe prolonged nausea and vomiting during pregnancy (extended into the second trimester, known as SNVP) and mental and cognitive problems as well as brain morphology. The subjects were 10,710 children aged 9–11 years mainly from the Adolescent Brain Cognitive Development (ABCD) study, while emotional and psychiatric findings were validated using 2,092,897 participants from the Danish National Cohort Study. SNVP was found to be significantly correlated with emotional and psychiatric problems and reduced global cognitive ability in children. SNVP is associated with low cortical area and volume, particularly in the cingulate cortex, precuneal cortex, and superomedial prefrontal cortex. These lower cortical areas and volumes play an important role in the relationship between SNVP and mental and cognitive problems in children. In the Danish national

cohort, severe nausea and vomiting during pregnancy were significantly correlated with an increased risk of behavioral and mood disorders in children. SNVP is closely correlated with mental and cognitive problems in children and is mediated by brain structures [51–57]. Dr. Fei Li's team conducted a randomized clinical trial of a high-risk *in utero* rescue intervention to evaluate the neurodevelopmental outcomes in healthy full-term infants at 545 days (18 months) who received milk fat globule membrane, bMFGM, and bovine lactoferrin in a 365-day infant formula. Of the 451 included infants (228 in the control group; 223 in the MFGM + LF group), 291 completed study feeding and the Bayley-III test at 365 days (148 in the control group; 143 in the MFGM + LF group). The MFGM + LF group had higher average cognitive (+8.7), language (+12.3), and motor (+12.6) scores; no difference was observed at Day 545. There was a significant improvement in overall development assessment from Day 120 to Day 275 and attention on Day 365. Few differences were found in neurodevelopmental outcomes on Day 545. However, scores on the Chinese Communication Development Scale suggested that the scores of infants and young children in the intervention group improved significantly on Day 545; scores were higher in the MFGM + LF group. The overall incidence of respiratory tract-related adverse events and diarrhea was significantly lower in the MFGM + LF group on Day 545. Clinical findings from RCTs support that early nutritional intervention can effectively promote the development of the nervous system. In this study we found that milk fat globule membrane can significantly improve cognitive, language, and motor scores in Bayley-III in the intervention group [58]. Thus, if the intervention is advanced to the gestation period, the fetus will receive the corresponding nutritional intervention *in utero*. During the sensitive period of nervous system development and formation, it will have a more significant effect on promoting nervous system development and improving the long-term cognition of birth outcome. Apparently, further large-scale experimental studies are required to verify this hypothesis.

8.3.2 Early Intervention

8.3.2.1 Perinatal Folic Acid Supplementation for Prevention and Treatment of Autism Spectrum Disorders

Maternal vitamin deficiency during pregnancy is not consistently correlated with cognitive function in the offspring. Maternal vitamin D deficiency may be associated with the risk of autism spectrum disorder (ASD) and intellectual disability (ID) in the offspring. Furthermore, maternal vitamin deficiency is also associated with an increase in NTDs, the incidence of which can be reduced by folic acid (FA) supplementation, as described above. Therefore, folic acid and multivitamin supplementation is commonly recommended for pregnant women. Epidemiological studies have reported inconsistent association between maternal multivitamin or folic acid supplementation before and during pregnancy and the risk of ASD in the offspring. A recent high-quality study included children born after 2003 to determine maternal vitamin use during 270 days before delivery. Of the 45,300 children studied (22,090 girls and 23,210 boys; mean age at the end of follow-up was 10.0 years), 572 (1.3%) were diagnosed with ASD. Maternal use of folic acid and/or multivitamin supplements before pregnancy was correlated with a statistically significant lower likelihood of ASD in the offspring compared to no use (RR, 0.39; 95% CI, 0.30–0.50). Maternal use of folic acid and/or multivitamin supplements during pregnancy was correlated with a statistically significant lower likelihood of ASD in the offspring compared to no use (RR, 0.27; 95% CI, 0.22–0.33). The corresponding RRs were estimated as use of folic acid before pregnancy (RR, 0.56; 95% CI, 0.42–0.74), use of folic acid during pregnancy (RR, 0.32; 95% CI, 0.26–0.41), use of multivitamin supplements before pregnancy (RR, 0.36; 95% CI, 0.24–0.52), and use of multivitamin supplements during pregnancy (RR, 0.35; 95% CI, 0.28–0.44). This study of 45,300 children showed that children born to mothers who took FA and/or multivitamin supplements before and/or during

pregnancy had a lower risk of ASD compared to no early intervention. Sensitivity analyses examined the risks in different intervals, controlled for different confounding factors, and examined the assumptions of the analyses on which the statistical analyses were based, without weakening the observed risk reduction overall. The association between the use of multivitamin supplement and ASD risk was similar in the offspring of males and females. However, due to the small sample size, the power of the analysis for the offspring of females was low. Folic acid deficiency before pregnancy is therefore correlated with adverse childhood outcomes and ASD characteristics. In the study, after adjusting for maternal vitamin deficiency, the reduction in the risk of ASD in the offspring remained after maternal FA and multivitamin supplementation. Future studies on the underlying biological mechanisms can help us to understand the potential regulatory mechanisms in the possible causes of ASD with folic acid and multivitamin supplementation. The results of the present study are consistent with those of the Norwegian Birth Cohort study, showing that maternal FA use during the first 4 weeks of pregnancy and 8 weeks after pregnancy is correlated with a reduced risk of ASD in the offspring. This period is sensitive to the development of the central nervous system, including the closure of the neural tube, and is correlated with the development of basic brain structures. In this study, the researchers also point out that maternal use of FA and multivitamin supplements in the first 2 years of pregnancy was correlated with a reduced risk of ASD in the offspring. Maternal use of FA and multivitamins before and during pregnancy is similar in reducing the risk of ASD. Thus, folic acid and multivitamin supplementation during pregnancy can effectively reduce the incidence of ASD [33, 59].

In summary, the intervention during pregnancy and even before pregnancy is the window period of *in utero* pediatrics. The treatment of *in utero* pediatrics can not only promote the development of the nervous system but also effectively prevent neurodevelopmental disorders, yielding twice the result with half the effort.

8.3.2.2 *In Utero* Fetal Surgery

In utero surgical procedure of fetal neurological diseases is currently mainly focused on fetal encephalocele/meningocele and fetal hydrocephalus.

8.3.2.2.1 Encephalocele/ Meningomyelocele

Fetal surgeries to repair various malformations are originated in animal models of dogs in the 1930s. Until 1980, Harrison et al. discovered standard animal models suitable for fetal surgery. Based on the study of standard animal models, fetal surgery has made great progress, which can be not only applied to fetal surgery for congenital diaphragmatic hernia, sacrococcygeal teratoma, and pulmonary congenital cystadenoma malformation, but it is also technically feasible for fetal surgical repair of spina bifida. The theory of fetal intervention for meningomyelocele is based on the "two-hit" hypothesis. The first is the primitive embryo neural tube dysplasia, resulting in spinal cord exposure to amniotic fluid during pregnancy, then the secondary damage to nervous tissues. Through animal experiments, it has been confirmed that the fetus with meningomyelocele is affected by amniotic fluid, direct trauma, or fluid pressure due to the direct exposure of spinal cord tissue to the *in utero* environment, or these three causes interact with each other, resulting in both chemical and mechanical damage to the *in utero* spinal cord, thus affecting fetal neurological function. Fetuses with meningomyelocele had normal hindlimb movement on early prenatal ultrasonography, but lower extremity dysfunction was found after birth, indicating that secondary injury to the spinal cord *in utero* caused postnatal dysfunction, and exposed neural placodes were seen in autopsies of stillbirths and aborted fetuses. Children with other NTDs, with thin film or skin covering the lesion, have better neurodevelopment after birth compared to children with meningomyelocele, as evidenced by the damage to the exposed nerves by the *in utero* environment. Walsh et al. proposed the theory of "hindbrain recovery." Compared to the fetal skull in the second trimester, the degree of myelination of the neonatal skull

is more than a dozen times higher, so the repair in the second trimester can improve the cerebrospinal fluid circulation dynamics at an early stage, and the degree of myelination is low, and the skull plasticity is high. After that, the central nervous system of the fetus has good conditions for structural function recovery, and the technical feasibility is favorable.

Based on the "two-hit" hypothesis, fetal surgical techniques are applied to children with MMC for early intervention. The exposed spinal cord is re-encapsulated into the spinal canal during *in utero* surgery to reduce secondary neural tissue injury due to spinal cord exposure to the intrauterine environment, so as to achieve the purpose of improving neurological function and restoring normal neurological development as much as possible. The "two-hit" theory provides a theoretical basis for surgical intervention of MMC in fetal period to improve prognosis.

Based on the "two-hit" hypothesis, surgical intervention should be performed as early as possible to reduce the long-term exposure of spinal cord tissues to *in utero* damage. Therefore, the timing of prenatal diagnosis determines the choice of surgery time. Alpha-fetoprotein (AFP) is the first serum marker used to screen for fetal NTDs. Seventy-five percent to 80% of MMC fetuses are at 16 weeks of gestation. It is found that the screening with AFP in the first trimester by maternal serology is low in sensitivity. Some research statistics have found that fetuses with open spina bifida did not experience elevated AFP at 8–13 weeks of gestation. Once elevated maternal serum AFP is found, amniocentesis is further performed to examine AFP and cholinesterase in amniotic fluid to confirm the diagnosis. Ultrasonography can accurately detect open spina bifida in the skull and spine of the fetus. Ultrasonography has been reported to detect fetal spina bifida as early as 12 weeks of gestation, but most of them are diagnosed in the second trimester. Fetal ventricular enlargement, lemon head, and banana cerebellum are all signs suggestive of open spina bifida, while longitudinal examination of the fetal spine can depict abnormal widening between the pedicles or kyphoscoliosis. Continuous lateral examination of each vertebral segment can see the complete neural arch surrounding the spinal canal, which is necessary to rule out open spina bifida. Combined with the increase of maternal serum AFP and ultrasound results, MMC is mainly diagnosed in the second trimester of pregnancy between 18 and 22 weeks. For surgical intervention, more sensitive detection methods may be needed in the future if early diagnosis is to be made.

Most fetuses can be born alive and healthy without any intervention for MMC during the fetal period, but the disability rate correlated with it due to neurological dysfunction is very serious. Thirty percent of children die of complications from the respiratory system, urinary system, and central nervous system before they reach adulthood. In the past, the treatment of MMC tended to surgically repair spinal defects after birth and perform active and massive rehabilitation; in recent years, through numerous clinical trials and observations in animal models, fetal surgical intervention can save the neurological function of some affected children, and 19–25 weeks of gestation are the ideal time. The first case of prenatal MMC hysterotomy and repair was performed in the United States in 1997. In the beginning, it was only to repair the neural tube to avoid internal damage to the spinal cord, but unexpectedly, in addition to reducing spinal cord damage, it also reversed the occurrence of hernias in the back of the brain, the enlargement of the ventricles, and the formation of hydrocephalus, reducing the VP shunt rate by about 80–90%. The same experimental results were confirmed successively.

The National Institutes of Health initiated a multicenter, prospective randomized clinical trial. The first case of open surgery for *in utero* repair of fetal MMC began in 1997, and the NIH recruited a total of 183 cases from 2003 to 2010 and randomized them into 2 groups for open surgery for *in utero* repair and postnatal surgery for repair, respectively. At the same time, a MOMS system was established to the safety and effectiveness of the surgery. This article focuses on introducing surgical procedures and postoperative management related to *in utero* open surgery for MMC and the comparison with traditional postnatal surgical repair. The specific case inclusion criteria

were (1) singleton pregnancy; (2) the boundary of meningomyelocele located between T1 and Sl; (3) MRI evidence of hindbrain hernia formation; (4) gestational age between 19.0 and 25.9 weeks; fetus with normal karyotype; (6) US citizens; and (7) maternal age of at least 18 years [60, 61].

In the MOMS study, the participating teams during surgery included neurosurgeons, pediatricians, obstetricians, cardiologists, maternal-fetal pharmacists, anesthesiologists, and nursing staff to communicate with each other before and during surgery to ensure that the umbilical cord was safe without torsion or shedding during surgery and the fetus was warm, sufficient in blood supply, and no bradycardia and no circulatory failure during surgery. The temperature in the operating room was maintained at 26 °C. A fetal temperature detector was placed *in utero* during surgery, and glucocorticoids were given to the mother after 23 weeks of pregnancy to promote fetal lung maturation.

Anesthesia for *in utero* repair surgery was performed by intramuscular injection of a mixture of anesthetics and muscle relaxants. Transabdominal hysterotomy was performed, and the incision was selected in combination with ultrasound exploration of the location of the placenta. After laparotomy, the site of fetal MMC lesion was exposed to the uterine incision. Intraoperative ultrasound monitoring was applied to ensure the normal fetal heart rate, and intrauterine surgery was similar to the repair process of meningomyelocele after birth. First, the thin cyst wall around the lesion with no nerve fiber adhesion was cut. If the nerve fibers adhering to the cyst wall needed to be carefully dissected, the spider membrane surrounding the nerve substrate was entered, and the residual epithelial tissue was removed from the neural placodes. Studies have shown that residual epithelial tissue may increase the formation of epidermoid inclusion cyst. Except connective tissue fibers in the capsule can be cut off, all cords need to be detected whether they are bulging nerve tissues by acupuncture anesthesia instrument, which are preserved. The stripped nerve tissues are sent back to the spinal canal and covered with biofilm, which may play a role in barrier and avoiding spinal cord tethering. However, it is not confirmed at present that the dura mater is covered and sutured tightly. In most cases, the dura mater layer is insufficient and cannot be sutured and covered with dura mater repair material. The principle of surgery is to prevent cerebrospinal fluid leakage and not damage the nerve. At the end of surgery, it is necessary to place the umbilical cord and stop bleeding completely. During surgery, a catheter is placed in the uterine cavity and continuously injected with warm sodium lactate Ringer's solution, which can maintain the surface temperature of the fetus, avoid compression of the umbilical cord, and prevent dry skin. When the uterus is closed, warm sodium lactate Ringer's solution is injected into the uterine cavity until the depth of amniotic fluid pocket is in the normal range (determined by intraoperative ultrasound). At the same time, nafcillin and vancomycin are injected into the uterus. The uterus is closed using a uterine stapler, with the nail made of absorbable material. The traditional stapler is confirmed in animal experiments to cause infertility and make the embryo unable to implant [25, 26, 62].

From 2003 to 2010, 183 cases of MOMS were randomized into 2 groups. They were compared to the clinical trial of *in utero* repair and postpartum surgery in children with MMC. The results showed that the mortality rate at 1 year after birth in the prenatal surgery group was significantly lower, and the ventriculoperitoneal shunt rate was also lower compared to the postpartum surgery group (40% vs. 82%); the results of the Bayley assessment and independent walking ability assessment at 30 months after birth were significantly better in the prenatal surgery group compared to the postpartum surgery group (42% vs. 21%); during the first 12 months after birth, there was no hindbrain hernia in the surgery group (36%) and only 4% in the postpartum surgery group. Other studies have shown that the prenatal *in utero* surgery group made great progress in improving the size of the posterior cranial fossa and brain stem function, and that there was no obvious improvement in bladder function. Although the prognosis of the prenatal surgery group was satisfactory, there were more disadvantages in terms of surgical complications in the prenatal surgery group compared to the postpar-

tum surgery group: (1) The incidence of spontaneous rupture of membranes was high, oligohydramnios was likely to occur, and the incidence of preterm delivery was also higher compared to the postpartum surgery group. (2) In the prenatal surgery group, the average number of gestational weeks was 34.1 weeks, and 13% of fetuses were born earlier than 30 weeks; in the postnatal surgery group, the number of gestational weeks was 37.3 weeks, and no fetuses were born earlier than 30 weeks. (3) One-fifth of fetuses in the prenatal surgery group developed respiratory distress syndrome, which may be associated with premature delivery, so it was necessary to timely promote fetal lung maturation. In one fourth parturients, partially or completely dehiscent of the uterus occurred during delivery, since the muscle layer was very weak at the prenatal surgical scar. However, no maternal death occurred in both groups. The study results also showed that fetal surgery was easy to cause uterine dehiscence and uterine scar formation, which may affect the reproductive function in the future.

8.3.2.2.2 Hydrocephalus

"Hydrocephalus is a disorder of the cerebrospinal fluid circulation due to various reasons [63]. Prenatal ultrasonography can detect the early signs of fetal hydrocephalus—widened lateral ventricles as early as 18–20 weeks of gestation. The width of the lateral ventricles (6–8 mm) is generally relatively stable in the second and third trimesters. It is generally considered that the lateral ventricle dilates when the width of the lateral ventricle is ≥10 mm. Lateral ventricles >15 mm in width are severe and are often referred to as "hydrocephalus." Simple hydrocephalus without any other pathological finding is named isolated hydrocephalus.

The most common *in utero* surgery is fetal ventriculo-amniotic shunt (VAS). In addition, there are also fetal ventriculostomy and ventriculocentesis, but they are gradually being phased out due to poor surgical results. Therefore, it has set off a wave of scholars from all over the world to study the surgical treatment of *in utero* hydrocephalus. Due to the wide range of early surgical indications and poor diagnostic techniques, once severe fetal hydrocephalus is found and there is a tendency of progressive development, *in utero* surgery is performed when medical conditions permit. There are widely accepted standards domestically and abroad on the criteria for fetal *in utero* surgery, that is, the requirements for carrying out fetal surgery proposed by the International Fetal Medicine and Surgery Society: (1) accurate diagnosis and staging of fetal diseases and prenatal completion of fetal karyotyping and molecular diagnosis of genes, except for malformations, to clarify the natural outcome and prognosis of fetal diseases; there is no effective postnatal treatment; (2) the effectiveness of fetal surgery has been confirmed, that is, it can reverse the adverse effects of fetal diseases; (3) surgery should be performed in the fetal medical center with a multidisciplinary team, after ethical discussion, and fully informing the pregnant women and their families of the advantages and disadvantages of intrauterine treatment and the short- and long-term risks to the mother and fetus. The basic ethical principle for fetal surgery is that sufficient evidence is available to prove the surgical procedure is beneficial to the fetus, and the risks to the pregnant woman and fetus are acceptable.

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The Digestive System

9

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9.1 Fetal Upper Gastrointestinal Obstructions

9.1.1 Overview

Upper gastrointestinal obstruction refers to a series of diseases that occur in the digestive tract above Treitz's ligament due to various causes, including the esophagus, stomach, and duodenum. During fetal development, when the duodenum is obstructed due to structural malformations and functional abnormalities caused by pathological reasons, the amniotic fluid swallowed by the fetus cannot smoothly enter the duodenum and distal intestine, causing amniotic fluid to accumulate locally and resulting intestinal dilatation. The incidence of duodenal obstruction is about 1/2500–1/10,000 of live births. It is one of the most common causes of congenital gastrointestinal malformations in neonates, accounting for about half of the incidence of neonatal intestinal obstruction. The typical imaging manifestation during the fetal period is "double-bubble sign," that is, the dilated duodenum is connected to the gastric vacuole to form a "double-bubble" appearance. The common causes include duodenal atresia/stenosis, annular pancreas, and intestinal malrotation, all requiring postnatal surgical treatment. Prenatal diagnosis is helpful to assess the severity of the disease and provides more accurate prenatal counseling for the subsequent treatment options [1, 2].

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9.1.2 Typical Cases

9.1.2.1 Case Presentation

This is a 27-year-old pregnant woman at 31 weeks of gestation. Prenatal ultrasonography showed a cystic structure in the central abdominal cavity above the bladder, increased amniotic fluid, and possible obstruction of the duodenum or upper jejunum (Fig. 9.1). Amniotic fluid microarray showed no abnormality. She was referred to the Maternal and Fetal Medicine Center. Fetal MRI showed dilatation of the stomach and duodenum throughout (Fig. 9.2). After multidisciplinary consultation with obstetrics, pediatric surgery, neonatology, and medical imaging, she was diagnosed with possible fetal gastrointestinal malformation (distal duodenal obstruction). Multiple ultrasound examinations thereafter revealed duodenal dilatation with increased amniotic fluid. At 39 weeks + 1 day of pregnancy, the fetus was delivered by cesarean

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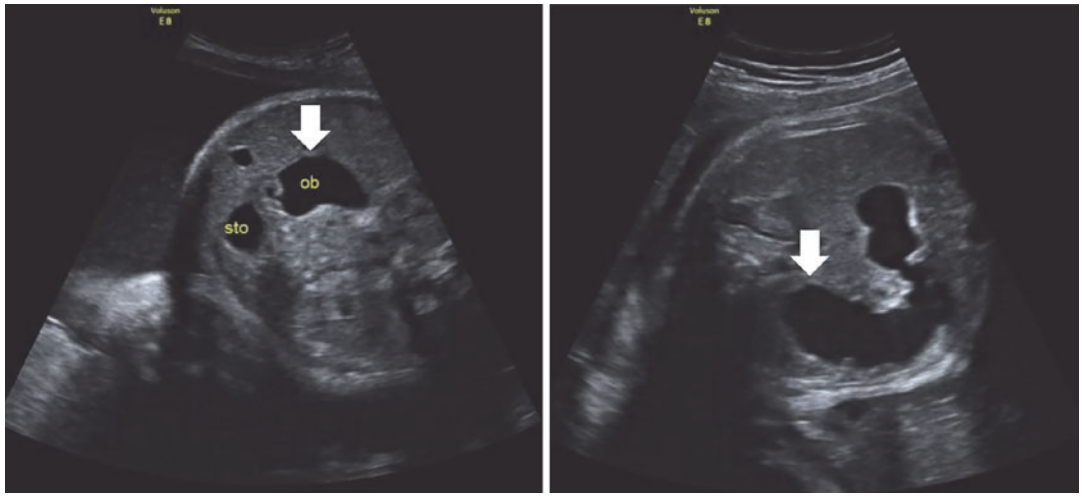

Fig. 9.1 Prenatal ultrasound image: duodenal dilatation (as indicated by the arrow)

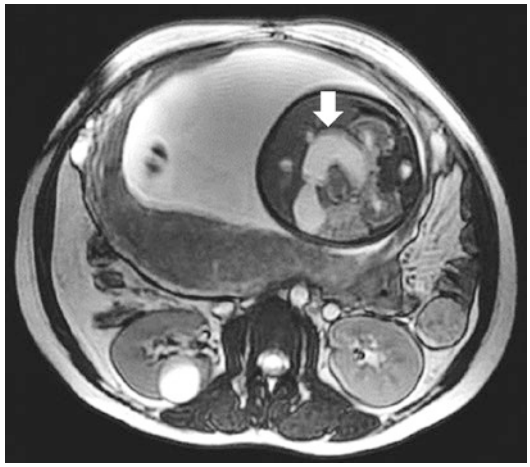

Fig. 9.2 Fetal MRI image: duodenal dilatation (as indicated by the arrow)

section due to premature rupture of the fetal membrane, with the birth weight of 3580 g and the Apgar score of 8′–9′–9′.

After birth, the baby was transferred to NICU for further examination. Abdominal radiography and contrast-enhanced ultrasonography of the upper gastrointestinal tract revealed duodenal obstruction, and the remaining tests were remarkable. Laparotomy was performed 2 days after birth. During the surgery, the duodenum was significantly dilated, the distal bowel was collapsed, and longitudinal opening of the duodenum showed typical duodenal web with central perforation at the junction of the dilated stricture. The septum was removed, and the intestinal canal was sutured horizontally. It was also found that the bowel was twisted 180° clockwise, which was diagnosed as intestinal malrotation. Bowel restoration and Ladd's procedure were performed to correct intestinal malrotation, and a jejunal feeding tube was indwelled. The postoperative diagnosis was duodenal atresia type I and intestinal malrotation. On the second day after surgery, sugar water was injected through the jejunal feeding tube. The baby was gradually transitioned to baby milk and fed orally. Three weeks after surgery, the baby was discharged. After 3 years of follow-up, the child had regular food intake, no vomiting, regular bowel movements, and normal growth and development.

9.1.2.2 Prenatal Diagnosis

The diagnosis of duodenal obstruction during the fetal period depends entirely on methods such as ultrasonography or MRI, with the prenatal detection rate about 52–59%. Increased amniotic fluid and duodenal dilatation are the main manifestations of prenatal imaging abnormalities for duodenal obstruction. Studies have reported that increased amniotic fluid occurred in about 30–40% of cases. The onset and severity are directly related to the severity of duodenal obstruction and also associated with other con-

genital malformations affecting amniotic fluid absorption. The occurrence and development of "double-bubble sign" are related to fetal swallowing, development, and maturation of gastric circular muscles and gastric peristalsis function. It is difficult to diagnose fetal duodenal structural malformations by ultrasound before 20 weeks of gestation. Generally, typical abdominal "double-bubble sign" does not appear until the second and third trimesters. We have observed 41 cases with "double-bubble sign" during the fetal period. The accuracy rate of diagnosis of duodenal obstruction after birth was 97.6% (40/41), of which 21 cases (52.5%) were annular pancreas (1 case concurrent with intestinal malrotation), 16 cases (40%) with duodenal atresia (2 cases concurrent with intestinal malrotation), and 3 cases (7.5%) with initial jejunal atresia [3–9].

In this case, prenatal ultrasonography revealed duodenal dilatation with increased amniotic fluid. Fetal MRI confirmed dilatation of the stomach and throughout duodenum, suggesting that the obstruction site was located at the distal end of the duodenum or the beginning of the jejunum. Further examination and evaluation were performed for surgery after birth. During the surgery, it was found that the child had duodenal septum atresia and intestinal malrotation. Both diseases may cause duodenal obstruction manifestations during the fetal period, but it is still difficult to identify the specific cause of duodenal obstruction by relying prenatal examinations alone, which should be comprehensively judged in combination with other postnatal examination results.

9.1.2.3 Concurrent Malformations

Studies have pointed out that about 38–69% of duodenal obstruction is concurrent with other malformations, of which congenital heart malformation is the most common, accounting for about 31–48%; about 37% of cases with chromosomal or genetic abnormalities, of which trisomy 21 is the most common, with an incidence of about 32–46%. Duodenal atresia occurs in about 3–5% cases of trisomy 21, and the rest also include ZIC3 mutation and 4q22.3 microdeletion. In addition, duodenal obstruction with congenital esophageal atresia, anorectal malformation, renal malformation, and spinal limb malformation have also been reported. Some cases had multiple concurrent malformations, such as VACTERL syndrome. When duodenal obstruction occurs, there may be multiple structural malformations of the bowel. For example, duodenal atresia or annular pancreas and intestinal malrotation may occur at the same time. Children should be evaluated before and after birth for these concurrent malformations with a very high incidence, especially chromosomal abnormalities, and should be taken seriously [10–16].

9.1.2.4 Surgical Treatment

Once duodenal obstruction is clearly diagnosed, it is necessary to perform surgery within the time limit after birth. The surgical methods can be individualized based on the specific conditions of children to select open or laparoscopic surgery. Attention should also be paid to concurrent digestive tract malformations and surgical treatment for correction. Duodenal atresia and intestinal malrotation in this case are good illustration of intraoperative correction for both structural deformities. In recent years, reports of laparoscopic surgery for duodenal obstruction have also been increasing year by year. Intraoperative placement of a transanastomotic feeding tube through the anastomosis can facilitate early postoperative nutritional support, reflecting the concept of Enhanced Recovery After Surgery (ERAS), promoting the recovery of gastrointestinal function, reducing the occurrence of cholestasis and other related complications [12, 17–19].

9.1.2.5 Prognosis

The overall prognosis of duodenal obstruction is good, with a cure rate of about 86.5–97%, and the cause of death is associated with factors such as concurrent malformations, septicemia, premature birth, and low body weight. Studies have reported that cases diagnosed in the early antenatal period have a higher mortality rate than those diagnosed after birth (34% vs. 0%), and the difference is directly related to the difference in concurrent malformations between the two groups. Some studies have also pointed out that concurrent

Down's syndrome or other serious malformations (especially complex congenital heart disease, etc.) has a higher risk of late death [10, 17, 18].

9.1.3 Research Progress

With the continuous improvement of prenatal diagnosis technology and the progress of auxiliary instruments and detection means, prenatal evaluation tends to be perfect. Not only has the prenatal diagnosis rate been improved, but scholars are also exploring methods such as ultrasound and MRI examinations for more accurate diagnosis and evaluation of duodenal obstruction before delivery. Due to the high incidence of duodenal obstruction with chromosomal abnormalities, most of which are trisomy 21 syndrome, on the basis of routine amniotic fluid and umbilical cord blood tests, noninvasive testing methods are used to detect such aneuploid chromosomal abnormalities. Multicenter, large-sample, high-level prospective studies should be conducted to develop a more optimal management strategies before and after delivery for clinical use [18, 20–26].

9.2 Esophageal Atresia

9.2.1 Overview

Congenital esophageal atresia (EA) is a severe congenital malformation of the digestive tract characterized by interruption of esophageal continuity, with or without esophagotracheal fistula. The incidence is about 1.97–2.43/10,000, ranking among the top three in the incidence of gastrointestinal malformations. The occurrence of the disease is mainly related to impaired primitive esophageal penetration at week 4 to week 6 of embryonic stage, as well as esophageal and tracheal septation insufficiency. The specific etiology is unclear and may be associated with multiple factors such as environmental teratogenicity, drugs, inflammation, angiodysplasia, and genetic inheritance. More than 50% of children with EA have multiple malformations, including congenital heart disease, urinary malformations, and chromosomal abnormalities. It is commonly expressed as "VACTERL," which also increases the complexity of EA treatment. Early diagnosis and accurate assessment of the disease are very important to improve survival and reduce postoperative complications [27–32].

9.2.2 Typical Cases

9.2.2.1 Case Reports

A 30-year-old pregnant woman was found to have a fetal ventricular septal defect during prenatal examination at 23 weeks of pregnancy. The aortic arch isthmus was slightly smaller, and fetal development was consistent with the weeks of gestation, indicating fetal cardiac malformations. Amniotic fluid microarray showed no abnormality. At 27 weeks of gestation, prenatal examination showed fetal ventricular septal defect, aortic coarctation, and polyhydramnios (amniotic fluid index 322). Fetal MRI revealed sac-like dilatation of the cervical esophagus, unclear gastric bubbles, polyhydramnios, and aortic coarctation (Fig. 9.3). After multidisciplinary consultation such as obstetrics, pediatric surgery, medical imaging, and neonatology, the diagnosis of fetal congenital esophageal atresia with congenital heart disease was proposed. Thereafter, she underwent prenatal examination, and the fetus was delivered by cesarean section at 39 weeks of pregnancy due to obstetric factors, with the birth weight of 2470 g and the Apgar score of 9′–10′–10′.

After birth, the baby was transferred to NICU. Esophagography and chest and abdominal X-ray showed that the proximal esophagus was blind, and gas shadows were observed in the gastrointestinal tract. The baby was diagnosed as esophageal atresia type III. Echocardiography and cardiac contrast-enhanced CT (Fig. 9.4) revealed aortic coarctation (2.5 mm), patent ductus arteriosus (3.2 mm), atrial septal defect (5.6 mm, 5.3 mm), ventricular septal defect (membranous part, 6.9 mm, 5.7 mm), and pulmonary arterial hypertension. After the multidisciplinary consultation with pediatric sur-

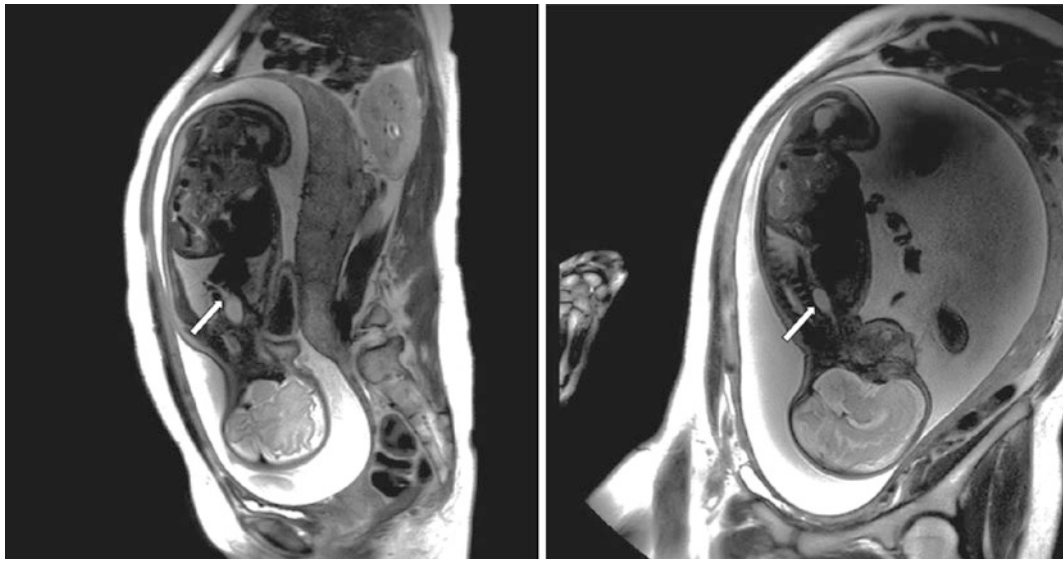

Fig. 9.3 Fetal MRI. Left panel: marked narrowing of the aortic arch isthmus; right panel: cystic dilatation of the esophagus (as indicated by the arrow)

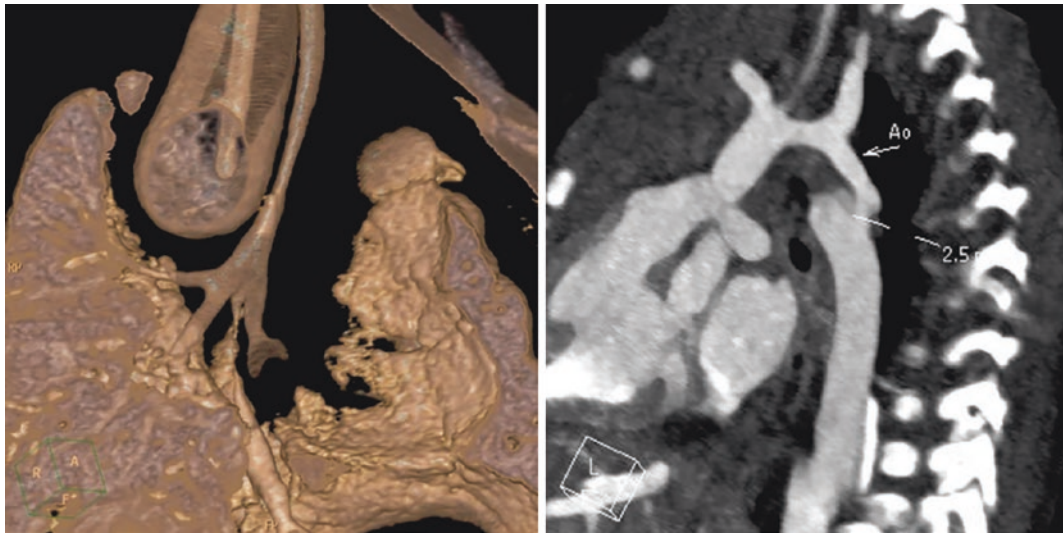

Fig. 9.4 Reconstructed image of chest enhanced CT. Left panel: the proximal esophagus is blind end and stomach tube shadows are observed inside, and the distal esophagus is connected with the trachea. Right panel: aortic arch stenosis

gery, pediatric cardiology and surgery, anesthesiology, and pediatric intensive care medicine again, it was decided to correct esophageal malformations by surgery first, followed by cardiac malformation correction surgery after the situation was stable. Six days after birth, thoracoscopic esophagotracheal fistula ligation and esophageal end-to-end anastomosis were performed under general anesthesia. The surgery was uneventful. One month after birth, correction for coarctation of the aorta, repair for atrial septal defect, and ligation of ductus arteriosus were performed. The child was followed up for 1.5 years. Esophagography showed no obvious stenosis. The oral feeding was smooth, and the dietary spectrum and nutritional status were the same as

those of children of the same age. Repeated echocardiography showed normal cardiac function and good correction of structural malformations.

9.2.2.2 Prenatal Diagnosis

Some children with EA can be diagnosed during the fetal period. Some signs of prenatal ultrasonography, such as proximal esophageal blind pouch sign, small gastric bubble or no gastric bubble, and increased amniotic fluid, suggest the possibility of esophageal atresia. The typical manifestations of fetal EA may be detected by ultrasonography before 20 weeks of gestation. Nevertheless, the diagnosis of prenatal EA is still very challenging. Research has indicated that prenatal ultrasound showed small or no gastric bubbles and increased amniotic fluid. The diagnosis rate is only about 40–56%. Another study revealed that ultrasound had a sensitivity of 31.7% and a specificity of 99% in the diagnosis of EA. For patients with abnormal prenatal ultrasound, MRI can be combined, which can help to improve the diagnostic rate. A clear diagnosis before delivery can help parents choose a medical center that has the conditions to treat EA, and avoid a series of problems during the transfer of the sick child after birth. Based on the fact that EA may be concurrent with chromosomal and genetic abnormalities, once esophageal atresia is suspected prenatally, amniotic fluid or umbilical cord blood puncture tests are of positive significance for genetic evaluation [33–40].

In this case, signs of polyhydramnios and no gastric bubbles were during prenatal ultrasound, and fetal cardiac malformations were also found. Supplementary fetal MRI is performed after ultrasound reveals abnormalities. Complete imaging data are helpful for medical imaging, obstetrics, and pediatric surgeons to comprehensively assess the condition and plan prenatal management options. Follow-up ultrasound continuous dynamic examination also helps to observe the condition of the fetus and grasp the conditions in a timely manner.

9.2.2.3 Treatments

Congenital esophageal atresia requires surgical treatment. Further evaluation before surgery includes (1) to determine the specific typing and judge the distance between the proximal esophagus and distal esophagus and (2) to understand the concurrent malformations. EA is divided into five types, with different treatment strategies for different types. For type I and type II EA, proximal esophagus and distal esophagus are often far away. One-stage esophageal anastomosis is impossible for most of them. Gastrostomy is required first, followed by radical surgery after the distance between the proximal esophagus and distal esophagus is close. Esophagography can clearly show the location of the proximal esophagus and whether there is an esophagotracheal fistula. For type I and type II EA, because the proximal esophagus and distal esophagus are blind ends, the location of the distal esophagus cannot be identified during prenatal or postnatal examination and can only be detected during surgery. In recent years, bronchoscopy before or during surgery has received more and more attention. Bronchoscopy can show the location and number of esophagotracheal fistulas and can detect other tracheal malformations such as tracheomalacia and tracheal stenosis, which is of great value in planning surgical methods. In addition, if there are other concurrent malformations such as severe congenital heart disease and digestive tract malformations that may have a serious impact on the short-term survival of children, the treatment plan for concurrent malformations should be considered comprehensively. The concurrent malformations can be treated at the same time as EA surgery if necessary [24, 41].

In this case, the child with EA was concurrent with aortic constriction, atrial septal defect, ventricular septal defect, and other severe cardiac malformations. It was assessed that the cardiac malformation might have an impact on the survival of the child. Therefore, after EA was corrected and stabilized, the cardiac malformation was treated within a limited period of time, and the child recovered well. The treatment process of this child was well-planned, reflecting the advantages of early diagnosis and multidisciplinary teamwork for such complex cases [42].

9.2.2.4 Prognosis

The overall cure rate of congenital esophageal atresia is 85–98%. At present, it is believed that the main factors affecting the prognosis of children with EA are birth weight less than 1500 g and concurrent severe congenital heart malformations. More recent studies have pointed out that chromosomal abnormalities have a greater impact on the survival time and long-term quality of life of children with EA. Severe postoperative complications such as severe gastroesophageal reflux and esophageal stenosis also adversely affect long-term quality of life. A series of expert consensus in Europe and the United States recommends that follow-up should be performed as planned until adulthood [32, 43–46]. The cases in this chapter were regularly followed up as planned. No serious postoperative complications were found, and there were no significant abnormalities in eating and nutritional status.

9.2.3 Research Progress

There has been great progress in the comprehensive diagnosis and treatment of congenital esophageal atresia, but there are still many unknowns about the disease, such as pathogenesis, underlying gene defects, and signaling pathways. Research in these areas will contribute to a deeper understanding of the disease.

Relevant clinical studies focus on the following aspects, including prenatal diagnosis and evaluation, surgical treatment, management of complications, and improvement of the quality of life. With the advancement of science and technology, more new detection and treatment methods have been created. The biochemical study of prenatal amniotic fluid hopes to further enhance and improve the prenatal diagnosis rate and disease prognosis assessment of EA. The implementation of advanced ultrasound technology, such as dynamic esophageal patency assessment (DEPA) and improvement of instruments and equipment, is helpful to improve the diagnostic rate of ultrasound, but large-scale prospective studies are still required to confirm its effectiveness. In the comprehensive management of EA, the multidisciplinary diagnosis and treatment model play an important role in prenatal and postnatal management as well as long-term follow-up [27, 47–51].

9.3 Congenital Small Intestinal Atresia

9.3.1 Overview

Small intestinal atresia is a common congenital structural malformation of the digestive tract in pediatric surgery and a common cause of neonatal intestinal obstruction [52]. The incidence is about 1 in 5000, with no significant gender difference. There are many theories about the causes of small intestinal atresia [53]. Surgery is the only radical treatment for small intestinal atresia. In recent years, with the improvement in the knowledge with the manifestations of the disease in the fetal period, the prenatal diagnosis rate of small intestinal atresia has been further improved. At the same time, the cure rate of intestinal atresia is more than 90% due to the development of neonatal monitoring and anesthesia technology. However, some children with critical intestinal atresia have a certain impact on their long-term prognosis due to postoperative complications such as short bowel and poor long-term bowel function recovery after surgery. For congenital digestive tract malformations, early diagnosis during fetal period and early management after birth may help to improve prognosis [54].

9.3.2 Typical Cases

9.3.2.1 Case

A 30-year-old pregnant woman was found that the fetal intestinal tract was dilated during prenatal ultrasound examination at 35 weeks of gestation, with the widest point of 23.4 mm (Fig. 9.5), accompanied by polyhydramnios. Fetal magnetic resonance imaging (MRI) revealed a well-filled fetal gastric bubble with marked dilatation of the duodenum and jejunum, the widest about 1.9 cm (Fig. 9.6). The prenatal diagnosis was possible

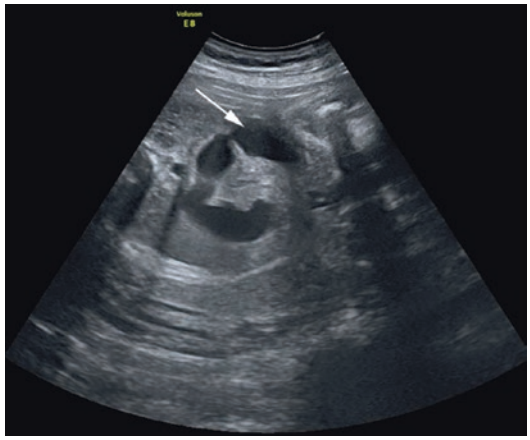

Fig. 9.5 Fetal ultrasound image showing bowel dilatation (as indicated by the arrow)

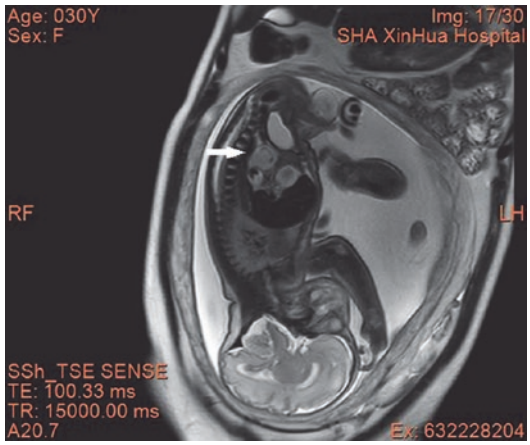

Fig. 9.6 Fetal MRI showing bowel dilatation (as indicated by the arrow)

jejunal atresia. After the multidisciplinary consultation with obstetrics, pediatric surgery, neonatology, anesthesiology, and medical imaging, the child was delivered by elective cesarean section at 38 + 4 weeks of gestation due to obstetric factors. The baby was also transferred to the PICU and treated with fasting and gastrointestinal decompression. At the same time, various preoperative examinations were completed, and an upright abdominal radiography showed gastrointestinal obstruction, and exploratory laparotomy was performed on the second day after birth. During the operation, membranous atresia of the jejunum at 50 cm from Treitz's ligament was observed, and no obvious atresia was observed in the distal small intestine and colon. Resection of the abnormal intestine and intestinal anastomosis was performed. The child recovered smoothly after surgery, was fed orally 3 days after surgery, and was discharged on the tenth day after surgery. The child grew and developed as a normal child of the same age.

9.3.2.2 Prenatal Diagnosis

The accuracy of diagnosing intestinal atresia by prenatal ultrasound still varies greatly in different centers. Currently, there is no uniform standard [55]. For example, polyhydramnios and bowel dilatation can all be used as a basis for diagnosis [56]. Although duodenal atresia is usually detected by the presence of the "double-bubble" sign before delivery, prenatal diagnosis of jejunal and ileal atresia remains difficult. Many factors can limit the diagnosis rate, such as the week of pregnancy, the number of ultrasound examinations, the experience of the ultrasound doctor, etc., all of which may be variables for the accuracy of prenatal diagnosis of small intestinal atresia [57].

Intestinal dilatation and polyhydramnios in the third trimester are thought to be associated with jejunal and ileal atresia. However, the diagnostic specificity of these two remains to be further studied, and other congenital abnormalities of the gastrointestinal tract such as meconium ileus, colonic atresia, Hirschsprung disease, and anal atresia may also be present with associated manifestations. However, these signs of imaging abnormalities are still of important significance. It enables fetuses to be evaluated and treated in a timely manner after birth, which is of great significance for the disease management. It is therefore important to determine the accuracy and diagnostic criteria for the detection of small intestinal atresia by ultrasound for the perinatal management of fetuses with small intestinal atresia [58–60].

MRI can better visualize the location and severity of bowel dilatation, and scans with different sequences can better distinguish different findings of the small intestine and colon. The normal small intestinal canal is filled with amniotic fluid and shows high-intensity signal on T2

sequences. The proximal and distal small intestines may have different findings on MRI. The jejunum usually shows high-intensity signal on T2 sequences and low-intensity signal on T1 sequences. Before 32 weeks of gestation, since meconium is rich in protein and moves slowly, the distal small intestine can still show high-intensity signal on T1 sequence. In fetuses with intestinal atresia due to meconium retention, proximal dilated intestinal loops may also show high-intensity signal on T1 sequence [61].

9.3.2.3 Prenatal Intervention

Small intestinal atresia is a structural abnormality of the digestive tract, so surgery is the only curative treatment. Usually, fetuses with intestinal atresia do not pose a serious threat to the survival of the fetus before delivery, so prenatal intervention is not required. Prenatal genetic testing can help rule out critical syndrome. Dynamic prenatal monitoring can keep up with the change of disease course, determine the progress of the disease, estimate the possible risks, and provide the basis for the development of a reasonable treatment plan after delivery in a timely manner, which has great value for clinical diagnosis and treatment [62].

9.3.2.4 Postnatal Assessment and Management

For children with fetal intestinal distension and suspected small intestinal atresia before delivery, the clinical symptoms and abdominal signs should be closely observed after birth to observe whether there are visible structural malformations of the digestive tract such as anorectal malformation, etc. At the same time, adequate gastrointestinal decompression should be given, and the expulsion of meconium should be observed, while the upright radiography should be completed. If necessary, selective gastrointestinal radiography and other examinations can be performed according to the condition to further confirm the diagnosis. It is necessary to pay attention to the water-electrolyte and acid-base balance and correct it before surgery. Surgical treatment should be performed within a limited period of time.

9.3.2.5 Treatments

The treatments of small intestinal atresia include initial preoperative preparation and surgical correction. Surgery is the only effective way to radically manage small bowel atresia [12]. Children with small intestinal atresia diagnosed before birth and confirmed after birth should be treated with fasting, gastrointestinal decompression, and correction of acid-base and water-electrolyte balance, and surgical preparation should be performed as soon as possible. The surgical method of children with intestinal atresia depends on the site of atresia. Resection of intestinal atretic segment and definitive intestinal anastomosis are the most ideal methods. Some children may have serious dilatation of the proximal intestinal duct of the atresia, so it may be necessary to consider appropriate tailoring or other forming methods for anastomosis. It is important to be alert to the possibility of multiple intestinal atresia and to fully explore all intestinal canals at the time of surgery [63].

9.3.2.6 Prognosis

The prognosis of intestinal atresia is usually good, with an overall survival rate close to 90%. However, severe premature delivery, respiratory distress syndrome, severe combined malformation, and short bowel syndrome will seriously restrict the prognosis of children. Although complications related to intestinal atresia are common, the improvement of surgical techniques and the development of neonatal perioperative management have greatly improved the cure rate and quality of life of children with intestinal atresia.

9.3.3 Research Progress

There are various theories on the formation mechanism of small intestinal atresia. At present, it is believed that small intestinal atresia may be an acquired lesion, which is caused by fetal intestinal ischemic necrosis due to vascular damage for certain reason. In general, the closer the location of vascular damage at the proximal end, the wider the range of intestinal lesions may be. The fetal intestine is sterile. After local necrosis

of the small intestine, the necrotic bowel tissue will be absorbed, leaving both distal and proximal blind ends, which may be accompanied by mesenteric defects. Some reports suggest that this type of atresia is reproduced by ligation of mesenteric vessels in experimental animals [64]. Causes of damage to fetal intestinal blood vessels include volvulus or midgut volvulus, intussusception, internal hernia, and blood supply interruption to segmental mesentery. Some cases are caused by the disruption of the fetal intestinal blood supply by underlying intestinal lesions. In addition, lesions prone to induce mesenteric thrombosis may also lead to insufficient intestinal blood supply to the fetus. Therefore, some scholars believe that risk factors for small bowel atresia include cystic fibrosis, abdominal fissure, midgut volvulus, hereditary thrombophilia, etc. All of these pathological factors may lead to potential thrombosis [64]. However, in clinical practice, it has been found that many children with small intestinal atresia do not have these risk factors, so genetic factors may also be involved in the pathogenesis of intestinal atresia.

Although the majority of cases of intestinal atresia are sporadic, there are reports suggesting familial cases of apple-peel intestinal atresia, indicating the possibility of genetic factors involved in the formation of small intestinal atresia [65]. In addition, researchers have reported a rare familial syndrome of multiple intestinal atresia (type IV). It has also been found that some cases of small intestinal atresia are concurrent with severe combined immunodeficiency (SCID) in children with mutations in *TTC7A* gene [66]. Intestinal atresia is also present in syndromes caused by genetic defects, such as Stromme syndrome, and patients present with jejunal atresia with ocular abnormalities and microcephaly [67].

Some children with small bowel atresia still have poor prognosis due to intestinal motility disorder after restoration of intestinal continuity. This phenomenon suggests that small intestinal atresia may also have defects in the intestinal tract itself. Decreased contractile fibers of smooth muscle have been reported in the proximal intestinal canal, but this phenomenon is not significantly associated with impaired motility. Significant changes in the morphology and density of Cajal cells have also been reported in the proximal and distal atretic intestinal canal [68]. The phenomenon may be related to intestinal dilatation, which leads to segmental motility disturbance of proximal intestinal canal. In addition, the reduced number of neuronal cells and fibers in the proximal atretic intestinal canal may lead to delayed neuronal development. It has also been shown that the defective expression of CD117-positive intestinal Cajal cells in the proximal intestinal tract may help to understand postoperative motility disturbance [69]. These phenomena may suggest that the extent of resection during intestinal resection in children with intestinal atresia may require further research.

9.4 Congenital Anorectal Malformation

9.4.1 Overview

Anorectal malformation (ARM) is the most common congenital digestive tract disease in neonates, with a wide variety of complex pathological changes and different manifestations in male and female. It can be manifested as anal atresia or combined with various forms of fistulas such as rectum, urethra, bladder, perineum, and vagina. The incidence of ARM in newborns is 1/1500–1/5000. The incidence is roughly equal in male and female, slightly higher in male. ARM is often accompanied by one or more malformations in other systems, and 28–70% patients are accompanied by other system malformations [70]. Among the syndromes associated with ARM malformation in clinical practice, VACTERL syndrome is the most common, and the main features include spinal defects, ARM, heart malformation, esophageal atresia with or without tracheoesophageal fistula, renal defects, and limb malformations.

At present, ARM is thought to be caused by the combination of genetic and environmental factors. Most of ARM is considered to be a sporadic disease, and there are few reports of familial cases.

9.4.2 Typical Cases

9.4.2.1 Prenatal Examination

The childbearing history of the patient was 0–0–0–0. Fetal movement appeared at 16 weeks of gestation and was good till present. The pregnant woman took prenatal examination regularly. The screening for Down's syndrome showed low risk, and the birth defects tests were unremarkable. Two prenatal ultrasonography examinations at 34 and 36 weeks of gestation revealed an anechoic area in the lower abdomen of the fetus and possible enlarged bladder. The pregnant woman visited the MDT clinic for fetal abnormalities in our hospital. Ultrasound revealed fetal bladder enlargement, bladder wall thickening, mild hydronephrosis of both kidneys, and rectal dilatation of 25 mm. Fetal MRI showed hydronephrosis and dilatation of the left renal pelvis and calices and the upper end of the left ureter, and marked enlargement of the bladder with rectal dilatation (Fig. 9.7). After multidisciplinary consultation, the diagnosis of possible congenital anorectal malformation concurrent with multiple malformations was proposed.

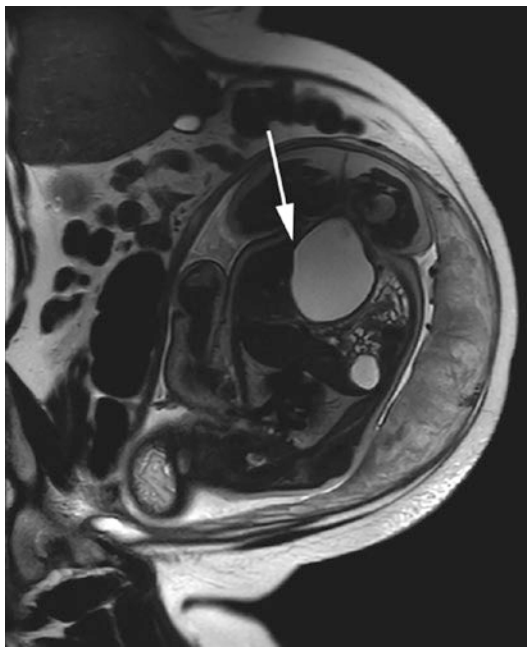

Fig. 9.7 Fetal MR imaging suggesting significant enlarged bladder with rectal distension (as indicated by the arrow)

9.4.2.2 Postpartum Conditions

The baby was delivered by cesarean section at 40 weeks of gestation due to obstetric factors, with the body weight of 3980 g, clear amniotic fluid, and unremarkable umbilical cord. Apgar score was 9–10–10. After birth, the baby was transferred to the neonatal intensive care unit, and meconium mixture was seen in the urine throughout.

Postnatal physical examination revealed a slightly distended abdomen, an empty left scrotum, no anus at the normal anal opening, and no obvious pigmentation in the anal pit. The inverted radiography at 24 h after birth revealed high aproctia, and echocardiography was unremarkable (Fig. 9.8). Abdominal ultrasound showed that the left renal pelvis was separated by 11 mm, with no other findings. Congenital anorectal malformation (possible rectovesical fistula) and left hydronephrosis were diagnosed.

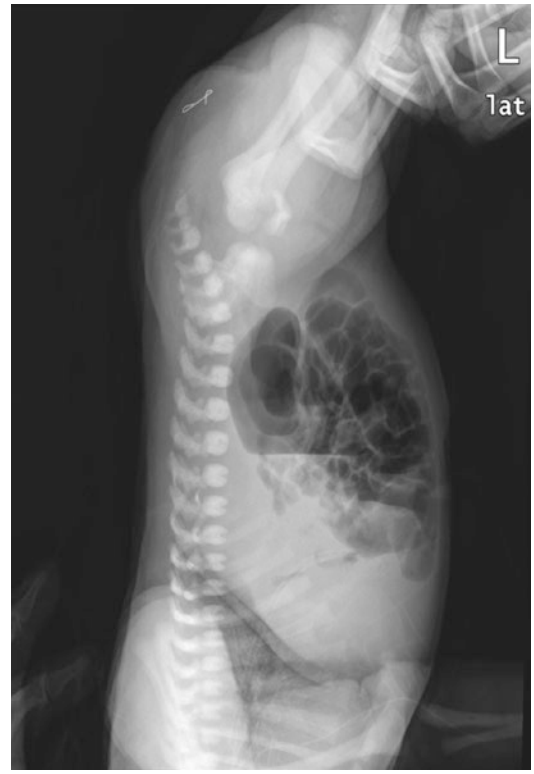

Fig. 9.8 The inverted radiography at 24 h after birth showing the blind end of the rectum located above the line between the center of the pubic bone and the tip of the coccyx

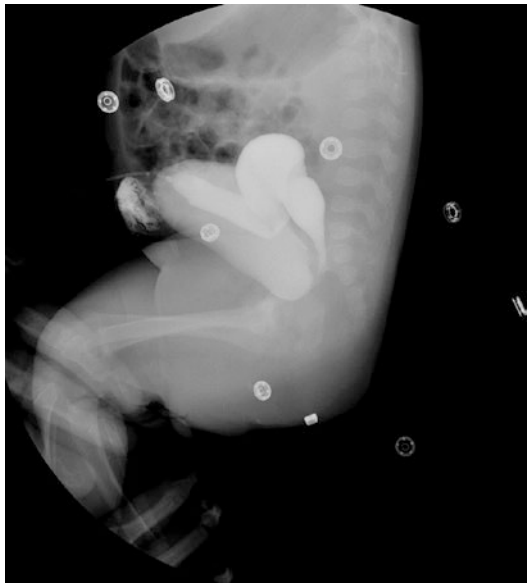

Fig. 9.9 Contrast enhanced colonography at the blind end of rectum showing rectobladder neck fistula (as indicated by the arrow)

9.4.2.3 Surgical Treatment

Double-lumen colostomy of the sigmoid colon was performed on the next day after birth. The baby was admitted for evaluation 3 months after birth. Contrast enhanced colonography showed a rectobladder neck fistula and laparoscopic-assisted anoplasty was performed (Fig. 9.9). Closure of sigmoid colostomy was performed 10 months after birth.

9.4.3 Prenatal Diagnosis

9.4.3.1 Ultrasonographic Findings

It is difficult to diagnose ARM with prenatal ultrasonography. The ultrasonographic findings for different ARM vary greatly. For instance, ARM with urinary fistula formation, urine may enter the rectum continuously from the bladder or urethra through the fistula. When the amount of fluid entering the rectum exceeds the reabsorption function of the colon, there may be sudden accumulation of fluid in the intestinal lumen, resulting in significant rectal and colonic dilatation [71–73]. Due to the mixture of meconium and urine, calcifications or stones are formed in the intestinal lumen, or meconium enters the bladder to form calcifications or stones in the bladder.

The possibility of concurrent malformations should also be considered, since anorectal malformations are mostly associated with multiple organ structural malformations, such as VACTERL syndrome. Fetal rectal dilatation, hydrocolpos, and other related malformations were found during prenatal ultrasonography, all of which suggested that the fetus may have anorectal malformations. It is also necessary to focus on the examination of the fetal heart, spine, trachea, and kidney [74].

9.4.3.2 MRI Findings

For some children with ARM, the fetal MRI may show a dilated rectum filled with meconium. If the rectum and urinary tract communicate, abnormal fluid may be observed in the rectum, which is abnormally low intensity on T1 and high intensity on T2. Fetal MRI can also measure the distance between the rectal notch and the bladder neck [75–77]. The assessment of fetal rectouterine notch is helpful in differentiating high anorectal malformations from cloacal malformations. Again, close attention should be paid to the possibility of concurrent malformations [78–81].

9.4.4 Prenatal Intervention and Assessment During Neonatal Period

If a fetus is suspected with ARM on prenatal examination, ultrasonography should be performed to rule out related malformations. Due to the increased risk of chromosomal abnormalities, amniotic fluid or umbilical cord blood puncture chromosome examination and diagnostic genetic testing should be performed [82, 83]. If early diagnosis before delivery is feasible, multidisciplinary comprehensive evaluation, parental counseling, and preparation should be carried out, and concurrent abnormalities should be screened. Some patients with major malformations and chromosomal abnormalities may select appropriate time to terminate pregnancy under the guidance [84].

Prenatal intervention is generally not required for fetuses suspected with ARM unless concurrent with polyhydramnios or with multiple malformations. This disease will not change the indications for delivery. Vaginal delivery is generally an option, and it is also not necessary to change the timing of delivery [85]. Since newborns should be evaluated and treated after delivery, delivery should be performed at a medical institution with neonatal surgery department and neonatal intensive care unit.

Generally, the diagnosis of ARM in the neonatal period is not difficult, but it is important to accurately determine the location of the blind end of the rectum and whether there is a fistula within 24 h after birth in order to take reasonable treatments in the neonatal period [86]. In baby boys with ARM, the clinical presentations include an anal opening or no anal orifice. Sometimes, a recto-cutaneous fistula at the perineum suggests low ARM. If no anal orifice presents in the perineum and there is no significant pigmentation, it suggests high possibility of intermediate or high ARM. If a single orifice in the perineum and urine or feces excreted from that orifice are found in a normal female infant, cloacal malformation or a higher rectovaginal fistula is considered. If two orifices are found in the labia, most of them are middle and high ARM with rectovaginal fistula. If three orifices are found in the labia, it is a rectovestibular fistula. If an orifice presents in the connection between the posterior perineum and the normal anal orifice, it is a rectovaginal fistula [87].

Inverted radiography at 24 h after birth can be performed to identify the position of the blind end of the rectum, and the type of ARM can be identified according to the position of the gas at the end of the rectum. MRI is very important for neonates scheduled for stage I surgical treatment, which can determine the distance from the blind end of the rectum to the anus, evaluate pelvic floor muscle development, and determine the location of the rectal fistula. It is also helpful to understand whether there are any malformations in the lumbosacral and caudal vertebrae and whether there are abnormalities such as Currarino syndrome. Tests such as B-ultrasound, CT, heart color ultrasound, or MRI can be used to find out if there are any concurrent malformations [88].

9.4.5 Postnatal Management, Surgical Treatment, and Prognosis in the Neonatal Period

For children suspected with ARM based on prenatal examination and postnatal appearance examination, fasting, gastrointestinal decompression, and other symptomatic treatment should be given immediately after birth. After comprehensive examination and evaluation of the type of ARM, male children with ARM should seek one-stage anoplasty for rectal perineal fistula or membranous atresia of the anus. Colostomy should be completed within 48 h after birth in male children with other types of ARM. The vast majority of women with ARM can maintain defecation through the fistula. If the defecation through the fistula is found after birth by appearance examination, fistula dilatation can usually be given to assist defecation, and radical surgery is performed 3 months after birth. The treatment for anal membranous atresia is the same as ARM in male infants. For those who wait 24–48 h after birth to find no fistula or the fistula is too small to maintain fecal output through dilation and lavage, colostomy may be performed, and anoplasty may be performed electively at 3 months of age.

Most children with ARM follow the sequence of "colostomy-anoplasty-colostomy closure" for surgical treatment. Ninety percent of children with ARM are treated with small posterior sagittal anorectoplasty. Rectobladder neck fistula and partial rectourethral fistula at prostate part are indications for laparoscopic-assisted anorectoplasty.

The prognosis of different types of ARM is different. The prognosis of low ARM is good, and those with sacrococcygeal or spinal cord abnormalities have a certain impact on defecation function. Defecation and urination function after cloacal malformation surgery depends on the length of the common channel. About 25% of children with ARM have varying degrees of fecal incontinence

after surgery and can achieve nearly normal quality of life under long-term bowel management [89].

9.4.6 Research Progress

As one of the gastrointestinal malformations with the highest incidence in newborns, ARM is a hot topic of research on how to improve the diagnosis rate during prenatal examinations and identify serious malformations such as defective cavity malformations and VACTERL syndrome as much as possible to guide pre-pregnancy intervention, so as to avoid serious effects on long-term quality of life caused by severe postnatal malformations. In recent years, the application of three-dimensional ultrasound combined with high-frequency technology and three-dimensional fetal magnetic resonance technology has improved the screening rate of ARM prenatal examination to a certain extent, especially the prenatal diagnosis rate of defective cavity malformations [90]. Moreover, the improved diagnosis ability of prenatal ARM will allow the treatment of ARM become a seamless sequential treatment model between fetus and newborn to further change the treatment and prognostic outcome of ARM.

9.5 Hirschsprung Disease

9.5.1 Overview

Hirschsprung disease (HSCR), also known as aganglionosis, is one of the most common causes of neonatal intestinal obstruction due to the lack of ganglion cells in the submucosa and in the intestinal wall, resulting in a spasmodic stenotic state of the intestinal segment and secondary dilatation and hypertrophy of the colon [91]. The disease has a familial genetic predisposition, which is the result of a variety of gene effects and environmental factors [92]. At present, the possible pathogenic genes under study are RET, GDNF, NRTN, ECEI, EDN3, EDNRB, SOX10, ZFHX1B, and PHOX2B. In familial HSCR patients, RET gene mutation accounts for 50%, and sporadic cases account for 15–20% [71, 93, 94].

The rate of other associated malformations in children with HSCR was 16–32%. The most common were urinary system malformations (3–5%) including hydronephrosis and renal hypoplasia, and the others included congenital heart disease (1%) and anorectal malformation (2.5–3.4%). About 3–5% of patients with HSCR will also have Down's syndrome. About 3–5% of patients with HSCR are concurrent with Down's syndrome [95]. At present, some genetic syndromes have been reported in children with HSCR, such as Waardenburg syndrome, Von Recklinghausen syndrome, Smith-Lemli-Opitz syndrome, multiple endocrine gland tumors type 2, and Bardet-Biedl syndrome [96].

9.5.2 Typical Cases

9.5.2.1 Prenatal Examination

The childbearing history of the patient was 1–0–1–1. Fetal movement appeared at 18 weeks of gestation. The pregnant woman received routine prenatal examination regularly, and amniocentesis karyotyping showed no abnormalities. At 35 weeks of gestation, obstetrical examination at a local hospital revealed fetal bowel dilatation of 20 mm. She visited our MDT clinic for fetal malformations, and ultrasonography at 38 weeks of gestation revealed colonic bowel dilatation of 21.8 mm (Fig. 9.10), and MRI revealed intestinal dilatation in the middle and lower abdomen, mainly in the colon (Fig. 9.11). In 2013, one fal-

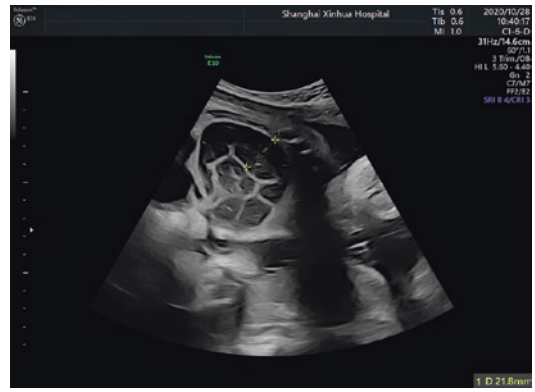

Fig. 9.10 Fetal ultrasonography at 38 weeks of gestation revealing bowel dilatation

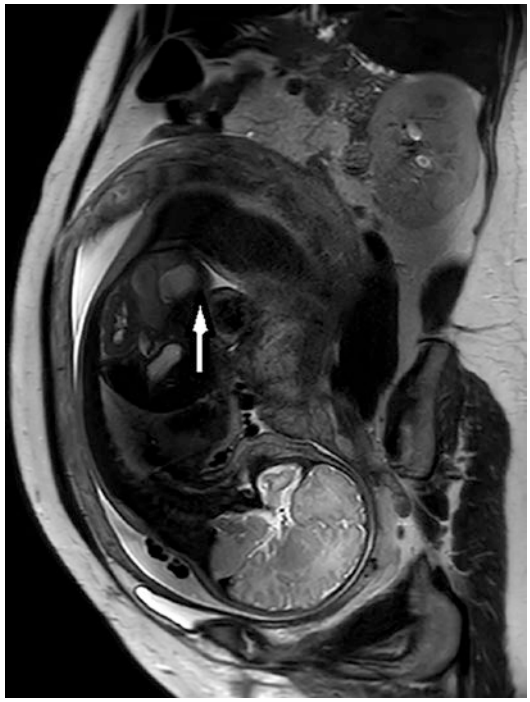

Fig. 9.11 Fetal MRI revealing ectocolon (as indicated by the arrow)

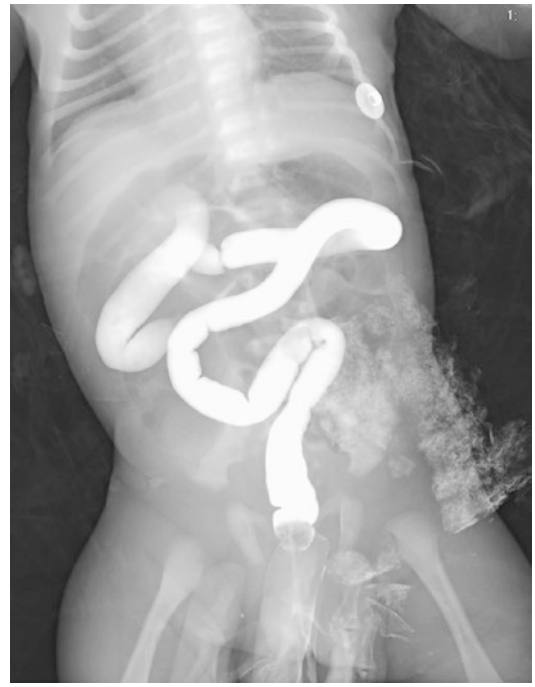

Fig. 9.12 Postnatal colonography showing small spasms in the whole colon

lopian tube was removed due to ectopic pregnancy. In 2016, a baby boy was born by cesarean section and died of HSCR.

9.5.2.2 Postnatal Conditions

The child was delivered by cesarean section at 38 weeks of gestational age, G3P2, with the birth weight of 3150 g. The neonatal Apgar score was 10–10–10. After birth, the child was transferred to the neonatal intensive care unit for observation, and a small amount of dark green meconium was discharged after the application of glycerol enema. One day after birth, the child developed progressive abdominal distension and was given gastrointestinal decompression, glycerol enema for relaxing the bowels, cleansing enema, and anti-infection treatment, after which the abdominal distension was not significantly relieved. Physical examination showed the abdomen was markedly distended, the abdominal veins were exposed, the gastrointestinal type was not obvious, and the bowel sounds were weak, 1–2 counts/min. Anal appearance examination showed no abnormality, anorectal examination showed no obvious anal stenosis, rectal ampulla was empty in the abdomen, and there was no obvious burst exhaust, and defecation after the finger was pulled out. Barium enteroclysis was performed 5 days after birth, which showed that the whole colon was small and barium was not discharged on 24-h delayed radiography, considering the possibility of total colonic aganglionosis (Fig. 9.12). Whole exon examination of the parents and the child revealed no significant abnormalities.

9.5.2.3 Surgical Treatment

Relevant examinations were completed after admission, and laparoscopic exploration + multi-punch intestinal biopsy + enterostomy was performed 5 days after surgery. During the operation, the ascending colon and transverse colon were slender. Multi-punch pathological biopsy of the whole digestive tract was taken, and ganglion cells were observed in the intestinal wall 70 cm from the beginning of the jejunum. No ganglion cells were observed in the other multi-punch

pathological biopsies of the jejunum, ileum, and whole colon. Thus, double-lumen jejunostomy was performed at this site. Postoperative diagnosis: total colonic aganglionosis. The child underwent partial small bowel resection + ascending colon-transverse colon-descending colon resection + jejuno-descending colon side-to-side anastomosis (Martin procedure) at 13 months of age. After surgery, symptomatic treatment such as enteral + parenteral nutrition therapy and anti-infective treatment was given, and the child recovered well.

9.5.3 Prenatal Diagnosis

9.5.3.1 Ultrasonographic Findings

HSCR is rarely diagnosed in the fetal period, and its ultrasonographic findings lack specificity, making prenatal diagnosis difficult. Its ultrasound findings include multiple dilated bowel loops, polyhydramnios, diffuse fetal intestinal dilatation, increased bowel echogenicity and increased abdominal circumference, and other sonograms [78, 97–99]. In some cases, intestinal dilatation is progressive and continuously extends to the distal colon. It has been reported that the dilated intestinal tract of the colon in the fetus with HSCR is "S"-shaped and "C"-shaped, with little peristalsis, poor sound transmission in the bowel, and is full of strong patchy echoes, uneven and low echo characteristics [81, 100].

9.5.3.2 Fetal MRI Findings

Fetal MRI is helpful in the differential diagnosis. Dilated intestinal tract of the fetus can be seen at the T2-weighted coronal position, mainly in the periphery of the abdomen, with widening of the bowel space [101–103]. It has been reported that MRI rapid sequence technique is helpful to reveal normal fetal colon and congenital colon lesions. However, due to the non-specificity of intestinal dilatation, it is difficult to confirm the diagnosis before delivery [104].

Most of the manifestations of prenatal HSCR are nonspecific and can be confused with those including small bowel and colon atresia or stenosis, as well as defective cavity malformations and anal atresia. The definitive diagnosis usually requires postnatal examination [105–109].

9.5.4 Prenatal Intervention and Assessment During Neonatal Period

Suspected HSCR during pregnancy generally does not require special treatment, and it is not necessary to change the timing and mode of delivery. Genetic testing during pregnancy is recommended to rule out possible combined malformations and genetic problems, and karyotype analysis is recommended to rule out conditions such as Down's syndrome [110, 111]. A multidisciplinary team evaluation is recommended to communicate and interact with parents, explain the onset and development of the condition, and ease the patient's emotional and psychological stress [112].

The suggestive role of prenatal examinations makes the early postnatal diagnosis and early management possible, largely reducing serious complications due to delayed diagnosis. In the neonatal period, about 80% of HSCR may present with typical clinical symptoms [113, 114]. The main manifestation is that only a small amount or no meconium is passed within 48 h after birth, including vomiting and abdominal distention, and there is significant improvement after relaxing the bowels with glycerol enema, anal dilation, and enema. On physical examination, the abdomen is highly distended, the abdominal wall is thinned, and the abdominal wall veins are revealed. Anal examination and digital rectal examination during the neonatal period are helpful in the diagnosis [115, 116].

Barium enema in children with HSCR can show stenotic, transitional, and dilated segments. However, for neonates and children with long-segment HSCR, since the colonic dilatation proximal to the lesion is not significant and there is little difference in contrast with the stenotic segment, a negative barium enema test in newborns cannot be used as a basis for ruling out HSCR diagnosis [117].

Rectal anal inhibitory reflex is of great value in the diagnosis of HSCR, and its diagnostic accuracy can reach more than 90% [118]. However, in normal newborns, especially premature infants, the rectal anal inhibitory reflex may not occur within 2 weeks after birth due to imperfect development of the enteric nervous system. Therefore, if the first examination is negative, another examination 14 days later can be considered to help the diagnosis [119].

Histological examination of the entire rectum is the gold standard for the diagnosis of HSCR pathological sections lack ganglion cells in the intermediate myenteric plexus and submucosal plexus, accompanied by nerve fiber hyperplasia. Therefore, pathological examination is necessary to confirm the diagnosis in children with a high clinical suspicion of HSCR [120].

9.5.5 Postnatal Bowel Management, Surgical Treatment, and Prognosis

For those with symptoms during the neonatal period, supportive treatment such as laxation, anal dilation, cleansing enema, intravenous nutrition, and infection prevention are required. Intestinal perforation, which is prone to colonic lavage in newborns, should be avoided. Care should be taken to adjust the water-electrolyte and acid-base balance. Colostomy should be considered for children with poor general condition, poor bowel cleansing effect, or suspicion of TCA. Surgical treatment is the only radical treatment for HSCR. Currently, stage I HSCR treatment has become the mainstream procedure. Under the condition of good intestinal management, stage I radical resection 2–3 months after birth is appropriate [121, 122].

The majority of children with HSCR have both good short-term and long-term prognosis after surgery. Megacolon-associated enterocolitis is the most common and serious complication after HSCR surgery and requires attention and timely intervention. The most common long-term complications are constipation and defile feces, and whether there is an effect on urination and sexual function, which requires long-term follow-up.

9.5.6 Research Progress

Although the treatments for HSCR are mostly effective, there is still a lack of effective methods for accurate prenatal diagnosis. In recent years, based on family studies of HSCR cases, combined with GWAS and whole-exon/genome-wide sequencing techniques, a series of genetic sites that may be involved in HSCR and potential pathogenic genes for related syndromes have been discovered. With the popularization of non-invasive prenatal DNA screening and sequencing technology, it is expected that HSCR cases will be detected early in future precise prenatal screening to initiate an early intervention model after birth and reduce the mortality rate HSCR serious neonatal complications (gastrointestinal perforation, megacolon enterocolitis).

9.6 Congenital Short Bowel Syndrome

9.6.1 Overview

Congenital short bowel syndrome (*CSBS*) is a rare hereditary disease of the digestive tract. In 1969, Hamilton et al. first identified two cases of children with CSBS concurrent by intestinal malrotation [123]. Prenatal ultrasound in some children shows bowel dilatation, but there are no specific changes. The length of the small intestine after birth in children with CSBS (about 50 cm on average) is significantly shorter than that of normal infants of the same age (about 190–280 cm) [124]. The main clinical manifestations are chronic diarrhea, abdominal distension, bilious vomiting, and malnutrition, and its incidence is <1/1,000,000 [125]. In recent years, the survival rate of children with CSBS has increased from 28.5% to 75% due to the development of intestinal rehabilitation, parenteral nutrition, and multidisciplinary treatment teams [125].

The underlying etiology and molecular mechanism of CSBS are not clear. Currently, the possible etiological hypotheses are as follows: firstly there are defects in midgut development from the fifth to the tenth week of the embryonic stage. Dorney et al. first proposed that if the primitive midgut cannot be fully accommodated in the umbilical cord lumen throughout pregnancy, it will lead to a shortening of its length because of the limited extension and rotation process of the small intestine [126]. Secondly, the primitive midgut is damaged by ischemia during *in utero* development due to vascular obstruction, resulting in a shortening of the length of the intestine [127]. Thirdly, neuronal dysplasia or diffuse abnormal delamination of the smooth muscle in the small intestine causes abnormal reduction in intestinal peristalsis and affects the normal extension of intestinal development. Fourthly, chromosomal anomalies, such as chromosome 4 rearrangement, looping, and chromosomes 2 and 11 translocation, but the functional meaning of these chromosomal abnormalities is unknown. Fifthly, autosomal recessive forms of coxsackie and adenovirus receptor-like membrane protein (*CLMP*) mutations or deletions [128]. Sixthly, X-linked Filamin A (*FLNA*) mutations [129].

Until now, there is no radical treatment for CSBS. The treatment is mainly to maintain growth and development with parenteral nutrition, while gradually increasing the amount of nutrients in the intestines to promote compensation for length and function of the small intestine, so as to better absorb nutrients. With the establishment of multidisciplinary diagnosis and treatment model of intestinal failure and the optimization of nutritional support programs, the quality of life of children with CSBS has been greatly improved.

9.6.2 Typical Cases

This is baby boy more than 2 months old, who was admitted to our hospital due to "more than 2 months after surgery for intestinal atresia with slow weight gain." Ultrasound at 28 weeks of gestation revealed lower abdominal intestinal dilatation of the fetus; regular review of color Doppler ultrasound revealed progressive dilatation of the intestinal canal of the fetus. The child was delivered by cesarean section at 34 weeks + 3 days of gestational age, with the birth weight of 1940 g. Abdominal distension gradually worsened after birth, so "intestinal atresia resection and intestinal anastomosis" was performed on the second day after birth. Intraoperative findings showed small intestinal atresia at 40 cm from the Treitz's ligament, distended bowel, slender colon, and unobstructed water injection, and end-to-side anastomosis of the small intestine and colon was performed. Postoperative pathology: Intermuscular ganglion cell were observed in intestinal wall. After the operation, the child still had recurrent abdominal distension, unable to defecate spontaneously, and had feeding difficulties. Therefore, more than 1 month after birth, the child underwent "exploratory laparotomy + intestinal adhesiolysis + small intestinal atresia T-shaped ostomy." Intraoperative findings: the original anastomosis was seen about 60 cm from the Teitz's ligament, and the intestinal canal at the distal end of the anastomosis was slender. The original anastomosis was resected followed by T-shaped ostomy. Postoperative pathology: intermuscular ganglion cells in the intestinal wall were easily seen, and the development was fair. After surgery, the abdominal distension was slightly improved, but the stoma output was more. When the child transferred to our hospital (more than 2 months after birth), the oral intake of deeply hydrolyzed formula milk (enteral nutrition) was 540 mL/day (45 mL/occasion, q2h), ostomy output was 150–200 g/day, and anal stool volume was 20 g/day.

Physical examination: conscious, good reaction, and thin subcutaneous fat. The abdomen was slightly distended and soft to touch. Yellow watery stools were observed in the fistula bag in the right lower quadrant, and the intestinal canal at the fistula was ruddy; the gastrointestinal type was not identified; the liver and spleen were not enlarged; the bowel sounds were three beats/min. The cardiopulmonary system and nervous system were unremarkable.

Nutrition assessment was performed after admission: a 2-month-old male, weighing 2.26 kg (weight-for-age *Z* value = −6.39) and 48.5 cm in length (length-for-age *Z* value = −5.77).

Given that the child had the disease during the fetal period and had recurrent symptoms after birth, whole exon sequencing was performed. Due to the short length of small intestine, small absorption area, and large daily intestinal content output, oral feeding was changed to continuous infusion via nasogastric tube (22.5 mL/occasion, qh), and parenteral nutrition support was also given. After adjusting the nutrition feeding method, the daily fistula output was not significantly reduced, so the feeding volume was gradually reduced to 8 mL/h, and there was still no improvement. Then, the whole exon sequencing results returned: *CLMP* (c.206G > A, p.R69H) mutation. Combined with medical history, previous evaluation, and examination, the current diagnosis is congenital short bowel syndrome, postoperative intestinal atresia, severe malnutrition and prematurity. Due to the large amount of fistula output and more than 3 months since the last operation, the child underwent "intestinal resection + terminal ileum-ascending colon Bishop-Koop procedure" at more than 4 months of age to increase the effective length and absorption area of the intestine. After the operation, according to the abdominal symptoms, signs, and fistula output of the child, the nutritional regimen was gradually adjusted to infusion once every 3 h, with a milk volume of 23 mL each time (lasted 2 h); at the same time, the parenteral nutrition was gradually reduced. At the age of more than 8 months old (more than 3 months since the last operation), the daily formula milk volume increased to 50 mL/occasion (lasted 1 h), once every 3 h; the fistula output was 5–10 g/day, the anal stool volume was about 100 g/day, and the child underwent "colon Bishop-Koop procedure + intestinal resection and intestinal anastomosis." After surgery, the daily formula milk was gradually increased to the preoperative amount, stool 2–3 times a day, total amount 100–150 g/day, and yellow thin stool; nutritional status improved (10 months of age, weight 4.8 kg, weight-for-age *Z* value = −5.37). The general condition was stable, and the child went to a local hospital for continued intestinal rehabilitation according to the current treatment plan.

9.6.3 Research Progress

CLMP encodes a transmembrane protein that is expressed throughout all developmental stages of the small intestine. Van Der Werf et al. identified biallelic loss-of-function mutations in the *CLMP* gene in seven (Fig. 9.13) CSBS patients from five independent families, establishing a causal relationship between *CLMP* mutations and autosomal recessive CSBS [128]. As an adhesion molecule, CLMP co-localizes with tight junction protein [128], which is involved in cell proliferation regulation, and *CLMP* mutations may lead to small intestinal shortening by affecting small intestinal cell proliferation during small intestinal development. However, the results of an in vitro cell experiment showed that mutant CLMP (V124D) mislocalized to the cytoplasm, but did not affect the survival, proliferation, and migration processes of this cell line, while not altering the intercellular tight junction structure [130]. Currently, only one study has reported a mouse model with *CLMP* defects. This model has a high mortality rate at birth and early postnatal period. However, the typical shortening phenotype of the small intestine was not observed in CSBS patients, but intestinal malrotation and severe hydronephrosis

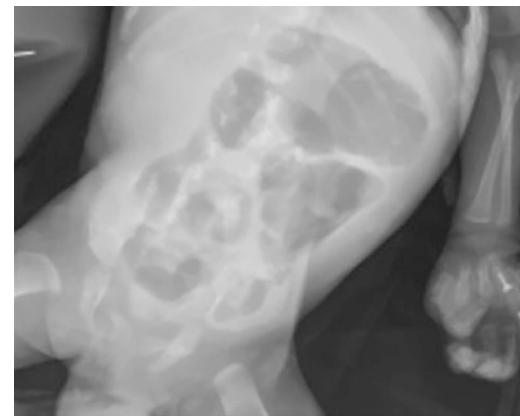

Fig. 9.13 Findings of upright abdominal radiography

are found [131]. How *CLMP* functions during the development and extension of the small intestine remains to be further investigated.

Filamentous protein A, encoded by the *FLNA* gene, is a cytoskeletal protein that binds to actin and regulates cell shape by cross-linking actin filaments. It plays an important role in signal transduction and cell migration processes when cells respond to environmental changes [132]. *FLNA* mutations are associated with a variety of diseases, including intestinal pseudo-obstruction [133], periventricular nodular heterotopia [134], otopalatodigital syndrome types I and II [135], Melnick-Needles syndrome [135], and X-linked cardiac valvular dysplasia [136]. A previous study by Van Der Werf et al. found that, in family with CSBS, the patients were all males, consistent with an X-linked recessive pattern of inheritance. The investigators also noticed that the symptoms of patients in this family were very similar to the clinical manifestations of X-linked CIPO patients due to *FLNA* mutations. Hence, they sequenced the *FLNA* candidate gene in this family and found a 2-bp deletion mutation (c.16-17delTC), and this mutation co-segregated with the involvement of members in this family, confirming *FLNA* as the pathogenic gene for CSBS for the first time [129]. Male patients with CSBS have been found to have two base deletion mutations (c.65-66delAC) in exon 2 of the *FLNA* gene [133, 137].

Compared to patients with *CLMP* mutations, patients with *FLNA* mutation have a relatively long small intestine, about 55–235 cm, so the age of diagnosis will also be delayed, between 1 day after birth and 15 years old. Negri et al. [125] conducted a systematic review of CSBS patients in 2020. A total of 61 children were included between 1969 and 2019, with a mean small bowel length of 58.24 cm. 98.4% of patients were concurrent with malrotation of intestines. Eighteen children had *CLMP* or *FLNA* gene mutations. Wang Ying et al. [138] found that Chinese CSBS population also had *CLMB* and *FLNA* gene mutations, and a total of nine children were included in this study, of which five children had *CLMP* gene mutation and one had FLNA gene mutation. In addition, six patients were concurrent with malrotation and two patients were concurrent with intestinal atresia, with a mean total small bowel length of 51.7 cm. It has been reported [139] that 3/4 of children with CSBS died within 1 year of age due to infection secondary to malnutrition or liver failure, and most of the children with CSBS were found to have moderate lower height and weight during follow-up.

Therefore, *CLMP* gene and *FLNA* gene mutations have been shown to lead to the development of congenital short bowel syndrome, but further genetic and molecular mechanism studies are still required in order to establish individualized intestinal rehabilitation. Appropriate and standardized nutritional support can improve the prognosis of children with CSBS.

9.7 Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome

9.7.1 Overview

Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) is a rare and serious congenital disease in children. In 1976, Berdon et al. first reported five cases of MMIHS in children. The main symptoms were nonobstructive bladder and bowel dilatation and decreased small colon and intestinal peristalsis [140]. One study included 227 cases of MMIHS reported from 1976 to 2011, with a survival rate of 19.7%. The most common causes of death were septicemia, multiple organ failure, and malnutrition [141]. A national survey in Japan counted MMIHS cases from 2001 to 2010, and the 5- and 10-year survival rates were 63% and 57%, respectively [142].

At present, various hypotheses have been proposed regarding the pathogenesis of MMIHS: The first is vacuolar degeneration in smooth muscle cells and intestinal myopathy [143]. The second is ganglion cell dysplasia [144]. The third is abnormalities in interstitial cells of Cajal. Lack of interstitial cells of Cajal can lead to poor intestinal peristalsis and voiding dysfunction [145]. The

fourth is genetic mutations. Mutations such as ACTG2 (γ-smooth muscle actin), MYH11 (myosin heavy chain 11), MYLK (myosin light chain kinase), MYL9 (myosin regulatory light chain 9), and LMOD1 (leiomodin1) affect the normal contractile function of smooth muscle cells, resulting in slowed intestinal peristalsis [146–150].

Prenatal diagnosis is conducive to early identification of MMIHS, timely and correct intervention and treatment, and improvement of patient prognosis. The possibility of MMIHS should be considered when the prenatal ultrasonography revealed that the fetus has a huge bladder, or if the amount of amniotic fluid is normal or increased in the second trimester of pregnancy, the bladder is severely dilated, with or without hydronephrosis, especially in female fetuses [151]. However, gastrointestinal abnormalities, including gastric dilatation (second trimester) and intestinal dilatation (third trimester), are rarely found via prenatal ultrasonography. In addition, it is found that fetuses with MMIHS have lower urinary sodium and phosphorus levels and higher calcium levels, which are associated with muscle contraction defects [152]. After birth, the main manifestations are abdominal distension, vomiting, difficulty in spontaneous urination and defecation, and poor nutritional status. Plain abdominal radiography can detect gastric and intestinal dilation. Gastrointestinal angiography can detect poor gastric motility, poor gastrointestinal rotation, and a small colon. Bladder ultrasound can detect signs such as bladder dilation and hydronephrosis.

At present, there is no effective treatment for MMIHS. Symptomatic treatment (gastrointestinal decompression, enema, intermittent catheterization, etc.) and nutritional support play a key role in the treatment of MMIHS, and some children may require enterostomy to relieve symptoms.

9.7.2 Typical Cases

A female child at the age of 2 years and 8 months visited our hospital due to "after surgery for Hirschsprung disease and recurrent abdominal distension for more than 1 year." Ultrasound at 33 weeks of gestation revealed enlarged bladder. The child was delivered by cesarean section at 39^{+1} weeks of gestational age, with the birth weight of 2750 g, and normal passage of meconium and urine. Supplementary foods were added at 4 months of age, and abdominal distension occurred immediately after food intake, which could be relieved spontaneously, with no abnormalities in urine and defecation. At the age of 7 months (3 months with supplementary food), abdominal distension was aggravated. Difficult defecation developed and required glycerol enema to assist defecation. At the age of 1 year and 3 months, the patient visited a local hospital due to "recurrent abdominal distension with difficult defecation," and "Hirschsprung disease" was considered. The patient underwent radical surgery for Hirschsprung disease, and pathology revealed few ganglion cells in the myenteric plexus of the intestinal wall. After surgery, the child still had recurrent abdominal distension and was unable to defecate spontaneously. Therefore, the contrast-enhanced ultrasonography of upper gastrointestinal tract was checked and revealed intestinal obstruction and poor gastrointestinal motility; whole exon sequencing was also done. The doctor recommended enterostomy to relieve abdominal distension symptoms, while the parents chose conservative treatment, and the patient was discharged. After discharge, the parents gave the child intermittent enema (once every 5–6 days) and glycerol enema (once every 2–3 days) for defecation; the child had occasional vomiting, recurrent abdominal distension, and poor weight gain. When the child visited our hospital, she orally took 500–600 mL/day of whole protein formula and a small amount of supplementary food every day.

Physical examination after admission: conscious, good response, thin subcutaneous fat, distended abdomen, soft to touch, obvious tympany sounds, the gastrointestinal type not identified, the liver and spleen not enlarged, negative shifting dullness, weak bowel sound, 0–1 beats/min. The cardiopulmonary system and nervous system were unremarkable.

Whole exon sequencing results showed *ACTG2* do novo (c.769C > T, p257R > C). On admission,

relevant examinations were completed: upright abdominal radiography (Fig. 9.14) revealed two elevated septa, dilated intestinal canal in the upper abdomen, and air-fluid level. Ultrasound of urinary system was unremarkable.

Nutrition assessment after admission: female, 2 years and 8 months old, height: 83 cm (height-for-age *Z* value = −2.73), weight: 10 kg (weight-for-age *Z* value = −2.22)

Diagnosis on admission: MMIHS and malnutrition of moderate degree

After admission, the child was fed with frequent small amounts of formula (200–400 mL/day) and was given parenteral nutrition support; domperidone was given to promote gastrointestinal motility; nasogastric tube was indwelled for gastrointestinal decompression (200–600 mL/day, yellowish green); intermittent urinary catheterization and transanal saline enema were performed; stable internal environment was maintained. Upper gastrointestinal radiography (Fig. 9.15) revealed gastric dilatation and poor peristalsis; therefore, a nasojejunal feeding tube was indwelled, and enteral nutrition was fed by the nasojejunal route. According to the abdominal symptoms, signs, and stool conditions, the formula was gradually increased to 130 mL/occasion (maintained for 2 h), once every 3 h; at the same time, the parenteral nutrition was slowly reduced until discontinuation. The child was then discharged and followed up.

After discharge from hospital, the child's jejunal tube slipped out into the stomach cavity twice. The main symptom was that after the formula was fed in a tube, the undigested milk could be extracted through the nasogastric tube; the plain abdominal radiography at the hospital showed that the tip of the jejunal feeding tube was located in the gastric cavity. Considering that the child needs long-term nutritional support, and in order to improve the quality of life of the child and family, the nasogastric tube and nasojejunal tube were changed to percutaneous endoscopic gastrostomy jejunostomy (PEG-J) (Fig. 9.16): G tube for gastrointestinal

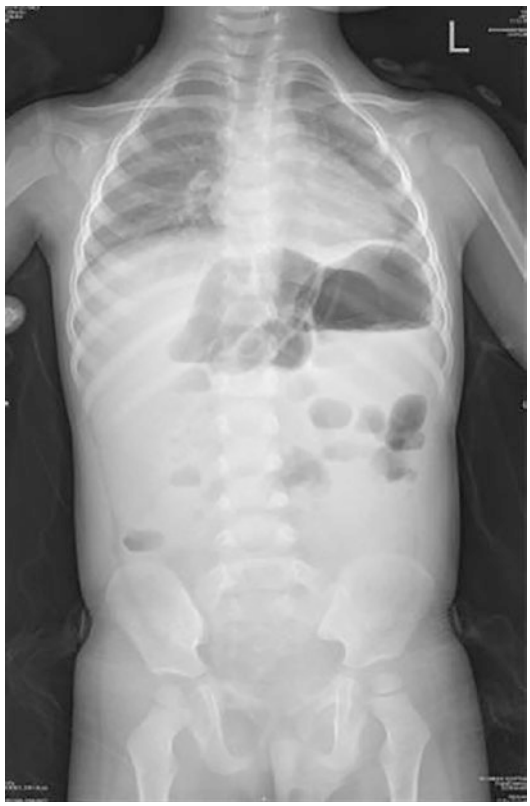

Fig. 9.14 Upright abdominal radiography

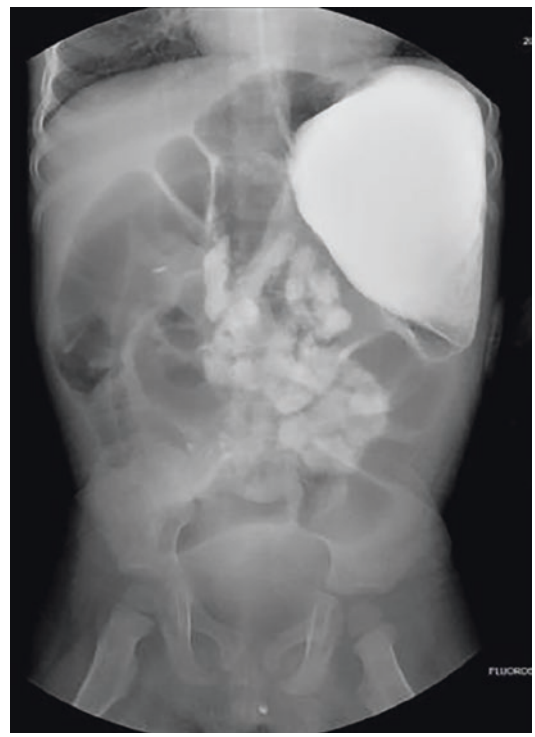

Fig. 9.15 Upper gastrointestinal tract contrast

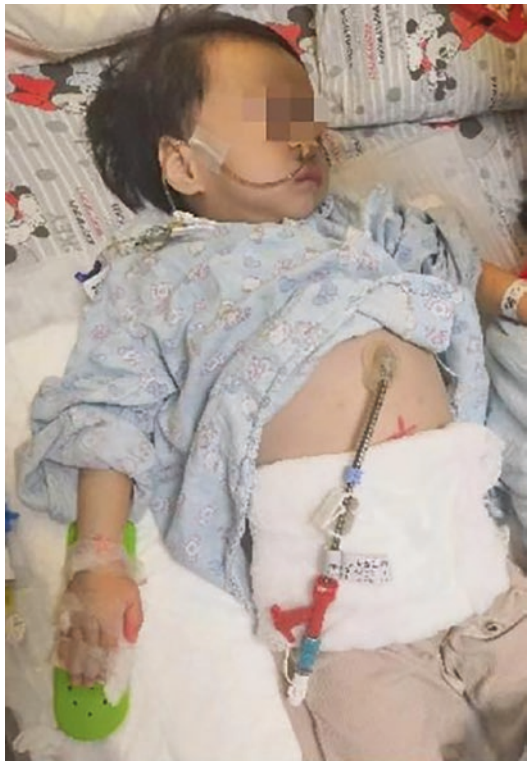

Fig. 9.16 PEG-J

decompression and J tube for infusion of enteral nutrition. No complications occurred during or after surgery such as local wound infection, pneumoperitoneum, gastrointestinal perforation, and bleeding. G tube sometimes had poor decompression effect and was assisted by intermittent indwelling nasogastric tube. With the above diagnosis and treatment, the abdominal distension symptoms were gradually reduced; the nutritional status was improved (at 3 years and 4 months of age, weight 12 kg, weight-for-age Z value = −1.59). A small amount of oral porridge, minced pork, eggs, and other foods could be taken orally, and the types of diet were gradually diversified.

9.7.3 Research Progress

Smooth muscle actin gamma 2 encoded by *ACTG2* is the predominant actin subtype found in visceral smooth muscle and is involved in SMC contraction by organizing into F-actin. Lehtonen et al. [153] identified *ACTG2* as the pathogenic gene for familial visceral myopathy in a family that had several members diagnosed with this disease. In addition, this gene is also the most important contributing factor for pediatric intestinal pseudo-obstruction (PIPO)/MMIHS, with approximately 44% of PIPO/MMIHS patients carrying *ACTG2* mutations [154]. The vast majority of *ACTG2* mutations are heterozygous de novo missense mutations which are inherited in an autosomal dominant manner within the family. In rare cases, homozygous loss-of-function mutations in *ACTG2* may also lead to severe visceral myopathy with autosomal recessive inheritance within the family [155]. More than 30 *ACTG2* mutations have been identified to date, most of which are missense mutations [156]. In vitro cell-based assays demonstrated that mutant *ACTG2* protein could not integrate into F-actin effectively, and the contractility of cells overexpressing mutant *ACTG2* protein was significantly lower than that of wild-type cells [146, 157]. It should be noted that the severity and manifestations of the disease can vary significantly even among different members from the same family who harbor identical *ACTG2* mutations [158].

Billon et al. [159] recently reported a non-consanguineous family in which megabladder was found in both the first and second fetuses, and whole exon sequencing revealed that both fetuses carried compound heterozygous mutations in *PDCL3* and that *PDCL3* mRNA expression in lung tissue disappeared. *PDCL3* is a binding protein for the protein chaperone CCT, which promotes actin folding into the native G-actin conformation which in turn polymerizes into F-actin [160], and both are highly expressed in colonic and bladder smooth muscle, so it is speculated that *PDCL3* mutations may cause MMIHS.

ACTG2 gene mutations are the main pathogenic gene of MMIHS, accounting for approximately 44.1% of all genetic factors. Assia et al. [158] discovered that 33 patients from 53 families had *ACTG2* gene mutations. Wei Zhiliang et al. [161] reported that 39 children with chronic

intestinal pseudo-obstruction underwent whole exon sequencing and 21 children developed missense mutations in the *ACTG2* gene. In addition, *MYH11*, *MYLK*, *LMOD1*, and *MYL9* genes have also been identified to be involved in the pathogenesis of MMIHS [156]. However, there are still about 55% of MMIHS cases, with no pathogenic gene identified.

Since no effective treatments are available for MMIHS yet, the prognosis of patients is poor. The mortality of children in the first year of life is 90%. With the advent of total parenteral nutrition and intestinal transplantation, the survival rate has improved (about 56%) [142]. Small bowel transplantation in children with MMIHS has been reported [162] to have a 3-year survival rate of approximately 50%. Huang et al. [163] reported that an 8-year-old boy was able to be completely weaned from parenteral nutrition 4 years after bowel transplantation.

In summary, genetic factors play a crucial role in the pathogenesis of MMIHS. Active implementation of *in utero* ultrasound diagnosis (screening for giant bladder and other symptoms) and *in utero* genetic diagnosis (screening for pathogenic gene mutation of MMIHS) can help to achieve the purpose of eugenics and reduce pressure on families and society. Further identification of new MMIHS pathogenic genes and implementation of pathogenic mechanism studies will provide a scientific basis for genetic diagnosis and genetic counseling of MMIHS and then provide potential targets for MMIHS prevention and control strategies.

9.8 Choledochal Cyst

9.8.1 Overview

Choledochal cyst is characterized by extrahepatic and/or intrahepatic bile duct dilatation. The pathogenesis is mainly based on two theories: "anomalous arrangement of pancreaticobiliary duct" and "congenital biliary stricture" [164], but its molecular pathogenesis remains poorly understood. The prevalence of choledochal cyst in Europe and the United States has been reported to be 1 in 13,000, whereas the prevalence in East Asian children is much higher. With the continuous improvement in equipment and technology for prenatal examination, most choledochal cysts can be diagnosed in the fetal period, promoting early and appropriate surgical intervention and reducing the occurrence of complications [165–167].

9.8.2 Typical Cases

9.8.2.1 Cases

This is a 28-year-old pregnant woman. At 23 weeks + 2 days of gestation, ultrasonography showed a cystic echo in the fetal right side of the abdominal cavity, size of 27.7 * 15.1 mm, with a clear border and a smooth cystic wall. Repeated ultrasonography at 28 weeks + 5 days of gestation showed a fetal upper abdominal cystic structure of 38 * 18 mm (Fig. 9.17) with a wall thickness of 2.2 mm, accompanying the portal vein, considering the possibility of cystic dilatation of the bile duct. Further fetal magnetic resonance imaging (MRI) showed a fetal hepatic hilar lesion of 23 * 19 * 28 mm, which was closely correlated with the common bile duct, considering type I choledochal cyst (Fig. 9.18). She visited our hospital and repeated MRI at 34 weeks + 6 days of gestation revealed a cystic T2WI high-intensity signal below the liver in the

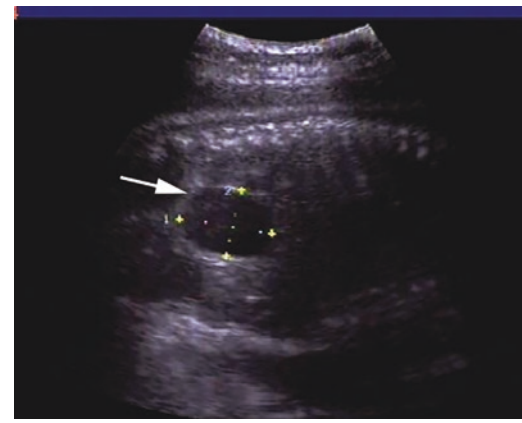

Fig. 9.17 Fetal ultrasonic image: cystic mass below porta hepatis (as indicated by the arrow)

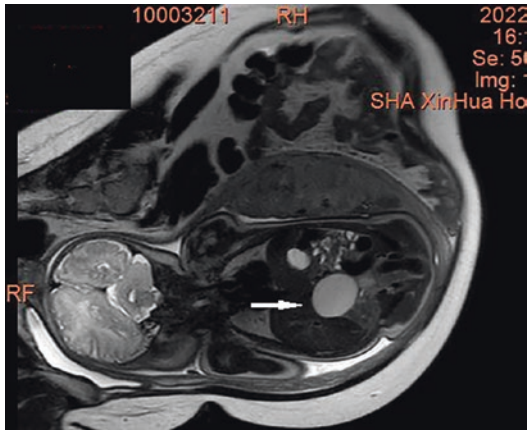

Fig. 9.18 Fetal MRI: cystic mass below porta hepatis (as indicated by the arrow)

right abdomen of the fetus, measuring about 3.2 cm × 3.0 cm, which appeared to communicate with the common bile duct and was diagnosed as possible fetal choledochal cyst (type I). Repeated ultrasonography at 38 weeks + 6 days of gestation showed a fetal abdominal mass with the size of 43 * 28 mm. After the multidisciplinary consultation with obstetrics, pediatric surgery, neonatology, anesthesiology, and imaging medicine, the child was delivered by cesarean section at 39 weeks + 1 day of gestation due to obstetric factors. At the same time, the child was transferred to PICU. After the tests for various biochemical indicators and imaging examinations, the child was diagnosed as choledochal cyst. Since cyst continued to enlarge and there were manifestations of biliary obstruction and risks of cyst perforation, laparoscopic choledochal cyst resection and Roux-en-Y biliary-enteric anastomosis were performed 7 days after birth. The child recovered smoothly after surgery, tried oral feeding 3 days after surgery, and was discharged on the eighth day post operation. After discharge, the patient was followed up for many times, and the growth and development were similar to normal children of the same age.

9.8.2.2 Prenatal Diagnosis

Ultrasonography is currently the preferred technique for prenatal diagnosis of choledochal cysts. According to the literature, prenatal ultrasonography can detect choledochal cysts as early as 15 weeks of gestation, with an average diagnosis time of 27 weeks of gestation [168]. The prenatal ultrasonographic features of choledochal cysts include (1) the diameter of the common bile duct is more than 3.1 mm; (2) the cyst is usually located in the lower boundary of the liver or interhilar region and is fully separated from the gallbladder; (3) the cyst wall is smooth and slightly thickened, and the cyst shows an anechoic dark area with irregular shape and no bleeding in the cyst; (4) typical features: the cyst communicates with the intrahepatic bile duct and gallbladder; and (5) the cyst can grow and enlarge with pregnancy [169, 170].

Fetal MRI is another noninvasive method for diagnosing choledochal cysts. The SSFSE sequence by MRI shows the dilatation of the common bile duct, along with normal gallbladder structure, common hepatic duct, and intrahepatic bile duct. At the same time, the "emptying" phenomenon of cysts helps to distinguish between cysts and blood vessels [170, 171].

9.8.2.3 Prenatal Intervention

Choledochal cyst resection and cholangiojejunostomy are the only radical treatment for choledochal cyst. Since the condition does not pose a serious risk to the survival of the fetus before delivery, no prenatal intervention is required. Dynamic prenatal ultrasound follow-up to understand the trend of cysts can provide a basis for the development of appropriate treatment plan after delivery [172].

9.8.2.4 Postnatal Assessment and Management

The common manifestations of choledochal cysts include abdominal pain, abdominal mass, and jaundice, but these symptoms rarely occur at the same time during the neonatal period [173]. In the neonatal period, biliary obstruction often manifests as jaundice. In severe cases, clay-like stools may occur, and symptoms such as vomiting may also occur if the cyst is large, and a few children may present with early cyst perforation and require emergency surgery [174–176].

Imaging is the key measure for further diagnosis after birth. The diameter of the common bile duct in the neonatal period is about 1–2 mm, so abnormal dilatation of the extrahepatic biliary tract often provides us with a basis for diagnosis. The diagnosis can be further confirmed if anomalous arrangement of pancreaticobiliary duct is found by MRCP, ultrasound. or CT.

Pathological sections of choledochal cysts show the thickening of the cyst wall, manifesting as staggered arrangement of dense connective tissue and smooth muscle chains, and different degrees of inflammatory cell infiltration under the microscope. In the neonatal period, the inflammatory manifestations are often mild. The older the child, the more severe the degree of inflammation. Intestinal metaplasia of the cyst wall is seen in some children, and ulceration of the cyst wall is common. Choledochal cysts are at risk of carcinogenesis.

9.8.2.5 Treatments

Surgery is the only effective treatment for choledochal cysts. Complete cyst excision, Roux-en-Y biliary-enteric reconstruction should be performed. Sometimes, due to recurrent episodes of cholangitis, the cyst wall is tightly adherent to the portal vein and cannot be safely removed. The anterior wall can be removed, the posterior wall mucosa can carefully electrocauterized and then biliary-enteric H-J reconstruction can be performed. In some cases, the surgical approach can be selected according to the location and classification of the cyst. For example, simple cystectomy or diverticulectomy can be performed for type II choledochal cyst, and reconstruction is required if it is associated with significant biliary stricture. Type III choledochal cysts can be managed with endoscopic sphincteroplasty, sphincteroplasty alone, cystectomy plus sphincteroplasty, or pancreaticoduodenectomy.

9.8.2.6 Prognosis

The overall prognosis of choledochal cyst after radical surgery is good. Perioperative complications mainly include bile leakage, pancreatic duct damage, etc. Long-term complications include anastomotic stricture, bile duct dilatation, bile duct stones, etc. However, the overall incidence of complications is not high. For children with severe bile duct obstruction and severe liver function impairment before surgery, fibrosis or even cirrhosis may already exist in the liver, and the prognosis may be greatly affected. Multiple intrahepatic bile duct dilatation (type V), also known as "Caroli" disease, may require liver transplantation in the long term. Therefore, early diagnosis and timely management are critical.

9.8.3 Research Progress

For the pathogenesis of choledochal cyst, anomalous arrangement of pancreaticobiliary duct is currently the main etiological theory [177]. In patients with anomalous arrangement of pancreaticobiliary duct, the sphincter of Oddi cannot regulate the function of the pancreatic bile duct confluence, leading to bidirectional reflux. Pancreatic juice mixes with bile and activates pancreatic enzymes in pancreatic juice, which can cause bile duct injury and cholangitis, leading to bile duct dilatation. However, anomalous arrangement of pancreaticobiliary duct can be found in only about 50–80% of patients with choledochal cysts in clinical practice, so further studies are required for its etiology. There is evidence that genetic factors play an important role in the pathogenesis of choledochal cysts. For example, the occurrence of some choledochal cysts is familial hereditary. Some cases of choledochal cysts are found to be associated with familial adenomatous polyposis (FAP) [178], while Caroli, type V choledochal cyst, is usually associated with the pathogenesis of autosomal recessive polycystic kidney disease (ARPKD) or autosomal dominant polycystic kidney disease (ADPKD) [179]. Gender and ethnic differences in the incidence of choledochal cysts have also been reported. Choledochal cysts, if untreated, have a tendency to become cancerous in adulthood, and there are also changes in genetic factors during disease progression. TP53 and RBM10 mutations have been found in patients with choledochal cysts and bile duct carcinoma [180], KRAS amplification is also found. The

expression of inducible nitric oxide synthase (iNOS) is also significantly increased in patients with choledochal cysts, which may lead to biliary mucosal hyperplasia, inflammation, and malignant transformation of the biliary mucosa [181].

Although recent genomics and transcriptomics studies have provided new clues about the pathogenesis of choledochal cysts, our research on the etiology of the disease and the potential mechanism of malignant transformation is still in its infancy. The application of CRISPR/Cas9 gene-editing technology and human iPS cell and liver organoid culture technology may provide help in the functional evaluation of genomic variants in choledochal cysts and research on dysregulation of genetic pathways.

Due to genetic heterogeneity and its underlying complex pathological mechanism, the pathogenesis of choledochal cysts can show complex gene polymorphisms. Therefore, it is necessary to further study the pathogenesis of choledochal cyst, comprehensive analysis of the huge database of genomic variants, and the study of their association with clinical manifestations and malignant potential. Artificial intelligence algorithms can identify genetic variants and abnormal transcriptomic profiles associated with different subtypes of choledochal cysts in the analysis of massive data from different reported genomes, transcriptomics, and clinical information, which provides great help for us to understand its potential pathogenesis and mechanism of malignant transformation.

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Respiratory System

10

Wei-Hua Pan, Jian-Hua Zhang, Wei-Peng Wang, Yi Wang, Jing-Yang Li, and Jun Wang

10.1 Obstruction of the Upper Airway

10.1.1 Introduction

Congenital upper airway obstruction (UAO) is a group of congenital lesions, which originate from oral cavity, pharynx, neck and even the entrance of main airway and pose a potential risk of airway obstruction to children [1]. The common congenital UAO includes hemangioma of the floor of mouth, cervical teratoma, cervical lymphangioma and laryngotracheomalacia [2–5]. The sensitivity of prenatal ultrasonography in the diagnosis of fetal head and neck tumors has been established with the continuous development of ultrasound imaging technology in the past three decades. High-field magnetic resonance imaging (MRI) has further made it possible to identify the fetal neck tumors and their relationships with the surrounding tissues and organs during pregnancy.

Clinically, CHAOS (Congenital High Airway Obstruction Syndrome) is used to refer to a series of upper airway obstructive malformations by neonatal surgeons [6]. Although such malformations are hardly associated in their pathogenesis basis, they share a common feature, i.e., occurrence of neonatal airway obstruction during the delivery resulting in asphyxia or even death.

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10.1.2 Case Reports

10.1.2.1 Case 1

A 29-year-old pregnant woman was found to have a huge solid cystic mass in the fetal neck, accompanied by polyhydramnios on prenatal examination at 31 weeks of gestation. She was then referred to a higher-level hospital for amniotic fluid reduction surgery at 32 weeks of gestation. Meanwhile, MRI was performed for the fetus and a soft tissue tumor with solid cystic component was confirmed in the fetal neck. It was preferentially considered to be a teratoma, which showed expansive growth, and pressed inward to the main bronchus of the neck, with a size of approximately 112 × 78 mm (Fig. 10.1). After multi-disciplinary consultation, the fetus was delivered by elective cesarean section at 38 weeks + 4 days of gestation, and then successfully placed with an endotracheal tube before cutting umbilical cord, Apgar Score 8/9/10, and referred to NICU immediately. After preoperative examinations, excision of the tumor in left neck was performed on the third day of birth. The tumor was located in the deep surface of left

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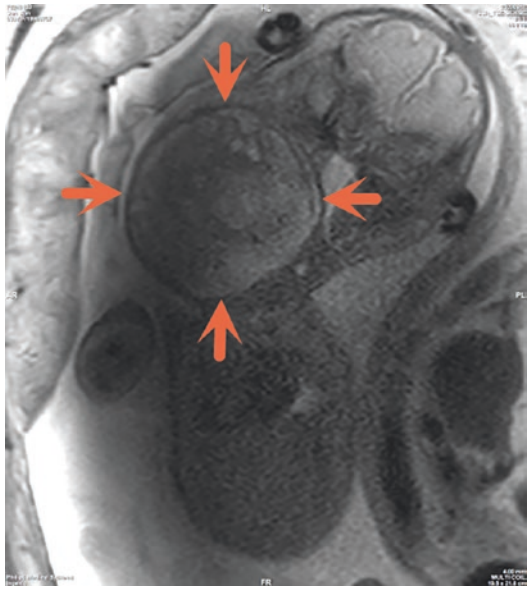

Fig. 10.1 Case 1: Fetal MRI images showed a huge solid cystic mass in left neck (range indicated by arrow) compressing main airway medially

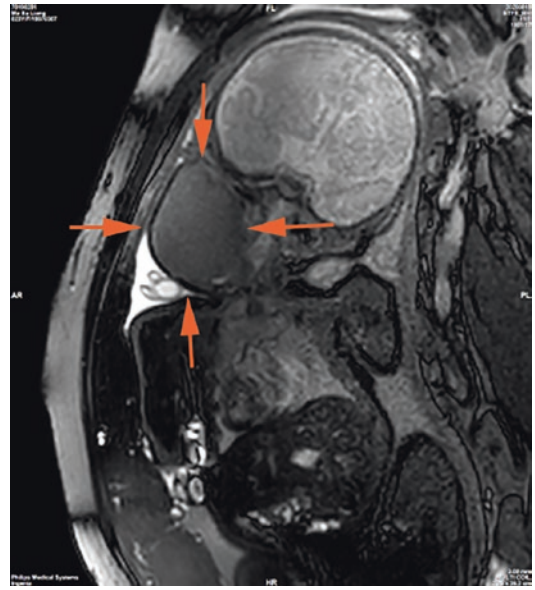

Fig. 10.2 Case 2: Fetal MRI images showed an exophytic cystic mass in left neck (range indicated by arrow) with airway centered and uncompressed

platysma muscle, with approximately 140 × 100 × 90 mm in size, solid component and intact envelope. It pushed left common carotid artery and internal jugular vein outward and compressed main bronchus inward. It was removed completely along the envelope.

The infant recovered to be stable after surgery with mechanically ventilation and lactation via nasogastric tube. Once the endotracheal tube was removed on the fourth day, the infant breathed spontaneously and was fed orally. On the tenth day, the infant was discharged with pathological findings suggesting a "mature teratoma". Outpatient follow-up after discharge showed that alpha-fetoprotein (AFP) decreased to normal levels on the third month after birth. The child is now 2 years old. No recurrence is indicated by ultrasound and AFP throughout the follow-up. Growth and development are comparable to normal children of the same age.

10.1.2.2 Case 2

A 34-year-old pregnant woman was found to have an anechoic mass in left neck of the fetus on prenatal examination at 36 weeks + 3 days of gestation, suggesting a cystic lymphangioma in the fetal neck, with a normal volume of amniotic fluid. A fetal MRI was performed simultaneously and showed that the mass was approximately 88 × 45 × 72 mm in size, cystic, exophytic growth, with no compression of the trachea in the center (Fig. 10.2). Based on the multi-disciplinary diagnosis, the pregnancy was terminated by elective cesarean section at 38 weeks + 2 days and a 3450 g male infant was successfully delivered with routine umbilical cord cutting and an Apgar score of 10/10/10. A cystic mass of 90 × 100 mm was observed in left neck, with low tension and intact surface skin. Then the infant was transferred to NICU immediately for supervision and preoperative preparation, during which the infant was fed orally without oxygen inhalation.

The resection of lymphangioma in left neck was performed on the seventh day after birth. During the operation, the tumor was found to be located subcutaneously, invading the anterior cervical muscle and encasing the internal jugular vein. After separating the cyst and surrounding tissue the tumor was removed completely. The infant was fed orally without oxygen inhalation after surgery and discharged on the eighth day with a pathological diagnosis of lymphangioma.

Outpatient follow-up was performed regularly. The child is 2 years old now, and the ultrasound shows no recurrence. Growth and development were comparable to normal children of the same age.

10.1.2.3 Prenatal Diagnosis

Oral cavity, pharynx and neck structures of the fetus are relatively fixed, and prenatal ultrasonography is highly sensitive to the diagnosis of structural malformations. In both cases above, fetal neck tumors were detected in routine fetal ultrasonography in the third trimester, by which the nature and size of the tumor as well as its relative relationship with surrounding tissues and organs were initially identified. As a useful supplement to ultrasonography in fetus with abnormal head and neck structures, MRI can provide clinicians, especially obstetricians and pediatric surgeons, with more visual imaging information for diagnosis. In Case 2, the water signal of the tumor on MRI was obvious, excluding the possibility of solid mass. Combined with its exophytic growth characteristics, lymphangioma should be considered preferentially in the diagnosis; whereas in Case 1, the fetal MRI showed that tumor signal presented solid change and fluid signal mottled, with obviously expansive growth and intact envelope, so the teratoma could be diagnosed at the earliest time. Both MRI diagnoses matched with postoperative pathology.

10.1.2.4 Risk Assessment of the Fetus

Risk assessment is an important part of routine prenatal diagnosis to identify potential risks of survival or disability at any time point of continued pregnancy, delivery and after birth. Risk assessment for fetal upper airway obstructive diseases focuses on evaluating the impact of tumor on continued development during pregnancy, and the possibility of tumor ulceration during delivery, and complete or partial airway obstruction after delivery.

In Case 1, the tumor grew expansively and compressed the airway significantly, causing deviation of main bronchus to the opposite side. Meanwhile, increase of amniotic fluid volume further indicated that compression caused by the tumor had affected the normal circulation of the fetal amniotic fluid through trachea and esophagus, so it was necessary to maintain placental circulation during delivery in order to gain time to implement tracheal intubation for the newborn. In contrast, ultrasound and MRI images in Case 2 showed an exophytic growth of the neck tumor with the airway in the middle and the amniotic fluid volume at a normal level. The size of the tumor was not significantly different from that of Case 1, but tracheal intubation preparation was not required during deliver.

10.1.2.5 Treatments

Clear fetal diagnosis and risk assessment before birth provide reliable information for rational sequential treatments from fetus to newborn and to childhood. The diagnosis and evaluation also include screening for structural malformations of other systems and organs and chromosomal genetic testing, so as to improve the diagnosis and assess the overall maternal-fetal risk. It is especially important for fetuses with obstructive lesions in the upper airway detected by prenatal examination.

In both cases above, malformations were not found in other organs and systems. The fetus in Case 1 received amniotic fluid reduction treatment at 32 weeks of gestation since amniotic fluid was abnormally increased due to the obstruction of the airway and esophagus by the tumor. Meanwhile, the multi-disciplinary consultation definitely confirmed the risk of intrapartum airway obstruction in the fetus, and an elective cesarean delivery with fetal tracheal intubation while maintaining the placental blood supply was planned. In contrast, tracheal intubation was not prepared during delivery for the infant in Case 2, since the tumor with an exophytic growth did not pose a risk of airway obstruction and the amniotic fluid volume was maintained at a normal level. The therapy strategy above also required multi-disciplinary consultation for clarification.

Given that the lesions were first detected in the third trimester of pregnancy, in both cases, amniocentesis or umbilical cord blood puncture to improve genetic testing had lost its time window and were not performed.

10.1.2.6 Prognosis

The prognosis of neonatal UAO depends not only on the nature of lesion, but also on the tendency to cause airway obstruction during delivery, which further worsens the prognosis of such neonatal defects. However, continuous development in prenatal diagnostic techniques have advanced the definitive diagnosis of upper airway obstructive lesions to the fetal period. Risk assessment and rational sequential treatments by multidisciplinary consultation can effectively avoid the risk of fetal edema and growth retardation, and greatly reduce perinatal mortality caused by airway obstruction due to the lesion during delivery. The prognosis of these birth defects will then much depend on the nature of the lesion itself.

In Case 1, the risk of fetal growth retardation was effectively avoided by the amniotic fluid puncture and reduction procedure, considering that the polyhydramnios was in late pregnancy. And placental circulation was maintained during delivery until the airway was opened by tracheal intubation, which successfully prevented occurrence of intrapartum asphyxia. Pathological examination of the resected tumor confirmed a mature teratoma and AFP was at a normal level with no recurrence on ultrasound during 2 years of follow-up. All these results indicated a well prognosis. The follow-up was continued.

10.1.3 State-of-Art of the Research

The delivery of neonates with congenital high airway obstruction syndrome (CHAOS), although rare, often astounds the obstetrician [6]. In the absence of safe airway measures, delivery of such fetuses will inevitably result in hypoxia, brain damage and even death. As a clinical syndrome, CHAOS contains intrinsic and extrinsic obstructions. Intrinsic obstruction includes pharyngolaryngeal atresia, pharyngolaryngeal valve, tracheal atresia and pharyngolaryngeal cyst, while extrinsic obstructions are caused by tumors in oral cavity, head, neck or thoracic inlet, which oppress or block the airway. The common tumors include hemangioma, teratoma, lymphangioma, and occasionally neuroblastoma [7–9].

With the progress of prenatal diagnostic techniques, especially the wide application of fetal ultrasound, MRI and genetic diagnosis, in utero monitoring of fetal growth and development has matured and been systematized [10–12]. CHAOS has achieved a breakthrough from diagnosis to evaluation and treatment based on prenatal diagnosis by detection measures mentioned above and multi-disciplinary consultation of obstetrics, medical imaging, pediatric surgery, etc. When possible UAO in the fetus is predicted, preservation of maternal-fetal circulation can effectively avoid the risk of neonatal hypoxia due to airway obstruction during delivery, and thereby improve the survival and prognosis of children with such congenital defects [13].

The detection of fetal UAO relies primarily on ultrasound images including direct and indirect basis. Space-occupying lesions such as hemangiomas, lymphangioma and teratomas can be clearly detected by ultrasonography, and therefore UAO can be diagnosed preliminarily based on their acoustic signal characteristics [11, 14]. The indirect basis includes mainly secondary lesions resulting from UAO. As a result of airway obstruction, excretion of amniotic fluid produced by the fetal lung is restricted and ultrasonic image manifestations such as dilatation of tracheobronchial tree, enlargement of lung lobe and diaphragmatic abduction may gradually appear. The indirect basis is particularly important for fetal upper airway obstructive lesions such as pharyngeal atresia, pharyngeal valves and tracheal atresia, where direct fetal ultrasound signs are difficult to manifest. Fetal MRI is now widely used for further diagnosis of UAO in fetuses with high suspicion of that on ultrasound because MRI images are clearer and more intuitive, which compensates for the deficiency of ultrasound. Therefore, fetal MRI is more acceptable to clinicians [12].

The overall assessment of fetuses with CHAOS has become a consensus, including presence of concomitant structural malformations in other tissues and organs, growth retardation, and genetic chromosomal defects. Ultrasound, MRI and whole genome analysis by amniocentesis or cord blood puncture are commonly used for screening. Space-occupying lesions on airway, especially tumors

with rich blood supply such as hemangioma and teratoma, are prone to cause fetal hydrops, thus closer surveillance of fetal development is necessary [15, 16]. At present, multi-disciplinary treatment model established under the consultation mechanism of departments of obstetrics, pediatric surgery, neonatology, imaging and anesthesia has gradually become a consensus, which is the key to ensure the quality of life from pregnancy to delivery and in the long-term future.

With the development and improvement of prenatal diagnosis and multi-disciplinary treatment evaluation, the diagnosis and treatment of fetal UAO have been progressively standardized from the potential chaos state. The consensus in the management of CHAOS during delivery is using the *Ex-Utero* Intrapartum Treatment (EXIT) procedure, which ensures fetal airway before completion of delivery and umbilical cord clamping while keeping feto-maternal circulation [17]. Thus, sequential therapy strategy with great rationality is achieved, and finally bring a better quality of life to children with CHAOS.

10.2 Congenital Diaphragmatic Hernia

10.2.1 Introduction

Congenital diaphragmatic hernia (CDH) is a congenital anomaly resulting from a developmental defect in the diaphragm leading to herniation of abdominal organs into the thoracic cavity, causing pathological changes such as mediastinal displacement and pulmonary dysplasia. Its incidence is approximately 1/2000–1/5000 [18]. The etiology and pathogenesis of CDH are not yet clear. They are thought to be multifactorial with genetic, environmental, and nutritional factors playing a role [19]. Despite that great progress has been made in the diagnosis and treatment of CDH in recent years, the mortality of children with severe CDH is still as high as 50% mainly due to pulmonary dysplasia and pulmonary hypertension [18]. Some surviving children with CDH have complications in a long term, such as chronic lung disease, growth retardation and chest deformity, affecting their prognosis and quality of life [20–22]. Accurate assessment of CDH in early stage, formulation of treatment regimen, and prevention and treatment of postoperative complications are major challenges in the diagnosis and treatment of CDH [23]. Multi-disciplinary treatment (MDT), a multi-disciplinary collaboration during antepartum-intrapartum-postpartum-follow-up period for CHD children, may be an effective model to address this challenge.

10.2.2 In Utero Diagnosis and Sequential Therapy of CDH

10.2.2.1 In Utero Diagnosis and Treatment of Twin Pregnancy with One CDH Fetus

Case 1: The fetus with CDH was the smaller one of the twins, male and born prematurely at 36 weeks + 3 days of gestation with a weight of 1750 g. His mother was G1P2. Ultrasonography of the pregnant woman at 18 weeks of gestation showed a twin pregnancy with a gastric bubble located in the left thoracic cavity of one fetus. Ultrasonography at 26 weeks of gestation in our hospital showed gastric bubble-, intestinal- and partial liver-like echoes in left thoracic cavity, right axis deviation, and a lung-to-head ratio (LHR) of 0.65 in one of the twins (Fig. 10.3).

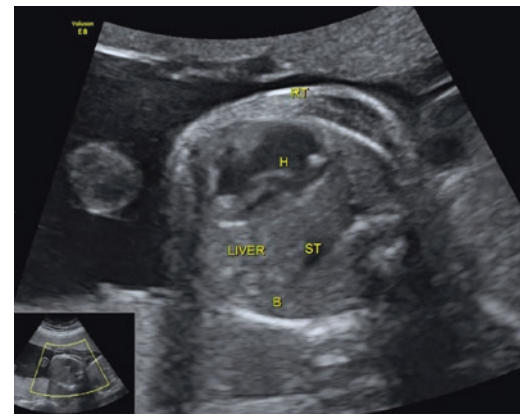

Fig. 10.3 Ultrasound showed gastric bubble-, intestinal- and partial liver-like echoes in left thoracic cavity

Echocardiography revealed pulmonary atresia, ventricular septal defect, aorta originating from the right ventricle, and moderate tricuspid regurgitation (Fig. 10.4). MRI showed stomach, intestinal canal and part of the liver in left thoracic cavity in one of the twins (Fig. 10.5).

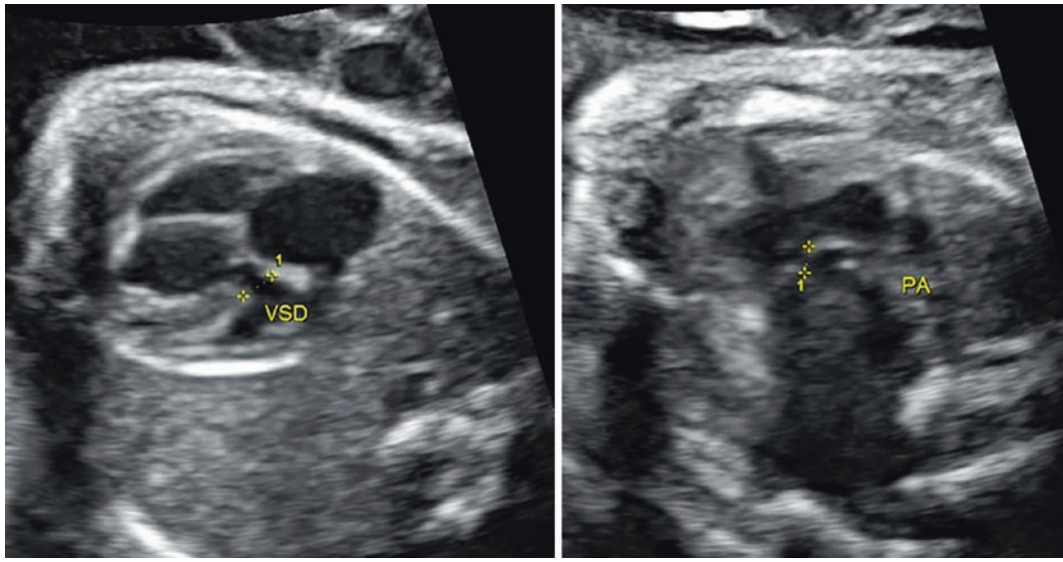

Fig. 10.4 Echocardiography indicated pulmonary atresia, ventricular septal defect, aorta originating from the right ventricle, and moderate tricuspid regurgitation

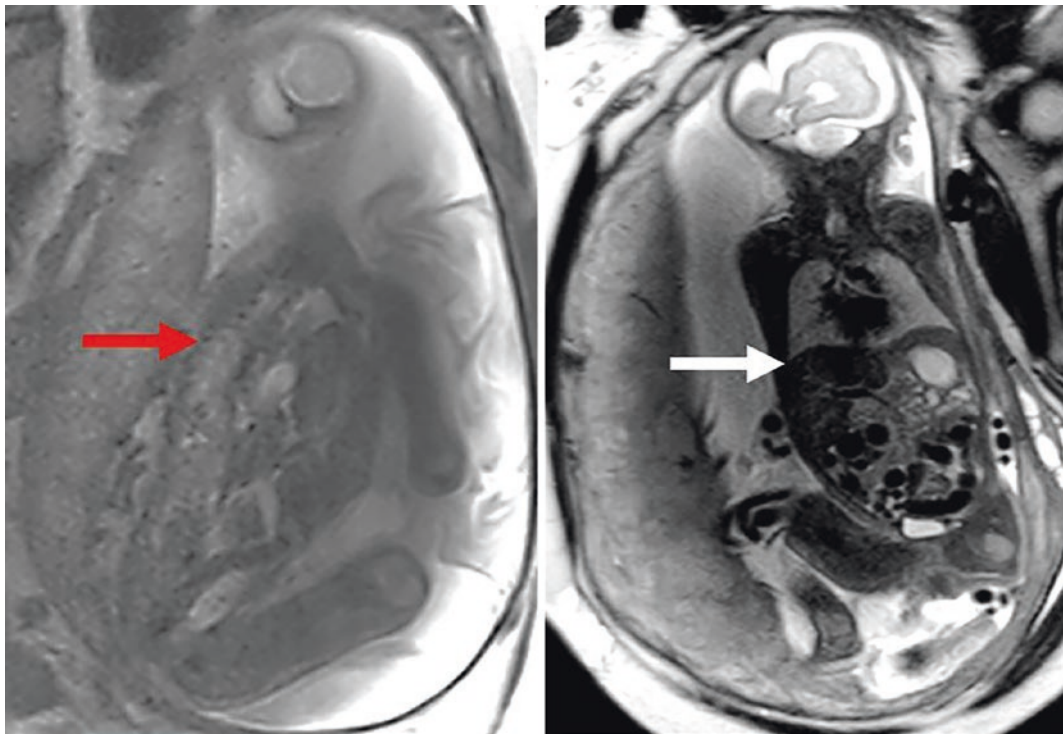

Fig. 10.5 Fetal MRI revealed a twin pregnancy with stomach, intestinal canal and part of the liver in left thoracic cavity of one fetus (red arrow), and abdominal organs not herniated into the thoracic cavity of the other fetus (white arrow)

The infant was immediately treated with tracheal intubation, oxygen supply by pressurized balloon, chest compression and gastric tube indwelling without umbilical cord transection at birth. Echocardiography showed pulmonary atresia, ventricular septal defect, atrial septal defect, aortic overriding, patent ductus arteriosus, and enlargement of right atrium and ventricle. The family requested to give up any treatment after thorough consultation.

10.2.2.2 In Utero Diagnosis and Treatment of CDH with Genetic Abnormalities

Case 2: Ultrasonography at 23 weeks + 5 days of gestation in another hospital showed possible fetal diaphragmatic hernia, possible ventricular septal defect, and low risk for Down's screening in the second trimester. The pregnant woman was then referred to our hospital for further examination and evaluation. Ultrasound indicated fetal left diaphragmatic hernia (gastric bubble and partial intestine-like echoes in left thoracic cavity, right axis deviation, LHR: 1.11); echocardiography showed ventricular septal defect. After a careful evaluation of the disease, a MDT team, organized by pediatric surgery department of our hospital and composed of obstetrics, pediatric endocrinology and genetics, and medical imaging, recommended to improve the cord blood puncture and genechip test before next evaluation. Results of genechip test of the pregnant woman suggested Kleefstra Syndrome type 1 (chromosome 9q34.3 deletion and chromosome 17p13.3p13.2 duplication). Geneticists believed the outcome of the syndrome to be serious based on genetic test results, and recommended careful consideration of fetal retention. As a result, the pregnant woman underwent pregnancy termination after an approval by the ethics committee.

10.2.2.3 In Utero Diagnosis and Sequential Therapy of CDH

Case 3: A male patient, born at 38 weeks + 6 days of gestation with a weight of 3800 g. His mother was G4P1. Ultrasonography at 22 weeks of gestation in another hospital showed fetal left diaphragmatic hernia (LHR: 0.82, O/E LHR: 35%). Ultrasonography at 25 weeks of gestation in another pediatric hospital showed left diaphragmatic hernia (LHR: 0.7, O/E LHR: 27%). In utero intervention, namely Fetal Endoscopic Tracheal Occlusion (FETO), was recommended to his family. Then his family came to our hospital for prenatal consultation. Ultrasound showed fetal left diaphragmatic hernia (partial intestine-like echoes and gastric bubble in left thoracic cavity, liver located in abdominal cavity, LHR 1.28, O/E LHR: 50.60%). Echocardiography showed right axis deviation and approximately normal intracardiac structures. MRI suggested that a large amount of intestinal canal could be seen in left thoracic cavity of the fetus and the liver was located in the abdominal cavity (Fig. 10.6). Departments of Pediatric Surgery, Obstetrics, Neonatology and Medical Imaging in our hospital formed a MDT team to evaluate the condition of the fetus. After that, continuing pregnancy was recommended and relevant risks, therapy regimen and long-term prognosis were informed.

After birth, the infant was given supportive treatments such as tracheal intubation and gastric tube indwelling. Apgar score was 8, 9 and 9 points at 1, 5 and 10 min, respectively. The infant was then transferred to the Department of Pediatric Emergency and Critical Care Medicine for supportive treatments including fasting, gastrointestinal decompression, ventilator-assisted ventilation and sildenafil administration. On the second day after birth, breathing and circulation were stable, and thoracoscopic repair of diaphragm was performed. The infant recovered well and was discharged on the 19th day after surgery. Outpatient follow-up was performed regularly since discharge. Growth and development were comparable to normal children of the same age over 3 years after surgery. Only pulmonary function test showed mild obstructive ventilatory dysfunction.

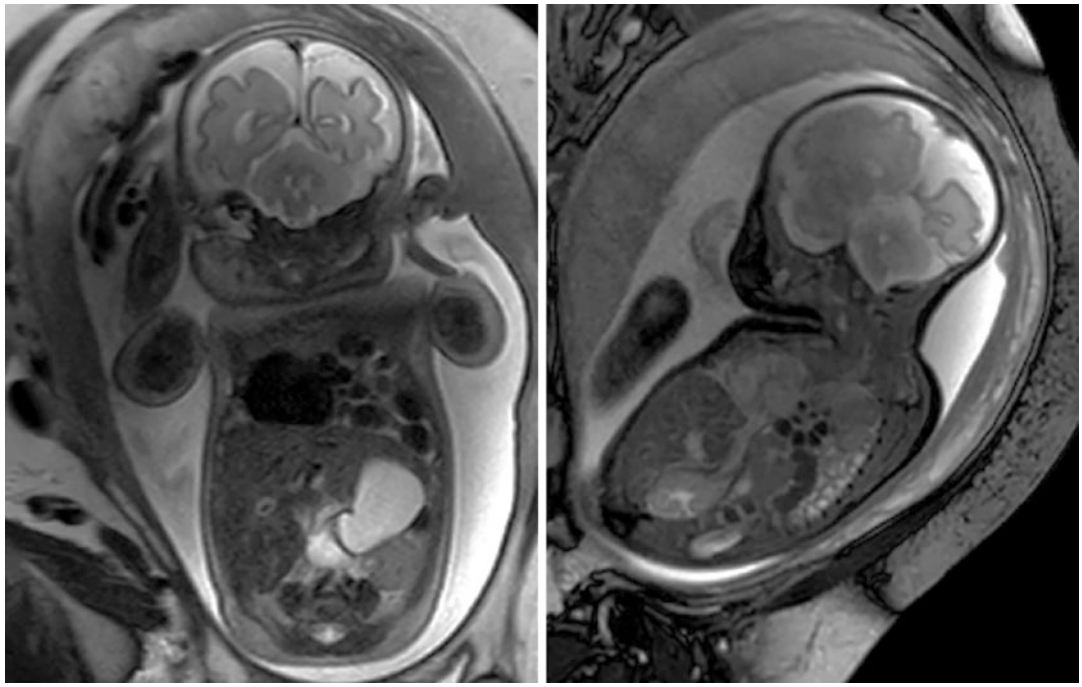

Fig. 10.6 Fetal MRI suggested a large amount of intestinal canal in left thoracic cavity and right axis deviation

10.2.3 Research Progress

10.2.3.1 Prenatal Examination and Assessment

Diagnosis and evaluation of the condition and prognosis of CDH in the early fetal period is helpful to direct prenatal counseling, in utero intervention and postpartum treatment regimen. Ultrasonography is an important examination for prenatal screening and diagnosis of CDH, with about 2/3 of CDH detected in the second trimester [24]. Multiple indicators related to the severity of CDH, including LHR, observed to expected LHR (O/E LHR), liver location and combined malformations can be obtained by ultrasonography. O/E LHR and liver herniation are generally accepted internationally as important indicators for evaluation of fetal lung dysplasia in CDH. An O/E LHR <25% or <45% was considered to be severe pulmonary dysplasia in left or right CDH, respectively [25, 26]. However, the accuracy of LHR and O/E LHR metrics depends on the measurement experience and learning curve of sonographers [27]. Beginners were able to measure LHR and O/E LHR more accurately after testing at least 70 CDH cases [27]. In Case 3, the LHR and O/E LHR of the same fetus measured by ultrasound in three hospitals were quite different, which affected the assessment of fetal condition and prenatal counseling, suggesting that more accurate measurements should be obtained in experienced maternal-fetal medicine centers.

Fetal MRI is useful to determine fetal lung development and liver location [28]. MRI can measure total fetal lung volumes (TFLV) and quantify the volume of herniated liver into the thorax, which is helpful to determine the type of herniated intrathoracic organs, the presence of hernial sac, mediastinal compression, etc. Our studies showed that CDH fetus with a hernia sac or a mediastinal angle <30.7° had a better prognosis [29, 30]. In the 3 clinical cases presented here, prenatal MRI effectively assessed the type of herniated intrathoracic organs and mediastinal displacement in children with CDH.

Cardiac dysfunction is another important prognostic factor in children with CDH [31], and about 20% of CDH are concomitant with con-

genital cardiac malformations [32]. Therefore, fetal echocardiography should be performed after detection of CDH to identify whether there are complicated congenital cardiac malformations. In Case 1, prenatal echocardiography revealed severe congenital heart malformations in one fetus with CDH, suggesting a poor prognosis in prenatal evaluation. In Case 2, cord blood puncture and genechip test revealed diaphragmatic hernia, ventricular septal defect and Kleefstra syndrome. The prenatal MDT team recommended pregnancy termination due to its poor prognosis. Studies have reported that CDH children associated with important structural malformations and/or genetic abnormalities have a worse prognosis than those with isolated CDH [33]. Therefore, genetic testing should be performed in fetuses with CDH to assess the presence of genetic abnormalities and other malformations.

A twin pregnancy with CDH in one fetus is rare and has only been reported in case reports. In a retrospective study of 142 CDH children in our Department, 11 twins with one CDH fetus were found [34]. The mortality rate of CDH in one of the twin is significantly higher than that in singletons, which may be due to the fact that CDH in one of the twins are prone to premature delivery and low birth weight. Therefore, prenatal monitoring should be strengthened, and it is recommended that the pregnant woman should be admitted to the hospital at 32 weeks of gestation to monitor the changes of maternal-fetal conditions [34]. In the prenatal evaluation, some CDH fetuses in twins are found to have severe pulmonary dysplasia with a poor prognosis. However, to ensure the growth and development of the normal fetus in twins, it is generally recommended continuing pregnancy and no intervention for the fetus to avoid affecting the development of normal fetus. It is a challenge for clinicians to improve the prognosis of CDH fetuses without affecting the development of normal fetus in the twins.

10.2.3.2 In Utero Intervention

In utero treatment may be an option for CDH fetuses with severe pulmonary dysplasia [25]. FETO is the main strategy to promote lung development in CDH fetuses. Its theory is to place an inflated latex balloon in fetal trachea through the fetoscope, obstruct the trachea, and allow secretions accumulated in the airways, thereby promoting lung morphology and structural development [35]. Interventions are generally recommended at 26–28 weeks of gestation. It should be carried out in centers with extensive experience in fetoscopy and accurate assessment of lung development for CDH fetuses and by a specialized MDT team composed of physicians and nurses from pediatric surgery, obstetrics, fetal medicine, and anesthesiology [36].

FETO can improve the clinical outcomes of children with severe CDH [37–40]. In a multicenter, randomized, controlled study, FETO at 26–28 weeks of gestation effectively improved the survival rates of CDH fetuses with severe pulmonary dysplasia [36]. However, survival improvement disappeared when FETO was postponed to 30–32 weeks of gestation even for CDH fetuses with moderate pulmonary dysplasia [41]. In addition, FETO is associated with complications such as premature rupture of membranes and preterm delivery [42]. Therefore, surgical indications should be controlled when performing FETO. Some investigators have developed new technology and equipment to optimize FETO. Chiara et al. used an injectable, degradable hydrogel to block the trachea prenatally, avoiding a second procedure to relieve the obstruction [43]. Basurto et al. applied a smart tracheal occlusion device to obstruct outflow of lung fluid. It was equipped with a magnetic valve, which can be opened under the influence of magnetic field around any magnetic resonance scanner. Thus, maternal-fetal risk is reduced by relief of tracheal obstruction and avoidance of the second invasive operation [44]. Pulmonary hypertension is one of the important factors affecting the prognosis of children with severe CDH [45]. No studies have shown the effect of FETO on pulmonary vascular development, and it will be explored in the future [46].

10.2.3.3 Intrapartum Management

The obstetricians decide the delivery mode based on the maternal-fetal examination results. The overall principle is to take the fetal condition into account under the premise of ensuring maternal safety. Before delivery, pediatric surgery, anesthesiology, pediatric emergency and critical care medicine and other related multidisciplinary departments enter the delivery room to wait for delivery, and complete the preparation of various medical devices and equipments, such as intubations, oxygen, negative pressure suction machine, transfer ventilator, incubator, etc. Once the fetus is delivered, a series of procedures, including tracheal intubation, oxygen supply after airway clearance, and gastrointestinal decompression by indwelling gastric tube are performed before cutting the umbilical cord. Then the infant is urgently transferred to the department of pediatric emergency and critical care medicine using a transport ventilator for further treatment [47].

10.2.3.4 Postpartum Management

Examinations should be made immediately after delivery of CDH fetus, including blood gas analysis, anteroposterior and lateral chest radiography, echocardiography, hemodynamic monitoring, etc. Prognostic indicators for CDH children are collected and used to guide the selection of postpartum treatments, which include ventilator-assisted ventilation mode, administration of sildenafil and nitric oxide inhalation. In a recent study, we established a model composed of 5 indicators, including 1-min Apgar score, birth weight, type of diaphragmatic hernia, location of liver, and $PaCO_2$ in blood gas analysis for postpartum risk assessment. It can be used to predict the prognosis of children and assess the severity of their disease. The surgery is not performed until respiratory and circulatory status of the child is relatively stable. Surgical methods include transabdominal diaphragmatic repair, thoracoscopic diaphragmatic repair, and so on [30]. The presence of complicated malformations in thoracic cavity and lung are observed during surgery and then the decision is made according to the intraoperative situation as for whether to deal with them together or not [31]. A comprehensive management for respiratory and circulatory system should be continued after surgery, such as ventilator-assisted ventilation and hemodynamic monitoring [30].

10.2.3.5 Long-Term Follow-Ups

Some of surviving infants with CDH have a multitude of long-term complications, such as chronic lung disease, gastroesophageal reflux, growth retardation, neurocognitive impairment and chest deformities, which affect their prognosis and quality of life [20–22]. We analyzed the data of 87 children with CDH retrospectively and found that 45 children developed postoperative complications (51.7%), including growth retardation (10.3%) and complications of respiratory (39.1%), digestive (12.6%), neurological (4.6%), and musculoskeletal (14.9%) system [22]. CDH children may have long-term pulmonary dysfunction after surgery, and this may persist into adulthood. We followed the results of pulmonary function test in 45 children with CDH after surgery and found that respiratory function was normal in 7 children (15.6%) and abnormal in 38 children (84.4%). Of 89 pulmonary function tests, 10 (11.2%) cases had normal tidal respiratory function, 62 (69.7%) cases had obstructive ventilation function disturbance, 6 (6.7%) cases had restrictive ventilatory dysfunction, and 11 (12.4%) cases had mixed ventilatory dysfunction [21]. Therefore, the child with CDH will be normally followed up after discharge to monitor the growth and development and the lung function recovery. The Pediatric Surgery Department in our hospital will collaborate with many departments such as Pediatric Respiratory Medicine, Child Healthcare and Follow-up Center to carry out the CDH multi-disciplinary follow-up. Follow-up is recommended at 3, 6, 9, 12, 18, 24 months, 3 years after birth, and annually thereafter. Follow-up includes chest X-ray, pulmonary function test, growth and development assessment, nutritional assessment, and multisystem comprehensive assessment and guidance such as dietary guidance and neurophysiological development assessment [48].

10.3 Congenital Cystic Lung Lesions (CCLLs)

10.3.1 Introduction

10.3.1.1 Classification

Congenital cystic lung lesions (CCLLs) are a group of rare congenital pulmonary dysplasia caused by the emerging of tracheal and bronchus abnormality or branch dysplasia during the embryonic period. CCLLs are cystic space-occupying lesions with air, liquid or air-liquid mixture in the lungs. CCLLs can occur in different parts of bronchial branches at different developmental stages.

Depending on the origin of fetal dysplasia, CCLLs are mainly divided into congenital pulmonary airway malformations (CPAM), bronchopulmonary sequestration (BPS), and other rare types. The subtypes of CCLLs share some common embryology and clinical manifestations but each has its own characteristics.

10.3.1.2 Congenital Pulmonary Airway Malformations (CPAM)

CPAM is the most common type of congenital cystic lung lesions and is characterized by abnormal overgrowth of the bronchioles in a single lobe of unilateral lung, especially the terminal bronchioles. The lesional lobes are markedly enlarged, appearing like a multilocular honeycomb structure with disordered arrangement. The pathogenesis may be signal transduction disorder between the epithelial cells and the underlying mesenchymal cells in the embryonic period is disturbed, which leads to a lack of normal alveoli in the lungs and excessive proliferation of the pulmonary mucus glands, resulting in lung mass [49, 50]. The morbidity of CPAM is about 1/35,000 to 1/7200 live births, and it is increasing gradually [50, 51]. For a long time, CPAM was also called congenital cystic adenomatoid malformation (CCAM). Stocker raised the concept of CPAM in 2002 [52]. According to gross and histological manifestations of the lesions, CPAM was further classified into 5 types: types 0, 1, 2, 3, and 4, of which Types 1 and 2 were the most common types (about 70% to 85%).

10.3.1.3 Bronchopulmonary Sequestration (BPS)

BPS, also commonly called pulmonary sequestration (PS), is another common congenital cystic lung lesion besides CPAM. BPS is a mass of non-functional lung tissue, which is different from normal trachea and bronchi. It is generally believed that because the vessels connected with the aorta remain in the embryonic development, and normal aorta and pulmonary artery do not enter the primitive arterial plexus, the systemic circulation arteries supply the non-functional lung tissue [53, 54]. Pulmonary sequestration is classified into intralobar and extralobar sequestration. The intralobar sequestration is more common and accounts for about 75%. The lesion is mostly located in the posterior basal segment of the left lower lung and in the paravertebral position and it is often accompanied by pleural adhesions. Infection symptoms are more severe. The extralobar sequestration is rare, which is mostly located in the posterior basal segment of the left lower lung and may also be located in the mediastinum [55, 56].

10.3.1.4 Other Rare Lesions

Relatively rare CCLLs include: bronchogenic cyst (BC), alveolar cyst (AC), congenital pulmonary lymphangiectasis (CPL). Because of their rarity, a combination of imaging and pathological comprehensive analysis is often required for definitive diagnosis [57, 58].

10.3.2 Prenatal Diagnosis

10.3.2.1 Prenatal Diagnostic Techniques and Standards

CCLL can be detected by prenatal ultrasonic screening at 18–22 weeks of gestation in the majority of fetuses, presenting as hyperechoic, hypoechoic (anechoic) or complex echogenic masses, with an overall sensitivity of 94% and specificity of 95.3% [59] Ultrasonography has become the first choice for prenatal diagnosis and

follow-up of CCLL because of its convenience, high reproducibility and visible blood supply source.

In addition to observing the lesion itself, prenatal ultrasonography is used to detect serious systemic complications such as hydrops fetalis. Hydrops fetalis presents excessive accumulation of fetal extracellular fluid due to impaired venous return caused by too large fetal lung focus volume and compression of vena cava. The diagnostic criteria for hydrops fetalis were the presence of at least 2 of the following 5 items: ① Skin edema (≥ 5 mm); ② Placenta thickening (>6 cm); ③ Abdominal effusion; ④ Pleural effusion; ⑤ Pericardial effusion and/or hydramnios. Although the incidence of hydrops fetalis is low, the mortality is as high as 82–93%. Hydrops fetalis is an important cause of induced labor and in utero death in fetuses with CCLL [60].

Fetal magnetic resonance imaging (fMRI) can better visualize the chest structure, lesion shape and associated malformations of children with CCLL, and can also evaluate the fetal lung volume, define the border of lesion and its adjacent relationship, which is helpful for prenatal comprehensive evaluation of the fetus. Therefore, fMRI can be used for the diagnosis of high-risk fetuses who are not clear by ultrasound, need further identification of the type or location of the lesion, need prenatal treatment, or need to define whether they are accompanied by other systemic malformations [61].

10.3.2.2 Risk Assessment of Fetus

Ultrasonography is the first choice for prenatal diagnosis of CCLL, and is the main method for regular prenatal follow-up. Some specific criteria or imaging features can be used to assess fetal risk. The most common measure is the fetal CCAM volume ratio (CVR) proposed by Cromblehlome et al. in 2002, which is calculated as: (lesion length × height × width × 0.523)/head circumference [62]. The larger the CVR value is, the larger the relative volume of the lesion is and the more obvious the thoracic space occupying effect is. Some studies have analyzed the relationship between CVR value and prognosis and found that when the CVR is >1.6, there is a risk of hydrops in the fetal period; when the CVR is ≤0.91, there is a lower risk of hydrops fetalis; when the CVR is <0.56, the negative predictive value of fetal hydrops is 100%, and the prognosis is good. It should be noted that the CVR value will change with the increase of gestational age. Therefore, the determination of CVR values at different periods will help to accurately evaluate the outcome of fetus and guide the follow-up of pregnant mothers [63].

10.3.3 Treatments

10.3.3.1 Prenatal Intervention

Most of fetuses with CCLL found prenatally can be delivered smoothly, and few of them experience fetal hydrops due to the compression by lesions, which has a great risk of death. Fetal intervention treatment of CCLL can reduce the mortality at fetal birth to a certain extent. Many large case series have shown that when the CVR is ≥1.6 with or without large cysts, there is an 80% chance of fetal hydrops, and antenatal steroid administration to the mother can prevent the growth of lesion and alleviate the edema [64]. However, it must be noted that for some specific types of CCLL, such as macrocystic CPAM, antenatal intramuscular injection of steroid to mothers is ineffective. Therefore, these patients often need amniocentesis for amniotic fluid decompression or pleural amniotic shunt [65, 66].

Fetoscopic or open fetal surgery to destroy or resect the lesion may be considered when the mass is large. For example, when the CVR is ≥1.6, there are significant compression symptoms or mediastinal displacement, or there is a risk of hydrops fetalis, and amniocentesis shunt is not effective so that the risk of prenatal or postnatal death is high [67]. Recently, EXIT treatment of complicated and severe CCLL fetuses has been carried out with close cooperation of multi-disciplines. The tumor is resected under the condition of maintaining the fetal-placental circulation, and then the umbilical cord is cut off to start the spontaneous breathing of the newborn, which avoids the respiratory distress caused by severe lung compression and poor expiration.

The survival rate of the operation is nearly 90% [68, 69].

10.3.3.2 Postpartum Sequential Therapy

To date, surgical resection of CCLL after birth remains the treatment of choice. However, there are still controversies about the timing, mode and extent of surgical resection. Most investigators believe that some types of CCLL, such as small cystic CPAM, may resolve spontaneously. Moreover, as early surgery has high risk, it is recommended not to undergo the surgery temporarily but perform follow-up observation. However, considering the risk of complications (recurrent infections, airway compression, pneumothorax, etc.) in asymptomatic children at any time during follow-up, more and more studies have shown that operations on small infants are safe, with controllable complications [70]. Therefore, a new expert consensus in China suggests that operations be recommended in asymptomatic children between 6 months and 1 year of age [71].

In recent years, with the development of thoracoscopic surgery/robotic surgery and the equipments, thoracoscopic surgery/robotic surgery has the advantages of lower surgical trauma, less limitations in shoulder joint activity and thoracic development, shorter hospital stays, less visible incision than an open survey. It is now increasingly accepted by doctors [72, 73]. Some studies have shown that the medium and long-term pulmonary function after surgery is well recovered in children with CPAM, and there is no significant difference between children with CPAM and normal children [74, 75].

10.3.4 Case Report

10.3.4.1 Case 1

A 43-year-old pregnant woman at 24 weeks + 1 day of gestation, ultrasonography in another hospital showed space-occupying lesions in the left thorax of the fetus, the possible diagnosis was congenital cyst adenomatoid malformation of the lung, and the pregnant women was transferred to our hospital for further examination. Ultrasonography results: slightly strong echo mass of 31 × 15 × 20 mm was observed in the left thoracic cavity, CDFI: no aortic flow signal was observed, and the CVR was 0.6. Clinical diagnosis showed space-occupying lesions in the left lung of the fetus, with a high possibility of CPAM (Fig. 10.7). Fetal echocardiography examination showed that the heart shifted slightly to the right and the intracardiac structure was roughly normal. fMRI examination was performed at 28 weeks of gestation, which showed normal amniotic fluid volume and placenta, no fetal edema, normal development, and local signal increased in the left lung, considering high possibility of CPAM (Fig. 10.8). The MDT team

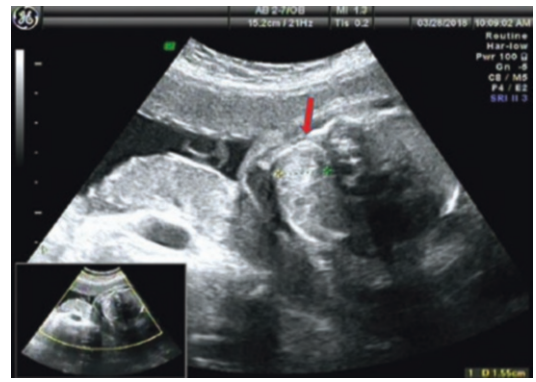

Fig. 10.7 Fetal ultrasound examination shows a slightly strong echogenic mass in the left thoracic cavity, with a high probability of CPAM

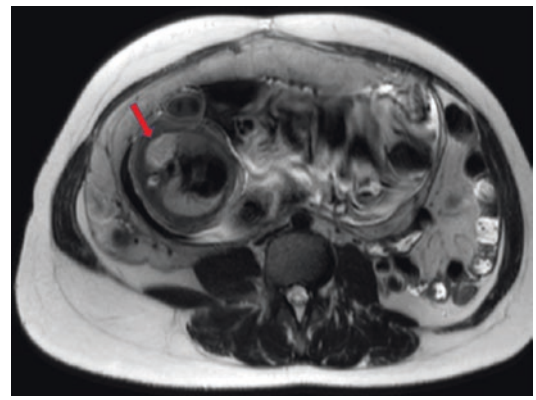

Fig. 10.8 Fetal magnetic resonance imaging demonstrates localized increased signal intensity in the left lung, with a high probability of CPAM. The size of lesion was smaller than that 4 weeks ago

of our hospital performed a comprehensive evaluation on the condition of the fetus, and considered that the fetus was stable without hydrops fetalis-related manifestations. It was recommended to continue the pregnancy, and inform the pregnant woman of relevant risks, relevant treatment regimen and long-term prognosis after delivery of the fetus. The fetus was delivered in our hospital at 37 weeks + 5 days of gestation, G2P2, BW: 3200 g, without cyanosis and asphyxia after birth, and APGAR score: 10/10/10.

The infant was given mixed feeding after birth, and had the same growth and development as a normal child of the same age. Chest CT examination was performed in the outpatient department at 3 and 6 months after birth, respectively, indicating CPAM in the left lower lung (Fig. 10.9). At the age of 6 months, the infant underwent thoracoscopic left lower lobectomy after examination in the pediatric surgery department of our hospital. During the operation, different sizes of cystic changes were found in the left lower lung, with gas and no liquid in the cyst, and mild adhesion between the left lower lung and its surroundings was observed. The lesion was completely resected under thoracoscopy. The operation lasted for 60 min without blood transfusion. The chest tube was removed on postoperative day 2 and the child was discharged on postoperative day 6. Pathological findings confirmed type 2 CPAM. The child was followed up for 3 years, and the imaging reexamination showed good recovery without residual or recurrent lesions.

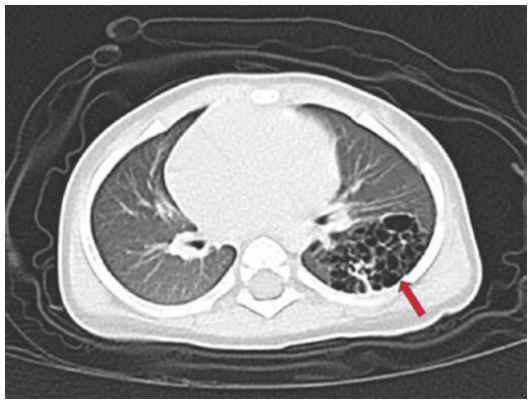

Fig. 10.9 Chest CT examination results at 6 months after birth: multiple cystic lucent shadows with patchy shadows and small nodule-like shadows are observed in the left lower lung, and no abnormal blood flow signal is observed after enhancement, considering CPAM in the left lower lung

10.3.5 Research Progress

Because in utero interventions are complex, carry a high risk, and involve family, social, ethical and other related issues, there is no major breakthrough in intrauterine treatment of CCLL in recent years. Clinical research still focuses on the severity of fetal disease and prognosis assessment, in order to provide reference for clinical treatment. In addition to the CVR mentioned above, lung-thorax transverse-area ratio (L/T) has been proposed to distinguish CPAM from BPS during fetal life in recent years. It has been found that the L/T value of PS significantly increases in late pregnancy, while the L/T value of CCAM slightly decreases. Therefore, continuous monitoring of L/T is of great significance in predicting the pulmonary function of infants with CPAM or BPS after birth [76, 77]. A retrospective analysis was performed in 28 neonates with CPAM, and they were divided into three groups based on the severity of CPAM: mild (n = 7), moderate (n = 13) and severe (n = 8). The investigators found that all infants with severe disease had fetal hydrops and polyhydramnios during fetal period, and the final L/T value was less than 0.25. Thus, when a fetus with CPAM presents with polyhydramnios, hydrops fetalis and L/T < 0.25, the risk of severe dyspnea or death after birth is increased [78].

In summary, with the development of various technologies, the prenatal diagnosis of CCLL is continuously improved, and imaging methods such as ultrasound and MRI can provide evidence for the CCLL evaluation in fetal period. Further study on the relationship between the imaging parameters of CCLL and its changes after birth and long-term prognosis will be helpful for clinicians to make a reasonable treatment regimen.

10.4 Congenital Chylothorax

10.4.1 Introduction

Congenital chylothorax is a congenital anomaly caused by the accumulation of chyle (lymph) in the pleural space in the fetal period [79–81]. It accounts for about 65% of primary pleural effusion, and the incidence is about 1: 10, 000–15, 000. It is more likely to occur in male infants (male-to-female ratio 2:1) and is more common in the right side. Perinatal deaths due to fetal pleural effusion account for approximately 22% to 53% and has a tendency to increase. Therefore, prenatal identification and intervention of congenital chylothorax is of great importance.

10.4.1.1 Etiology

Chylothorax is usually associated with inadequate transport of chyle to the main circulation and can be classified as congenital or secondary depending on the etiology [82]. Drainage obstructed by the mass formed by congenital lymphatic dysplasia and the delayed lymphatic drainage are the important causes of congenital chylothorax in the fetal period. (1) Congenital pulmonary lymphangioma. Multiple lymphangiomas formed by local hyperplasia of well-differentiated lymphoid tissue infiltrates the lungs and other thoracic tissues; (2) Congenital lymphangiectasia. Diffuse dilation of the interlobular and subpleural lymphatic vessels may be caused by a primary developmental defect, or secondary to diseases such as congenital heart disease or pulmonary vein obstruction; (3) Congenital lymphatic dysplasia syndrome. It is an anomaly of lymphatic vessels of undetermined etiology that can cause congenital chylothorax. (4) Congenital lung malformation, congenital diaphragmatic hernia and other fetal chest abnormalities may be associated with congenital chylothorax. Chylothorax may also be associated with chromosomal abnormalities (such as Turner and Noonan syndromes), X-linked myotubular myopathy, and missense mutations of integrin $\alpha_9\beta_1$. Secondary chylothorax of fetal origin is very rare. Thoracic duct rupture or injury caused by trauma can cause chylothorax; thrombus, tumor (lymphoma, neurogenic tumor, teratoma, etc.), and granulomatous infection (tuberculosis, etc.) of the superior vena cava or subclavian vein compress the thoracic duct and so on, causing its proximal pressure to increase, over-expand, or even rupture, resulting in unilateral or bilateral chylothorax [83].

10.4.1.2 Clinical Manifestations and Evaluation

Fetal symptoms [84–87]: Symptoms are usually associated with pleural effusion. A large amount of chylous fluid in the pleural cavity during early pregnancy acts as a space occupying lesion that limits the normal development of the lungs, leading to pulmonary hypoplasia and pulmonary arterial hypertension. Fetal lung development may be normal when congenital chylothorax occurs during late pregnancy. Fetal cardiovascular function is also impaired, cardiac output is reduced, venous return is blocked, and anasarca occurs. The loss of protein reduces the swelling pressure in the vascular compartment and increases lymphatic leakage into the interstitial space. This non-immune heart failure is associated with an increased proportion of fetal death and a worse prognosis when it occurs before 24 weeks of gestation.

Prenatal evaluation: Prenatal examination can be used to determine the amount of pleural effusion in the fetal period, so as to focus on the evaluation of possible accidents during the delivery, and prepare a variety of emergency plans to prevent intrauterine distress, asphyxia, hypoxic-ischemic encephalopathy and even death.

Neonatal symptoms [84–87]: Respiratory distress, heart failure, etc. may occur at birth, and the newborn with chylous ascites may have abdominal and scrotal expansion and edema.

Postpartum management: Take timely measures including oxygen therapy and assisted respiration to deal with various possible symptoms, and give timely surgical treatment if necessary. Minimize the impact on the subsequent lung development and lung function of infants.

10.4.1.3 Diagnosis

Prenatal diagnosis [84–87]: Prenatal ultrasound has a high degree of identification of fetal pleural effusion, and pleural effusion puncture can be performed under the guiding of ultrasound. Chylothorax can be diagnosed if the total cell count of the puncture fluid exceeds 1000 cells/μL (of which at least 80% are lymphocytes) and triglycerides are above 1.2 mmol/L. Magnetic resonance (MR) may also be done prenatally.

Postpartum diagnosis [84–87]: (1) Chest X-ray and chest CT examination can show pleural effusion, and initially evaluate the volume and location of effusion; (2) MR lymphangiography can identify lymph nodes well, but has limited value in evaluating lymphatic vessels. Noninvasive lymphatic magnetic resonance imaging can accurately image the thoracic lymphatics without contrast agent; (3) Lymphoscintigraphy provides high-resolution imaging of the surrounding lymphatic vessels by using filtered 99mTc as a contrast agent and provides insight into lymphatic flow dynamics; (4) Lung biopsy is the gold standard for the diagnosis of congenital pulmonary lymphangiectasis. (5) Thoracoscopy is used for direct observation of the chyle leak point and detection of relevant abnormalities of the lymphatic system, including lymphatic malformations of soft tissues and organs, lymphangiomatosis kaposi, etc. (6) Related gene detection.

10.4.1.4 Treatments

10.4.1.4.1 Fetal Treatments

The goal of prenatal intervention is to mitigate the effects on lung development, achieve normal development as much as possible, and reduce the interference of effusion in venous return and cardiac function. Interventions include conservative monitoring, in utero fetal thoracentesis, thoracoamniotic shunt and in utero pleurodesis. If the amount of chylothorax is small, conservative observation can be performed first. Single in utero fetal thoracentesis guided by ultrasound can effectively resolve pleural effusion and prevent the development of further complications in some fetuses. Some fetuses require thoracoamniotic shunt, which provides continuous one-way drainage of fluid from the chest cavity into the surrounding amniotic cavity. The sonographic signs of pleural hypertension (diaphragmatic inversion) are helpful in determining the timing of surgery. There is a lack of sufficient evidence on the safety and efficacy of in utero pleurodesis with OK-432. Other interventions include pain management in pregnant women, fluid and electrolyte balance, nutritional management, blood pressure support, etc. [84–87].

10.4.1.4.2 Neonatal Treatments

The goal of neonatal treatment is to reduce the volume of chylothorax to keep the pleural space open and allow time for injured lymphatic vessels to heal or form sufficient collateral connections. Diagnostic thoracentesis is usually required, and an indwelling chest tube is necessary if excessive pleural effusion affects respiration or the effusion may recur. Other formulas containing somatostatin analogues (octreotide) for reducing lymphatic secretion, immunoglobulins and antibiotics for preventing the risk of systemic infection, nutritional support and medium chain triglycerides (MCT) can be directly absorbed into the portal system without lymphatic drainage, helping to reduce the production of chylous fluid and sealing leaks, etc. Critically ill patients may require assisted ventilation. Surgical intervention may be considered if conservative treatment is ineffective or has poor efficacy [84–87].

10.4.2 Cases Reports

A 30-year-old pregnant woman, with childbearing history of 0-0-0-0, regular pregnancy schedule, LMP 08-Nov-2021, EDC 15-Aug-2022, urine HCG test at more than 1 month after cessation of menstruation (+), and unobvious early pregnancy reactions (nausea and vomiting) occurred at 2 months of gestation. Prenatal ultrasonography showed that fetal development was basically consistent with fetuses of the same age, fetal movement was good since 5 months of gestation. Regular prenatal examination was performed, thin-layer liquid-based cytology test (−), fetal anomaly scan showed no abnormality, non-invasive prenatal gene test

showed low risk, and the growth was consistent with the gestational age. OGTT(−).

On 10 June 2022, ultrasound examination showed fetal right pleural effusion (free anechoic area 44 × 15 mm), and follow-up reexamination suggested that the amount of effusion gradually increased, and thoracentesis drainage and amniotic fluid reduction were performed 1 week later. During the operation, 1600 mL of amniotic fluid was reduced, and 30 mL of pleural fluid was drawn from the right costophrenic angle of the fetus by ultrasound-guided intercostal puncture. Ultrasound monitoring showed that the lungs were significantly expanded than before (Fig. 10.10). Half a month later, dexamethasone was given to promote fetal lung maturation. The next day, fetal thoracentesis, thoracic drainage tube placement, umbilical vein puncture and amniotic fluid reduction were performed. 1200 mL of amniotic fluid was reduced, 70 mL of pleural fluid was withdrawn from the fetus, a 3F double J tube was placed in the fetal thoracic cavity, and the other end was pushed into the amnion cavity. Five days later, amniotic fluid reduction was performed again, and about 1500 mL of amniotic fluid was drawn (Fig. 10.11). A female infant was delivered the next day due to "premature separation of placenta".

The child was G1P1, gestational age 34 weeks +2 days, cesarean section. Birth weight was 2670 g, and Apgar score was 7, 8 and 9 at 1, 5 and 10 min, respectively. Due to tachypnea and pleural effusion, the newborn was urgently transferred to hospital for oxygen administration by balloon pressure of endotracheal intubation.

Physical examination at admission: body temperature: 36.2 °C, pulse rate: 125 beats/min, respiratory rate: 35 times/min, SpO_2 93% (mechanical ventilation). The newborn had clear consciousness and poor response. The anterior fontanel was flat and soft, with size of 0.8 * 0.8 cm^2 and head circumference of 33 cm. The neck was soft, the trachea was in the middle, the breath sounds of the right lung were low, the breath sounds of the left lung were coarse, and no obvious dry and moist rales were heard in both lungs. Acceptable muscle tension of limbs, incomplete embrace reflex, foraging reflex (−), suction reflex (−), and grip reflex (−).

10.4.2.1 Laboratory Tests

Hematology parameters: WBC 10.78 × 10^9/L, N% 59.5%, L% 15.3%, HB 134 g/L, PLT 332 × 10^9/L, CRP < 1 mg/L.

Blood biochemistry: alanine aminotransferase 6.0 U/L, GGT 165.0 U/L, albumin 25.7 g/L, and lactic acid 4.6 mmol/L;

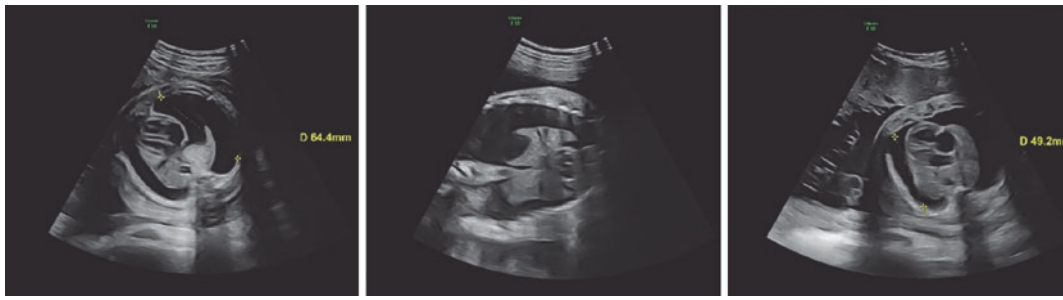

Fig. 10.10 Fetal ultrasound examination showed bilateral pleural effusion

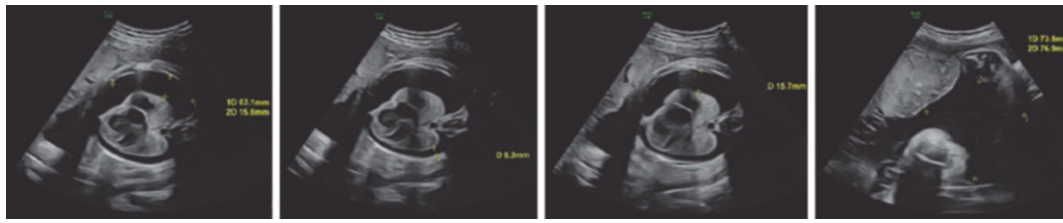

Fig. 10.11 Fetal ultrasound image showed improvement of pleural effusion

DIC: prothrombin time 12.3 s, partial thromboplastin time 63.8 s, and fibrinogen 1.77 g/L.

DD dimer 12.22 mg/L, fibrin (ogen) degradation product 22.42 mg/L.

Detection of hydrothorax and ascites: WBC count 9120 × 10^6/L, WBC differential multiple nuclei 15%, WBC differential mononuclear 85%, Rivalta protein qualitative test: positive (+), chlorine 103 mmol/L, glucose 3.9 mmol/L, quantitative test of body fluid protein: 49.2 g/L, LDH 169 U/L, qualitative test of chylothorax: negative (−).

Immunoglobulin: immunoglobulin G 1.75 g/L, immunoglobulin A < 0.28 g/L,

Chest X-ray at admission (7/7): increased and blurred markings in both lungs (Fig. 10.12).

Chest CT (7/27): Centrally symmetrical changes of bilateral bronchi, airway reconstruction showed right upper bronchial ostial stenosis and bilateral thoracic drainage (Fig. 10.13).

Diagnosis: neonatal respiratory failure, pleural effusion, chylothorax, neonatal pneumonia, premature baby, neonatal hypoproteinemia,

Diagnosis and treatment: reasonable anti-infection, oxygen inhalation, fasting, ventilator assisted ventilation, maintenance of water and electrolyte balance, IVIG and other symptomatic treatment. Octreotide was given from the 14th day of admission to reduce the production of pleural effusion, and gradually increased to 6 μg/kg × h and stopped on the 54th day after birth The child was given total parenteral nutrition during fasting period, and milk was provided on the 45th day after birth, after which the milk amount was gradually increased to be tolerable. Closed thoracic drainage was performed on the right side upon admission, and performed on the left side on the seventh day (Fig. 10.14). Bilateral closed thoracic drainage tubes were removed on the 55th day after birth. On Day 67, the child had normal body temperature, stable oxygen saturation without oxygen inhalation, and basically normal chest X-ray reexamination (Fig. 10.15), so the child was discharged.

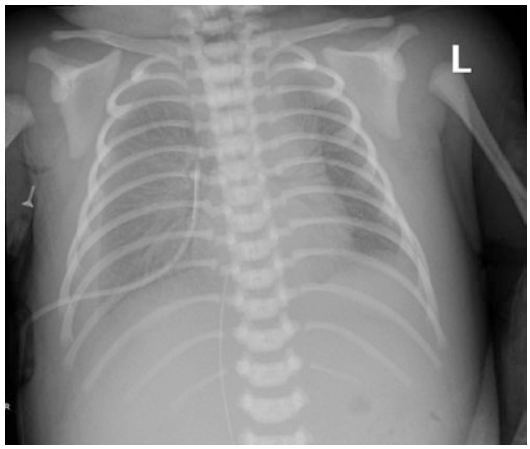

Fig. 10.12 Chest X-ray on the first day after birth (under right thoracic closed drainage)

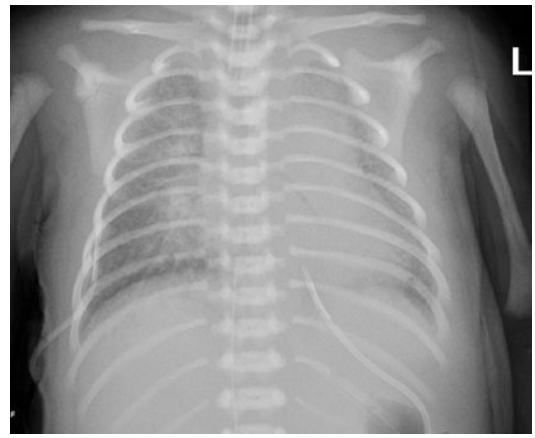

Fig. 10.14 Chest X-ray on the seventh day after birth (under bilateral chest drainage)

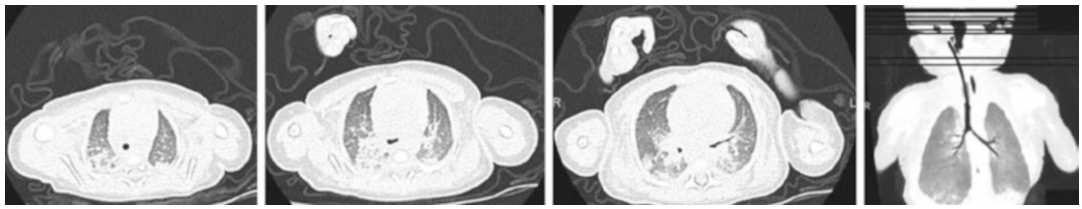

Fig. 10.13 Chest CT image

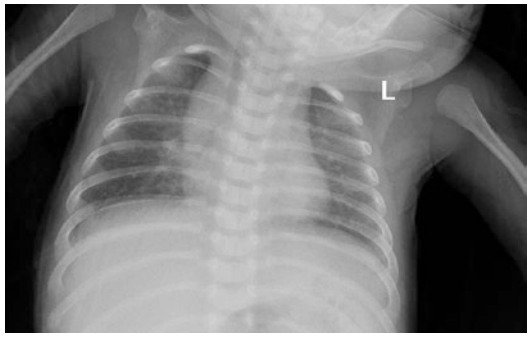

Fig. 10.15 Chest X-ray at discharge (67 days after birth)

10.4.3 Research Progress in Prenatal Diagnosis

Studies on lymphangiogenesis-related genes in chylothorax have focused on vascular endothelial growth factor receptor 3 (VEGFR3), protein tyrosine phosphatase non-receptor type 11 (PTPN11), integrin 9 (ITGA9), and forkhead box protein C2 (FOXC2), but no association of these genes with congenital thoracic duct malformations has been reported [88]. In addition, the detection of lymphangiopathy in newborns using oligonucleotide comparative hybridization with special arrays and SNP arrays is reportedly being carried out. The results are expected to provide more information for prenatal diagnosis.

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Urinary System

11

Sheng-Nan Liu, Yong-Dong Pan, and Guo-Feng Xu

11.1 Introduction

Congenital anomalies of kidney and urinary tract (CAKUT) are common fetal congenital malformations, accounting for 1% of all congenital malformations. The overall incidence of CAKUT is 0.3–0.6% [1]. CAKUT may have different clinical phenotypes, including renal cystic anomalies (such as polycystic renal dysplasia, polycystic renal disease, and obstructive cystic dysplasia), urinary tract obstruction-related diseases (such as renal hydrocele, posterior urethral valves, urorectal septum malformation sequence (URSMS), and megabladder), renal structural and positional anomalies (such as ectopic kidney, renal agenesis, kidney duplex, and horseshoe kidney), lower urinary tract anomalies (such as hypospadias), and associated malformations of the urinary tract (chromosome 17q12 deletion syndrome) [2].

Many factors may lead to fetal urinary system malformations, including genetics, genes, endocrine, and drugs. Early pregnancy screening can detect urinary system abnormalities, which can help clinicians make diagnosis and provide treatment as early as possible, to improve the prognosis and outcome.

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11.1.1 Normal Embryonic Development of the Urinary System

The urinary system is originated from the intermediate mesoderm of the early embryo. From Week 4 of embryonic development, the intermediate mesoderm on the lateral somite starts separating from the somite, forming a pair of cord-like cell clusters, with the rostral end developing into the nephrogenic segment and the caudal end developing into the nephrogenic cord. At Week 5 of the embryonic stage, the nephrogenic cord increases in size, protrudes from the posterior wall of the embryo body into the coelom, and forms a longitudinal bulge along both sides of the spine, known as the urogenital ridge. Then, a longitudinal groove appears in the middle of the urogenital ridge, separated into the lateral mesonephric ridge and the medial gonadal ridge.

The development of the kidney and ureter can be divided into 3 independent stages: i.e., pronephros, mesonephros, and metanephros. The pronephros (see Fig. 11.1) develops from Week 5 of the embryonic stage, and 7–10 pairs of transverse epithelial cell cords appear in the nephrogenic segment at the rostral end, forming the pronephric tubule, which extends caudally and connects at the lateral end to form a longitudinal duct (called the pronephric duct). By the end of the fourth week, all the pronephric tubules have been degenerated, and a small segment of the pronephric duct connected with the pronephric

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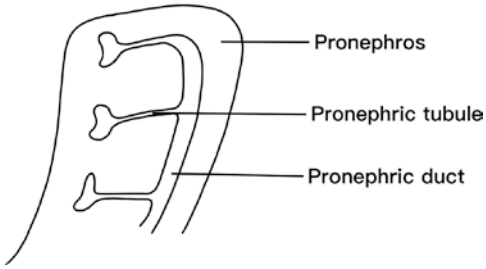

Fig. 11.1 Pronephros

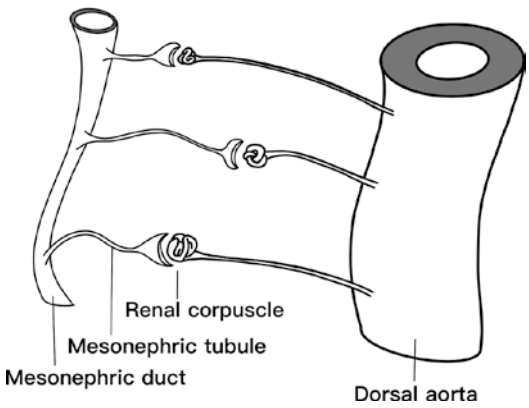

Fig. 11.2 Mesonephros

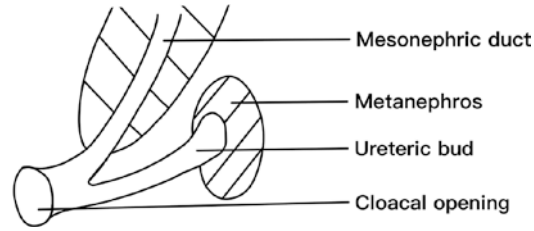

Fig. 11.3 Metanephros

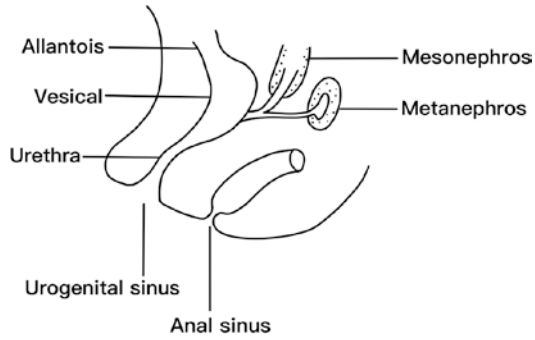

Fig. 11.4 Bladder and Urethra

tubules also have been degenerated, while the other parts are retained, called mesonephric duct. The development of mesonephros (see Fig. 11.2) starts at the end of Week 4. A single-layered cuboidal epithelium from the rostral end of the nephrogenic cord in the thorax and abdomen develops into an "S"-shaped tubule, called mesonephric tubules. The medial end of each transverse tubule is enlarged and caved into a double cup-shaped renal capsule. Capillary bulbs branching from the dorsal aorta are present in the capsule, together with renal capsules to form the renal corpuscle. The development of metanephros (see Fig. 11.3) begins during the development of the mesonephros, that is at the beginning of Week 5 of the embryonic stage. The metanephros is originated from the ureteric bud and the metanephrogenic blastema. The ureteric bud is a blind tube that grows dorsolaterally from the tail of the mesonephric duct near the cloacal opening. The ureteric bud extends into the metanephrogenic blastema, the top of which expands into the renal pelvis, and the trunk is the ureter. Through the induction of the ureteric bud, the mesoderm at the caudal end of the mesonephric ridge forms many dense cell clusters surrounded by the ureteric bud in a cap shape, forming the nephrogenic primordium, which then develops into nephrons.

The bladder and urethra are mainly differentiated from the cloaca and urogenital sinus. (See Fig. 11.4) During Weeks 4–7 of the embryonic stage, the urorectal septum extends caudally and separates the cloaca into a dorsal anal sinus and a ventral urogenital sinus. The urogenital sinus can be divided into upper, middle, and lower segments. The bladder develops from the upper enlargement. The middle segment, also known as the urethral segment, develops into the prostatic part or membranous part of the male urethra, or the female urethra. The lower segment forms the majority of the cavernous portion of the urethra or the vestibule of the female vagina.

11.1.2 Common Diseases and Etiological Factors for Congenital Anomalies of Kidney and Urinary Tract

Urinary system malformations present with different clinical phenotypes. Common urinary system malformations and their possible etiological factors are provided in this section.

11.1.2.1 Renal Cystic Disease

Fetal renal cystic disorders can be classified into four types based on Potter's classification: infantile polycystic kidney, multicystic dysplastic kidney, adult polycystic kidney, and obstructive cystic dysplastic kidney [3].

Multicystic dysplastic kidney (MCDK) is one of the most common renal dysplastic disorders in fetal life. The mechanism of renal obstruction begins in the early stage of embryonic development and interferes with the interaction between the ureteral bud and the metanephric blastema causing kidney dysplasia. Renal dysplasia is morphologically characterized by renal parenchyma with cysts of different sizes and numbers, with normal renal morphology hardly present, which may be accompanied by ureteral dysplasia or atresia [4].

The cause of multicystic dysplastic kidney is still unclear. Related studies have found that abnormal chromosome copy numbers, chromosomal deletion or duplication, and related pathogenic genes may be the pathogenic factors of MCDK. Currently identified causal genes include PAX2 (OMIM: 167409), SIX2 (OMIM: 604994), TCF2 (OMIM:189907) [5–10]. In addition, chromosomal abnormalities (such as 17q12 microdeletions, 22q13.31 microdeletions, 10q24.3 microdeletions, 10q24.3 microdeletions) may lead to MCDK. Among them, microdeletions and microduplications due to isolated MCDK, 17q12 and 22q11.2 structural aberrations are the most common chromosomal abnormalities [11]. MCDK has also been reported in related fetal anomaly syndromes such as Digeorge syndrome and Kallmann syndrome.

Multicystic dysplastic kidney mostly occurs in the unilateral kidney, but a recent study of 97 children with MCDK found that about 20% of these children also had structural abnormalities in the contralateral kidney [12]. The prognosis of children with MCDK mainly depends on the function of the unaffected kidney. Earlier ultrasound and genetic screening during pregnancy may preserve the unaffected kidney function in children with MCDK. It is important to monitor amniotic fluid volume during pregnancy because the normal volume may indicate temporarily normal renal function, while low volume or poor bladder filling on fetal ultrasound may indicate renal failure with a poor prognosis [2].

Obstructive cystic dysplasia is Potter type IV renal cystic disease, and ultrasound during pregnancy can detect renal hydrocele or ureteral dilatation, renal shrinkage, and renal parenchyma echo enhancement. If renal hydrocele is detected during pregnancy, the fetus should be thoroughly scanned to rule out other urinary system malformations or multisystem malformations. Early multidisciplinary consultation, including pediatric urology, can provide a detailed follow-up program for pregnant women and their families.

11.1.2.2 Urinary Obstructive Disorders

(1) Congenital hydronephrosis (CHn) refers to the renal collecting system dilatation that exists in the fetal period. The International Fetal Society defines it as a separation of the renal collecting system of more than 0.5 cm before 24 weeks of gestation and more than 1 cm after 24 weeks of gestation and in the neonatal period. Renal hydrocele is common in fetal ultrasound, but is mostly clinically insignificant, and can disappear spontaneously. Progressive and persistent renal hydrocele requires clinical monitoring and attention. The severity of renal hydrocele can be clinically graded by SFU and APD.

APD refers to the value of the anteroposterior diameter of the renal pelvis in the cross-section of the kidney. The APD is an objective and quantitative grading system for renal hydrocele. Renal hydrocele is diagnosed as APD $\geq$ 4 mm during the second trimester and $\geq$7 mm during the third trimester. During the second trimester of pregnancy, 4 mm $\leq$ APD < 7 mm is considered renal

hydrocele, 7 mm ≤ APD ≤ 10 mm is considered moderate renal hydrocele, and APD > 10 mm is considered severe renal hydrocele. During the third trimester of pregnancy, 7 mm ≤ APD < 9 mm is considered mild renal hydrocele, 9 mm ≤ APD ≤ 15 mm is considered moderate renal hydrocele, and APD > 15 mm is considered severe renal hydrocele [13].

SFU grading system is based on the renal pelvis, calyces, and kidney. SFU Grade 0 refers to no dilatation of the collecting system; SFU Grade 1 refers to no extrarenal renal pelvis dilatation; SFU Grade 2 refers to renal pelvis dilatation to extrarenal and major calyceal dilatation; SFU Grade 3 refers to SFU Grade 2 features + minor calyceal dilatation; SFU Grade 4 refers to SFU Grade 3 features + renal cortical thinning. A limitation of the SFU system is the difficulty in distinguishing segmental calyceal dilatation or parenchymal thinning [14].

Pathological renal hydrocele has many etiological factors, obstructive factors being the most common. They include vesicoureteral reflux, ureteropelvic junction obstruction (UPJO), ureteral orifice cyst, posterior urethral valves (PUV), and primary non-reflux megaureter. Rare factors include the ectopic ureter, ureterovesical junction obstruction (UVJO), and ureteral stricture.

The prognosis of fetal renal hydrocele mainly depends on the etiology of renal hydrocele, the onset of renal hydrocele, the separation degree of the collecting system, renal cortex atrophy, and fetal renal function [15, 16]. Clinicians decide the follow-up and treatment based on the separation degree of the collecting system in the fetal period. When the anteroposterior diameter of renal pelvis dilatation is >15 mm, high possibility of obstructive disorder is considered. It should be actively followed up after delivery and surgically treated if necessary; When the it is 10–14 mm, postpartum follow-up examination should be performed; When it is 4–10 mm, it may be normal or physiological and the follow-up observation during pregnancy is sufficient [17, 18].

Fetal lower urinary tract obstruction (LUTO) is a disorder of urinary tract excretion due to the posterior urethral valve, urethral atresia, or urethral stricture. Micturition disorder can lead to in utero oligohydramnios, which may result in fetal lung hypoplasia and increase neonatal death. In addition, severe lower urinary tract excretion disorders, and bladder function damage due to intervention may lead to renal dysplasia [19, 20]. Posterior urethral valves (PUV) are the primary cause of lower urinary tract obstruction in fetuses, with an incidence of 1/4000–1/8000. Soft tissue valves are present in the posterior urethra, which leads to urethral obstruction. Ultrasound diagnosis of posterior urethral valves is the main basis of prenatal diagnosis and can be detected during the first trimester of pregnancy. The dilated bladder neck can manifest as a funnel-like protrusion into the dilated posterior urethra (keyhole sign) by ultrasound. In addition, thickened bladder muscle trabeculae, and increased bladder thickness can be seen by ultrasound. Some children may present with ureteropelvic dilatation and oligohydramnios.

11.1.2.3 Renal Morphological and Positional Disorders

Ectopic kidney refers to the failure of the kidney to reach its normal position during metanephros development. The metanephros is supplied through branches of the abdominal aorta during development. With the degeneration of the lower branch-supplying vessels, the higher branch vessels are supplying for the kidney. When the degeneration of the lower branch vessels fails, the degenerated vessels may make an obstacle of the ascending of the kidney, resulting in a lower ectopic kidney. Excessive ascent may enter the chest cavity. Ectopic kidney can be divided into pelvic ectopic kidney, crossed ectopic kidney, and thoracic ectopic kidney according to their location [21]. Ectopic kidney is highly suspected when no renal echoes are detected in the normal renal area, and the ipsilateral adrenal gland shows a "supine" sign by ultrasonography [22]. Children with ectopic kidney are mostly concurrent with other urinary system malformations such as renal dysplasia, kidney duplex, ectopic ureteral orifice, and fused kidney.

Renal agenesis results from ureteral agenesis or failure to induce differentiation of the metanephric primordium into the metanephros during embryonic development, with an overall prevalence of 0.04% of postnatal unilateral renal agenesis [23]. Renal agenesis is mostly unilateral onset; if it is

bilateral, it could be a serious and fatal malformation. Once confirmed bilateral renal agenesis, the pregnancy should be terminated as early as possible. Previous research suggested that renal agenesis has a genetic predisposition. In addition, trisomy 21, Turner's syndrome, and chromosome 22q11 deletion syndrome are associated with chromosomal anomalies. Mutations in several genes have also been shown to be associated with the pathogenesis of renal agenesis, such as HNF-1β, PAX2, SALL1, SIX1, and EYA1 [24, 25]. During pregnancy, fetal renal agenesis can be diagnosed by ultrasound. Oligohydramnios often occurs if bilateral renal agenesis is present, which makes it difficult for ultrasound penetration. Both amnioinfusion and MRI can improve the diagnostic rate of renal agenesis. The prognosis of renal agenesis is closely correlated with concurrent malformations. For unilateral renal agenesis, the unaffected kidney is enlarged to compensate the body's urinary functions. When accompanied by extrarenal malformations, such as cardiac and digestive tract malformations, it indicates a poor prognosis.

Kidney duplex is also one of the common congenital anomalies of urinary system in children, which is often accompanied by a ureteral cyst, ectopic ureteral orifice, and ureteral reflux [26]. During embryonic development, double ureteral buds develop at the lower end of the mesonephric duct, or the top of the ureteral bud splits into two bundles, which might form a kidney duplex. The kidney duplex has different anatomical types, such as complete duplication of the ureter into the bladder, or the duplicated ureter joining the normal ureter in the middle before draining into the bladder. In prenatal diagnosis, MRI has definite value and advantage in the diagnosis of kidney duplex. The dilated renal pelvis, calyces, and ureter could be clearly displayed on MRI. Confused by bowel, clinicians have difficulty in identifying dilated ureter [27]. Due to its concurrent malformations, such as ectopic ureteral orifice and ureteral cyst, kidney duplex may cause recurrent urinary tract infection or urine leakage and other clinical manifestations, which require postpartum diagnosis or even surgeries.

Horseshoe kidney is one type of renal fusion disorder. During Weeks 5–8 of the embryonic stage, the lower renal grades on the left and right sides interconnect and fuse to form a horseshoe kidney. Horseshoe kidney can be diagnosed by ultrasound during pregnancy, which shows that both kidneys are in the prespinal position, and both kidneys are fused in front of the abdominal aorta and inferior vena cava in a "U" shape. Studies have shown no significant risk of genetic abnormalities in isolated fetal horseshoe kidney. Fetuses with Turner's syndrome and trisomy 21 have a higher probability of horseshoe kidney [28, 29]. Patients with horseshoe kidney generally do not require special treatment. However, when accompanied by disorders such as kidney stone, hydronephrosis, urinary calculi, or tumors, it is more difficult to manage due to the abnormal anatomy of the horseshoe kidney.

11.1.2.4 Genetic Syndromes Associated with Urinary System Abnormalities

Prune-Belly Syndrome (PBS) is a rare congenital malformation characterized by a triad of abdominal wall muscle defects, urinary tract abnormalities, and bilateral cryptorchidism. The incidence rate is about 1/50000 [30]. The etiology of PBS may be that during Weeks 6–10 of embryonic development, mesodermal abdominal wall and urinary system muscles stop developing due to external factors. In addition, some scholars believe that the etiology may include urethral obstruction or chromosomal aberrations. Because Prune-Belly Syndrome is often concurrent with multiple system malformations. Ultrasound diagnosis during the first trimester of pregnancy can help improve the prognosis. PBS ultrasound images show the following features: (1) a large cystic mass in the fetal abdominal cavity; (2) thin fetal abdominal wall. Early diagnosis of PSB should be distinguished from diseases with secondary enlargement of the bladder due to other lower urinary tract obstructions such as posterior urethral valves and urethral atresia. The prognosis of PBS is mainly associated with the presence or absence of concurrent malformations. After the early diagnosis of PBS, Ekwunife placed a drainage tube in the bladder of PBS children without other malformations and drained urine into the amniotic cavity to improve the pulmonary developmental environment and improve the prognosis of children [31].

11.2 Clinical Practice

11.2.1 Congenital Hydronephrosis (CHn)

11.2.1.1 Introduction

Congenital hydronephrosis is one of the common urinary system malformations in children, with the most common etiology of UPJO [32]. At prenatal visits, the incidence rate of renal hydrocele is about 1–2% [33–35]. It may have a major impact on the developing fetus when bilateral severe hydronephrosis is present, including subsequent development of fetal renal impairment, pulmonary dysplasia, and renal failure in the neonatal period [36]. However, with the rapid development of prenatal interventions, postnatal respiratory support, and renal replacement therapy, the short- and long-term outcomes in children with severe renal hydrocele during pregnancy have been further improved.

11.2.1.2 Case Report

A 31-year-old pregnant woman was found to have separation of the fetal bilateral renal collecting systems by ultrasonography at 13 weeks of gestation, 3 mm on the right and 5 mm on the left. Amniocentesis was also performed, and karyotype analysis showed no abnormalities. The patient was transferred to our hospital at 26 weeks of gestation because of continuous enlargement of the left renal hydrocele in a follow-up examination. Fetal magnetic resonance imaging showed poor right kidney morphology, multiple cystoid fluid signals, with the larger one about 25 × 15 mm, absence of normal left kidney structure, and a large cystoid signal in the left abdomen of about 75 × 55 mm, and severe renal hydrocele in the left kidney was suspected (Fig. 11.5). After multi-disciplinary consultation with obstetrics, pediatric urology, neonatology, anesthesiology, and imaging, the child underwent fetal renal puncture and drainage under ultra-

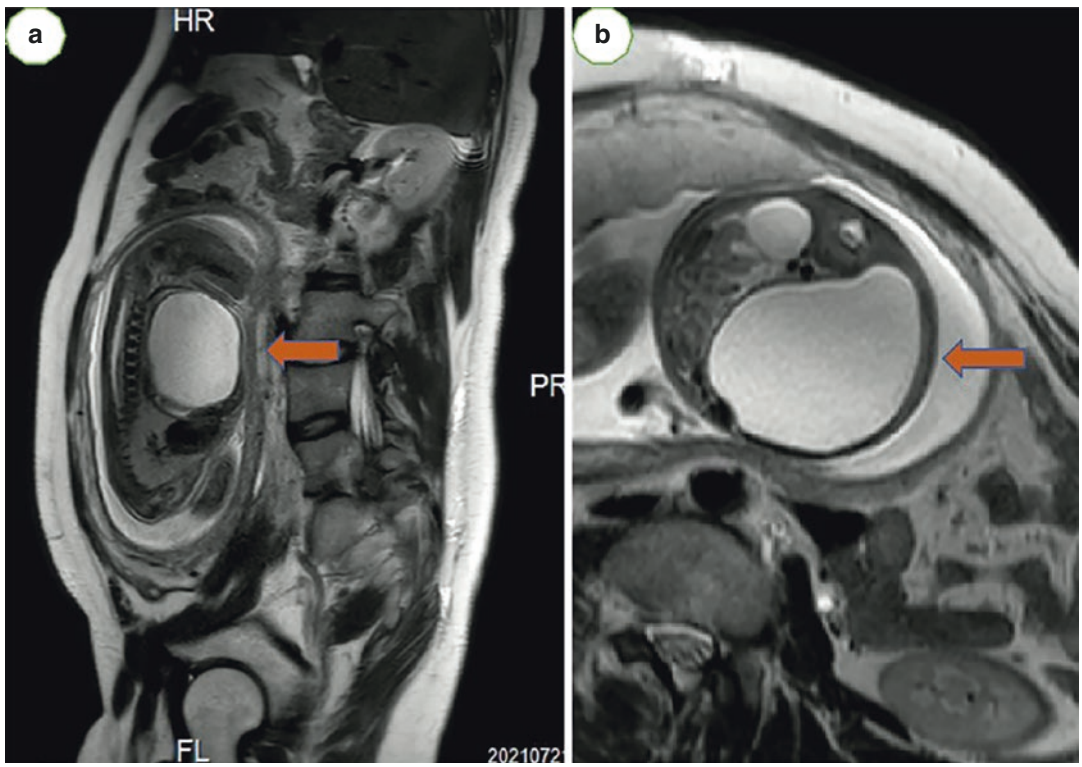

Fig. 11.5 Panel A shows Sagittal MR images of the fetus in A and cross-sectional images. The arrow indicates a large cystic signal in the left abdomen of the fetus, and compression and displacement of a part intestinal lumen in the fetus are shown in Panel B

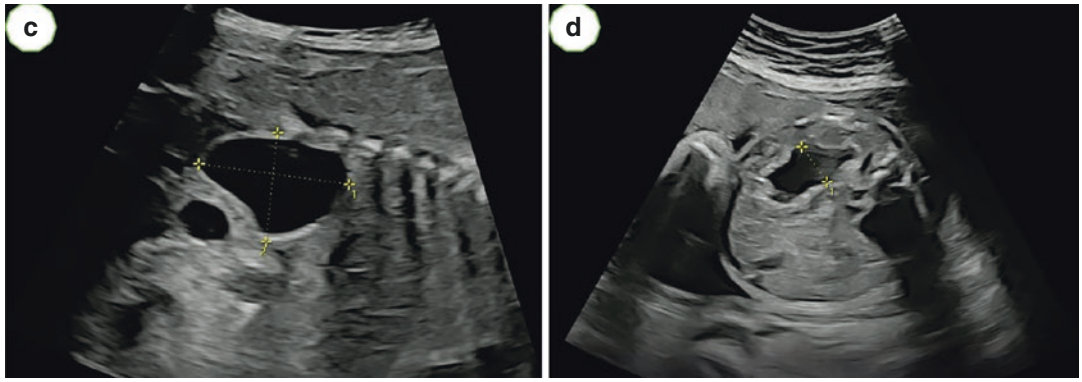

Fig. 11.6 Panel A shows ultrasound images before the first renal puncture drainage of the fetus. Panel B shows ultrasound images after 3 days of fetal first renal puncture drainage. The yellow dashed line shows the size of fetal left renal hydrocele, with left renal pelvis separation of 37 mm and 16.6 mm, respectively

sound localization at 27 weeks + 4 days of gestation. 100 mL of urine was drawn during the operation. Ultrasound monitoring showed that the left renal hydrocele basically disappeared. Routine inhibition of contractions and prevention of infection were performed postoperatively. Re-examination of ultrasound before discharge indicated a 16.6 mm separation of the left renal pelvis of the fetus (Fig. 11.6).

Ultrasound re-examination 2 weeks after puncture showed that fetal left hydronephrosis was significantly increased. Fetal renal puncture and drainage were performed successively at 28 weeks + 4 days, 30 weeks + 6 days, and 32 weeks + 2 days of gestation. Ultrasonography at 36 weeks +5 days of gestation showed two anechoic zone in the left kidney of the fetus of about 36 × 22 mm and 70 × 50 mm, and two anechoic zones in the right kidney of about 19 × 17 mm and 50 × 32 mm. After consultation, considering that continued pregnancy could lead to fetal renal impairment, endanger fetal life and prognosis, it was decided to induce labor with a water sac. Urinary ultrasound, magnetic resonance imaging, and radionuclide renography after birth showed congenital bilateral renal hydrocele, left ureteral cyst, and right kidney duplex. The child had urinary tract infection 10 days after birth, and underwent a cystoscopic laser ureterocele incision and puncture fistulization of the left perirenal cystic mass after multidisciplinary consultation. Since then, left perirenal infection have sustained, with poor drainage and non-functional left kidney. Then the left kidney and surrounding tissue resection was performed in June. The infection indicators returned to normal post-operation, and the child has been followed up regularly to date.

11.2.1.3 Prenatal Diagnosis

With the widespread and application of ultrasound antenatal screen, more congenital anomalies of urinary system can be identified prenatally [37]. At present, the diagnostic criteria of fetal renal hydrocele are not consistent domestic and abroad. Moreover, the timing of intervention and prognosis of fetal and neonatal renal hydrocele are controversial. Currently, the commonly used grading methods for renal hydrocele are the Society of Fetal Urology (SFU) grading system and antero-posterior-diameter (APD) of the renal pelvis. The APD grading system is mostly used in the evaluation of fetal renal hydrocele [38] However, it is still not clear or consistent enough for APD grading. It is most commonly accepted that APD ≥ 4 mm before 33 weeks of gestation or ≥7 mm after 33 weeks of gestation can identify 100% of fetuses with eventual impaired renal function or requiring surgery, as reported in the study by Corteville et al. [39]. Nguyen et al. [40] subdivided the APD grading of fetal renal hydrocele to three categories: mild, moderate and severe (Table 11.1). Therefore, as described herein, severe bilateral hydronephrosis is diag-

Table 11.1 Grading of Renal Hydrocele and Postpartum Urinary Disease Risk (adapted from Nguyen et al. [40])

	APD of the renal pelvis		Risk assessment of predictors
Grade	The second trimester (mm)	The third trimester (mm)	% (95% CI)
Mild	4–7	7–9	11.9(4.5–28.0)
Moderate	7–10	9–15	45.1(25.3–66.6)
Severe	>10	>15	88.3(53.7–98.0)

nosed if the anteroposterior diameter of the renal pelvis for bilateral renal hydrocele during the first trimester of pregnancy exceeds 15 mm.

11.2.1.4 Risk Assessment of the Fetus

The diagnosis and prognosis of fetal renal hydrocele is still a key and challenging part in prenatal counseling, and the accurate risk assessment plays a critical role in deciding whether to intervene, when and how to intervene in the follow-up treatment plan.

In general, ultrasound, magnetic resonance and other imaging examinations can be used to determine the grade or severity of fetal renal hydrocele, renal parenchyma, amniotic fluid volume, ureterectasis, renal length, bladder filling and other characteristic parameters [41]. In addition, fetal renal function can be evaluated by laboratory tests such as fetal urine biochemical indicators [42] and serum β-1 microglobulin [43]. In a meta-analysis predicting postpartum renal function, amniotic fluid volume and renal parenchyma were found to have the highest predictive value, with oligohydramnios having a sensitivity of 63% and a specificity of 76%, and renal parenchyma having a sensitivity of 57% and a specificity of 84% [44]. In this case, the fetal amniotic fluid volume during pregnancy was normal, while the left renal parenchyma was very thin. The ultrasound at 36 weeks +1 day of gestation showed that the left renal parenchyma thickness was only 3.3 mm, and the final outcome was surgical resection due to non-function.

11.2.1.5 Treatments

The common treatments of fetal renal hydrocele include pregnancy termination, expectant management, early delivery, and in utero treatments. In utero treatments can improve fetal survival rate and renal function to certain extent, but the selection of treatments is still controversial. Therapeutic measures reported in the literature include open fetal surgery, thoraco-amniotic shunt, PUV fetal cystoscopy laser ablation, and ultrasound-guided percutaneous vesico-amniotic shunt [45]. The most commonly used fetal intervention is vesico-amniotic shunt [46], which can correct oligohydramnios due to urinary obstruction by transferring urine to the amniotic cavity, promote the development of fetal kidneys and lungs, and ultimately improve the fetal disease course. However, to date, there are still insufficient data to support the benefits of fetal interventions [47].

11.2.2 Posterior Urethral Valves (PUV)

11.2.2.1 Introduction

Posterior urethral valves are the most common disorders in male congenital lower urinary tract obstruction, accounting for 2–3 per 10,000 newborns, with a prenatal diagnosis rate of up to 62% [48]. The embryology of the PUV is unknown. It may be resulting from the ectopia of the mesonephric duct to the cloaca [49]. The PUV can cause great damage to the urinary system, such as decreased urine output, oligohydramnios which lead to pulmonary hypoplasia during pregnancy. Even if timely treatment is given after birth to remove the posterior urethral valve and relieve obstruction, 40% patients still require drugs or intermittent clean catheterization to assist empty the bladder [50], and up to 30% of patients need lifelong renal replacement therapy [51].

11.2.2.2 Case Report

A 35-year-old pregnant woman showed no significant abnormalities on ultrasound during 11–14 weeks of gestation [52]. At 16 weeks of

gestation, ultrasound showed a distended fetal bladder (about 34 mm in width) with typical keyhole sign, mild renal hyperechogenicity with mild renal hydrocele, and amniotic fluid volume at the lower limit of normal, with no other systemic abnormalities found. Amniocentesis has indicated 46, XY karyotype. The condition of the fetal urinary tract deteriorated markedly after 2 weeks, and ultrasound showed an increased bladder width of 61 mm and a marked dilatation of the renal pelvis and calyces. After being informed of the prognosis and the prenatal intervention regimen, the pregnant woman agreed to undergo the balloon catheter dilatation procedure.

The pregnant woman was local anesthetized by intragluteal injection of fentanyl and atracurium. An 18-gauge needle was inserted into the fetal bladder under ultrasound guidance. A coronary catheter was inserted into the urethra, and a Maverick2 balloon catheter (2 mm in diameter and 9 mm in length) was inserted using the guide wire to dilate the fetal urethra. The balloon is inflated in the lower urethra of the fetus. Fetal urinary flow returned to normal immediately post-operation, and ultrasound examination 1 day post-operation indicated successful bladder decompression with normal amniotic fluid volume. The pregnant woman was currently at 27 weeks of gestation, with normal fetal amniotic fluid volume, and there was no evidence of urinary tract obstruction.

11.2.2.3 Prenatal Diagnosis

Posterior urethral valves can be diagnosed prenatally or postnatally, and no differences in outcome between prenatally and postnatally diagnosed PUVs have been identified [53]. In prenatal diagnosis, ultrasound is the most common method to evaluate fetal lower urinary tract obstruction, and magnetic resonance imaging is also being used increasingly. Prenatal ultrasound secondary to lower urinary tract obstruction usually presents with a dilated and thick-walled bladder and a dilated posterior urethra, combination of which to produce a "keyhole" appearance, the keyhole sign; The associated ureter hydronephrosis may be asymmetric, with unilateral ureter hydronephrosis in approximately 15% of cases with posterior urethral valves [54]. In the study by Liao et al. [55], it was noted that a megabladder found during the first trimester of pregnancy, with a bladder diameter of 7 mm or greater in the longitudinal direction, usually can resolve spontaneously, whereas it is unlikely to resolve when distended to >15 mm. Since the exact cause of fetal bladder distention could not be assessed by ultrasound, Welsh et al. [56] and Ruano et al. [57] evaluated the utility of fetoscopy in determining the cause of obstruction and concluded that direct fetal cystoscopy (83.3–100%) was more sensitive than ultrasound (62.5–63.6%) in the diagnosis of posterior urethral valves. These findings should be verified by ultrasound and other examinations after birth, and the diagnosis depends on micturition cystography.

11.2.2.4 Risk Assessment of the Fetus

Although oligohydramnios and bilateral cortical cysts make it difficult to determine the degree of prenatal renal dysplasia, they are statistically significant in predicting postpartum renal function, with a specificity of 67–75% for oligohydramnios and 89% for cortical cysts in predicting poor renal function (blood creatinine >1.2 mg/dL) at 1 year of age [58]. There have been controversies over the utility of fetal urinalysis, which include sodium, calcium ionized, chloride, osmolality, and β2 microglobulin, to further measure the degree of renal impairment. The results of a systematic review have shown that elevated fetal urine sodium, calcium, and β2 microglobulin concentrations did not significantly predict postpartum renal dysfunction [59]. It has also been suggested that serial analysis of fetal urine by bladder puncture every 48–72 h is more representative of renal reserve than analysis of single urine from fetuses with lower urinary tract obstruction [59]. In a latest study [60], 67 fetal urinary peptides were successfully used to predict postnatal kidney survival in fetuses with posterior urethral valves. The specificity and sensitivity were higher than traditional fetal urinalysis and ultrasound.

11.2.2.5 Treatments

The treatments of fetal lower urinary tract obstruction still constitute an unresolved and widely discussed issue, and the main goal of its prenatal intervention is to reduce postnatal morbidity and mortality. At present, there are two main techniques commonly used in various centers: vesico-amniotic shunting (VAS) and posterior urethral valve ablation under fetal cystoscope. Urethral stent implantation through the fetal bladder has also been reported [61]. In order to overcome the technical limitations of the fetoscopic procedure and avoid the complications inherent to the fetoscopy and VAS, urethroplasty was achieved in this case by using a balloon catheter in coronary angioplasty. The first advantage of this technique is less invasive and does not indwell a stent in the fetus. Secondly, the entire procedure is performed guided by ultrasound; a flexible and trackable catheter can be easily steered in all directions and inserted into a narrow space. Because the fetal urethra is only mechanically dilated, the catheter is removed immediately after the procedure, so there is no risk of thermal injury or long-term irritation to surrounding tissues.

11.3 Studies

With the advances in fetal medicine, in utero surgeries have gradually developed. The purpose of the in utero operation is to control the development of pathological changes in fetal tissues and organs, and improve the function of fetal tissues and organs to allow for normal fetal development and maturation. Common treatment methods include drugs, surgeries, and so on. With the development of minimally invasive techniques, in utero therapeutic shunts and fetal cystoscopy have been continuously used in prenatal intervention to improve urinary system hydrops during the embryonic stage, and can effectively improve the pre and postnatal survival of the fetus.

11.3.1 In Utero Surgeries of Urinary System

In utero shunt surgery has been an important method of in utero surgeries since its first application in the 80's. In 1997, Coplen found that persistent oligohydramnios was a risk factor for poor fetal prognosis through the previous study on operations of in utero fetal renal hydrocele. However, it was difficult to diagnose the cause of in utero renal hydrocele by in utero ultrasound. Only 47% of the perinatal survival rate achieves a breakthrough in the in utero treatments of the urinary system. However, 45% rate of complications also pose a great challenge to in utero surgery [62].

In utero treatments of fetal renal hydrocele have certain limitations. The incidence of fetal congenital urinary system malformation is about 1%, in which only 1/500 may lead to oligohydramnios and other complications. Therefore, more than 90% of fetuses with renal hydrocele do not require in utero surgery. For renal hydrocele with normal amniotic fluid volume, it should be monitored regularly by ultrasound follow-up. Then relevant treatments should be carried out after delivery if necessary. When amniotic fluid volume is moderately to severely reduced, the fetal prognosis should be fully evaluated. If severe organ hypoplasia occurs, pregnancy may be terminated, and labor induction can be performed as early as possible as clinically indicated. When there is no fetal impair of reneal function and the lung of fetalis mature, early delivery is recommended to provide early fetal treatments after birth. Most importantly, when the fetal kidney function is adequate, and the lungs are mature, in utero shunt surgery can reduce the pressure of urinary obstruction, and increase the amount of amniotic fluid, consequently promoting the development of lung maturity, and reducing the renal function injury [63].

Common urological-related in utero procedures include percutaneous fetal kidney puncture, percutaneous fetal vesico-amniotic shunt, pyelo-amniotic shunt and etc. This section mainly introduces the surgical methods, complications and prognosis of shunts and fetal cystoscopy.

11.3.2 Urinary System Shunt Procedures

The bladder-amniotic shunt is mainly used for fetal lower urinary tract obstruction to drain

through bladder drainage tube, increase amniotic fluid volume, promote fetal lung development and maturation, and reduce the damage of urinary tract obstruction to renal function. At present, the vesico-amniotic shunt is considered to contribute to improvement of perinatal survival. No studies have found statistical significance in long-term fetal renal function and survival rate. The optimal surgical timing for vesico-amniotic shunt is during 18–26 weeks of gestation. The procedure is performed by placing one end of a dual-lumen pigtail catheter in the fetal bladder and the other in the amniotic cavity under ultrasound guidance. Perfusion of the amniotic cavity before and after catheterization can help fill the amniotic cavity, thus improving the surgical field and increasing the accuracy of catheter placement. Previous studies have found that vesico-amniotic shunt has a high incidence of complications. The incidence of postoperative complications such as vesico-peritoneocutaneous fistula and displacement of the drainage tube is about 30–45%. In a cohort study of 246 cases with lower urinary tract obstruction, vesico-amniotic shunt significantly improved perinatal survival, with no significant difference in renal function and survival at 6 months after birth [64].

Pyelo-amniotic shunt is used for upper urinary tract obstruction. The procedure was ultrasound-guided insertion of a trocar into the amniotic cavity through abdominal puncture. Push the pigtail catheter through the cannula, distal to the renal pelvis and proximal to the amniotic cavity. The fetal abdominal wall was selected as the puncture site. Nassr et al. [65] concluded that the combination of fetal ultrasound parameters and urine biochemistry can predict the development of fetal renal function. Therefore, biochemical samples should be regularly collected during pyelo-amniotic shunt. By exploring the perinatal outcome of bilateral renal hydrocele after pyelo-amniotic shunt, Wu Wenjuan's team in China have found that the 28-day survival rate and operation rate in the in utero treatment group were higher compared to the conservative treatment group; while for the half-year survival rate, 1-year survival rate, and nephrectomy rate, no significant differences were observed [65]. Previous studies have shown that the conduction of pyelo-amniotic shunt in patients during pregnancy reduces the compression of fetal renal parenchyma, speculating that the increase of amniotic fluid volume can promote fetal lung development, and increase the survival rate of perinatal infants.

11.3.3 Fetal Surgeries with Cystoscopy

The first fetal cystoscopy was performed in 1954 by Westin guided by a hysteroscope to insert into the amniotic cavity. Since then, it has been used during in utero surgery, tissue biopsy and other procedures. The specificity of prenatal ultrasound for the diagnosis of fetal lower urinary tract obstruction is not high. While fetal cystoscopy, due to its direct vision, has certain advantages in the diagnosis of fetal lower urinary tract obstruction. The doctor inserted the fetal bladder layer by layer through the skin and subcutaneous tissue of pregnant women through intraoperative ultrasound localization, avoiding the placenta and fetus, and then performed the surgical operation [66]. There are no authoritative comparative data about the use of fetal cystoscopy with shunt surgery. Vinit has noted that there have been no studies demonstrating fetoscopy is more effective than shunting in relieving lower urinary tract obstruction [67]. For fetal cystoscopy (c), the most common complications are premature rupture of membranes and perineal fistula. In addition, placental and fetal damage, abortion, premature labor and amniotic cavity infection, as well as amniotic fluid leakage have occurred [68]. Compared with shunt, fetal cystoscopy only requires a single operation without multiple catheterizations, and it is easier to relieve the lower urinary tract obstruction and keep the bladder drainage unobstructed under direct vision. With the development of fetal cystoscopy, it may be further used in clinical practice. Its effectiveness and safety will be further verified.

11.3.4 Discussion

Taken together, urinary system-related in utero surgeries are mainly used for renal hydrocele. It can help urinary drainage and increase the amniotic fluid volume through shunts, fetal cystoscopy, etc., thus reducing kidney injury due to urinary tract obstruction. On the other hand, urinary drainage leads to an increase in amniotic fluid volume and promotes fetal lung maturation. In utero treatments have shown to significantly improve perinatal survival in fetuses with surgical indications, but no definitive findings have been found regarding long-term survival and renal function improvement.

The best timing for in utero surgery is from 20 to 30 weeks of gestation. The indications for operation include: (1) persistent bladder dilatation with upper urinary tract dilatation; (2) bladder outlet obstruction; (3) amniotic fluid volume decreased or too little; (4) obstruction affecting the development of kidney and lung. (5) megabladder; (6) severe bilateral renal hydrocele due to obstruction of the urinary tract [69]. Clinicians should fully understand the surgical indications, cautiously decide the in utero surgical method, and communicate well with the families. For facilities which may provide in utero treatments, multidisciplinary cooperations are required to ensure the safety of the mother and infant.

11.3.5 Sequential Managements of the Urinary System

The sequential managements of fetal urinary system malformation include prenatal examination and follow-up, prenatal treatments (drug and surgery), early cesarean section and timely termination of pregnancy. Postpartum managements include follow-up and treatments (drugs and surgery).

For congenital renal hydrocele, clinicians may follow the UTD grading proposed by the 2014 American Urological Association consensus for follow-up examinations (see Table 11.2) [70]. In addition, due to different etiological factors of renal hydrocele, the clinical treatments varied, that is, the diagnosis and management differs for different etiologies.

Table 11.2 Follow-up plan for congenital renal hydrocele UTDA Grade A1 to A3

	UTA A1	UTD A2-A3
Fetal period	If UTD A1 is diagnosed before 32 weeks of gestation, B-ultrasound examination should be performed once after 32 weeks of gestation. If UTD A1 is relieved, the follow-up could be stopped	Re-examination of B-ultrasound 4–6 weeks after initial diagnosis
After birth	If resolved, B-ultrasound for at least twice: after birth (1) >48 h to 1 month; (2) 1–6 months	B-ultrasound performed >48 h to 1 month
Others	Pay close attention if necessary	Expert consultation

Certain conditions, such as PUV or bilateral severe renal hydrocele, may require more appropriate treatments

Renal hydrocele due to different etiological factors requires different clinical diagnoses and treatments. In 2018, the Nephrology Group of the Shanghai Association of Pediatric Nephrologists proposed the "Expert Consensus on Early Management of Congenital Renal Hydrocele in Chinese Children". Clinical doctors should carry out the diagnosis and treatment according to guidelines for different etiologies [71].

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12 Endocrine System and Inherited Metabolic Diseases

Shi-Ying Ling, Li-Li Hao, Si Ding, and Lian-Shu Han

12.1 Introduction

12.1.1 Endocrine Disorders

The endocrine system is one of the most important regulatory systems of the human body, maintaining the integrity and stability of physiological functions. The thyroid gland is a vital organ of endocrine system and plays a key role in regulating basal metabolism, body growth, and the development of the nervous system during infancy through the synthesis and secretion of thyroxine.

The thyroid gland is originated from the endoderm. At 4 weeks of gestation, endodermal cells proliferate at the base of the primitive pharynx and migrate to the lower part of the neck to form a flask-like structure, that is, the thyroid primordium. The thyroid primordium gradually grows downward in the interstitium and is connected to the epithelium on the surface of the pharynx through the thyroglossal duct. The thyroglossal duct usually degenerates by 6 weeks of gestation. In a small number of people, the thyroglossal duct is completely or partially residual after birth, forming a thyroid cyst or fistula. Thyroid tissue may be retained at abnormal sites during migration, forming ectopic thyroid tissue, which can be found at the foramen cecum of the tongue, near the hyoid bone, or in the chest. During the development of the thyroid gland, neuroectodermal cells originated from neural crest fuse approaching the thyroid gland, evolving into thyroid parafollicular cells, and secreting calcitonin. At 11 weeks of gestation, the thyroid gland begins to store iodine and synthesize thyroxine, while the pituitary gland begins to secrete thyroid-stimulating hormone (TSH). At 18 weeks of gestation, the hypothalamic-pituitary-thyroid axis is completely formed.

Congenital hypothyroidism (CH) is most commonly caused by thyroid hypoplasia or dysplasia, followed by a deficiency in thyroid hormone synthesis. The manifestations of children with CH in utero vary from each other. For mild cases, it might be asymptomatic, while severe cases might show varying degrees of goiter. During the neonatal period, children may present with drowsiness, little crying, light crying, hoarseness, little movement, poor sucking force, bloated face, and abdominal distension, often concurrent with umbilical hernia. Infants and children with CH are mainly characterized by mental retardation and poor physical development. The diagnosis of CH is based on typical clinical symptoms, decreased free thyroxine (FT4) in serum and elevated TSH. The primary treatment is levothyroxine (LT4) supplementation.

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12.1.2 Inherited Metabolic Diseases

Inherited metabolic diseases (IMDs) are a group of genetic disorders with specific metabolic markers, which are usually caused by dysfunction of enzymes, transporters, or cofactors in the corresponding metabolic pathways due to genetic variants, resulting in abnormalities in the metabolism, synthesis, transport, and storage of specific metabolites in the body [1, 2]. Currently, more than 1000 types of IMDs have been identified. Although each disease is rare, its overall incidence is 1 in 800 [3]. IMDs can be classified in different ways. Based on the type of metabolites involved, IMDs can be divided into metabolic abnormalities of amino acids, organic acids, fatty acids, urea cycle-related products, carbohydrates, nucleic acids, and metal elements; based on the organelles affected by abnormal metabolism, they can be divided into lysosomal storage disorders, mitochondrial disorders, peroxisomal disorders etc. [2]. The in utero clinical presentations of IMDs are complex and nonspecific, often involving multiple organ injuries, and are mainly divided into three categories [4]: (1) deformities, such as head malformations, facial malformations, skeletal malformations, congenital heart diseases, and neural tube defects; (2) dysplasia, such as cortical heterotopia, cortical cysts, posterior fossa malformations, polycystic kidney, and hepatic cysts; and (3) other manifestations, such as intrauterine growth retardation, fetal edema, and hepatosplenomegaly. Postnatal clinical manifestations include metabolic encephalopathy, epilepsy, developmental delay, feeding difficulties, metabolic acidosis, severe hypoglycemia, and hyperammonemia. The onset time of IMDs symptoms is related to its metabolic type, mainly depending on the nature and accumulated concentration of harmful metabolites, the degree of impairment of enzyme activity due to mutations, etc. The severity is also associated with environmental factors, such as diet and infection [5]. Some disorders can be detected by ultrasound or MRI in utero [6]. For example, for lysosomal storage disorders and glycogen storage disease type IV, nonimmune fetal edema can be detected in utero; for glutaric acidemia type I, fetal lateral fissure loss, germinal stromal cysts, and severe macrocephaly can be detected in utero; for fetuses with cystinuria, intestinal hyperechogenicity can often be detected before the 36th week of embryonic age.

12.2 Clinical Practice

12.2.1 In Utero Diagnosis and Sequential Treatment of Congenital Hypothyroidism

12.2.1.1 In Utero Diagnosis of Congenital Hypothyroidism

Fetuses suspected of CH can be detected by ultrasonography in utero, the determination of TSH and reverse triiodothyronine (rT3) in amniotic fluid, and tests of maternal blood TSH levels. The diagnosis of fetal CH can be proposed if maternal blood TSH is normal, amniotic fluid TSH is elevated, and rT3 is decreased [7]. The accuracy of prenatal diagnosis is unclear and should be judged with caution.

12.2.1.2 The Case of In Utero Diagnosis of Congenital Hypothyroidism

Catarina et al. [8] reported a case where CH was successfully diagnosed during the fetal period. At 29 weeks of gestation, the ultrasonography showed that the bilateral thyroid glands of the fetus were significantly increased, the isthmuses were thickened, and the echo in the gland was thick; color Doppler flow imaging revealed that the blood flow in the bilateral thyroid lobes was significantly increased, and the blood flow rate in the superior thyroid artery was accelerated. At 32 weeks of gestation, ultrasonography showed that the fetal goiter was significantly enlarged. The amniotic fluid test showed increased TSH levels (3.53 uIU/mL, reference value 0.04–0.51 uIU/mL) and normal FT4 levels (0.3 ng/dL, reference range 0.1–0.77 ng/dL). Ten days later, the second amniotic fluid test showed TSH and FT4 levels were 1.69 uIU/mL and FT4 0.6 ng/dL, respectively. After multidisciplinary consulta-

tion, a male infant was delivered by cesarean section at the 38 weeks of gestation, with a body weight of 3480 g. The Apgar scores of this infant at 1 min and 5 min were 7 and 9, respectively, and he was admitted to the hospital after birth due to "dyspnea for 10 min." Cord blood TSH was significantly increased (> 715 uIU/mL, reference range 2.3 to 13.2 uIU/mL), and FT4 was decreased (0.2 ng/dL, reference range 2 to 5 ng/dL). Thyroid ultrasonography revealed a thyroid gland of 18 mm × 32 mm × 18 mm in the right lobe and 18 mm × 38 mm × 17 mm in the left lobe, confirming the diagnosis of prenatal goiter. The diagnosis of congenital hypothyroidism was confirmed in combination with the cord blood hormone results.

12.2.1.3 In Utero Treatment of Congenital Hypothyroidism

If fetal CH results from maternal administration of antithyroid drugs, pregnant women may lower the dose of antithyroid drugs to reduce the inhibition of antithyroid drugs on the fetal thyroid gland. If pregnant women are not taking antithyroid drugs, intra-amniotic injection of LT4 may be considered. Intra-amniotic injection of LT4 has been used in the treatment of fetal CH in some studies [8, 9]. However, LT4 injection is not currently available in China, making the treatment challenging. More studies are needed to further support whether appropriate maternal use or increase of LT4 is effective in the treatment of fetal CH.

12.2.2 In Utero Diagnosis and Sequential Treatment of Amino Acid Metabolism Disorders

Amino acid metabolism disorders are caused by genetic mutations that lead to enzyme defects, finally resulting in impaired metabolism of related amino acids and organ damage, with the brain, liver, and kidneys being most commonly affected. Children usually have no abnormal clinical manifestations during the fetal period and begin to show symptoms within the first year of life. Even if the fetus is a patient with amino acid metabolism disease, the excessive amino acids in the fetal body can be metabolized by the mother. Hence, there is no abnormality of amino acids in the amniotic fluid, making it impossible to determine whether the fetus is affected by amino acid testing in the amniotic fluid. Currently, diagnosis in utero can only be made by identifying the genetic variants through genetic analysis of the proband and parents. In our Department of Pediatric Endocrinology and Genetics, amniocentesis was performed in 499 pregnant women in families with a proband diagnosed with amino acid metabolism disorders. A total of 107 fetuses were diagnosed as affected, including 94 cases of phenylketonuria, three cases of maple syrup urine disease, one case of tyrosinemia, one case of citrullinemia, and two cases of ornithine transcarbamoylase deficiency. All pregnancies with affected fetuses were terminated. The mothers of the remaining unaffected fetuses continued pregnancy until delivery and have been followed up to date, with the children showing normal growth and development.

12.2.2.1 The Cases of In Utero Diagnosis of Amino Acid Metabolism Disorders

Case 1: The proband was a patient with phenylketonuria due to compound heterozygous variants in the *PAH* gene c.728G > A (p.R243Q) [maternal] and c.1068C > A (p.Y356X) [paternal]. The mother underwent prenatal diagnosis at 17 weeks of gestation. A heterozygous c.728G > A (p.R243Q) variant was detected in the amniotic fluid cell gene, and the fetus was diagnosed as a carrier of the *PAH* gene variant. The pregnant woman continued her pregnancy until term delivery, and the newborn had the heel blood screening of phenylalanine level < 120 umol/L. During the telephone follow-up, the family reported no obvious abnormalities in physical and growth examination of the infant at the age of 1, 3, and 6 months.

Case 2: The proband was a patient with phenylketonuria due to compound heterozygous variants in the *PAH* gene c.1068C > A (p.

Y356X) [maternal] and c.842 + 2 T > A [paternal]. The mother underwent noninvasive prenatal testing at 16 weeks of gestation. Circulating single-molecule amplification and resequencing technology (cSMART) was used to quantify and massively amplify the pathogenic loci of maternal plasma DNA alleles. The fetal genotype was determined by the percentage of allelic variations, and the fetus did not carry the above two variant loci. The result was consistent with the diagnostic result of amniocentesis.

In addition, noninvasive prenatal testing (NIPT) was performed in 33 fetuses in families with phenylketonuria in our department. The results of 32 NIPT were consistent with the results of interventional prenatal diagnosis, with only one false positive, achieving a sensitivity of 100% and a specificity of 96.15%. In summary, seven fetuses were affected, 18 were heterozygous carriers, and eight were normal fetuses [10].

12.2.2.2 The Methods of In Utero Diagnosis of Amino Acid Metabolism Disorders

Most fetuses with amino acid metabolism disorders have no specific manifestations in utero. Ultrasonography can provide evidence for the diagnosis of some amino acid metabolism disorders, while genetic testing is still an important basis for in utero diagnosis.

1. In utero manifestations: Most children with amino acid metabolism disorders often have no specific clinical presentations during the fetal period.
2. Ultrasonography: Some children with amino acid metabolism disorders may have abnormal presentations. For example, fetuses with cystinemia are often accompanied by colonic hyperechogenicity [11], and fetuses with serine synthase deficiency and asparagine synthase deficiency are often concurrent with microcephaly and in utero growth restriction [12–14].
3. Genetic testing: Prenatal genetic testing is an important diagnostic tool and can be divided into invasive and noninvasive prenatal diagnosis. For invasive prenatal diagnosis, chorionic villi cells can be collected from 9 to 12 weeks of pregnancy or amniotic fluid cells from 16 to 22 weeks of pregnancy for genetic analysis. For noninvasive prenatal diagnosis, the fetal-free DNA can be directly tested in maternal plasma. The commonly used noninvasive prenatal diagnosis techniques mainly include polymerase chain reaction (PCR) technique, cSMART, and second-generation sequencing technique. Since the direct variant detection technique fails to diagnose all cases, multiple ligation-dependent probe amplification (MLPA), combined with genetic polymorphism linkage analysis, should be used for prenatal diagnosis in some families.

12.2.3 In Utero Diagnosis and Sequential Treatment of Organic Acidemia

Organic acidemia is a group of diseases caused by the accumulation of organic acids and their bypass metabolites in the body due to the defective function of enzymes in the process of organic acid metabolism. Organic acidemia is mostly autosomal recessive, and the common disorders include methylmalonic acidemia (MMA), propionic acidemia (PA), isovaleric acidemia, glutaric acidemia type I (GA-I), and holocarboxylase synthase deficiency. Children with organic acidemia have varying degrees of in utero manifestations. Mild cases may be asymptomatic, while dysplasia can be seen in severe cases. Tandem mass spectrometry of acylcarnitine and gas chromatography mass spectrometry of organic acid in amniotic fluid are important methods for in utero diagnosis of organic acidemia. For example, MMA is presented as the increased levels of propionylcarnitine (C3), ratio of C3/acetylcarnitine (C2), methylmalonic acid, and methylcitric acid in amniotic fluid; PA is manifested as the increased levels of C3, ratio of C3/C2, levels of methylcitric acid, and 3-hydroxypropionic acid in amniotic fluid; and GA-I is shown as the increased levels of glutarylcarnitine (C5DC), ratio of C5DC/octanoylcarnitine (C8), and levels of glutaric acid in amniotic fluid. Genetic testing

is still an important basis for the diagnosis of organic acidemia. But if the causative gene of the proband is unclear, mass spectrometry tests of amniotic fluid acylcarnitine and organic acid are required for diagnosis.

In the past 10 years, mass spectrometry has been used in our hospital to detect acylcarnitine and organic acids in amniotic fluid for prenatal diagnosis of 731 pregnant women with familial organic acidemia. Among them, the probands included 564 cases with MMA, 92 cases with PA, 56 cases with GA-I, and 8 cases with isovaleric acidemia. The positive rates were 21.8%, 20.7%, 21.4%, and 25%, respectively. A total of 157 fetuses were prenatally diagnosed with organic acidemia, and most pregnant women chose to terminate the pregnancy. There was only one case of isovaleric acidemia in a pregnant woman who was not diagnosed at screening and chose to continue the pregnancy; the fetus was born with mildly increased blood isovaleric carnitine and urine isovaleric glycine, confirming the diagnosis of isovaleric acidemia; the rest of the fetuses judged as not affected were born and followed up normally [15–18].

12.2.3.1 The Cases of In Utero Diagnosis of Organic Acidemia

12.2.3.1.1 Methylmalonic Acidemia

Case 1: The proband was diagnosed with cblC-type MMA and found only one variant in c.394C > T in the *MMACHC* gene. At 17 weeks of gestation, 20 mL amniotic fluid was drawn from her pregnant mom, of which 2 mL was used for homocysteine detection, 3 μL for tandem mass spectrometry, and 2 mL for gas chromatography mass spectrometry. Amniotic fluid cells from the remaining amniotic fluid were cultured for 2–3 weeks and collected for genetic testing. The results showed that only one genetic variant (c.394C > T in the *MMACHC* gene) was detected in the fetus, making it difficult to determine whether the fetus was affected. But the C3 level (8.53 μmol/L, reference range 0.3–3.0 μmol/L), ratio of C3/C2 (0.66, reference range 0.05–0.25), methylmalonic acid level (9.16 mmol/mmol Cr, reference range 0–0.8 mmol/mmol Cr), methylcitric acid level (0.18 mmol/mmol Cr, reference range 0–0.3 mmol/mmol Cr), and homocysteine level (11.6 μmol/L, reference range 1.10–4.10 μmol/L) were significantly higher than the reference values in amniotic fluid. Therefore, the fetus was diagnosed as MMA, and an induced abortion was performed.

Case 2: The proband was diagonsed with cblC-type MMA and found only one variant of c.217C>T in the *MMACHC* gene. At the 18 weeks of gestation, 20 mL amniotic fluid was drawn from pregnant women for mass spectrometry, homocysteine examination, and genetic testing. The results showed that only one genetic variant (c.217C > T in the *MMACHC* gene) was detected in the fetus, making it hard to determine whether the fetus was affected. Since the C3 level (0.72 μmol/L), ratio of C3/C2 (0.10), methylmalonic acid level (0 mmol/mmol Cr), methylcitric acid level (0 mmol/mmol Cr), and homocysteine level (4.10 μmol/L) were normal in amniotic fluid, the fetus was ruled out as a patient with MMA. The pregnancy continued, and the fetus was born healthy, with normal blood C3 level, ratio of C3/C2 and homocysteine level, and the urine methylmalonic acid and urine methylcitrate being also normal. During the follow-up to date, the child's growth and development are the same as those of the same age.

12.2.3.1.2 Propionic Acidemia

Case 1: The proband was a patient with PA due to a homozygous variant c.1118 T > A in the *PCCA* gene. Prenatal amniotic fluid mass spectrometry and genetic testing were performed at 16 weeks of gestation. The results showed increased C3 level (16.55 μmol/L, reference range 0–5.0 μmol/L), ratio of C3/C2 value (0.92, reference range 0–0.3), and methylcitrate level (0.74 mmol/mmol Cr, reference range 0–0.5 mmol/mmol Cr), and normal 3-hydroxypropionic acid level (1.10 mmol/mmol Cr, reference range 0–35 mmol/mmol Cr) in amniotic fluid. Hence, the fetus was diagnosed as PA. The results of genetic testing showed that the fetus carried the same homozygous variant c.1118 T > A in the *PCCA* gene as the proband. Induced abortion was performed.

Case 2: The proband was a PA patient with compound heterozygous variants c.337C > T [paternal] and c.866G > C [maternal] in the *PCCB* gene. Prenatal amniotic fluid mass spectrometry and genetic testing were performed at 17 weeks of gestation. The results showed that only one genetic variant (c.337C > T in *PCCB* gene) was detected in the fetus, making it difficult to determine whether the fetus was affected. Since the amniotic fluid level of C3 (1.32 μmol/L), ratio of C3/C2 (0.06), 3-hydroxypropionic acid (0 mmol/mmol Cr), and methylcitric acid level (0 mmol/mmol Cr) were normal, propionic acidemia was ruled out. The pregnant woman continued her pregnancy and gave birth to a healthy infant, whose blood C3 level, ratio of C3/C2.and urine 3-hydroxypropionic acid, and methylcitric acid levels were within normal range. During the follow-up to date, the child's growth and development are the same as those of same-age children.

12.2.3.1.3 Glutaric Acidemia Type I

Case 1: The proband was a patient with GA-I. Prenatal amniotic fluid mass spectrometry and genetic testing were performed at 16 weeks of gestation. The results showed the C5DC level (3.56 μmol/L, reference range 0–0.3 μmol/L), ratio of C5DC/C8 (19.77, reference range 0–4), and glutaric acid level (95.41 mmol/mmol Cr, reference range 0–2.5 mmol/mmol Cr) were increased in amniotic fluid. The fetus was diagnosed as affected. The results of genetic testing showed that the fetus carried the same two variants of the *GCDH* gene as the proband. Induced abortion was performed.

Case 2: The proband was a patient with GA-I (carrying only one variant c.533G > A in *GCDH* gene). Prenatal amniotic fluid mass spectrometry and genetic testing were performed at 16 weeks of gestation. The results showed that the fetus carried only one variant c.533G > A in the *GCDH* gene identical to the proband, making it impossible to determine whether the fetus was affected. However, the C5DC level (2.85 μmol), C5DC/C8 ratio (22.28) and glutaric acid level (266.02 mmol/mmol Cr) in amniotic fluid were significantly higher than the reference. Hence, the fetus was diagnosed as GA-I, and induced abortion was performed.

Case 3: Prenatal diagnosis was performed at 17 weeks of gestation in the primipara, and two *GCDH* variants c.300G > A (maternal, clinical significance unknown) and c.383G > A (paternal, possible pathogenic) potentially associated with GA-I were detected in the amniotic fluid cells. But its correlation needs to be further clarified, and it was not possible to determine whether the fetus was affected by GA-I. Based on the C5DC level (0.37 μmol/L), ratio of C5DC/C8 (3.64), and glutaric acid level (2.73 mmol/mmol Cr), the fetus was judged as not affected. The pregnant woman continued her pregnancy. The blood C5DC level and urine glutaric acid level of the fetus were normal after birth. During the follow-up to date, child's growth and development are the same as those of same-age children.

12.2.3.2 The Methods of In Utero Diagnosis of Organic Acidemia

The in utero manifestations in fetuses with organic acidemia are usually nonspecific, but amniotic fluid specific acylcarnitines and organic acids are elevated in different disorders. Thus, the detection of amniotic fluid specific metabolites is an important method for prenatal diagnosis of organic acidemia. For pregnant women with clear genetic variants in the proband, genetic testing can be used as an important basis for the diagnosis.

1. In utero manifestations: The fetal presentations of organic acidemia vary from asymptomatic in mild cases to developmental abnormalities in severe cases, such as growth retardation, facial abnormalities, microcephaly, congenital heart disease, and dilated cardiomyopathy in children with MMA [19]. For fetuses with GA-I, abnormally enlarged head circumference may be observed, and MRI reveals frontotemporal atrophy, sylvian fissure, widened subarachnoid space, and subependymal cysts [20, 21].

2. Tests of acylcarnitine and organic acids in amniotic fluid: Amniotic fluid is collected from pregnant women at 16–20 weeks of gestation. Determination of acylcarnitine in amniotic fluid by tandem mass spectrometry and organic acid in amniotic fluid by gas chromatography mass spectrometry are important methods for the diagnosis of organic acidemia. Amniotic fluid C3 level, ratio of C3/C2, and methylmalonic acid and methylcitric acid level are increased in fetuses with MMA; amniotic fluid C3 level, ratio of C3/C2, and methylcitric acid and 3-hydroxypropionic acid level increased in fetuses with PA; and amniotic fluid C5DC level, ratio of C5DC/C8, and glutaric acid level increased in fetuses with GA-I. These methods are rapid, accurate, and highly specific, but it is necessary to be careful for possible false negatives in gas chromatography-mass spectrometry [15, 17, 18].
3. Tests of amniotic fluid homocysteine: The detection of amniotic fluid homocysteine level by immunofluorescence polarization technique can be used to assist in the prenatal diagnosis of MMA combined with homocysteinemia [22].
4. Enzymatic tests: The enzyme activity can be measured on amniotic fluid cells or chorionic villi tissue, such as biotinidase activity detection for children with biotinidase deficiency, but this method is time-consuming and laborious, thus being rarely used in clinical practice.
5. Genetic testing: In utero genetic testing methods can be divided into invasive (with amniotic fluid cells or chorionic tissue as test sample) and noninvasive testing (with maternal blood as test sample). However, diagnosis cannot be made if the genetic variant site of the proband is unclear or if only one variant site is detected. Therefore, it is recommended to perform amniotic fluid metabolite detection combined with genetic testing for the clinical diagnosis of fetuses suspected of organic acidemia to provide reasonable genetic counseling and reproductive selection.

12.2.3.3 In Utero Treatment of Organic Acidemia

Early in utero medication may improve the prognosis of some children with organic acidemia. For example, oral or intramuscular administration of vitamin B12 and oral administration of biotin during pregnancy are effective for fetuses with vitamin B12-responsive methylmalonic acidemia and with holocarboxylase synthetase deficiency, respectively [23]. Fetuses were born without abnormalities, and the postnatal metabolites were within normal range, but disease-related complications may still be present [24, 25]. Currently, there are limited reports of in utero treatment of organic acidemia, and its long-term prognosis still need further studies in large samples.

12.2.4 In Utero Diagnosis and Treatment of Fatty Acid Oxidation Disorders

Fatty acid oxidation disorders are a group of relatively common inherited metabolic diseases, all of which are autosomal recessive disorders. Fatty acid β oxidative metabolism is impaired due to defects in the function of enzymes or transporters in the pathway when fatty acids enter the mitochondria for beta-oxidative metabolism. Such disorders mostly occur after birth and mainly manifest as hepatic disease, cardiomyopathy, and muscle disorder. In utero developmental abnormalities are found mainly in fetuses with carnitine palmitoyltransferase II deficiency and multiple acyl-CoA dehydrogenase deficiency [26, 27]. The diagnosis of such disorders generally depends on the analysis of relevant enzyme activities and genetic testing.

In our Department of Pediatric Endocrinology and Genetic, 17 pregnant women with probands who were diagnosed with fatty acid oxidation disorders underwent prenatal genetic diagnosis via amniocentesis, including 7, 5, 3, and 2 probands diagnosed with primary carnitine deficiency, multiple acyl-CoA dehydrogenase deficiency, and very long-chain and short-chain

acyl-CoA dehydrogenase deficiency, respectively. Among them, five pregnant women carried pathogenic compound heterozygous variants or homozygous variants of related disorders, and all of them chose to terminate the pregnancy. The remaining 12 pregnant women continued their pregnancies. The fetuses were born normally, and their growth and development after birth were similar to children of the same age.

12.2.4.1 The Methods of In Utero Diagnosis of Fatty Acid Oxidation Disorders

For fetuses with Dandy-Walker malformation or other brain development disorders, the possibility of fatty acid oxidation disorders should be considered, especially for those with polycystic kidney and fatty liver. The diagnosis still requires enzyme activity testing and genetic testing.

1. In utero manifestations: Common presentations include polycystic kidney and brain dysplasia (including ventricular enlargement, hydrocephalus, ventricular calcification, corpus callosum agenesis, Dandy-Walke malformation, etc.) and may also be associated with growth retardation, microcephaly, unusual facies, cardiomegaly, and congenital heart disease [27–29]. They are more common in cases with carnitine palmitoyltransferase II deficiency and multiple acyl-CoA dehydrogenase deficiency.
2. Enzymatic test: The corresponding enzyme activity can be determined on amniotic fluid cells or chorionic tissues.
3. Genetic testing: It is necessary to identify two pathogenic variations of disease-related genes in the proband. Pregnant women may undergo choriocentesis at 9–12 weeks of gestation to collect chorionic cells for genetic testing, or may receive amniocentesis at 16–22 weeks of gestation to collect amniotic fluid cells for genetic testing.
4. Tandem mass spectrometry: Analysis of amniotic fluid acylcarnitine by tandem mass spectrometry can assist in diagnosis. For instance, increased short-medium-chain acylcarnitine and long-chain acylcarnitine are observed in the amniotic fluid mass spectrometry in fetuses with multiple acyl-CoA dehydrogenase deficiency and very long-chain acyl-CoA dehydrogenase deficiency, respectively [30]. However, this method may have some false negatives and should be judged with caution [28].

12.2.5 In Utero Diagnosis and Sequential Treatment of Lysosomal Disorders

Lysosomal storage disorders (LSDs) are a group of diseases in which biological macromolecules such as nucleic acids, proteins, lipids, and mucopolysaccharides cannot be degraded properly, thus being stored in lysosomes due to defective function of lysosomal enzymes caused by genetic variants. Most LSDs are autosomal recessive, while a few are X-linked recessive. Common LSDs include mucopolysaccharidosis, Gaucher's disease, GM1 gangliosidosis, Fabry's disease, and Pompe's disease [31].

Our hospital has been engaged in the prenatal diagnosis of LSDs for many years. More than 20 LSDs have been diagnosed and treated with the help of genetic and enzymatic examinations. During the process of testing, amniotic fluid was drawn, and amniotic fluid cells were cultured for the extraction of enzymes and DNA, and the proband's clinical phenotype and parental genotype were referred. In the 38 fetuses that underwent prenatal diagnosis in our department, there were 17 noncarriers and 9 carriers of one relevant pathogenic variant, and the pregnancy could be continued. The remaining 12 fetuses carried two relevant pathogenic variants or were hemizygous, of which 11 fetuses were diagnosed as LSD with consistent enzymatic and genetic results, and the pregnancy was terminated.

12.2.5.1 The Cases of In Utero Diagnosis of Lysosomal Disorders

12.2.5.1.1 Mucopolysaccharidosis Type II

Case 1: The proband was a patient with mucopolysaccharidosis type II who was hemizygous for the *IDS* gene variant (c.620_621insT, p.

Q207Hfs*21). Amniotic fluid was drawn from the pregnant woman at 16 weeks of gestation, and cells were cultured to determine iduronate sulfatase activity. Genomic DNA was collected from amniotic fluid cells for fetal sex identification and *IDS* genetic variation testing. The results showed that iduronate sulfatase activity was significantly decreased in fetal amniotic fluid cells (0.49 nmol/4 h·mg, reference range 31–110 nmol/4 h·mg). The male fetus carried the *IDS* genetic variation consistent with the proband and was diagnosed with mucopolysaccharidosis type II. The family chose to terminate the pregnancy.

12.2.5.1.2 Gaucher's Disease

Case 1: The proband was a patient with Gaucher's disease due to compound heterozygous variants in c.680_681delinsGG (p.N227R) [paternal] and c.1448 T > C (p.L483P) [maternal] in the *GBA* gene. Amniotic fluid was collected from the pregnant woman at 18 weeks of gestation for prenatal diagnosis, and amniotic fluid cells were cultured for β-glucosidase activity and *GBA* gene testing. The results showed that the fetus had significantly decreased β-glucosidase (4.93 nmol/h·mg, reference range 55–231 nmol/h·mg) and carried the same two *GBA* gene variants as the proband and therefore was diagnosed with Gaucher's disease. The family chose to terminate the pregnancy.

12.2.5.1.3 Niemann-Pick Disease

Case 1: The proband was a patient with Niemann-Pick disease and carried compound heterozygous variants in the *SMPD1* gene: c.649delG (p.E217Rfs*40) [maternal], c.1497_1498delinsAC (p.Y500H) [paternal]. Amniotic fluid was drawn from the pregnant woman at 16 weeks of gestation, and amniotic fluid cells were cultured for enzyme activity and genetic testing. The results showed decreased acid sphingomyelinase activity in fetal amniotic fluid cells (11.38, reference range 84–285 nmol/17 h·mg). The fetus had two variants consistent with *SMPD1* gene of the proband and therefore was diagnosed with Niemann-Pick disease. The family chose to terminate the pregnancy.

12.2.5.2 The Methods of In Utero Diagnosis of Lysosomal Disorders

LSDs are the most common IMDs with in utero abnormalities. In utero diagnosis of these disorders can be made by imaging, enzyme activity tests, and genetic testing.

1. *In utero* manifestations: Fetal hydrops and ascites are the most common *in utero* presentations of LSDs and are often detected in the second trimester [6]. Other *in utero* manifestations include growth restriction, hepatosplenomegaly, thick neck (normal karyotype), adrenal calcification, fetal movements decreased, stiff epiphyseal line, etc. [29].
2. Enzymatic testing: The disease can be further diagnosed by the detection of specific enzyme activities in chorionic villi, amniotic fluid, and umbilical cord blood, of which amniotic fluid is the most commonly used fetal sample. And the detection of enzyme activities in amniotic fluid supernatant and cultured amniotic fluid cells is an important tool for the diagnosis of LSDs.
3. Genetic testing: Most LSDs are autosomal recessive disorders. DNA from fetal samples can be extracted for genetic testing. For example, *GBA* genetic variation is detected for Gaucher's disease, *ASAH1* variation for Fabry's disease, *GALC* variation for Klebsiella disease, and *GAA* variation for Pompe's disease [2]. Since most of the LSDs are severe and difficult to treat, termination of pregnancy is mostly adopted for affected fetuses diagnosed prenatally. and reasonable genetic counseling and reproductive selection are performed based on the results of genetic testing.

12.2.6 In Utero Diagnosis and Sequential Treatment of Glycogen Storage Diseases

Glycogen storage diseases (GSDs) are a group of genetic disorders that are characterized by specific enzymatic defects involving the synthesis or

degradation of glycogen. Most of these disorders have no abnormal manifestations in utero. Enzyme activity analysis and genetic testing are the main methods for the diagnosis of such disorders.

In our hospital, 162 pregnant women from families with GSD probands were diagnosed by prenatal gene diagnosis via amniocentesis, including 75 cases with GSD type II, 47 cases with GSD type IA, 17 cases with GSD type III, 10 cases with GSD type IB, 6 cases with GSD type IX c, 4 cases with GSD type VI, and 3 cases with GSD type IXa. A total of 44 cases carried pathogenic compound heterozygous variants or homozygous variants of genes associated with corresponding diseases. Pregnant women chose to terminate pregnancy, and the remaining 118 pregnant women whose fetus carried one variant or did not carry relevant pathogenic variants continued pregnancy. The fetuses were born without abnormalities, and their postnatal growth and development were similar to infants of the same age.

12.2.7 The Methods of In Utero Diagnosis of Glycogen Storage Diseases

Since GSDs usually has no abnormal clinical manifestations during the fetal period and enzyme activity detection is very limited, genetic testing becomes the main basis for in utero diagnosis.

1. In utero manifestations: Most of these disorders have a postnatal onset, while for onset during the fetal period, it is mainly seen in children with GSD type IV, such as polyhydramnios and decreased fetal movements during pregnancy. Fetal hydrops is the main clue to for diagnosis, and other possible abnormal findings include in utero growth retardation, face malformation, joint flexion contracture, limb deformity, and intraventricular hemorrhage [32].
2. Enzymatic tests: Amniotic fluid cells or chorionic tissue are usually used to determine the corresponding enzyme activity, but the enzymatic detection technique is relatively sophisticated. For children with GSD type Ia, there are no defects of glucose-6-phosphatase in amniotic fluid cells or chorionic tissue, and enzyme activity should be measured by fetal liver biopsy [33]. Hence, enzymatic tests are rarely used at present.
3. Genetic testing: It is necessary to identify two pathogenic variants in the proband. Chorionic villus tissue is generally collected at 9–12 weeks of gestation, or amniotic fluid cells are collected at 16 to 22 weeks of gestation for genetic testing.

12.3 Research Progress

12.3.1 Progress in Intrauterine Treatment of Endocrine Disorders and Inherited Metabolic Diseases

Advances in prenatal screening and molecular diagnostics allow the early diagnosis of many IMDs in pregnancy and make it possible for early intervention and treatment. Currently, in utero treatment methods including fetal endoscopy and open fetal surgery can only temporarily repair some fetal structural malformations. In contrast, in utero cell transplantation or gene therapy allows earlier in utero treatment of fetal IMDs. The advantages include: (1) It can deliver larger doses per unit body weight because the fetus is small, (2) immune responses to grafts can be avoided since the fetal immune system is immature and immune tolerant, (3) substances can be easily delivered to more target organs because some physical barriers in the fetus are weak, and (4) it can avoid some irreversible perinatal pathological damages [34, 35]. Although in utero cell transplantation or gene therapy has not been used in clinical settings, significant progress in intrauterine cell therapy has been made in overcoming transplantation and immunological barriers in mouse and large animal models, and fetal gene therapy has been demonstrated as an effective way to prevent the onset of IMDs in animal models [36].

12.3.1.1 In Utero Cell Transplantation

In utero cell transplantation (IUCT) is an ideal way to treat IMDs by delivering corrective donor cells to the fetus before the onset of clinical symptoms of the disease to avert irreversible damage. Among the cells used for transplantation, stem cells are predominant, which rapidly produce a large number of normal cells in the recipient by virtue of their strong proliferation and differentiation abilities, and have lower immunogenicity. Hematopoietic stem cells (HSCs) are the most studied and widely used stem cells in clinical practice. Fetal HSCs are able to differentiate into all blood cell lineages, and their differentiated offspring can express major histocompatibility complex (MHC) antigens and participate in the development of the immune system, inducing immune tolerance to grafts [37]. Furthermore, HSCs migrate on a large scale during fetal development, which enables donor cells to effectively implant, differentiate, and expand in the naturally occurring migration processes to reconstitute new hematopoietic systems and correct diseases [38]. Moreover, compared to postpartum hematopoietic stem cell transplantation, the fetal bone marrow in the first and second trimesters of pregnancy has a larger space to receive engraftment of donor cells without the requirement for myeloablative regimen [39]. At present, successful clinical cases are limited to immunodeficiency disorders such as X-linked severe combined immunodeficiency, and the in utero hematopoietic stem cell transplantation (IUHCT) fails to demonstrate a therapeutic effect for most disorders [35]. In this context, researchers have made some progress in the in utero treatment of IMDs in rodent or large animal models by optimizing the type of transplanted cells and the route of transplantation.

In a previous study, considering that IUHCT could only induce transient chimerism in immunocompetent mice and primates, investigators provided in utero treatment to cats with α-mannosidosis using monocytes. By the ultrasound-guided intraperitoneal transplantation, donor monocytes were transplanted in the brain, liver, and spleen and persisted (up to 125 days), with α-mannosidase activity dozens of times higher than in the untreated models. However, the enzyme level and cell count still did not achieve clinical efficacy, and further optimization of experimental strategies was required to improve the effect [40].

Norimasa Ihara's research group used maternal immune-matched allogeneic donor cells to avoid inducing intrauterine immune responses and delivered a larger cell dosage via intravenous injections to overcome the competitive inhibitions of recipient HSCs, thus achieving lifelong transplantation of donor HSCs in various tissues such as the brain. Mice with mucopolysaccharidosis type VII treated with this IUHCT, although having lower serum enzyme activities, had significantly increased survival, improved bone structure, and restored reproductive performance compared to the untreated controls [41].

Brendan H. Grubbs's team used a rat model of Crigler-Najjar syndrome lacking UDP-glucuronosyltransferase (UGT1A1) as a recipient. They transplanted the human amniotic epithelial cells (hAECs) with hepatic differentiation potential into the liver of a second trimester fetus by ultrasound-guided IUCT. On a postnatal day 21, anti-human mitochondria-positive cells were detected in the liver of recipient rats, but the number of pups that died before parturition was higher than expected. Moreover, the short observation time window and the small size of experiments also do not allow a comprehensive evaluation of treatment effects [42].

In utero enzyme replacement therapy (IUERT) and IUHSCT have been investigated in mice with mucopolysaccharidosis type VII (MPS VII) at 14.5 days of embryonic age. Intrahepatic injection of recombinant human β-glucuronidase (IUERT) mice showed higher enzyme activity in several tissues after birth, the survival rate was significantly improved, and the infused enzyme was successfully delivered to microglia and reduced neuritis. The grip strength of mice was significantly increased. In addition, IUERT could prevent the production of postpartum anti-enzyme antibodies. The investigators also treated MPS VII mice with IUHSCT. This method can cross-correct hepatic Kupffer cells and improve multiple tissue phenotypes, and reduced inflam-

mation in the vicinity of donor microglia was observed in chimeric mice [43].

From the above studies, it can be seen that although the current IUCT for IMDs can successfully achieve donor input and enzyme activity recovery, the transfer efficiency and the degree of enzyme activity recovery are still limited, accompanied by problems such as high embryonic lethality. These problems should be addressed by further optimization of experimental conditions.

12.3.1.2 In Utero Gene Therapy

In utero gene therapy (IUGT) is a method to treat disorders through their genetic roots before birth by counteracting or replacing malfunctioning genes in damaged cells of the fetus [44]. Traditionally, IUGT is administrated by introducing exogenous genetic material into the embryo or fetus, allowing the cell to produce a sufficient amount of normal proteins. Later on, gene editing technology developed rapidly, and nucleases such as transcription-activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs), or clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins (CRISPR-Cas) system can be used to achieve targeted changes in the endogenous genome. IUGT can be divided into in vitro and in vivo methods. The former is to temporarily remove the sick cells from the fetus, correct the genetic defect in vitro, and then return them to the body; the latter is to introduce exogenous DNA and/or gene editing systems directly into cells in vivo. A comparison of the two methods shows that in vivo IUGT is superior since in vitro IUGT requires to inject of a large number of modified cells into the fetus through transplantation, which puts forward higher requirements for the survival and proliferation ability of the transplanted cells [45]. It should be noted that all available IUGT methods for fetuses have only been studied in animal models, and more stringent efficacy and safety tests are required before clinical application.

The key to the success of in utero gene therapy is the ability to effectively deliver genetic material to target organs and cell groups. The capacity of different organs/cells to receive viral vectors depends on a variety of factors, including the vector serotype, the route of vector delivery, and the developmental stage of the recipient at the time of vector injection [34]. For example, after the onset of fetal respiratory movement in the third trimester of mice pregnancy, the viral vector injected into the amniotic cavity can target the lung epithelium relatively specifically by means of amniotic fluid "inhalation" [46]. Before the formation of keratinized skin layer in the first trimester, the viral vector injected into the amniotic cavity can effectively target skin progenitor cells [47]. Other injection routes include intramuscular injection, intracerebral injection targeting the central nervous system, and vitelline vein injection targeting the liver. Minimally invasive delivery methods used in large animal models and humans include ultrasound-guided intraperitoneal, intracardiac, and umbilical vein injection, ultrasound-guided intraventricular injection, ultrasound-guided or minimally invasive intratracheal, or intra-pulmonary parenchymal injection.

IMDs have various indications for IUGT: (1) monogenic disorders, which often involve only one variant site; (2) prenatal diagnosis, which can be performed, and (3) disorders that can cause irreversible damage to organs during the perinatal period. Some progress has been made in IUGT research in IMD animal models. Waddington et al. reconstituted neuronal glucocerebrosidase expression by intracranial injection of AAV vectors into fetuses in a mouse model of neuronal Gaucher's disease with *Gba* variant, effectively restored motor ability, significantly improved survival, and reduced neuritis and neurodegeneration in mice [48]. In a subsequent study in mice with hereditary tyrosinemia type I, a third-generation base editor 3 (BE3) was injected into the vitelline vein and was delivered into embryonic hepatocytes by adenoviral vectors to introduce a nonsense mutation of *Hpd* gene in the hepatocyte in utero, effectively alleviating the accumulation of toxic metabolites due to downstream *Fah* gene variants and rescuing fatal liver failure [49]. In addition, in utero AAV9 delivery of adenine base editor (ABE) successfully corrected the causative variant of the *Idua* gene in the mouse model of mucopolysaccharidosis I Hurler syndrome (MPS IH), which significantly

improved the survival and rescued disease phenotypes in multiple organs, such as muscle, bone, and heart [50]. Although IUGT has good efficacy in some animal models of IMDS, it is still in the early stages of research. Safety issues such as nonspecific mutations resulting from the introduction of genetic material, germ cell transmission and adverse effects on organ development, and complex ethical issues still require more extensive and detailed research at the preclinical stage.

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Motor System

13

Wen-Yuan Sui and Jun-Lin Yang

13.1 Introduction

13.1.1 Early Development of the Spine and Spinal Cord

The development of the spine and spinal cord starts at the third week of gestation. At this stage, the embryo consists of two layers of cells called the blastoderm. On about 15th day of gestation, a groove is formed in the midline of the blastoderm and gradually lengthens. This groove is called the primitive groove. The primitive groove gradually deepens at the rostral end of the embryo and extends to the caudal end. The resulting depression is called the primitive pit, and the cells surrounding the primitive pit are called the primitive knot. The rostral end of the embryo eventually forms at the primitive pit and primitive knot. The entire structure (primitive pit, primitive knot and primitive groove) is called primitive streak. The primitive streak forms the longitudinal axis of the embryo, distinguishing the left and right sides of the embryo. Thus, at the third week of gestation, the embryo develops the rostral/caudal, left/right, and ventral/dorsal direction. The epiblast cells proliferate and migrate through the primitive streak to form a three-germ layer embryo. Epiblast cells migrate to replace the hypoblast cells, forming the endoderm. While the epiblast cells continue to migrate to the area between the epiblast and endoderm to form the mesoderm. At this point, the epiblast cells are also renamed as ectoderm. Two structures are formed at the midline of the mesoderm—the anterior notochordal disc and notochordal process. The notochordal process is originally a hollow mesodermal tube, and it continues to develop into a solid structure called notochord. Notochord induces vertebral body formation, and when the vertebral body is formed around the notochord, nucleus pulposus is formed. After the development of notochord, three structures have been formed in the mesoderm, that is, the paraxial mesoderm, intermediate mesoderm, and lateral plate mesoderm. Paraxial mesoderm is located adjacent to the notochord, with its cells able to form somites, which will be differentiate into bone, voluntary muscle, and skin. The intermediate mesoderm differentiates into the urinary and reproductive systems. The lateral plate mesoderm can be divided into ventral and dorsal layers. The ventral cells will differentiate into mesothelial tissues of internal organs and the dorsal layer cells will differentiate into skin and body wall.

13.1.2 Formation and Differentiation of Somites

As mentioned above, bones, voluntary muscles, and neck and trunk skin originate from the

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somites. On approximately 20th day of gestation, somites begin to appear in pairs. The somites originate from the paraxial mesoderm and develop from rostral to caudal end at a rate of three to four pairs each day. Initially, there are 42–44 pairs of somites formed on the lateral surface of the notochord. Subsequently, five to seven pairs of somites at the caudal end will degenerate, and finally form 37 pairs of somites. The first to fourth pairs of somites at the rostral end will form the occipital bone, craniofacial bone, and bony structures of the inner ear. The 5th–12th pairs of somites form the cervical vertebrae (since the first cervical somite participates in the development of the occipital bone, only seven of the eight pairs will form the cervical vertebrae). The 13–24th pairs of somites form the thoracic vertebrae, the 25–29th pairs form the lumbar vertebrae, and the 30–34th pairs form the sacral vertebrae. The three pairs of somites at the caudal end form the caudal vertebrae. The position of somites in the embryo determines the anatomic structure of the spine and peripheral nervous system. With the development of the embryo, the somites gradually develop into several substructures. Each substructure eventually differentiates into a specific tissue structure. The first substructure to appear is called the hypostome. The bony joints eventually form the bony structure of the spine. The sclerotome is formed after the appearance of a cavity in the midsection of the somite near the notochord and neural tube. After the cavity ruptures, the loose core cells within it migrate and surround the notochord and neural tube. The cells surrounding the notochord and neural tubes are called sclerotomes. The ventral sclerotomes surrounding the notochord develop into the vertebral body, while the dorsal sclerotomes surrounding the neural tubes develop into the vertebral arch. The normal development of the vertebral body and vertebral arch is associated with the induced differentiation of sclerotomes by the notochord and neural tubes. Incomplete closure of the neural tube may affect the normal cellular signaling transduction and the induced differentiation of the sclerotomes, resulting in the congenital malformation of spinal dysraphism. Spine bifida is an incomplete closure of the vertebral arch, which leads to the exposure of nerve tissue. Spina bifida occulta refers to incomplete closure of the vertebral arch only. However, when spina bifida is profound, tissue within the nerve tubes may protrude and connect to the skin. The protruding nerve tissues are surrounded by the membrane and form a hernia. If the hernia contents contain only the spinal membrane (dura and arachnoid membranes), it is called a spinal meningocele. If the hernia contents contain nerve tissue and the spinal membrane, it is called a myelomeningocele. Once the sclerotome is formed and approaches the notochord and the neural tubes, it begins to divide to facilitate the extension of the spinal nerves from the neural tubes into their respective segments. When the division of the sclerotome is completed, the caudal half of the rostral sclerotome merges with the rostral half of the causal half of the sclerotome to form the vertebra precursor. The division and refusion of the sclerotome may explain why there are eight pairs of cervical nerves but only seven cervical vertebrae. The rostral part of the sclerotome of the first cervical segment participates in the formation of the occipital bone, while its caudal part together with the rostral part of the sclerotome of the second cervical segment forms the first cervical vertebra. The first cervical nerve extends out from the above regions of C1, the second cervical nerve extends out between C1 and C2, and the eighth nerve extends out from C7/T1. The sclerotome cells surrounding the notochord develop into the fibrous ring of the intervertebral disc, while the surrounded notochord forms the early-stage nucleus pulposus. With the child development, the fibrocartilage cells gradually replace the original notochord cells in the nucleus pulposus.

13.1.3 Development of Central Nervous System

During early embryonic development, two key structures are formed in the mesoderm: the anterior notochord disc and the notochord process. The anterior notochord disk may induce the ectodermal cells to form the neural plate. Under the

inductive factors produced by the anterior notochord disk, neural plate cells proliferate from the rostral side to the caudal side and differentiate into neuroectoderm. The rostral side of the neural plate is relatively wider and differentiates into the brain, while the caudal end is relatively thinner and differentiate into the spinal cord. The caudal end of the neural plate lies above the notochord and is flanked by somites. This structure allows the caudal end of the neural plate surrounded by the sclerotomes that have differentiated into the spinal canal, and it continues to differentiate into the spinal cord. During neurulation, the neural plate involutes, and the bilateral tissues fold and fuse at the midline, thus forming the neural tube. Once the neural tube has formed at the midline, it facilitates the separation of the ectoderm, which differentiates into a three-layered structure. The inner layer is the lamina ventricularis, which is the closest to the lumen of the neural tube. Lamina ventricularis contains neuroepithelial cells, which can differentiate into central nervous system cells. The first generation of cells formed by the proliferation of neuroepithelial cells is neuroblasts, which eventually form the neurons of the central nervous system. Once formed, neuroblasts migrate from the lamina ventricularis into the mantle layer and eventually develop into the gray matter of the central nervous system. At the fourth week of gestation, the neuroblasts in the mantle layer form a pair of columnar structures on the abdomen and back. The cells of the dorsal spine form the connecting neurons that connect the motor neurons of the ventral spine with the sensory neurons of the dorsal root ganglia. The neurites that outgrow from neuroblasts extend peripherally to form the third layer of the neural tube (the marginal layer) and eventually develop into the white matter of the central nervous system.

13.1.4 Ossification of Vertebral Body

The cartilaginous centers begin to appear in the mesoderm-forming spine precursors at approximately the sixth week of gestation. Initially two cartilaginous centers appear within the vertebral body and subsequently fuse into one at the midline. If only one cartilaginous center is present, a hemivertebra will be formed, resulting in congenital scoliosis. Subsequently, the cartilaginous centers of the vertebral arch, transverse process and spinous process appear successively, therefore completing the cartilaginous process of the spine. Each vertebra has three primary ossification centers: one for the vertebral body and the other two for the vertebral arch. Vertebral ossification occurs first in the lower thoracic and upper lumbar regions. Vertebral ossification progresses more rapidly in the caudal spine, while vertebral arch ossification progresses more rapidly in the cervical spine. It precedes the ossification of the cervical vertebral body, and the cervical plate begins to ossify around the eighth week of gestation. In the lumbar spine, the lamina first becomes ossified and fused in the midline and then develops toward the cephalic end. Once ossification is completed, the lamina will not fuse with the vertebral body; in contrast, a cartilaginous joints region will be retained between the two. The cartilaginous joints region is formed to adapt to the enlargement of the spinal canal during the development of the spine, and it will eventually disappear by the age of 6. After birth, secondary ossification centers will appear at the ends of the transverse, spinous, and bony processes loops and eventually fuse and disappear by the age of 20–30 years [1–4].

13.1.5 Classification of Congenital Scoliosis

Congenital scoliosis (CS) deformity is usually manifested by stiffness, and some types of CS may further affect spinal balance. It is critical to predict the timing of rapid progression of scoliosis. Correct classification of spinal deformities is necessary to accurately predict the progression of scoliosis. CS can be divided into three categories: formative disorder, segmental disorder, and mixed disorder. MacEven has classified congenital spinal malformations, which were later modified by Winter. This classification method was accepted by the Scoliosis Research Society in

1968. Classification according to Winter et al. [5].

(1) No classification: presence with multiple types of defects: no typical category; (2) fusion of ribs; (3) partial disorder in one side of a single vertebral body: this results in a wedge or trapezoidal defect: potential pedicle defect; (4) the complete disorder in one side of a single vertebral body: a hemivertebra is formed; (5) bilateral segmental disorder; lack of intervertebral disc between adjacent vertebrae; (6) unilateral segmental disorder: Unsegmented bone bridge occurs, which may involve two or more vertebral bodies, or may only involve the vertebral body or the posterior part of the spine.

Vertebral body formation disorders are due to partial absence of the vertebral body. Anterior, anterolateral, posterior, posterolateral, and lateral sides of the vertebral body may be involved. Formation barrier can be partial formation barrier or complete formation barrier. Cuneiform vertebra is an incomplete formative disorder. Two pedicles are present in the malformed vertebral body, but one pedicle is hypoplastic. A hemivertebra is a complete vertebral defect. A deformed vertebral body has only one pedicle and one half of the vertebral body. There are three types of hemivertebrae [6, 7]: fully segmented, partially segmented, and unsegmented. Fully segmented hemivertebrae have growth plates at the rostral side and the caudal side of the vertebral body. In this case, the fully segmented hemivertebrae continue to grow, which has a marked effect on spinal balance. Similarly, the unsegmented hemivertebrae are not separated from the rostral and caudal vertebrae, and the hemivertebrae have limited growth potential and thus have little impact on spinal balance. A partially segmented hemivertebra has a disc on one side of the vertebral body and is fused with the adjacent vertebral body on the other side. In the case of multiple jumping hemivertebrae, the growth on one side of the hemivertebra is offset by that on the contralateral hemivertebra of the spine. The hemivertebrae are separated by at least one vertebral body, which is common in the thoracic spine. Poor segmentation blocks the segmentation between the vertebral bodies. Bone bridges between the vertebral bodies disturb the growth on the same side of the spine, resulting in tethering effect. If the bone bridge is bilateral (block vertebra), the effect on spinal balance is relatively minor. Mixed deformities refer to the presence of both poor segmentation and forming disorder in one patient. These patients are often at risk of rapid progression.

Because only 30% of the spine is ossified at birth, it is difficult to determine whether a vertebral deformity is present at birth. In general, the posterior anatomy of the spine is often overlooked in the classification of CS. Knowledge with the anatomy of the posterior part of the spine is very helpful in determining the surgical plan. Posterior anatomy may appear completely normal; however, exposure of neural structures may appear due to laminar fusion or incomplete laminar closure. Therefore, preoperative three-dimensional (3D) CT reconstruction of the spine to get acquainted with the posterior structure of the spine is very valuable. Nakajima et al. emphasized the importance of 3D structural studies in patients with CS, highlighting the possibility of anterior and posterior anatomical defects in CS. They classified posterior anatomic abnormalities in patients with a single malformed vertebral body into bilateral pedicle and unilateral pedicle groups, which were further classified according to abnormalities in laminar formation. In some patients with multiple deformities, they observed the fusion of the vertebral arch and concluded that 3D CT can observe various morphological abnormalities in the posterior aspect of the deformed vertebral body [5–7].

13.1.6 Evaluation of Patients with Congenital Scoliosis

The main physical examinations performed in patients with CS include a detailed spinal and neurological examination, radiological evaluation, and possible co-existence of other deformities.

Because the growth of the spine is the focus of CS, the physical examination begins with recording the patient's sitting height, standing height, and body weight. The growth of children requires additional attention because there is a strong link between the growth and the progression of scoliosis. Congenital spinal deformities can lead to spinal imbalance, so the patient's coronal and sagittal balance should be recorded. Coronal and sagittal spinal balance, tilted pelvis, tilted head, and shoulder balance should be carefully recorded, and stiffness in lateral bending should be evaluated. Deformities of the ribs may coexist with deformities of the spine, so deformities of the ribs should be documented. Respiratory function of the lung can be evaluated by pulmonary function tests to evaluate the potential restrictive respiratory dysfunction. A detailed neurologic examination includes muscle strength, skin sensation, abdominal wall reflexes, and tendon reflexes to rule out spinal dysraphism. The patient's back should be carefully examined for abnormal hair, lipomas, skin indentation, and pigmentation, which may be the first signs of intraspinal disorders. Physical examination such as calf asymmetry, talipes cavus, talipes equinovarus, and vertical talus are all signs of spinal dysraphism. It is necessary to conduct these detailed examinations of the lower extremities [8–11].

13.1.7 Imaging Examination of Congenital Scoliosis

Imaging examinations are required to identify pathological abnormalities of the spine, make classifications, and develop surgery plan. Routine radiological examination is important to evaluate the type of the malformations. Infants should be placed in the supine position during radiographic photography. Posteroanterior and lateral radiography can be performed when the child can stand up. Cobb angle measurements in CS patients are sometimes difficult because of the deformed end plates and deformed pedicles. However, the type of deformity, the severity of scoliosis, and the growth potential of the vertebral deformity can be observed using clear radiographs. Meanwhile, clear radiograph is also very important to follow up the progress in CS. The relative size of the different intervertebral spaces can be observed by X-ray to assess the growth potential of the deformed vertebrae. If the intervertebral space is narrow and poorly demarcated, the growth potential of the spine is limited. If the intervertebral space is clear and wider and the intervertebral disc is morphologically normal, it may have a high potential for growth and progress in CS. Even though the traditional evaluation method of CS is radiography, it is difficult to distinguish the small-sized deformities that overlap with malformed vertebral bodies as well as those deformities with complex structures. For patients requiring surgery, more detailed imaging techniques are necessary. CT and MRI are essential for patients with complex spinal deformities undergoing internal fixation. Three-dimensional CT examination is the first choice for the detection of bony structural abnormalities and deformities. For complex deformities, 3D CT should be performed, but it is not recommended during follow-up. Because of its sensitivity and noninvasiveness, MRI has become the first choice to detect the intraspinal canal disorders. The indications of MRI include neurological symptoms identified during physical examinations, such as muscle weakness, anesthesia, and abnormal rectal bladder function, and abnormalities of the skin directly over the spine, including deep sunken skin, hair on the skin surface, or neuropathic pain in the lower extremity and back, lumbosacral kyphosis, and widened inter-pedicle distance. MRI examination is essential for patients with spinal correction and internal fixation. The incidence of genitourinary malformation in patients with CS is 18–40%. Hence, patients with CS should undergo urinary ultrasound examination. Since the incidence of congenital heart disease in patients with congenital spinal deformity is 26%, it is important to perform a detailed cardiac examination and cardiac ultrasound examination [12–16].

13.1.8 Congenital Spinal Deformity Concurrent with Other Deformities

The spine develops during fourth to sixth week of gestation along with the genitourinary, musculoskeletal, and cardiovascular systems. Thus, many CS patients also exhibit abnormalities in other organ systems. These anomalies may be isolated or associated with VACTERL syndrome (vertebral anomalies, anorectal atresia, cardiac anomalies, tracheo-esophageal fistula and/or esophageal atresia, and renal and limb anomalies), and the musculoskeletal system should be closely examined for other anomalies of the cervical spine (Klippel-Feil syndrome), upper extremities (e.g., Sprengel malformation or radial defects), and/or lower extremities (e.g., hip dysplasia) [17]. Of these children, 20–40% have genitourinary anomalies, which are usually with only anatomical abnormalities, but with normal kidney function. However, kidney ultrasound or spinal magnetic resonance imaging (MRI) for kidney evaluation is recommended for all these patient, and 18–26% CS patients have cardiac anomalies [18]. Ventricular septal defect is the most common presentation. Echocardiographic evaluation by a cardiologist is necessary prior to any surgical procedure. Neuroaxial abnormalities occur in up to 40% of CS patients. A variety of abnormalities can be observed, including diastematomyelia, intradural lipoma, syringomyelia, Chiari malformation, and tethered cord. Shen et al. recently reported a 43% incidence of intraspinal anomalies in 226 CS patients, with diastematomyelia being the most common [19]. In this study, patients with thoracic hemivertebra and/or segmentation and failure in mixed diagnosis tend to have intravertebral abnormalities. Recent studies have shown that CS patients and rib deformities have a significantly higher incidence of intraspinal deformities than those without such deformities [8].

13.2 Clinical Practice

13.2.1 Intrauterine Surgical Intervention for Hemivertebral Malformation

Intrauterine surgical intervention for hemivertebral malformation has not been reported worldwide. As surgical intervention for hemivertebra anatomically involves deep tissues, which are adjacent to spinal cord's neural tube structure, vertebral segmental artery, and thoracoabdominal major vessels, it is relatively sophisticated and traumatic. Thus, intrauterine treatment is still in the exploratory stage. However, in the perspective of eugenics, the intrauterine diagnosis of hemivertebra is increasingly mature. Both ultrasound and intrauterine MRI can facilitate the diagnosis of hemivertebra in early stage and guide the continuous medical consultation and medical intervention for the follow-up diagnosis and treatment of children with hemivertebra according to the characteristics of malformation (multiple involved systems concurrent with the malformation).

13.2.2 Conservative Treatment of Hemivertebral Deformity: Case Report

Compared to crankshaft phenomenon of the early stage long-segment fusion and the potential complications of impaired respiratory function, as well as the complications associated with growth-friendly techniques, derotation brace has been described as an effective "time-buying strategy" for congenital scoliosis. Satisfactory clinical results can be obtained by using brace in some patients (Figs. 13.1, 13.2, 13.3, 13.4, 13.5, 13.6, 13.7, 13.8, 13.9, and 13.10).

Treatment of congenital scoliosis with brace: Case 1.

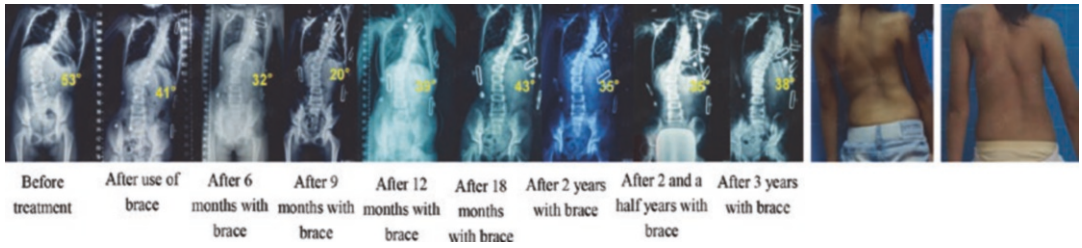

Fig. 13.1 A 3-year-old girl diagnosed with congenital hemivertebra with Cobb angle of 53° was treated with brace for 5 years, and the Cobb angle was controlled to 38°, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 2.

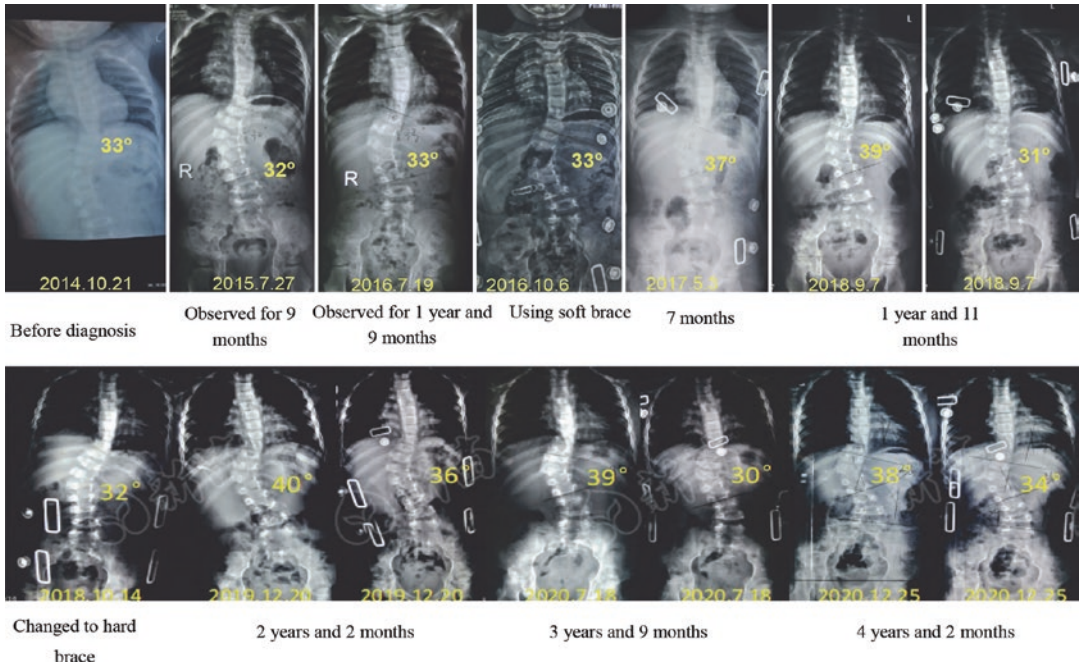

Fig. 13.2 A 4-year-old boy diagnosed with congenital hemivertebra combined with butterfly vertebra with the Cobb angle of 32° used soft and hard braces alternately for 4 years and 2 months, and the Cobb angle was controlled to 34°, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 3.

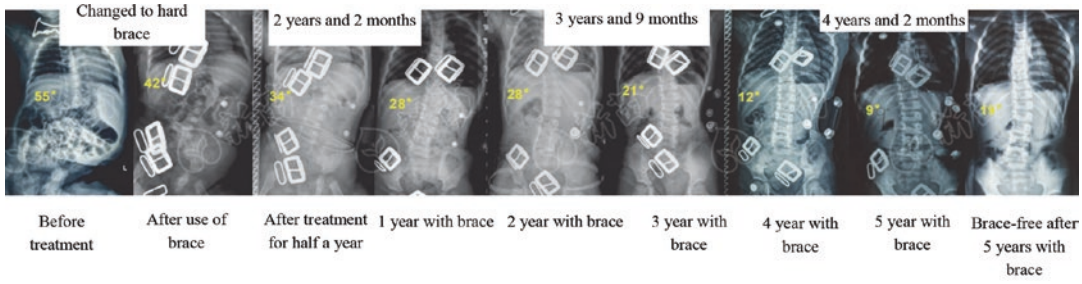

Fig. 13.3 A 3-month-old boy diagnosed with congenital hemivertebra with Cobb angle of 55° used soft and hard braces alternately for 5 years, and the Cobb angle was controlled to 18°, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 4.

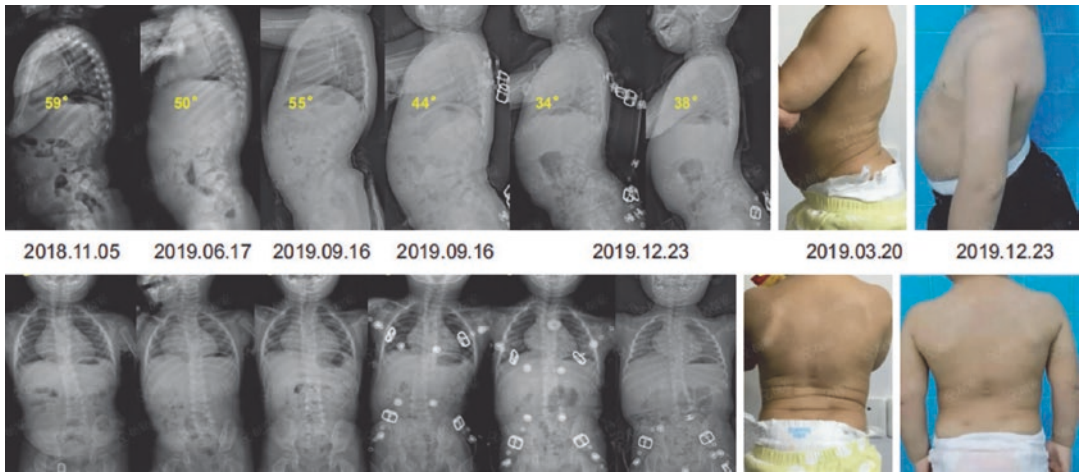

Fig. 13.4 A 1-year-old boy diagnosed with congenital hemivertebra with scoliosis Cobb angle of 55° used soft and hard braces alternately for 5 years, and the Cobb angle was controlled to 38°, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 5.

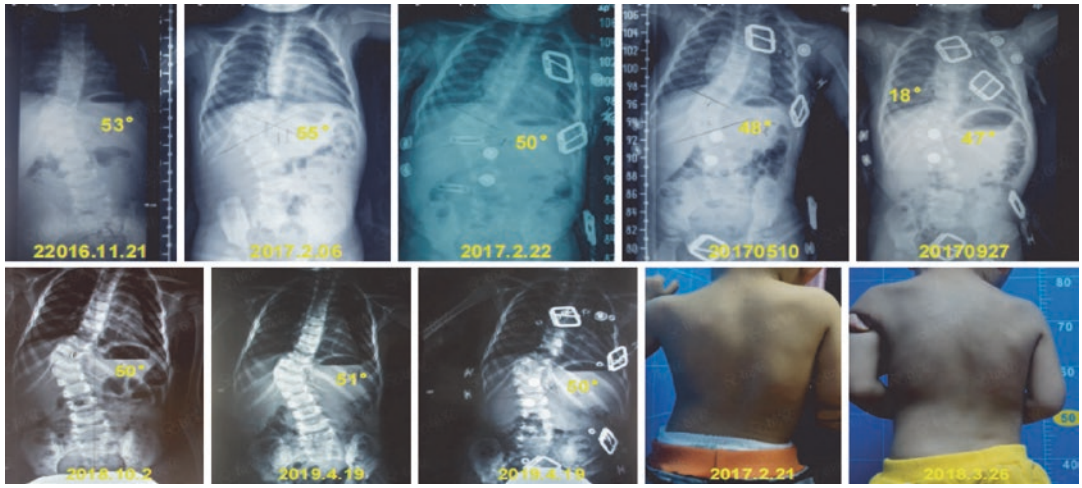

Fig. 13.5 A 1-year-old boy diagnosed with congenital hemivertebra with Cobb angle of 53° used soft and hard braces alternately for 2 years and 6 months, and the Cobb angle was controlled to 53° without significant aggravation, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 6.

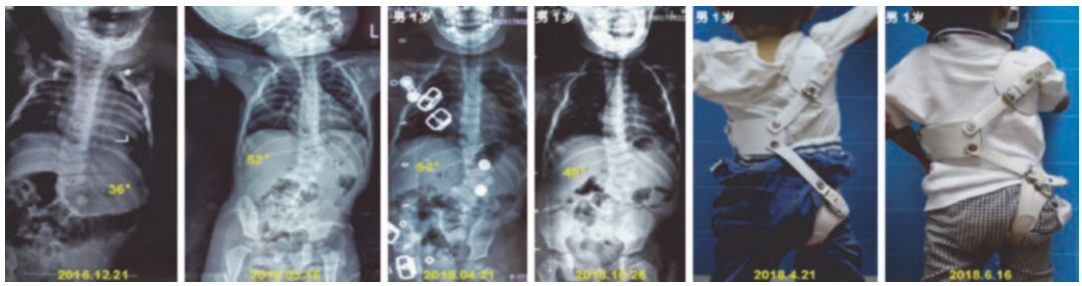

Fig. 13.6 A 2-year-old boy diagnosed with congenital hemivertebra with the Cobb angle of 34° used brace for 2 years, and the Cobb angle was controlled to 45°. Considering the age, it is planned to further postpone the surgery date, and currently, the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 7.

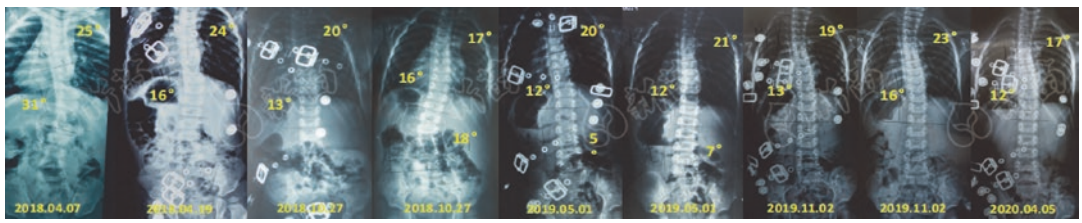

Fig. 13.7 A 1-year-old girl diagnosed with congenital hemivertebra with Cobb angle of 25° and lumbar curvature of 31° used soft and hard braces alternately for 2 years and 7 months, and the Cobb angle was controlled to 12° without significant aggravation, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 8.

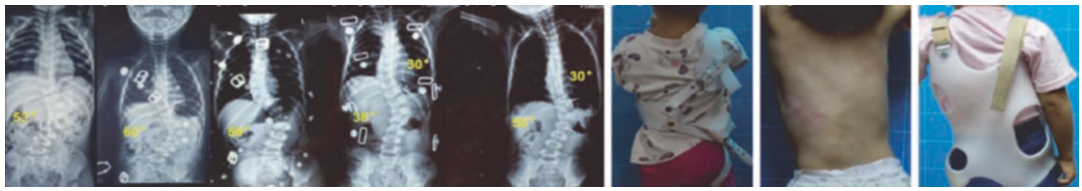

Fig. 13.8 A 1-year-old girl diagnosed with congenital hemivertebra with Cobb angle of 53° used brace for 3 years, and the Cobb angle was controlled to 13° without significant aggravation, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 9.

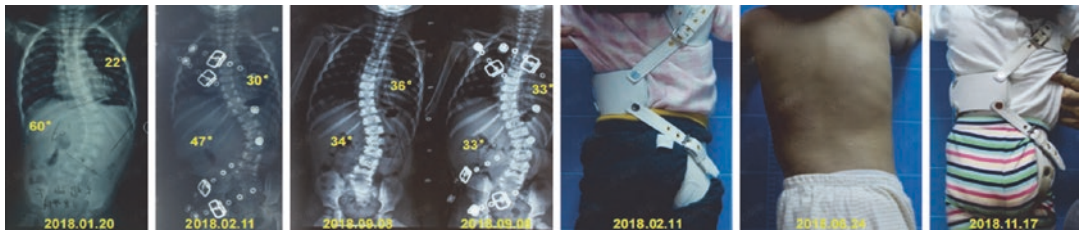

Fig. 13.9 A 1-year-old boy diagnosed with congenital hemivertebra with a Cobb angle of 60° used for 1 year, and the Cobb angle was controlled to 33° and with ongoing improvements, and the brace maintenance treatment is still ongoing

Treatment of congenital scoliosis with brace: Case 10.

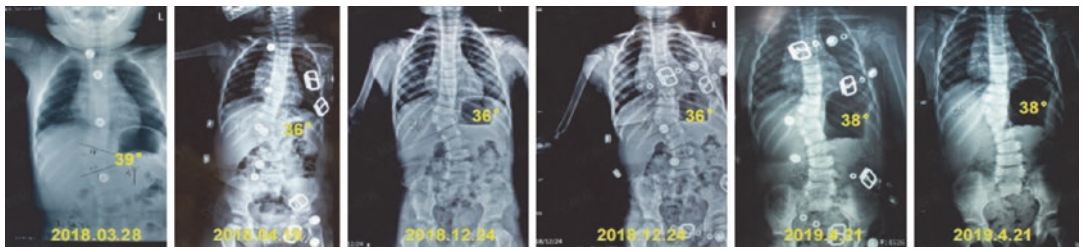

Fig. 13.10 A 1-year-old girl diagnosed with congenital hemivertebra with Cobb angle of 39° used for 1 year, and the Cobb angle was controlled to 33° without significant aggravation, and the brace maintenance treatment is still ongoing

13.2.3 Surgical Treatment of Hemivertebral Malformation: Case Reports (Figs. 13.11, 13.12, 13.13 and 13.14)

Surgical treatment for congenital scoliosis: Case 1.

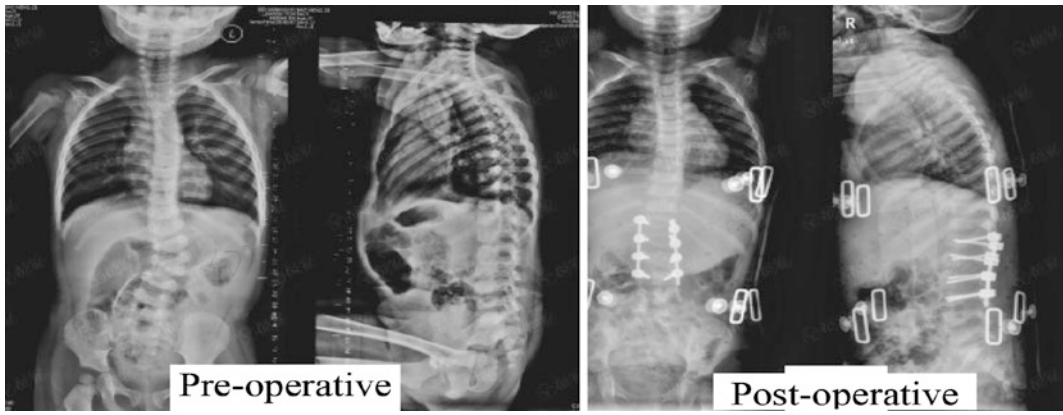

Fig. 13.11 A 1-year-3-month-old girl diagnosed with congenital hemivertebra with a pre-operative scoliosis Cobb angle of 47° was treated with hemi-vertebral resection

Surgical treatment for congenital scoliosis: Case 2.

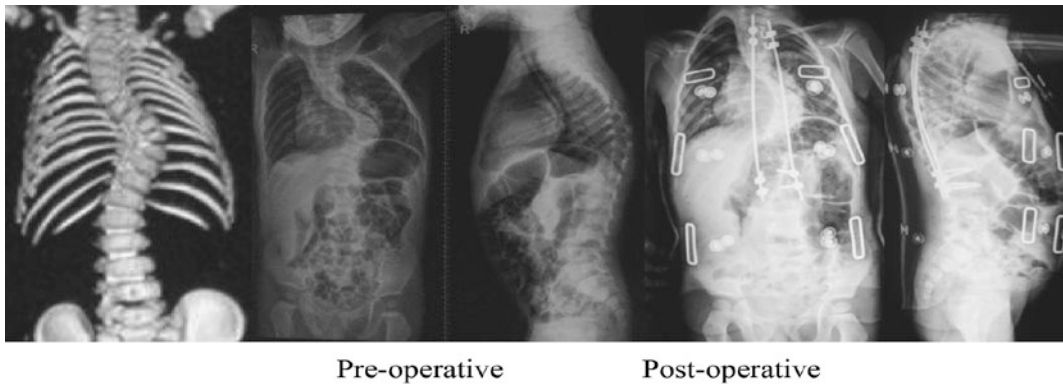

Fig. 13.12 A 2-year-old girl diagnosed with congenital hemivertebra underwent growing-rod technique surgery

Surgical treatment for congenital scoliosis: Case 3.

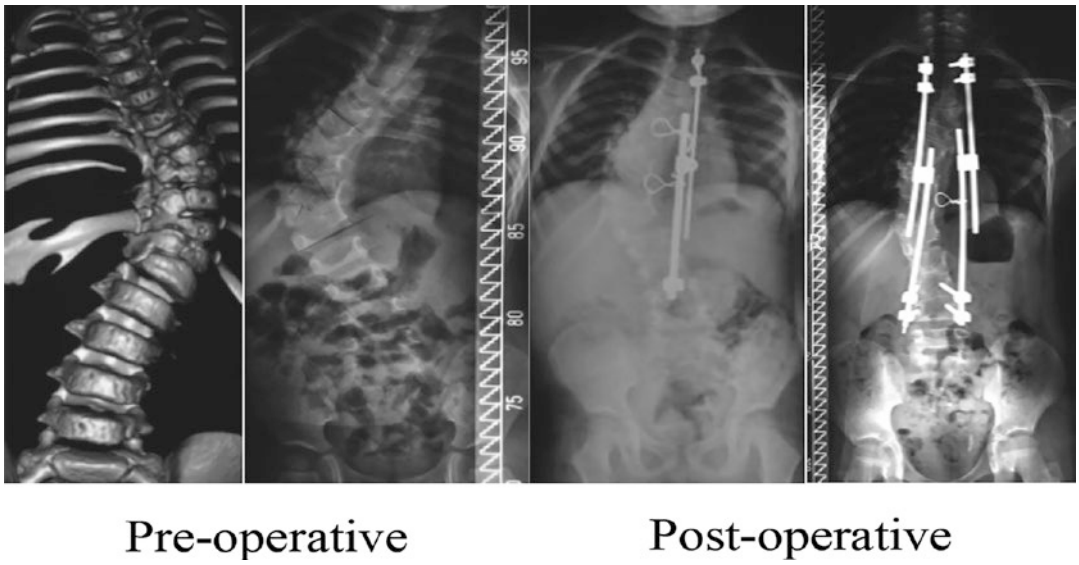

Fig. 13.13 A 4-year-old girl diagnosed with congenital hemivertebra with continuous aggravation underwent growing-rod technique surgery

Surgical treatment for congenital scoliosis: Case 4.

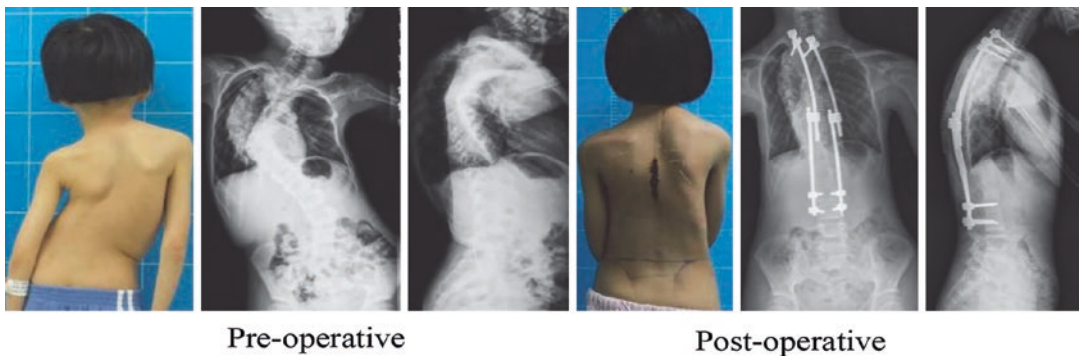

Fig. 13.14 A 6-year-old boy diagnosed with congenital hemivertebra with continuous aggravation underwent growing-rod technique surgery

13.3 Research Progress

13.3.1 Natural History of Hemivertebral Deformity

It is challenging to determine which type of congenital scoliosis progresses rapidly. In general, 25% patients with congenital scoliosis have no progression, 25% have slow progression, and 50% have rapid progression. The determinants of progression include the type of congenital scoliosis, location, and patient age [20]. Normal longitudinal growth of the spine is the sum of the growth of the upper and lower end plates of the vertebral bodies. The presence and quality of the intervertebral disk space around the abnormal vertebral segment may predict the potential of asymmetric growth, since a normal intervertebral disk often can predict the progression in lateral curvature [2]. Fully segmented hemivertebrae have a higher potential of progression, and the presence of identifiable discs indicates growth potential.

McMaster and Ohtsuka [16] reported progression rates in patients with different types of congenital spinal anomalies. The deformity with the highest risk of progression is unilateral fusion combined with contralateral hemivertebra, followed by unilateral fusion, hemivertebra, and wedge vertebra. And lumpy vertebral body is the least likely type to cause any significant deformity. "Hemivertebral displacement" occurs when hemivertebrae on one side of the spine is balanced by that on the other side and separated by a normal vertebral body, which is most common in the thoracic spine. Intuitively, the asymmetric growth potential should balance out under such circumstances. However, these malformations progress in up to 30% of patients [21]. Regarding the age of patients, the progression of scoliosis is generally most rapid before the age of 5 and during the peak growth period in adolescents, with the curvature of the thoracolumbar spine progressing more rapidly compared to the upper thoracic spine [22].

13.3.2 Etiological Study of Hemivertebral Deformity

The specific cause of congenital scoliosis has not been determined. Environmental and genetic factors, vitamin deficiencies, chemicals, and medications, alone or in combination, have all been associated with the development of vertebral abnormalities. Studies of the known causes of these vertebral deformities have shed lights on the etiology of congenital scoliosis. Whatever the cause, physiological damage occurs early in the embryonic period, long before the development of cartilage and bone. The resulting defects may lead to complete or partial fusion or underdevelopment of the vertebral bodies, which in turn may cause progressive curvature during the children growth and development. Congenital scoliosis is a multifactorial disease. Genetic and teratogenic factors play an important role in the development of CS. Vertebral deformities may exist alone or in combination with deformities in heart, kidney, or spinal canal. CS may combine with some recessive chromosomal diseases, such as Alagille, jar-cho-levin, Klippel-Fiel, Goldenhar, Trisomy 18, diabetic embryopathy, and VACTERL (spinal, cardiac, renal and limb anomalies, anal atresia, tracheal fistula, esophageal fistula) [23, 24]. The use of anti-epileptic drugs during pregnancy may lead to CS. Carbon monoxide (CO) and hypoxia are considered common teratogenic factors in congenital spinal malformations. CO is a common teratogenic factor. CO is a colorless, odorless gas, and it binds to hemoglobin with an affinity more than 200–300 times greater than oxygen. Therefore, CO binds readily to hemoglobin in the lung and is difficult to dissociate from hemoglobin in the surrounding tissues; thus, this may interfere with tissue oxygenation. CO can cross the placental barrier, but how CO causes spinal deformities remains unclear, and there are many studies on the correlation between CO and spinal deformities. Animal studies in rats and rabbits have shown that maternal exposure to CO during pregnancy

may lead to spinal and rib deformities. Loder et al. found that 70% of rats with spinal deformities had a CO exposure of 600 ppm on the ninth gestational day. The dose and duration of CO exposure may be the key factors, with the most pronounced effect occurring on the ninth gestational day in mice at the CO exposure of 600 ppm, which corresponds to the fourth gestational week in human embryonic development. Hypoxia is one of the pathogenic factors in experimental animal models [25–27]. These reports show a correlation between vertebral and rib deformities and duration and dose of CO exposure. Spinal deformities in rats demonstrated as vertebral segmentations and formation disorders, which are similar to those in humans. Based on experimental studies in mice, it is speculated that a series of candidate genes, such as Wnt3a, PAX1, DLL3, and Sim2, may be the cause of spinal deformity. Variation in these genes may lead to variation in early somite, resulting in rib fusions and developmental disorders in the anterior side of the spine and defects in dorsal neural arch formation [28].

13.3.3 Progress in Clinical Treatment of Hemivertebral Deformity

In patients diagnosed with CS, attention should be paid to patient age, status of spinal balance, and the type of deformity. If the patient's scoliosis might progress rapidly, such as unilateral hemivertebra combined with poor contralateral segmentation, early treatment is warranted, regardless of the patient's age. If the spine deformity is unlikely to progress, such patients should be followed up regularly, using follow-up radiograph and changes in the Cobb angle to monitor the progression of scoliosis.

13.3.3.1 Observation

Patients with spinal deformities can be followed up once every 4–6 months if the spine is in balance, and rapid progression is rare in patients with hemivertebrae and blocked vertebrae. The spinal balance and the changes in Cobb angle should be monitored. Each reexamined radiograph should be compared with the earlier ones to see if the lateral curvature has any progression [29].

13.3.3.2 Brace

For crankshaft phenomenon of the early stage long-segment fusion and the potential complications of impaired respiratory function, as well as the complications associated with growth-friendly techniques, derotation brace has been described as an effective "time-buying strategy" for congenital scoliosis [30]. Demirkiran et al. [31] reported that patients with progressive congenital scoliosis, described as "long curvature and multiple anomalous vertebrae," were treated with derotation brace, and surgery could be delayed by an average of 26.3 months. Patients enrolled in this study were 1–6.6 years of age. Baulesh et al. [32] reported the outcome of a similar derotation brace for the treatment of non-idiopathic scoliosis, and the surgery was delayed for an average of 2 years. These "delaying" strategies are effective considering the high complication rate associated with long-term treatment based on invasive growth-friendly technique.

13.3.3.3 Growth Regulation

Using in situ fusion (growth block on the convex side) without pedicle or vertebral nails to achieve the purpose of growth block is a treatment option that is more suitable for patients with normal growth potential on the concave side of the lateral curvature. Patients aged less than 5 years with lateral curvature <70° and without kyphosis or lordosis are ideal candidates for growth regulation surgery. Growth block on the convex side is an ideal procedure in the absence of obvious signs of skeletal maturation, but the outcome of this procedure is hard to predict [33–35].

13.3.3.4 Growth Retention/ Stimulation

The growing rod technique is used for treatment of idiopathic scoliosis or similar spinal deformities, which mainly relies on distraction to correct the deformity. However, a thorough review of the literature on growing rod technique published in recent years shows that this method has already been used in CS patients [36]. In a recent multi-

center study, 19 patients with CS who underwent the surgery with the growing rod technique obtained a 31% correction of lateral curvature of T1–S1 and annual extension of 12 mm over a 2-year follow-up period. In addition, the lung-to-thoracic space ratio increased from 0.81 pre-operation to 0.94 post-operation [37, 38]. It is noteworthy that the patient did not experience perioperative neurological injury. The growing rod correction technique is safe and effective in pediatric patients due to the flexibility in the deformed area of congenital scoliosis in children. Moreover, this method is also indicated for cases in which the deformed segment is too long to be resected or those with congenital spinal deformity combined with compensatory curvature. It is one of the thoracic expansion techniques; therefore, it is mainly used in patients with spinal deformities. If a patient has a fused, combined, or isolated thoracic respiratory insufficiency syndrome, in other words, if the main disorder is in the thorax, the treatment to address the thoracic deformity is the optimal route, that is, the thoracic extension therapy [39, 40].

13.3.3.5 Reconstruction

Hemi-vertebral resection is considered a valuable treatment for isolated congenital hemivertebrae. Initially, the resection site was closed with gypsum. Then, the posterior fixation hook was used, which was followed by pedicle screw fixation [41]. Chang et al. [42] reported successful long-term treatment results of hemi-vertebral resection and short-segment fusion using pedicle screws in children under 10 years of age over a mean follow-up period of 11.4 years. This study demonstrated a very high rate of correction of the main curvature (mean 75%) and compensatory curvature (30–78%), with no crankshaft phenomenon during the follow-up period. The CT scan showed no stenosis of the spinal canal, and the vertebral body height was similar to that of the adjacent unfixed segments. Olgun et al. [43] reported that the growth velocity of the vertebral body and spinal canal was not affected by pedicle screw fixation in young children. Chang et al. [42] advocated early hemi-vertebral resection in combination with posterior short-segment fusion with pedicle screws because patients who underwent the surgery before the age of 6 had better deformity correcting effect without any negative impact on vertebral or spinal canal growth compared to patients aged 7–10 years.

13.3.3.6 Prospects of New Directions, Technologies, and Methods

During the last decades, basic research and pharmaceutical research have had a great impact on the surgical field. With the discovery and research of *Helicobacter pylori* and the use of proton pump inhibitors, the landscape of surgical treatment of peptic ulcer has changed. In the past, such diseases were mainly treated by surgery, but now, almost all of them are treated by drug therapy. Unfortunately, so far, such advances have not been common in the treatment of spinal deformities.

Since the mid-1980s, the widespread use of MRI has changed the diagnostic spectrum of early-onset scoliosis. The use of MRI allows the diagnosis and treatment of deformities and anomalies in the spinal canal. MRI has also reduced the number of diagnoses of idiopathic scoliosis, such as Chiari malformation, tethered spinal cord, syringomyelia, diastematomyelia, and other difficult-to-diagnose disorders of the spinal canal. The increasing complexity of diagnosing the causes of early-onset scoliosis has paralleled the rapid advances in genetics and embryology in this field. New syndromes are being recognized, with new variants being discovered.

Translational research has raised the prospect of new treatment norms for early-onset scoliosis at the molecular level. For instance, studies have found that mutations in genes associated with folic acid metabolism have led to the use of routine oral folic acid therapy in women during their first trimester of pregnancy. Thanks to such studies, the incidence of neural tube defects such as anencephaly, herniation, and spina bifida have been significantly reduced. Such a disease due to multiple factors, like nutritional deficiencies and genetic predisposing factors, is also seen in other early-onset scoliosis. For instance, infantile early-onset scoliosis with genetic predisposing

factors, improper posture in the cradle, and other treatments may also lead to the development of a clinical syndrome. The molecular-level study of Marfan's syndrome has brought hope to new drug therapies for the disease. Treatment with TGF-B antibody induced rehabilitation of the vascular disorders in Marfan-gene knockout mice. While it may not be this specific antibody in humans, the use of other TGF-β antagonists may indicate a new era in drug therapy rather than surgical treatment of Marfan's syndrome. With the deepening in the study of idiopathic scoliosis genes, responsible genes can be sequenced, and variant genes can be identified. The vast majority of genetic disorders leading to syndromes with spinal deformities are also associated with chromosomal abnormalities like Down's syndrome or monogenic disorders such as achondroplasia and Prader-Willi syndrome. Progressive Charcot-Marie-Tooth Disease (CMT) is a neurological disorder, which is often associated with spinal deformities. Among them, 70% have one copy of chromosome 17, resulting in increased peripheral myelin protein production. This is the main reason for the progressive nature of the disease. Further studies may facilitate the emergence of novel drug treatments.

Muscular atrophy and muscular dystrophy are the consequences of complex genetic variations, many of which may lead to early-onset scoliosis. A deep understanding of the molecular mechanisms of these variations and diseases can bring bright prospects for further research in drug therapy. Recent studies have gained more insights on the development of congenital scoliosis. Based on the fact that molecular and genetic disorders may lead to vertebral segmentation and formation disorders, we will eventually discover the prophylactic measures for preventing scoliosis in the first month of pregnancy, thereby reducing the incidence of congenital scoliosis and reducing the severity of the deformity. Juvenile idiopathic scoliosis may be a polygenetic disorder in nature. The markers that predict scoliosis progression in adolescent idiopathic scoliosis cannot play the same role in the progression of infantile idiopathic scoliosis. Other polygenetic disorders are mainly tumors, including breast cancer, certain types of colon cancer, and glioblastoma. Translational studies have helped us to understand that molecular pathways of certain mutations contribute to the formation and development of spinal deformities, which provides an opportunity to develop new treatments.

Knowledge with the pathogenesis of idiopathic scoliosis and other diseases that lead to early-onset scoliosis offers hope that presymptomatic treatment or early medical treatment can result in better clinical outcomes. Such studies may involve multidisciplinary researchers, including molecular biologists, geneticists, biostatisticians, epidemiologists, and other specialists. Clinical relevance and guided studies are critical, and it is the responsibility of the clinicians. In addition, clinicians play a critical role in identifying and recruiting patients for conducting clinical studies for a specific disease. In the foreseeable future, blood stem cell transplantation and umbilical cord blood stem cell transplantation with pre-preserved blood may be used to treat some life-threatening diseases. Patients with early-onset scoliosis secondary to neuropathy (cerebral palsy, spinal cord injury, etc.) may be a suitable study population for neural cytokine and ectodermal stem cell transplantation. The authors believe that the use of recombinant proteins and enzymes may play a role in the treatment of some early-onset scoliosis, which is currently commonly used in the treatment of disorders with lysosomal accumulation, such as Gauchier, Fabry, and mucopolysaccharidosis. Bone marrow transplantation and enzyme replacement therapy have allowed patients with Hurler syndrome to survive to adulthood. However, there are still issues to be addressed in skeletal dysplasia and specific spinal deformities associated with mucopolysaccharidosis. The aggregation of glycosaminoglycans in bone and soft tissue is challenging to manage. Its correlation with glycolipids, esterified cholesterol, and GM2 and GM3 gangliosides is complex, which may lead to disorders in the vascular, central, and peripheral nervous systems and tissues of mesodermal origin. More molecular biological studies are warranted to explain certain metabolic abnormalities that cause local instability and even fatal compli-

cations in thoracolumbar kyphosis and upper cervical stenosis. Novel surgical and nonsurgical treatment will continue to play a role in the treatment of early-onset scoliosis. Assessing the efficacy of these treatments may be a profound challenge. Well-controlled, multicenter studies are helpful to the evaluation of novel therapies. This is particularly important for treatment of rare syndromes such as mucopolysaccharidosis. The optimal treatment can be discovered by establishing a database on mucopolysaccharidosis treatment.

Early-onset scoliosis includes many different specific diagnoses, and an accurate diagnosis can help to better understand the natural history of early-onset scoliosis of different etiologies, thus facilitating the evaluation of treatment efficacy. Different phenotypes of the same disease and different genetic abnormalities under the same phenotype have caused the prognostic and outcome studies of early-onset scoliosis questionable. Continuous advances in basic research will help to develop more specific criteria for clinical diagnosis of early-onset scoliosis and novel treatment strategies for better clinical outcomes in clinical settings.

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Reproductive System

14

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Each development stage of human reproductive system is involved with a sophisticated and complex regulatory network. The main organs of the reproductive system (testis/ovary) are originated from the intermediate mesoderm and the subsequent urogenital ridge during embryonic development. The development of external genitalia is closely correlated with the synthesis and function of male hormones. Many congenital disorders related to the differentiation and development of reproductive system are correlated with hereditary factors. This chapter will start from the gonadal development and focus on the hereditary factors contributing to fetal disorders of reproductive system development.

14.1 Normal Embryonic Development of Genitalia and Related Factors

The primordial germ cells migrate from the yolk sac endoderm to the genital ridge from the fourth to fifth week, to form the undifferentiated primordial gonad at about five to six weeks after fertilization. During the early embryonic stage, when male and female reproductive systems are comparable, this period is called the undifferentiated stage of reproductive organs. At the seventh week after fertilization, the primordial gonad of male with XY karyotype is differentiated into the seminiferous tubule and the interstitial cells of the embryo testis (Leydig cell) under *SRY* and *SOX9* genes. In male embryos, the Leydig cells can secrete androgen and interact with its receptors. The Wolffian duct (also called the embryonic duct of the mesonephros) further develops into the epididymis, the seminiferous duct, and seminal vesicles of the embryo. Meanwhile, anti-Müllerian hormone (AMH) secreted by the Sertoli cells will lead to the degeneration of the Müllerian ducts [1]. At 13–16 weeks after fertilization, the primordial gonad of females with XX chromosome karyotype is differentiated into embryonic follicles, thecal cells of embryonic follicles and stromal cells under WNT4, FOXL2, and other factors. Thus, the embryonic ovarian organ is formed. For women, in the absence of androgen and AMH, the Müllerian ducts further develop to form the fallopian tubes, uterus, cervix, and vagina. In the process of gonadal differentiation, multiple genes are involved in temporal and spatial regulations (Fig. 14.1). Any genetic defect involved in the above regulations may lead to errors in gonadal differentiation, resulting in disorders of sexual development or abnormalities in sperms/ova.

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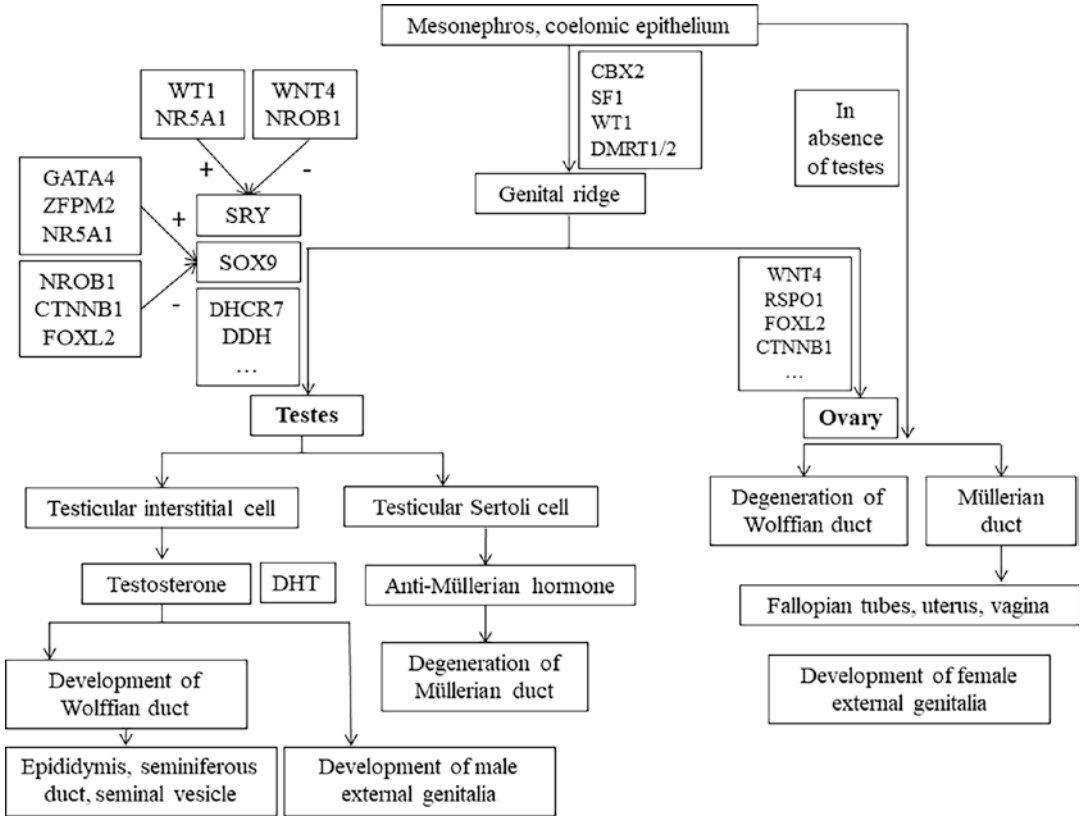

Fig. 14.1 Genetic mechanisms of human sex determination

14.1.1 Main Factors Related to the Development and Differentiation of Testis and External Genitalia

14.1.1.1 SRY

In mammals, sexual development occurs in two distinct and successive stages: sex determination and sex differentiation. In the process of male sex determination, the expression of *SRY* gene located on the Y chromosome initiates the cascade expression of genes in the Sertoli cells, which ultimately drives the morphological differentiation of the testis. *SRY* gene is a regulatory gene that plays a primary role in sex determination. The mRNA levels in the fetus with 46, XY karyotype are upregulated at the urogenital ridge seven weeks post-conception and drive the development of the bipotential gonad into testes [2]. After translation, *SRY* is transferred to the nucleus and binds to the enhancer region of *SOX9*, to drive the differentiation and proliferation of Sertoli cells and the seminiferous tubule tissue of the testis. In the process of sex differentiation, the testes secrete testosterone, dihydrotestosterone, and anti-Müllerian hormone, leading to the development of the male internal and external genitalia (prostate, seminiferous duct, penis, and scrotum) and the degeneration of the Müllerian ducts.

14.1.1.2 SOX9

SOX9 is the second major gene involved in male sex determination, and it encodes an *SRY*-related transcription factor. Expression of SOX9 is essential for testis differentiation, and it acts synergistically with SRY and NR5A1 transcription factors. SOX9 binds to its own promoter, forming a positive feedback loop that maintains high levels of SOX9 expression.

14.1.1.3 NR5A1

NR5A1, also referred as the steroidogenic factor-1 or SF-1, encodes an orphan nuclear receptor that plays an important role in the development of the hypothalamic-pituitary-gonadal-adrenal axis. In the Sertoli cells, NR5A1 acts synergistically with the transcription factor GATA4 at initial formation of the testis. It also binds to the SRY promoter to upregulate the expression of SRY. SF-1 can be detected in the primordial reproductive ridge 32 days after fertilization in human embryos. When the testes can be morphologically recognized, SF-1 is mainly confined to the Sertoli cells of the sex cord and subsequently expressed mainly in the Leydig cells. In addition to the gonad, SF-1 is also expressed in the ventromedial hypothalamic nucleus and pituitary gonadotropin cells.

14.1.1.4 NROB1

NROB1, also known as DAX1, is the nuclear receptor transcription factor that plays an important role in the development of human adrenal cortex and gonad. It is expressed in adrenal cortex, gonad, pituitary, and ventral median nucleus of hypothalamus. In individuals with XY karyotype, there is only a single copy of this gene. The presence of gene duplication mutation will lead to NROB1 overexpression, which may inhibit testicular differentiation. The gene duplication mutation of NR0B1 will result in a dose-dependent XY gonadal dysgenesis and a female phenotype. One of its molecular pathogenic mechanisms found in NROB1 transgenic XY mice is through the direct repression of NR5A1-mediated *SOX9* transcription. In females with the 46XX karyotype, the presence of two fully functioning copies of the *NR0B1* gene are essential to prevent testicular formation. Generally, loss-of-function mutation in NROB1 will lead to hypogonadotropic dysgenesis with primary adrenal insufficiency in males with the 46XY karyotype.

14.1.1.5 GATA4 and ZFPM2

Heterozygous mutations in the *GATA4* gene may lead to 46, XY disorders of sexual development. GATA4 is also associated with congenital heart disease, suggesting that GATA4 plays a role in gonadal and cardiac development. In the mouse model, *GATA4* mutation disrupted the connection between GATA4 and ZFPM2, resulting in abnormal testicular development. In the porcine model, GATA4 directly activated the SRY promoter, whereas in humans and mice, direct activation of SRY expression was observed only when the WT1 protein was also expressed. In the mouse model, it was found that mutations in *GATA4* or *ZFPM2* resulted in reduced interactions between GATA4 and ZFPM2 proteins, resulting in a decreased ability of any gene (independently or co-expressed) to activate the transcription of target genes such as *AMH*, *SRY*, and *SOX9* [3].

14.1.1.6 DMRT1

In the process of human fetal development, DMRT1 mRNA is detectable in both sexes by 11 gestational weeks, most abundant in Sertoli cell precursors during 10–20 gestational weeks; it is also expressed in oogonia and oocyte by 20 gestational weeks, and it decreases after meiosis [4]. DMRT1 is essential for the maintenance of the fate of Sertoli cells during testis differentiation. The expression of DMRT1 in testicular Sertoli cells of mice after birth maintains the high level of SOX9 expression, thus promoting the expression of testicle-specific genes in Sertoli cells and inhibiting the differentiation of ovarian-specific granulosa cells. Inactivating mutations in *DMRT1* cause these cells to differentiate into granulosa cells instead.

During early embryonic development, CBX2, SF1, WT1, and DMRT1/2 are involved in the regulation of the differentiation from mesonephros and coelom epithelium to genital ridge. During gonadal differentiation, *SRY* gene expression in human embryos with XY karyotype acts as a switch for testicular differentiation, driving the genital ridge to differentiate into testis. SRY is positively regulated by WT1 and NR5A1, while being inhibited by double concentrations of NROB1 and WNT4. SRY protein may activate some downstream genes such as *SOX9*. At the same time, it is also regulated by multiple transcription factors, which in turn initiates a network of testis gene expression and inhibits ovary-specific gene expression (WNT4 and RSPO1).

14.1.2 Major Factors Involved in Differentiation During Normal Development of the Ovary, Fallopian Tube, and Uterus

In response to regulating factors including WNT4, RSPO1, FOXL2, and others, the primordial genital ridge develops into an ovary in females with the 46, XX karyotype. In female embryos with the XX karyotype due to anorchia or loss of function of the testis, the Wolffian duct degenerates without the support of the high concentration testosterone. Meanwhile, the Müllerian duct develops due to the absence of the inhibition of anti-Müllerian hormone, and it is eventually differentiated into the oviduct, uterus, and vagina [5]. The meiosis of fetal germ cells to differentiate into oocyte and ovarian development involves the activation of gene pathways, including RSPO1/WNT4/β-catenin signaling, which is inhibited by SRY. In XX gonads, the Sertoli cell precursors accumulate β-catenin in response to RSPO1/WNT4 signaling transduction and inhibit the activity of SOX9.

14.1.2.1 WNT4

WNT4 plays a key role in the regulation of the Wnt/β-catenin signaling pathway. It also plays an important role in the regulation of mammalian gonadal differentiation and development. Fluorescence in situ hybridization (FISH) analysis reveals that Wnt4 transcripts were predominantly located in the cytoplasm of oocytes. WNT4 is a key regulator of mammalian ovarian development, with the highest levels of Wnt4 expression occurring during the embryonic stage [6, 7]. In mice, knockout of wnt4 may affect ovarian development and steroid synthesis and will lead to sex reversal in females [8].

14.1.2.2 RSPO1

RSPO1 (R-spondin 1) protein is a multipotent signaling ligand, with its key function of enhancing Wnt/β-catenin signaling transduction [9]. RSPO1 is a sex determining factor in female mammals, which plays a key role in reproductive organ development [10]. RSPO1 regulates the expression of gonadal differentiation-related factors via the β-catenin signaling pathway. It can also regulate the division and proliferation of primordial germ cells and the differentiation of somatic cells in gonad to resist the formation of testis, thus determining the female differentiation. Embryonic *RSPO1* mutations may result in impaired development of reproductive organs [11]. The mutations leading to sexual dysplasia identified so far are all involved in the highly conserved N-terminal cysteine-rich domain, which plays a key role in the activation of Wnt/β-catenin signaling pathway [12].

14.1.2.3 FOXL2

FOXL2 is a member of the forkhead box protein-encoding gene family, and its encoded transcription factor is evolutionarily highly conservative [13]. It is one of the genes with its expression first found to be upregulated in the ovarian development of female mice, suggesting that FOXL2 plays an important role in the process of early ovarian differentiation [14]. FOXL2, a nuclear protein expressed in ovarian follicular cells, is the earliest known marker in ovarian differentiation in mammals. It may play a role in ovarian cell differentiation and follicle development and/or maintenance [15, 16].

In addition, there are a variety of other molecules involved in the normal development of the ovary, fallopian tube, and uterus. During early embryonic development, HOX family genes play an important role in the differentiation of reproductive tract. Their transcription factors regulate the structure of the anterior-posterior axis of the Müllerian duct by regulating the corresponding positional information. Homologous expressed genes *HOXa9*, *HOXa10*, *HOXa11,* and *HOXa13* have been found to be expressed along the long axis of the Müllerian duct, and their expressions are overlapped in the mesenchyme of the local genital tract of female mice. The *Wnt* genes also play an important role in regulating the anterior-posterior axis and the radial axis. The expression of transcription factor P63 is the primary marker to distinguish the epithelial cells of the uterine, vagina, and cervix. The *CTNNB1* (catenin beta-1) gene is also involved in the regulation of

endometrial epithelial differentiation and plays an important role in maintaining the characteristics of the uterine epithelium.

14.2 Common Disorders and Pathogenic Genes of Congenital Reproductive System Anomalies

14.2.1 46, XY Gonadal Dysgenesis-Related Disorders

14.2.1.1 Testicular Hypoplasia

14.2.1.1.1 Complete Gonadal Dysgenesis (Swyer Syndrome)

Swyer syndrome is characterized by a female phenotype with ovarian degeneration and dysplasia of secondary sexual characteristics. The gonads are fibrous cords without follicles or normal germ cells, and they are at high risk of developing gonadoblastoma. The internal reproductive organs include bilateral fallopian tubes, uterus, and vagina. Females with Swyer syndrome have normal to tall stature. It is caused primarily by the mutations or deletions of the *SRY* gene on the Y chromosome. Another form of Swyer syndrome is gonadal dysgenesis with intact Y chromosome, which is caused by mutations or deletions/duplications of other genes that regulate gonadal differentiation (such as *SF-1, Dax-1, Wt-1* genes, etc.). It is resulting from testicular insufficiency, such as deficiency in anti-Müllerian hormone and androgens. The genital duct-derived organs are usually the uterus and fallopian tubes, and the external genitalia are under-masculinized [17, 18].

14.2.1.1.2 Partial Gonadal Dysgenesis

Patients with partial gonadal dysgenesis may present with female Turner syndrome signs (short stature, broad chest, cubitus valgus, etc.), ambiguous gender in external genitalia, and hypertrophy of clitoris. There is masculinization presentation in puberty. The gonads are often located in the abdominal cavity, with a cord-like gonad on one side and a malformed testis on the other. It may be induced by *DHH* gene mutation [18].

14.2.1.1.3 Common Disorders Leading to Male Gonadal Dysgenesis

Dax-1 Duplication Mutation

Dax-1 gene duplication mutation may induce gonadal dysgenesis in 46, XY males. The patients present with female external genitalia, with rare cases of reproductive duct-derived organs being the uterus and fallopian tube. The gonad is hypoplastic testis or ovary, the blood sex hormone level is decreased, and the gonadotropin level is increased. If the ovarian function is relatively normal, the sex hormone and gonadotropin levels are close to the normal female level, and the patient may combine with mental retardation, growth retardation, and facial-cranial malformation [18, 19].

Wt-1 Defect Syndrome

Wt-1 (Wilms' tumor suppressor 1) gene is expressed in the kidney, gonad, and primordial genital ridge. The missense mutation in exon 9 of *Wt-1* may lead to Denys-Drash syndrome, which is characterized by under-masculinization of external genitalia, cord-like gonad or hypoplastic testis, genital duct-derived organs being uterus and fallopian tubes, and with complicated renal lesions. Plasma gonadotropin level is increased, and gonadal hormone level is decreased. As the gonad is at high risk of developing blastoma, the probability of developing Wilms' tumor is about 4%. The splice site mutation in exon 9 of *Wt-1* gene will result in Frasier syndrome, with clinical manifestations including a cord-like gonad, genital duct-derived organs being dysplastic uterus and fallopian tube, and female external genitalia and may be concurrent with gonadoblastoma and renal lesions. *Wt-1* gene heterozygous deletion will lead to WAGR syndrome, which is characterized by Wilms' tumor; aniridia or iris malformation; urogenital dysplasia, including renal agenesis, horseshoe kidney, urethral atresia, hypospadias and cryptorchidism; and mental retardation [20].

SOX9 Defect

The main clinical manifestations include limb flexion and gonadal dysgenesis, which are

inherited as autosomal dominant inheritance disorders. Limb flexion includes long bone flexion, hypoplasia of scapula, pelvic deformity, small thorax, 11 pairs of ribs, cleft palate, macrocephaly, micromandible, orbital hypertelorism, and various degrees of cardiovascular and renal malformations. In three-fourth of patients, the gonads may develop to hypoplastic testes or ovaries, with the presence of the uterus and fallopian tubes, and the epididymis and seminiferous duct are absent or hypoplastic. Approximately 70% of patients have ambiguous external genitalia, and the remainder have female or male phenotypes [21].

SF-1 Defect

Mutations in the *SF-1* gene may lead to gonadal dysgenesis and adrenocortical insufficiency, which are manifested as female external genitalia, presence of uterus and fallopian tubes, gonadal dysgenesis, or absence of secondary sexual characteristic development during puberty, which are combined with manifestations of adrenocortical insufficiency [22].

14.2.1.2 Disorders in Androgen Synthesis or Androgen Dysfunction

14.2.1.2.1 Androgen Synthesis Disorder

5α-reductase Deficiency

It is an autosomal recessive disorder of sex differentiation due to deficiency of 5α-reductase resulting in insufficient androgen effect. 5α-reductase is a membrane protein located on the microsomes of target cells, which involves in the catalytic conversion of testosterone to the more potent dihydrotestosterone. In the process of the differentiation of external genital in a male fetus, dihydrotestosterone guides the bidirectional differentiation potentials of the genitalia primordium to differentiate toward the male direction, which is closely correlated with the development of scrotum and prostate. Male patients with 5α-reductase deficiency tend to develop feminization of external genitalia, which is clinically manifested as different degrees of sexual differentiation disorders, persistent urogenital sinus (blind-ended vagina), with clitoritis-like penis that can erect, with normal testes, seminiferous duct, and epididymides, but the prostate may appear as a form of residue. The testes may be located in the scrotum or groin, and the seminiferous duct and epididymis may open into the blind-ended vagina. At birth, the external genitalia present as a female phenotype, typically presenting with a pseudo-vaginal perineoscrotal hypospadias. During puberty, increased testicular secretion of testosterone drives male puberty development, testicular descent and enlargement, penis becoming longer and thicker, voice becoming rough, and muscularity. However, they may have sparse beard and pubic hair, armpit hair and body hair, a small prostate, and oligozoospermia. The patients have male personality and sexual awareness [20].

StAR Deficiency

Steroidogenic acute regulatory protein (StAR) transports cholesterol into the mitochondrial membrane, which is the first and rate-limiting step in the synthesis of steroid hormones. It is expressed in the adrenal gland and the gonads. Mutations in the *StAR* gene will result in cholesterol accumulation in adrenocortical cells and Leydig cells, and the resulting disruption of cell function may lead to congenital lipoid adrenal hyperplasia, which is inherited as an autosomal recessive disorder. The 46, XY male patients have female external genitalia, with a blind-ended vagina and absence of uterus and fallopian tubes. The gonads are presented as testes, which are located in the abdominal cavity, inguinal canal, or labia majora. After birth, due to the complete blockade of adrenocortical hormone synthesis, the newborn may have the manifestation of severe adrenocortical insufficiency [18, 23].

3β-hydroxysteroid Dehydrogenase Deficiency

3β-hydroxysteroid dehydrogenase (HSD3B2) is involved in the catalytic conversion of Δ5-steroids to Δ4-steroids. HSD3B2 deficiency will lead to the disorders in the synthesis of aldosterone, cortisol, and testosterone, resulting in the accumula-

tion of Δ5-steroids in the body. Among them, dehydroepiandrosterone (DHEA), a weak androgen, may lead to masculinization in female patients and varying degrees of feminization in male patients. There are two isozyme types of 3β-hydroxysteroid dehydrogenase, HSD3B1 and HSD3B2. HSD3B1 is distributed in peripheral tissues such as placenta, skin, and mammary gland, while HSD3B2 is distributed in adrenal gland and gonad. And the two enzymes are 93% homologous. The enzymatic activity of HSD3Bl is five times stronger compared to HSD3B2. Mutations in *HSD3B1* may lead to death of the fetus because of the inability to synthesize progesterone. Mutations in *HSD3B2* gene will result in adrenal and gonadal dysfunction, which is inherited as an autosomal recessive disorder. Male patients usually have micropenis with moderate to severe hypospadias in their external genitals, and adrenocortical crisis may occur after birth. Gynecomastia may occur during puberty, which is presumably attributed to the compensatory effect of HSD3B1 in peripheral tissues. In some patients, sufficient amount of testosterone can be generated to support penile development and spermatogenesis after glucocorticoid replacement therapy, due to the compensatory effect of HSD3B1 [20, 23].

17α-hydroxylase/17,20-lyase Deficiency

CYP17A1 has both of the functions of 17α-hydroxylase and 17,20-lyase. It exists in adrenal gland and gonad and is involved in the catalytic conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively. Mutations in *CYP17A1* may result in blockade of synthesis of cortisol and androgen, and the elevation of ACTH as feedback stimulates the increase in synthesis of deoxycorticosterone and corticosterone, further leading to hypernatremia and hypokalemia. The external genitalia of male patients often show micropenis with hypospadias. And a severe patient may have completely female external genital and blind-ended vagina, presence of epididymides and seminiferous duct, and absence of uterus and oviducts, and the gonads are testes locating anywhere in the descending process [20, 23].

17β-hydroxysteroid Dehydrogenase (HSD17B3) Deficiency

HSD17B3 is a testicular mitochondrial enzyme involved in the catalytic conversion of androstenedione to testosterone. This gene mutation may induce testosterone synthesis disorders. Most such patients have female external genitals. In a few patients, external genitals are ambiguous, with blind-ended vagina, presence of epididymis and seminiferous duct, and absence of uterus and fallopian tube, and the gonad is testis, which is often located in the groin. During puberty, with the increase in androstenedione, testosterone, estrone, and gonadotropin levels, patients may have masculinization, hairiness, voice change, and increased muscle mass. Some patients have different degrees of gynecomastia [20, 23].

14.2.1.2.2 Androgen Dysfunction (Complete/Partial Androgen Insensitivity Syndrome)

Androgen insensitivity syndrome is an X-linked recessive genetic disorder due to androgen receptor deficiency. Androgen receptors are the macromolecules that mediate the critical role of androgens in target cells. Defect in the genes encoding the androgen receptors is the main pathogenic cause. The development of the male embryo into a normal male phenotype requires, in addition to adequate testosterone secretion from the embryonic testes, the presence of androgen receptors on the primordium of the external genitalia and the mesonephric ductal structures such as the prostate in the embryo in order for these structures to develop into the tissues of normal male reproductive organs. Androgen insensitivity syndromes are resulting from defects of the androgen receptor itself or post-receptor defects. The patients may have different degrees of clinical manifestations, either completely female phenotype or subfertile males with normal male genitalia [20, 23], which can be divided into complete and incomplete types. Patients with complete androgenic insensitivity syndrome is born with a completely female phenotype, with a normal sized clitoris and scarce pubic hair with feminine distribution but with scant or absent axillary hair. The vagina is blind-ended, without uterus and adnexa.

The testis is not in fixed site, which may be located in the abdominal cavity or in the groin, even in the labia majora in a few cases. The clinical manifestations of incomplete androgen insensitivity syndrome are diverse, most of which are male or showing a male tendency appearance. The most common is the male phenotype at birth but with hypospadias, mostly with cryptorchidism, and without spermatogenic function. The Müllerian structures are absent, and mesonephric duct-derived organs can be present but with dysplasia [23, 24].

14.2.1.2.3 Leydig Cell Anergy Syndrome

The HCG/LH receptor on the testis is located on the membrane surface of the interstitial membrane of the testis. When HCG/LH binds to the receptor, it triggers the G protein to be allosteric, activates the cAMP-dependent protein kinase, and initiates the synthesis of testosterone through a cascade reaction. Leydig cell anergy syndrome is induced by mutation in the gene encoding the HCG/LH receptor, which prevents the testis from responding to HCG/LH. At the same time, the differentiation and development of Leydig cells depend on the stimulation of HCG/LH. The absence of HCG/LH will lead to the hypoplasia or absence of Leydig cells. Such patients do not have complete masculinization. The most severe cases have completely female appearance at birth, with urethral meatus and vaginal meatus, clitoris hypertrophy, or scroto-labial fusion, and incomplete testicular descent may be found in the groin, labia majora, or scroto-labial fusion. A hypoplastic epididymis and seminiferous duct may be present. Small penis with hypospadias can be seen in mild cases. Serum gonadotropin level is increased, and testosterone and estrogen levls are decreased. The testosterone does not respond to hCG stimulation test. The testes biopsies reveal lack of Leydig cells, but Sertoli cells are normal and have nearly normal seminiferous tubules and incomplete spermatogenesis [20, 23, 25].

14.2.1.2.4 Persistent Müllerian Duct Syndrome (PMDS)

It is mainly induced by mutations in the genes encoding anti-Müllerian hormone or the genes encoding anti-Müllerian hormone receptor and is named as persistent Müllerian duct syndrome type I and persistent Müllerian duct syndrome type II, respectively. The clinical manifestations are the same for these two types. Anti-Müllerian hormone (AMH) is secreted by Sertoli cells of the testis and induces the degeneration of the Müllerian ducts. If this hormone and its receptor are deficient, the Müllerian duct will not be degenerated and differentiated into the uterus and fallopian tubes. Patients with 46, XY generally have normal testicular development but with fallopian tubes and a uterus, cryptorchidism, and testes in the groin. Testes and fallopian tubes can also co-exist in the pelvic cavity [26].

14.2.1.2.5 Hypogonadotropic Hypogonadism

Hypothalamic or pituitary dysfunction due to various congenital or acquired factors may lead to decreased secretion of gonadotropins (FSH, LH), thus resulting in hypoplasia of the testis and decreased secretion of androgens. Clinical manifestations include testicular dysplasia, micropenis, and low levels of plasma gonadotropin and testosterone [17].

14.2.2 46, XX Gonadal Dysgenesis Disorders

The etiologies of 46, XX DSD include disorders associated with gonadal (ovarian) dysfunction, hyperandrogenism, and other structural abnormalities or syndromes [17].

14.2.2.1 Ovarian Dysgenesis

14.2.2.1.1 Ovotesticular Development Disorders

Ovotesticular disorder of sexual development is the co-existence of ovarian follicles and seminiferous tubules in the same patient. Specific phenotypes depend on relative gene expression patterns and the function of the gonad. Gonadal histological type can include ovary, testes, ovotestis, and dysgenesis types [18]. The underlying mechanism leading to ovotesticular disorders in 46, XX (SRY-) individuals may involve activation of

genes involved in testicular development in the absence of *SRY* and/or under-expression of ovarian/anti-testicular genes. Genes associated with ovotesticular developmental disorders include *NR5A1, SOX3, SOX10, WNT4, RSPO1*, etc. [27].

14.2.2.1.2 Testicular Sexual Development Disorder

There is a pseudo-autosomal region at the end of the short arm of X and Y chromosomes, which contains homologous genetic information, and exchange of genetic material occurs during meiosis pairing. *SRY* gene is located near the proximal end of the pseudo-autosomal region. Hence, if the short arm ends of X and Y chromosomes have unbalanced exchange including *SRY* gene, the 46, XX sex chromosome karyotype containing *SRY* may be generated, or the 46, XY karyotype without *SRY* may be formed [24]. In *SRY*-positive 46, XX patients, the dose of Y to X translocation is heterogeneous, from only encompassing the *SRY* region to possibly occupying 40% of the short arm of the Y chromosome. About one-third of patients have a cut point in the protein kinase gene region, and the more the short arms are translocated, the greater extent of masculinization the phenotype is. Approximately 80% of XX males are induced by Y to X translocations. The remaining 20% may involve mutations in other autosomal or X-linked genes, such as duplications of the *SOX9* locus or potential *SOX9* regulatory elements, or the presence of an underlying Y chromosome that is difficult to detect [26]. The patient's chromosome karyotype was 46, XX, with the clinical manifestations of male phenotype with small testis and (or) micropenis, cryptorchidism, and gynecomastia in some cases [18].

14.2.2.1.3 Gonadal Dysgenesis

Simple 46, XX gonadal dysgenesis syndrome is partly induced by defect of the FSH receptor (FSHR) located on 2p. Other etiologies are unknown and are presumed to be related to mutations in genes or receptor genes associated with ovarian organogenesis, such as mutations in primordial germ cell migration genes [24, 25]. Patients with this disease have a karyotype of 46, XX. The external genitalia are female, and other clinical manifestation include primary amenorrhea, no secondary sex characteristics developed by pubertal age, and normal height. Their gonads may be bilateral striated tissue, one side striated, contralateral underdeveloped ovaries, or bilateral underdeveloped ovaries. Sex hormone levels are reduced with elevated gonadotropin levels.

14.2.2.2 Clitoral Hypertrophy Due to Hyperandrogenism

14.2.2.2.1 Fetal Source Androgen Overload (21-hydroxylase Deficiency)

21-hydroxylase (*CYP21A2*) deficiency is the most common cause of the abnormal genital development in individuals with 46, XX gonadal dysgenesis. Approximately 95% of CAH are 21-hydroxylase deficiency caused by *CYP21A2* mutation, which will convert 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone substrates for the synthesis of cortisol and aldosterone, respectively. The classical type, with an incidence of about 1:15,000, can be divided to salt-wasting type and simple virilizing type. There is another more modest nontypical type. Female external genital abnormalities in classical salt-wasting 21-hydroxylase deficiency usually occur during the fetal or neonatal period, with external genital ranging from clitoral hypertrophy to perineal hypospadias to labial fusion. The extent of masculinization of the external genital can be so extensive that the appearance of external genitals of affected female infants resembles that of males with undescended testes on both sides. Unless determined by neonatal screening, infants with congenital adrenal hyperplasia typically presented with loss of weight gain, feeding difficulties, somnolence, dehydration, hypotension, hyponatremia, hyperkalemia, and masculinization of the external genitalia with vulvar pigmentation during the first two to three weeks after birth. It can be fatal when the diagnosis is delayed or missed. Current newborn screening has reduced mortality from acute adrenal insufficiency in this disease [28].

14.2.2.2.2 Fetal Placental-Derived Androgen Excess (Aromatase Deficiency, P450 Oxidoreductase)

Aromatase Deficiency

An autosomal recessive disease is caused by mutations in the *CYP19A1*, which encodes an aromatase. This enzyme is converted into estrogen in gonadal and extragonadal tissues, including the placenta. The placental tissue of aromatase deficient fetuses is unable to convert dehydroepiandrosterone sulfate from fetal adrenal glands to estrogen, with subsequent accumulation of its precursor androstenedione and testosterone. Affected female patients were born with an ambiguous phenotype of external genitalia, and their mothers developed masculine signs after the first trimester of pregnancy. Affected female patients may develop ovarian cysts in childhood, may not develop secondary sexual characteristics at puberty, and may develop primary amenorrhea and hyperandrogenemia [29]. If untreated, men and women with this disease will likely develop osteoporosis and tall stature.

P450 Oxidoreductase Deficiency

Cytochrome P450 oxidoreductases are electron donors for all microsomal P450 enzymes and other non-P450 enzymes. Mutations in this enzyme may affect the activity of enzymes involved in the synthesis of glucocorticoids, mineralocorticoids, and estrogens. The signs and symptoms range from mild to severe, depending on the mutation. Newborns with 46, XY may present with ambiguous external genitalia. Patients with severe P450 oxidoreductase deficiency may have skeletal deformities such as craniosynostosis, midface retrusion, forehead protrusion, arachnoid protrusion, bowing of femora, and radiohumeral synostosis, which is known as Antley-Bixler syndrome. Patients with the disease may also manifest with nostril atresia, mental retardation, and developmental retardation. The mothers of some of these newborns will have virilizing manifestations during pregnancy due to lack of aromatase activity [30].

14.2.2.2.3 Excess Maternal Androgens (Luteoma, Intake of Androgen Drugs)

Maternal Luteoma

Gestational luteoma is a benign, non-neoplastic lesion of the ovary. It is induced by the increased activity of luteinizing cells stimulated by the androgen that produces chorionic gonadotropin (HCG). Approximately two-third of female newborns born to masculinized mothers show some degree of masculinization [31].

Krukenberg's Tumor of Ovary

Krukenberg's tumor of ovary arises from the metastatic adenocarcinomas of gastrointestinal tissue or the breast. These tumors may produce androgens due to luteinization of the tumor stroma. During pregnancy, high-level androgen production due to elevated HCG levels may lead to masculinization of the pregnant woman and the female fetus [32].

Exogenous Androgen

In addition to gestational luteoma and androgen secretory tumor, maternal androgen hyperplasia during pregnancy may be induced by exposure to exogenous androgens. Some female fetuses may show masculinization of external genitalia at different degrees when the mothers are treated with progesterone for habitual abortion or other reasons in the first trimester of pregnancy. In addition, maternal intake of androgens is also a possible cause of fetal masculinization [33].

14.2.2.3 Others (Cloacal Exstrophy, Vaginal Atresia, MURCS Association, and Other Syndromes)

Other disorders due to the factors of nonchromosomal/nonhormonal abnormalities that lead to 46, XX gonadal dysgenesis include cloacal exstrophy, vaginal atresia, MURCS association, etc. Since the differentiation process in embryonic development is affected, the affected patients have reproductive tract developmental malformations, which are often accompanied by renal and

urethral malformations. And the routine treatment is surgical correction [20, 21].

14.3 Clinical Practice

14.3.1 Intrauterine Sex Determination and Management of a Fetus with Sex Chromosome Abnormality Found Prenatally by Noninvasive Method

14.3.1.1 Current Medical History

This is a 26-year-old woman with no family history of genetic disorders or other risk factors. During the second trimester of pregnancy, she requested the noninvasive prenatal testing (NIPT) as a primary screening test for fetal Down syndrome. The NIPT result showed a low risk for trisomy 21, trisomy 18, and trisomy 13, and additional analysis revealed a high risk of sex chromosome aneuploidy. Prenatal genetic counseling at 17 gestational weeks was recommended.

14.3.1.2 Prenatal Genetic Counseling

NIPT showed sex chromosomes aneuploidy. However, NIPT is a primary screening method, rather than diagnostic method. According to the previous data, the positive predictive value of sex chromosomes aneuploidy is 20–30%. Therefore, it is suggested that invasive prenatal diagnosis can be considered to confirm whether there are abnormalities in sex chromosomes.

14.3.1.3 Laboratory Tests and Results

14.3.1.3.1 Results of Chromosome Microarray Analysis of Amniotic Fluid Cells

The amniotic fluid cells were analyzed with chromosomal microarray-Affymetrix CytoScan 750k. The microarray results revealed there is an about 155 kb deletion involving Xp22.33q28 region (Fig. 14.2).

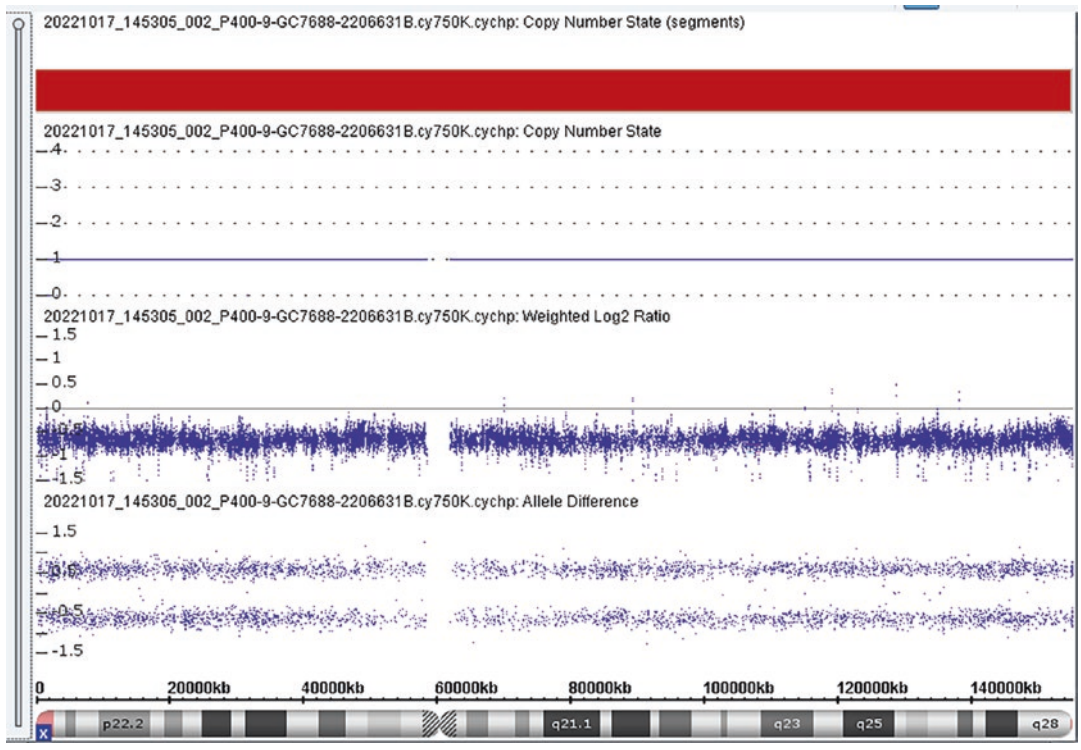

Fig. 14.2 Results of microarray study of DNA from amniocentesis

14.3.1.3.2 Results of Karyotype Analysis of Cultured Amniotic Fluid Cells

G-banded karyotyping on cultured amniotic fluid cells was conducted, in which a total of 50 metaphase cells were analyzed, showing a karyotype of 45, X (monosomy X) (Fig. 14.3).

14.3.1.3.3 Genetic Test of Sex Determining Gene (*SRY*)

The *SRY* gene was amplified by PCR using specific primers and analyzed by electrophoresis. The *SRY* gene, which is located at Yp, encodes the testis determining factor protein required for the development of male genitalia. If *SRY* genes are detected in the sample, a band with the size of 279 bp will be amplified by PCR. Therefore, PCR of amniotic fluid cell's DNA using primers for *SRY* produced a product of the expected size, confirming the presence of the *SRY* gene in this sample (Fig. 14.4).

14.3.1.3.4 Fluorescence In Situ Hybridization (FISH) Results

FISH was performed on interphase and metaphase cells in cultured amniotic fluid, using a probe specific for the centromere of the X chromosome (CEPX-green fluorescent labeling) and a probe specific for the sex-determining gene *SRY* in the short arm of the Y chromosome (red fluorescent label), with 500 cells counted. The results showed that 490 cells presented only one green, fluorescent signal marking the X chromosome (Fig. 14.5a), and ten cells presented *SRY* double-positive signals, with a proportion of 10/500 (2%) (Fig. 14.5b). The results showed monosomy of X in 98% cells, and 2% cells were *SRY* double positive. The FISH results showed a chimeric karyotype, with most of the cells missing one sex chromosome, and 2% of the cells were *SRY* double-positive, suggesting the presence of iso (Y) or idic (Y).

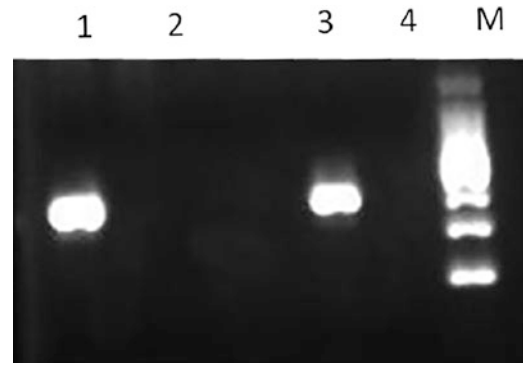

Fig. 14.4 PCR amplification electrophoresis of *SRY* in amniotic fluid samples. M marker; (1) amniotic fluid cells, (2) normal female, (3) normal male, (4) blank

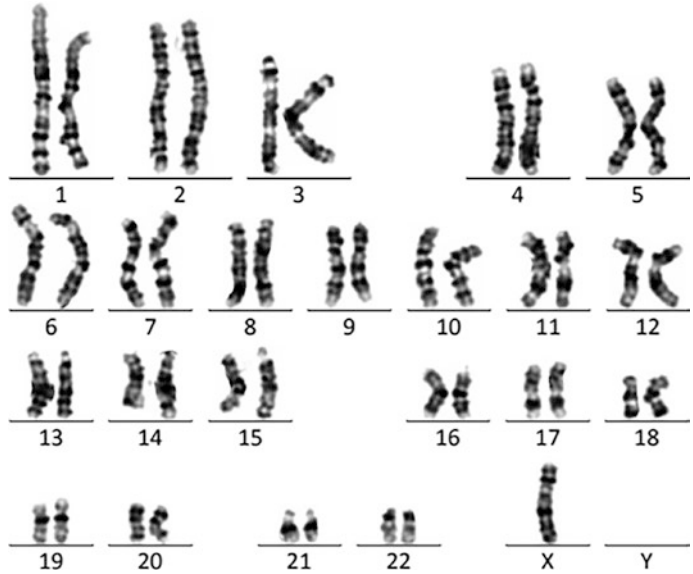

Fig. 14.3 G-banded karyotyping of cultured amniotic fluid cells

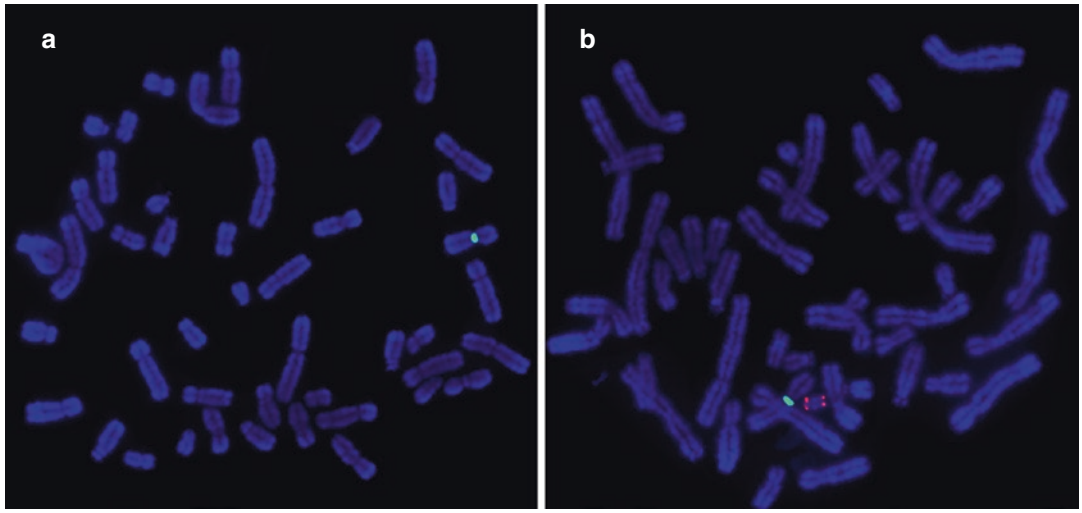

Fig. 14.5 Metaphase FISH results: FISH was performed using a probe specific for the centromere of the X chromosome (green) and a probe specific for the *SRY* site (red). (**a**) One Green signal in metaphase nuclei represents the 45, X cell line. (**b**) One green signal and two red signals in metaphase nuclei represent 46, X, iso (Y) or idic (Y)

14.3.1.4 Obstetrical Ultrasound Examination

The ultrasound examination reveals male external genitalia.

14.3.1.5 Post-Testing Consultation and Discussion

The chromosomal microarray analysis and karyotyping of the fetus showed the karyotype of 45, X, which was related to Turner syndrome. The main features of this syndrome include female appearance, short stature, webbed neck, cubitus valgus, underdevelopment of secondary sexual characteristics, congenital ovarian dysplasia, and primary amenorrhea. Most of the patients being infertile, some of the patients having mild intellectual disability, and some of the patients had congenital malformations in organs and tissues such as heart, kidney, and skeleton. Thus, the 45, X karyotype usually presents as a female appearance. But in rare cases, monosomy X patients are males, usually resulting from an unbalanced Y-autosomal translocation resulting in the retention of a short arm of the Y chromosome, which contains the sex-determining gene *SRY*, or the presence of a low proportion of the Y chromosome, which contains the *SRY* gene. The *SRY* gene, normally located at Yp, encodes the testis-determining protein and is essential for the development of the male genitalia. The fetus in this case showed male external genitalia determined by the ultrasound. The gender determined by karyotyping was inconsistent with the result of the ultrasound. Therefore, the presence of *SRY* and the presence of structural abnormalities involving the region where *SRY* is located should be further confirmed. Subsequent PCR confirmed the existence of *SRY* gene, and the FISH test with *SRY* site-specific probe further confirmed the existence of the region of *SRY* gene. Meanwhile, it was also confirmed that some of the cells were *SRY* double-positive, suggesting a chimeric karyotype. Taken together the results of chromosome microarray analysis and the FISH test, the karyotype was presumed to be 45, X/46, X, iso (Y), or idic (Y). This chimeric karyotype is commonly found in patients with gonadal dysgenesis, with high phenotypic heterogeneity. It may be manifested as Turner syndrome in women, as ambiguous external genitalia due to mixed gonadal dysgenesis, or as partial gonadal dysgenesis with the appearance of male external

genitalia. Meanwhile, the results of this case indicated that the prenatal routine test indicated 45, X, which was non-chimeric karyotype. However, due to the limitation of prenatal routine technology for low percentage chimerism, there was still the possibility of low percentage chimerism of Y chromosome or *SRY*. Hence, such cases should be comprehensively determined by combining the results of karyotyping, molecular test, FISH, and chromosome microarray analysis. Moreover, as the chimerism proportion is not directly related to the phenotype, it is challenging to predict the phenotype based on the abnormal proportion in the karyotype before the delivery. It is necessary to combine with ultrasound evaluation to estimate the possible phenotypic characteristics, so as to help evaluate the fetal prognosis and help the family decide whether to continue the pregnancy.

Combined with various results, the ultrasound result of the fetus suggested male external genitalia. Taken together with the results of genetic testing and literature retrieval, the fetus may be a male with partial gonadal dysgenesis or a normal male after birth. But the gonad has a risk of developing gonadal tumor, with an average probability of 15%. However, due to the structural abnormality of Y chromosome, the spermatogenic function will be affected, and the patient will not have childbearing potential in the adulthood. In addition, most cases will present with a short stature.

14.3.1.6 Follow-Up

The parents selected to continue the pregnancy and postnatal follow-up revealed male external genitalia.

14.3.2 Intrauterine Diagnosis and Management of Twin Pregnancy in Patients with Congenital Adrenal Hyperplasia

14.3.2.1 Current Medical History

A 37-year-old pregnant woman with 16 gestational weeks was diagnosed with twin pregnancy by ultrasound during pregnancy. The patient came for prenatal genetic counseling because her husband and daughter had 21-hydroxylase deficiency.

14.3.2.2 Prenatal Genetic Counseling

This is a 37-year-old woman for genetic consulting, who previously gave birth to a female child. Her daughter presented with vomiting, diarrhea, skin pigmentation, and clitoral hypertrophy after birth. Neonatal screening test showed increased 17- hydroxyprogesterone (17-OHP) (120 nmol/L), and *CYP21A2* gene analysis showed that she harbored homozygous c.518T > A (p.I173N) mutation and was diagnosed as 21-hydroxylase deficiency (21-OHD). In the following pedigree analysis, this woman carried a heterozygous mutation of c.518T > A (p.I173N), and her husband carried a homozygous mutation of c.518T > A (p.I173N). She requested for prenatal diagnosis. The amniotic fluid samples were taken from two fetuses (F1 fetus and F2 fetus) at 16 weeks of pregnancy, and genetic testing was performed.

14.3.2.3 Laboratory Tests and Results

14.3.2.3.1 Results of Genetic Test of *CYP21A2* and *SRY* Genes in Amniotic Fluid Cells

The amniotic fluid cells of F1 fetus and F2 fetus were analyzed using the STR polymorphism linkage analysis to rule out the maternal contamination. There was no maternal contamination discovered in the amniotic fluid samples. Then, F1 fetus and F2 fetus were tested for *CYP21A2* and *SRY* gene. The F1 fetus exhibited homozygous mutation c.518T>A (p.I173N) in *CYP21A2* gene and *SRY* gene (−). The F2 fetus had heterozygous mutation of c.518T > A (p.I173N) in *CYP21A2* gene with *SRY* gene (+).

14.3.2.3.2 Findings of Fetal Ultrasound (Fig. 14.6)

The F1 placenta was located in the posterior wall of the uterus, and the clitoris of the external genitalia was slightly hyperechoic. The F2 placenta was localized in the posterior wall of the uterine fundus, and the external genitalia was the male type.

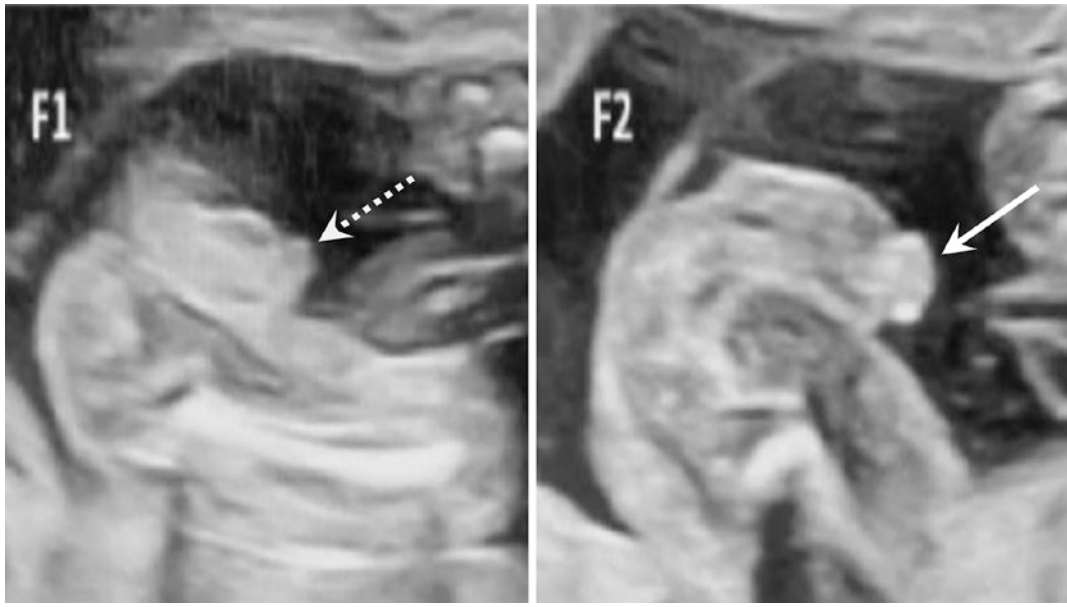

Fig. 14.6 Fetal ultrasound showed that the F1 placenta was located in the posterior wall of the uterus, and the clitoris of the external genitalia was slightly hyperechoic (dashed arrow). The F2 placenta was located in the posterior wall of the uterus fundus, and the external genitalia was a male type (solid arrow)

14.3.2.4 Post-Testing Consultation and Discussion

Congenital adrenal hyperplasia is a group of autosomal recessive hereditary disorders, due to the deficiency of various catalytic enzymes in the adrenal corticosteroid synthesis pathway, and the negative feedback of corticosteroid synthesis may lead to the hypersecretion of adrenocorticotropic hormone (ACTH); among them, 21-hydroxylase deficiency is the most common one. The daughter of the consulting patient had symptoms of vomiting and diarrhea after birth. Physical examination showed skin pigmentation and clitoral hypertrophy. The newborn screening results showed that 17OHP was obviously elevated. The *CYP21A2* genetic test showed that she carried homozygous mutation of c.518T > A (p.I173N). Therefore, the daughter was diagnosed with 21-hydroxylase deficiency and treated with hydrocortisone and 9 α-fludrocortisone, and her condition was stable at present.

The consulting patient's husband was 36 years old, with the height of 160 cm and weight of 82 kg. He complained that he had strong motor ability since childhood and had a history of precocious puberty. The consulting patient said that she became pregnant after she has been married for 3 months. Since then, she did not take any contraceptive measures but was not pregnant for 4 years. Laboratory tests performed at initial visit at his age of 36 years showed increased 17-OHP (213 nmol/L), ACTH (160 pg/mL), and androstenedione (>10 ng/mL). B ultrasound of the adrenal showed he had bilateral adrenal rest tumor. Due to harboring *CYP21A2* gene homozygous mutation of c.518T > A (p.I173N), the husband was diagnosed as nonclassical 21-OHD. Most adult males with nonclassical 21-OHD may no longer require medication. However, excessive testosterone can negatively inhibit FSH and LH, which may affect reproductive function in partial patients, and medication is required. The husband of the consulting patient was given oral hydrocortisone 20 mg/day after the diagnosis was confirmed. The consulting patient became pregnant 3 months later after her husband took the oral hydrocortisone. Combined with intrauterine ultrasound and prenatal genetic diagnosis of the amniotic fluid samples, F1 female fetus was identified as a 21-OHD patient,

and the F2 male fetus was identified as a 21-OHD carrier with the normal phenotype.

14.3.2.5 Follow-Up

The family chose fetal reduction of the fetus F1 at 20 weeks of gestation. Four weeks after the surgery, the fetus F2 developed normally, and the reduced fetus F1 was atrophied. F2 fetus was diagnosed as a carrier of 21OHD confirmed by gene diagnosis after birth, with normal clinical phenotype.

14.4 Research Progress

14.4.1 Common Environmental Risk Factors for Reproductive System Disorders of Intrauterine Origin

In the process of fetal development, critical molecular and cellular processes must respond to various hormones and other growth factors in a complete manner to enable the normal function of the fetus after birth. Chemicals or environmental disturbances in environmental exposure may affect these processes and may change the development and differentiation of gonads and external genitals as well as the postnatal endocrine function and other vital processes for reproduction, and some chemicals with different structures and effects have been shown to affect the synthesis and function of the hormones.

In recent years, the total birth rate has remained below the replacement levels (2.1 children per woman) in many countries worldwide. Decreases in the total birth rate may be correlated with male reproductive disorders, including testicular cancer, disorders of sexual development, cryptorchidism, hypospadias, low testosterone levels, poor semen quality, childlessness, altered sex ratios, and increased demand for assisted reproductive technologies. Reproductive disorders in adult males may occur in the uterus during the fetal period. Although they may be induced by genetic mutations, recent evidence suggests that these issues are often associated with environmental exposure (chemicals and other interfering endocrine substances) of the fetal testicles. Environmental factors can also adversely affect the adult endocrine system. These environmental factors may act directly or through epigenetic mechanisms as a result of increased environmental exposures due to modern lifestyles, and the effects of environmental exposure may persist for generations after exposure [33].

Polycystic ovary syndrome (PCOS) is one of the major endocrine disorders affecting women of childbearing age, but its etiology is still unclear. Some studies suggest that environmental factors, especially the intrauterine environment during the fetal period, play a key role in the development of PCOS. Androgens and endocrine-disrupting substances in the mother, such as bisphenol A, may contribute to the development of polycystic ovary syndrome in the fetus. Alterations in the uterine environment, including hormonal imbalances, may affect fetal gonadal development [34].

14.4.2 Progress in Prevention and Control Strategies for Hereditary Reproductive Disorders

In the primary prevention of hereditary reproductive disorders, it is of great significance to provide comprehensive guidance such as health education, pre-pregnancy care, and genetic counseling to couples preparing to give birth to children, especially conducting carrier screening among the high-risk groups. For couples who were found to carry pathogenic mutations in hereditary reproductive system diseases, genetic counseling and marriage and childbearing guidance should be conducted to evaluate the reproductive risks, select the best mode of reproductive and pregnancy management, and so as to prevent the birth of children with severe disorders and effectively reduce the risk.

The commonly used prenatal screening techniques include noninvasive DNA prenatal screening, etc. Carrier screening based on next-generation sequencing (NGS) technology is a new method for primary prevention of genetic

reproductive problems. The secondary prevention strategy for hereditary reproductive system disorders includes prenatal screening and prenatal diagnosis, especially on the basis of the clinical and genetic diagnosis of the proband. Genetic analysis for high-risk fetuses in families with genetic disorders through prenatal genetic diagnosis provides a practical and effective way for the prevention of these disorders. Prenatal diagnosis techniques include fetal ultrasound, karyotype analysis, fluorescence in situ hybridization, chromosome microarray analysis, Sanger sequencing, NGS, multiplex ligation-dependent probe amplification, etc. Currently, chromosome microarray analysis, NGS, and their derived technologies are rapidly developing and gradually become the mainstream technologies for prenatal diagnosis and genetic etiological diagnosis of birth defects [35]. The third level strategy in the three-level prevention and control plan for hereditary reproductive system disorders is neonatal disease screening. Considering the limitations in the screening techniques such as enzymology and metabolites through tandem mass spectrometry, gene sequencing especially the NGS will become an important method for screening genetic reproductive disorders.

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Magnetic Resonance Imaging (MRI) of the Fetus

15

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15.1 Introduction

15.1.1 Development History and Current Status of Prenatal Diagnostic Techniques of Fetal MRI

As early as 1983, Smith et al. [1] first introduced the application of magnetic resonance imaging (MRI) in obstetrics and fetal medicine by a letter to The Lancet. They performed MRI on six pregnant women preparing for labor induction. With T1-weighted imaging (T1WI) sequence and proton density-weighted imaging, they found that some measurements and judgments could be made and were consistent with the results of prenatal ultrasound results. However, there were some defects with fetal MRI at that time, including poor image quality and slow scanning speed. In 1995, Shi ZR et al. [2] from Changzheng Hospital of the Second Military Medical University first reported the application of fetal MRI in China. They used 0.35 Tesla (T) low-field MRI on 44 pregnant women in the second and third trimesters. Under the technical conditions at that time, it was still required to inject low-dose diazepam and use abdominal band fixation to reduce fetal movements for pregnant women before the examination to obtain high-quality MR images. By examining the brain morphological structure and signal changes of 42 normal fetuses, they found that fetal MRI had a high visible rate (≥74%) for cerebral hemispheres, cerebellum, brain stem, lateral ventricles, and eyeballs.

Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine began to perform fetal MRI in Shanghai Children's Medical Center under the leadership of Professor Zhu Ming and Professor Li Yuhua in 2003. In the same year, Shandong Medical Imaging Research Institute and Wuhan Tongji Hospital also began to carry out fetal MRI in clinical practice. These three hospitals were among the earliest institutions in China to carry out fetal MRI. At that time, 1.5 T MRI scanner has been used, with sedation no longer required. Therefore, fetal MRI entered the stage of clinical application. In the past 30 years, with the rapid development of imaging technology, the quality of fetal MR images has been significantly improved, and it has become one of the important prenatal imaging methods.

With regard to the regulation of fetal MRI techniques, academics domestic and abroad have published relevant guidelines. In 2014, multidisciplinary physicians in the United States jointly discussed and developed the "Guidelines for Fetal Imaging (2014)" [3]. The International Society of Ultrasound in Obstetrics and

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Gynecology (ISUOG) issued the "ISUOG Practice Guidelines for Fetal MRI" in 2017 on the basis of referring to the American Guidelines for Fetal Imaging and in combination with the results of its own investigation and research [4]. In China, the Chinese Medical Association organized experts to prepare and publish the "Chinese Expert Consensus on Fetal MRI" [5] in 2020 and comprehensively standardized the timing, safety, indications, scanning technology and diagnostic report, technical training, and management of fetal MRI.

15.1.2 Safety and Scope of Fetal MRI

15.1.2.1 Safety of Fetal MRI

As fetal MRI becomes more and more widely used in clinical practice, related safety issues have become the focus of everyone's attention. The safety of fetal MRI that requires attention in daily work is mainly related to the exposure to static magnetic field, thermal effect produced by radiofrequency magnetic field, noise, and the administration of gadolinium contrast agent.

So far, based on many safety studies on 1.5 T and 3.0 T MRI published domestic and abroad, the use of MRI has not been found to have any adverse effect on the mother or fetus. The latest guidelines in China, taking previous relevant clinical studies into account, state that MRI at field strength of 3.0T and below are safe for the fetus in the second and third trimesters. Although studies have shown that 1.5 T MRI for pregnant women in early pregnancy has no harm to the fetus, fetal MRI in early pregnancy is not necessary.

The thermal effect due to radiofrequency energy deposition is another focal issue for the safety of fetal MRI. The specific absorption ratio (SAR) value of radiofrequency is a quantitative indicator of tissue energy deposition. This indicator and the resulting thermal effect should be controlled. Krishnamurthy et al. [6] compared MRI at 1.5 T and 3.0 T in the same group of fetuses. The results showed that the SAR values did not exceed the threshold and that the SAR value of 3.0 T was lower, while the signal-to-noise ratio was higher. Normal mode is currently recommended for the scanning of pregnant women (SAR value <2.0 W/kg). In MRI examination, the International Electrotechical Commission (IEC) standards should be strictly followed, and the scanning duration should be shortened as far as possible. High SAR sequence and low SAR sequence should be scanned alternately to reduce potential risks.

To date, no evidence of postnatal hearing impairment due to MRI in fetuses at 3.0 T or less has been reported. Two large-scale studies by Ray et al. [7] and Strizek et al. [8] showed that MRI during pregnancy was not definitely related to fetal hearing impairment, and their studies also became an important basis for the development of international guidelines related to MRI during pregnancy.

A large study by Ray et al. [7] showed that the risk of invasive skin disorders such as nephrogenic systemic fibrosis (NSF), stillbirth, and neonatal death was relatively higher in the gadolinium-exposed group in the first trimester, while no significant adverse consequences were observed in the gadolinium-exposed group in the second and third trimesters. Available evidence suggests that exposure to gadolinium agents during pregnancy, especially in early pregnancy, may be of high risk. International and domestic guidelines do not recommend the administration of gadolinium contrast agents for MRI during pregnancy. The advantages and disadvantages should be fully weighed in clinical practice to reduce the unnecessary use of gadolinium agents [9].

15.1.2.2 Scope of Fetal MRI

Fetal MRI is a targeted (Grade IV) prenatal diagnostic imaging and is a supplemental test performed to confirm the results of ultrasound, or when ultrasound result indicates a possible but not definite diagnosis of abnormality. The ISUOG Practice Guidelines for Fetal MRI in 2017 ranked recommendations by evaluating the ability of fetal MRI to provide useful information beyond ultrasound. The recommendations for fetal MRI from high to low are posterior cranial fossa anomaly, corpus callosum anomaly,

diaphragmatic hernia, microcephaly, simple lateral ventricle dilatation, neural tube defects, pulmonary anomaly, lymphangioma, multiple malformations, twin-twin transfusion syndrome, skeletal dysplasia, cleft lip and palate, urinary tract anomaly, abdominal wall defects, history of abnormality with normal ultrasonography, monochorionic twins, and congenital heart disease. Among them, posterior cranial fossa abnormalities and corpus callosum abnormalities have absolute indications for MRI. Posterior cranial fossa abnormalities can be divided into different categories, with different prognosis. Due to its good tissue resolution, MRI can better display the anatomical structure of each lobe of the cerebellar vermis compared with ultrasound and rule out some cases where ultrasound indicates possible posterior cranial fossa abnormalities. Corpus callosum abnormalities include dysplasia of corpus callosum, agenesis of corpus callosum, and absence of corpus callosum. Due to the influence of fetal position, it is difficult to obtain three-plane images with ultrasound and also difficult in judging whether the corpus callosum is intact. Hence, fetal MRI is required to supplement the diagnosis. Compared with ultrasound, MRI is easy to display the presence and integrity of the corpus callosum with its multiplanar and multidirectional imaging and higher soft tissue resolution. Moreover, MRI can detect other combined malformations, which plays an important role in judging the prognosis.

15.1.3 Protocol of Fetal MRI

15.1.3.1 Timing of Fetal MRI

Fetal MRI is not recommended before 18 weeks of gestation in the Guidelines for Fetal Imaging (2014), ISUOG Practice Guidelines for Fetal MRI, or the Chinese Expert Consensus on Fetal MRI, since at this time, the fetus is small with frequent fetal movements. Some central nervous system structures such as the corpus callosum or cerebellum have not fully developed. The fetal MRI usually cannot provide more information than ultrasound before 18 weeks of gestation. In China, it is recommended that fetal MRI should be performed at or after 20 weeks of gestation, when better evaluation of the confirmed or suspected fetal abnormalities can be performed. The development of various fetal systems, especially the nervous system, is constantly changing as gestational age increases. Therefore, the timing of MRI examination and the examination results of various fetal systems must be combined with the development of different systems at gestational age. For example, neuronal migration has been completed by approximately 24 weeks of gestation, and the sensitivity of fetal MRI for the diagnosis of gray matter heterotopia is 77% after 24 weeks and 44% before 24 weeks [10].

15.1.3.2 Preparation Before Fetal MRI

First of all, it is necessary to rule out contraindications for MRI examinations, including absolute contraindications such as pacemakers and ferromagnetic implants, as well as relative contraindications such as claustrophobia. The applicant for fetal MRI should be an obstetrician, prenatal genetic counselor, or other legally registered medical staff. The application of MRI should include the information such as gestational age (last menstrual period), maternal history, family history, as well as the results of existing ultrasound or MRI. Before the MRI examination, an informed consent should be obtained from the mother with full discussion with the mother about the possible risks and benefits of fetal MRI, the purpose of the examination to confirm the results of the ultrasound examination, or to obtain additional information that was not found by the ultrasound. Pregnant women should choose a comfortable position, generally in the supine or left lateral decubitus position. Body-phased array coil with a large field of view is selected.

15.1.3.3 Selection of Fetal MRI Sequences

The most commonly used sequences for fetal MRI are single shot fast spin-echo (SSFSE) sequence, balanced steady-state free precession (BSSFP) sequence, T1-weighted imaging (T1WI), and diffusion-weighted imaging (DWI). T2-weighted imaging is the main sequence of

fetal MRI by using T2-weighted fast spin echo (SE) or steady-state free precession (SSFP) sequence. Of them, SSFSE sequence is the most commonly used in clinical practice and is applicable for all fetal systems but with poor display of the skeletal system. BSSFP sequence has fast imaging speed and high signal-to-noise ratio, but the tissue contrast is poor compared with SSFSE sequences, which is also applicable for various fetal systems. BSSFP sequence images of 3.0 T MRI are better in the display of skeletal or vertebral developmental malformations in fetuses. T1-weighted imaging can help show certain fetal tissue or fluid components, such as fat, hemorrhage, liver, and meconium. T he display of meconium can assist the diagnosis of bowel abnormalities and related complications such as meconium peritonitis. Apparent diffusion coefficient (ADC) value of DWI sequence can reflect whether the diffusion of water molecules in normal and abnormal fetal tissues is limited, help to characterize tumors, determine whether cysts contain more protein components, and find ectopic kidneys. Echo planar imaging (EPI) sequences can be used to visualize bone structure, calcification, and breakdown products of blood, indicating fresh, or old bleeding. The spin echo-EPI sequences have high signal-to-noise ratio and are currently the fastest acquisition sequences and can be used for imaging the fetal skeletal system. Thick-slab single shot hydrography sequence, a heavy T2 sequence, which can obtain images similar to magnetic resonance cholangiopancreatography (MRCP) and urinary tract hydrography, reflect the abnormal retention fluid of gastrointestinal tract and collecting system, and can well determine the expansion of effusion and the specific location of obstruction point, which is used for the diagnosis of gastrointestinal disorders and urinary tract hydrops in the fetuses. Other sequences such as fluid attenuated inversion recovery (FLAIR), susceptibility-weighted imaging (SWI), diffusion tensor imaging (DTI), blood oxygen level-dependent (BOLD), functional MRI, and magnetic resonance spectroscopy (MRS) can be selected and used as required.

At present, relevant guidelines point out that, since MRI is usually not the first-line examination but is performed when ultrasound suggests abnormality, or when prenatal ultrasonography is negative, but previous pregnancy or immediate family members have severe developmental abnormalities. The imaging and report should focus on the structure that is difficult to be evaluated by ultrasound to assist the diagnosis, thereby increasing the confidence of clinical decision-making and assisting prenatal and postnatal care.

15.2 Clinical Practice

15.2.1 Congenital Diaphragmatic Hernia

15.2.1.1 Case 1

Clinical Data

A 24-year-old female, G1P0. Prenatal Down's syndrome screening revealed low risk. Prenatal ultrasonography at 36 weeks of gestation showed fetal left diaphragmatic hernia, gastric bubble, intestinal tube, a very small amount of left lobe liver tissue in the left thoracic cavity, right shift of the heart with apex to the left, and lung-to-head ratio (LHR) of 2.23. During pregnancy, the woman had good mental state and appetite, good sleeping, and normal urination and defecation. The general indicators of the pregnant woman at admission: body temperature 37.0°C, pulse rate 80 bpm, respiratory rate 20 bpm, and blood pressure 110/74 mmHg (1 mmHg ≈0.133 kPa). The general condition of the woman during hospitalization was normal. Specialist examinations: fetal heart rate 144 bpm, fetal position LOA, good fetal movement, abdominal circumference 105 cm, uterine fundus 37 cm, fetal membranes: intact.

MRI Findings and Analysis

Fetal MRI was performed with cross-sectional breath-hold ultrafast balanced field echo sequence (BTFE-BH) and coronal ultrafast spin echo sequence (SSh-TSE SENSE) (Fig. 15.1a, b)

Surgery and Prognosis

The child was delivered at 38 weeks + 6 days by spontaneous delivery, with the birth weight of

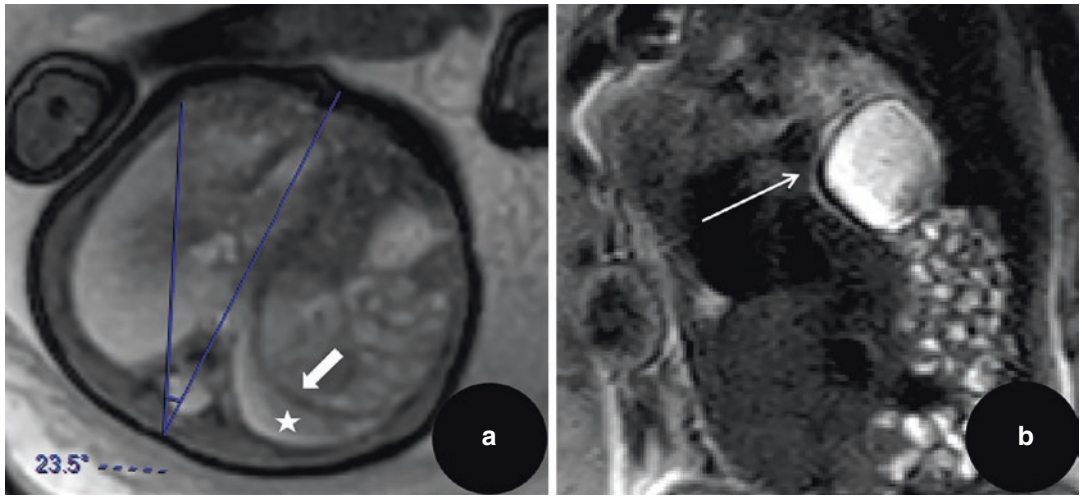

Fig. 15.1 (**a**, **b**) Cross-sectional breath-hold ultrafast balanced field echo sequence (BTFE-BH) image, coronal ultrafast spin echo sequence (SSh-TSE SENSE) image showing left diaphragmatic hernia, gastric bubble in the left thoracic cavity, heart compressed and shifted to the right, mediastinal shift angle of 23.5 degrees, and residual left lung tissue above the hernia contents. In this case, intraoperative findings included the diaphragmatic hernia with a hernia sac and the signs suggesting the presence of a hernia sac included: smooth lung-hernia interface ((**a**) indicated by thick arrow), the residual lung tissue compressed in a crescent shape ((**a**) indicated by open star), and arcuate effusion ((**b**) indicated by thin arrow) above the interface of the lung-hernia

3000 g, clear amniotic fluid, unremarkable placenta, umbilical cord around neck of one circle, Apgar score of 8 at 1 min, 9 at 5 min, and 9 at 10 min. After an initial resuscitation, the newborn's vital signs were stable. The newborn was transferred to the neonatal intensive care unit under tracheal intubation and pressurized balloon. After the condition was improved, the "thoracoscopic left diaphragmatic hernia repair" was performed. Intraoperative exploration showed weak diaphragm near the posterolateral side, with a defect size of about 4 × 3 cm and a hernia sac. The hernia contents were gastric bubble and intestinal canal. Ventilator-assisted ventilation and supportive treatment were continued after surgery. After 17 days, the infant was transferred to the pediatric general ward and discharged 2 days later.

15.2.1.2 Case 2

Clinical Data

A 28-year-old female, G5P2. The prenatal visits were irregular, and no prenatal birth defect screening was performed. Ultrasonography at 33 weeks of gestation revealed abnormal echogenicity of the left thoracic cavity of the fetus, possible left diaphragmatic hernia, and excessive amniotic fluid, with LHR of 1.96. During pregnancy, the woman had good mental state and appetite, good sleeping, and normal urination and defecation. The general indicators of the pregnant woman at admission: body temperature 36.6°C, pulse rate 80 bpm, respiratory rate 18 bpm, and blood pressure 112/70 mmHg (1 mmHg ≈0.133 kPa). The general condition of the woman during hospitalization was normal. Specialist examinations: fetal heart rate 155 bpm, fetal position LOA, good fetal movement, abdominal circumference 110cm, uterine fundus 34 cm, fetal membranes: intact.

MRI Findings and Analysis

Fetal MRI was performed with cross-sectional, sagittal breath-hold ultrafast balanced field echo sequence (BTFE-BH), and coronal SSh-TSE SENSE (Fig. 15.2a–c).

Surgery and Prognosis

The child was delivered at 39 weeks +2 days by spontaneous delivery, with the birth weight of

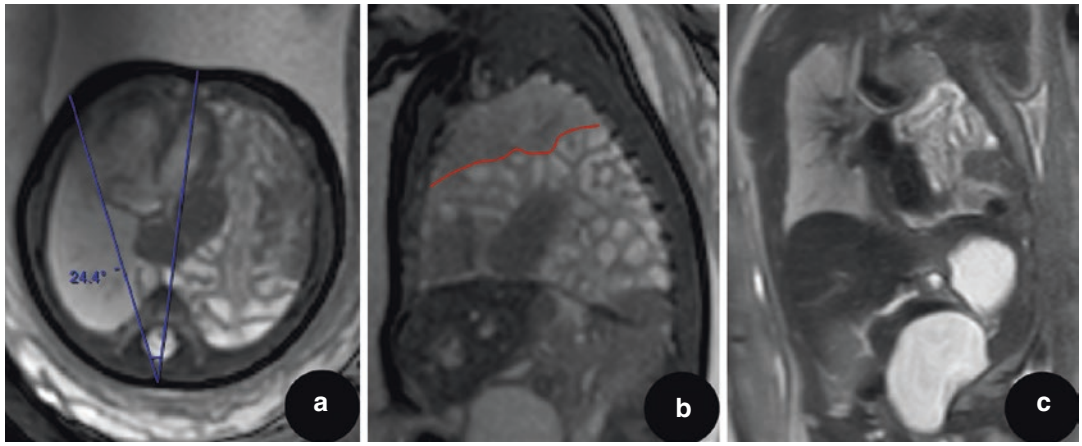

Fig. 15.2 (**a**–**c**) Cross-sectional, sagittal breath-hold ultrafast balanced field echo sequence (BTFE-BH) images and coronal SSh-TSE SENSE images, showing left diaphragmatic hernia, most of the left thoracic cavity occupied by the bowel, heart compressed and shifted to the right, mediastinal shift angle of 24.4° (**a**, **b**), residual left lung tissue above the hernia contents, and the gastric bubble in the abdominal cavity (**c**). In this case, intraoperative findings included no hernia sac in the diaphragmatic hernia, and the corresponding signs included: irregular shape of the lung-hernia interface ((**b**) red curve), no peripheral capsular sensation of the hernia, and residual lung tissue with uneven margins on the affected side

3000 g, clear amniotic fluid, unremarkable placenta, cord around neck, Apgar score of 9 at 1 min, 9 at 5 min, and 9 at 10 min. After initial resuscitation, the newborn's vital signs were stable. The newborn was transferred to the neonatal intensive care unit under tracheal intubation and pressurized balloon. After the condition was improved, the "thoracoscopic left diaphragmatic hernia repair" was performed. Intraoperative exploration showed posterolateral diaphragmatic defect, the size of the defect was about 2 × 3 cm, with no hernia sac, with the hernia content of intestinal canal. Ventilator-assisted ventilation and supportive treatment were continued after surgery. After 21 days, the child was transferred to the pediatric general ward and discharged 3 days later.

15.2.1.3 Case 3

Clinical Data

A 32-year-old female, G3P0. Down's syndrome screening during pregnancy revealed a normal fetus, and ultrasonography at 27 weeks of gestation suggested the possibility of fetal diaphragmatic hernia, LHR: 0.85. During pregnancy, the woman had good mental state and appetite, good sleeping, and normal urination and defecation. The general indicators of the mother at admission: body temperature 37.5°C, pulse rate 80 bpm, respiratory rate 20 bpm, blood pressure 120/80 mmHg (1 mmHg ≈0.133 kPa). The general condition of the mother during hospitalization was normal. Specialist examinations: fetal heart rate 150 bpm, fetal position LOA, good fetal movement, abdominal circumference 98cm, uterine fundus 35 cm, fetal membranes: intact.

MRI Findings and Analysis

Fetal MRI was performed with cross-sectional, coronal, and sagittal SSh-TSE SENSE (Fig. 15.3a–c)

Surgery and Prognosis

The child was delivered by cesarean section at 37 weeks +2 days, with the birth weight of 2450 g, clear amniotic fluid, racket placenta, spiral umbilical cord, Apgar score of 6 at 1 min, 7 at 5 min, and 7 at 10 min. After initial resuscitation, the newborn's vital signs were stable. The newborn was transferred to the neonatal intensive care unit under tracheal intubation and pressurized balloon. After the condition was improved, the "right diaphragmatic hernia repair" was performed. Intraoperative exploration and opening

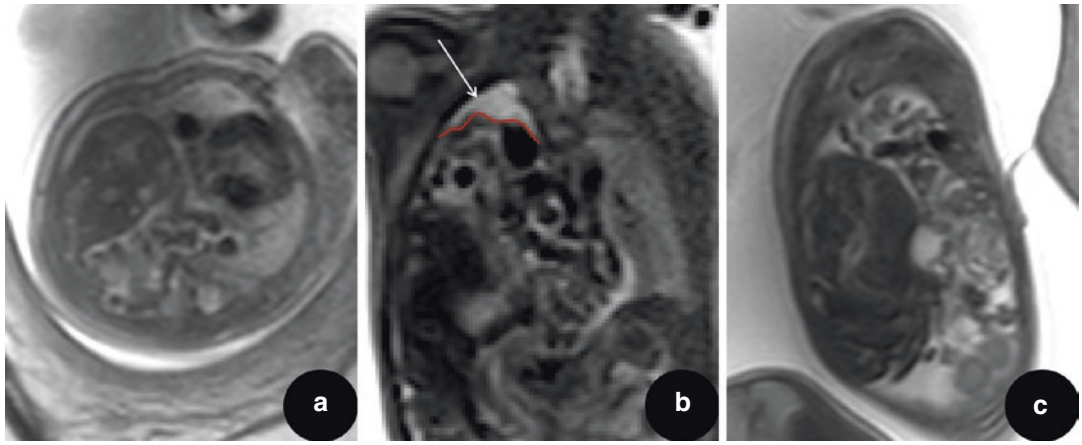

Fig. 15.3 (**a**–**c**) Cross-sectional, coronal, and sagittal SSh-TSE SENSE images showed right diaphragmatic hernia, no signal of normal lung tissue in the right thoracic cavity, liver, gallbladder and intestinal canal in the right thoracic cavity, perihepatic and peri-intestinal effusion, most of the heart located in the left thoracic cavity, and gastric bubble in the abdominal cavity (**a**–**c**). In this case, intraoperative findings included no hernia sac in the diaphragmatic hernia, and the corresponding signs included: no obvious residual lung tissue on the affected side, irregular shape of the upper edge of the bowel shadow ((**b**) red curve), and visible abdominal effusion ((**b**) fine arrow), which suggested the effusion located below the lung-hernia interface

of the abdominal cavity showed herniation of the right lobe of the liver, gallbladder, all small intestine, and part of the colon into the right thoracic cavity. After the intra-abdominal organs were included in the abdominal cavity, the diaphragmatic defect surface was about 4 × 3 cm, located posterolaterally, and the rest of the residual part was muscular tissue without a hernia sac. Three hours after the surgery, the child suddenly had oxygen saturation decreased to about 60%, heart rate decreased to 72 bpm, and was immediately given balloon pressurization and chest compression. Fifty minutes after emergency rescue, the child's heartbeat stopped, without spontaneous breathing, and the child died.

Prenatal Evaluation of Fetal Diaphragmatic Hernia by MRI

Congenital diaphragmatic hernia (CDH) is due to diaphragmatic dysplasia, resulting in varying degrees of herniation of abdominal contents into the thoracic cavity. Studies have shown that 4–12 weeks of gestation is a critical period for diaphragmatic development. Congenital diaphragmatic hernia will develop if the primitive thoracoabdominal membrane and intercostal muscles fail to fuse normally or the esophageal hiatus is relatively enlarged due to delayed descent of the gastric cavity. Pulmonary dysplasia and/or pulmonary hypertension are the leading causes of postpartum death in fetuses with isolated CDH. At present, prenatal ultrasound LHR is a key indicator for evaluating the prognostic risk of fetal diaphragmatic hernia, but it is greatly affected by the experience of the operator. However, prenatal MRI images can be repeatedly read and measured. The timing of clinical intervention and fetal prognostic risk can be comprehensively determined by evaluating the presence or absence of diaphragmatic hernia sac, measuring the observed/expected fetal lung volume (o/e FLV), correcting McGoon index, mediastinal shift angle, etc. The lateralization of the diaphragmatic hernia also has a certain correlation with the prognosis of the fetus. Left diaphragmatic hernia is more common, accounting for about 85%, with the herniated organs of stomach, spleen, small intestine, colon, left lobe of liver, left kidney and left adrenal gland, while the herniated organs of right diaphragmatic hernia are mainly liver, gallbladder, small intestine, colon, and right kidney. The prognosis of right diaphragmatic hernia is usually worse compared to the left diaphragmatic hernia. In addition, hepatic

herniation is an independent risk factor affecting the prognosis of congenital diaphragmatic hernia, and the severity can be quantitatively assessed by the liver-to-thorax ratio (LiTR).

15.2.2 Agenesis of Corpus Callosum

Clinical Data

A 33-year-old female, G2P1. The Down's syndrome screening during the second trimester revealed low risk, and ultrasonography at 28 weeks of gestation revealed widening of the lateral ventricles of the fetus. During pregnancy, the woman had good mental state and appetite, good sleeping, and normal urination and defecation. The mother had previously undergone cesarean section. The general indicators of the mother at admission: body temperature 36.8°C, pulse rate 74 bpm, respiratory rate 19 bpm, blood pressure 118/76 mmHg (1 mmHg ≈0.133 kPa). The general condition of the mother during hospitalization was normal. Specialist examinations: fetal heart rate 145 bpm, fetal position LOA, good fetal movement, abdominal circumference 91 cm, uterine fundus 25 cm, fetal membranes: intact.

MRI Findings and Analysis

Fetal MRI was performed with axial, coronal, and sagittal T2WI (Fig. 15.4a–c).

Genetic Testing and Prognosis

After admission, the mother completed various relevant examinations and underwent percutaneous umbilical cord blood puncture under ultrasound monitoring. The prenatal genome-wide microarray scan test report showed that a 267-kb deletion in the range of 7q11.22 was detected in the tested samples, with clinical facial abnormalities, developmental delay, mental retardation, growth retardation, and language delay. The mother subsequently underwent labor induction.

15.2.3 Dandy-Walker Malformation

Clinical Data

A 30-year-old female, G2P0. Prenatal ultrasonography at 16 weeks of gestation revealed a small fetal head (details unknown). Prenatal ultrasonography at 20 weeks of gestation revealed possible fetal cerebellar vermis dysplasia. During pregnancy, the mother had good mental state and appetite, good sleeping, and normal urination and defecation. The general indicators of the mother at admission: body temperature 36.3°C, pulse rate 93 bpm, respiratory rate 18 bpm, blood pressure 110/78 mmHg (1 mmHg ≈0.133 kPa). The general condition of the mother during hospitalization was normal. Specialist examinations: fetal heart rate 145 bpm, fetal position LOA, good fetal movement, abdominal circumference

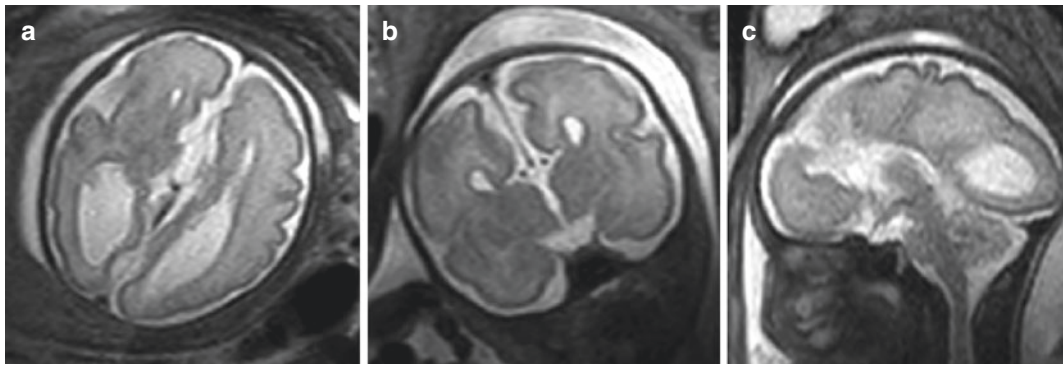

Fig. 15.4 (**a**, **b**) Axial and coronal T2WI showed separation of the lateral ventricles of the fetus, enlargement of the posterior horn of the lateral ventricle in the shape of "water droplet," widening of the longitudinal fissure cistern, and no callosal junction in the cerebral hemispheres. (**c**) Median sagittal T2WI showed no normal corpus callosum structure

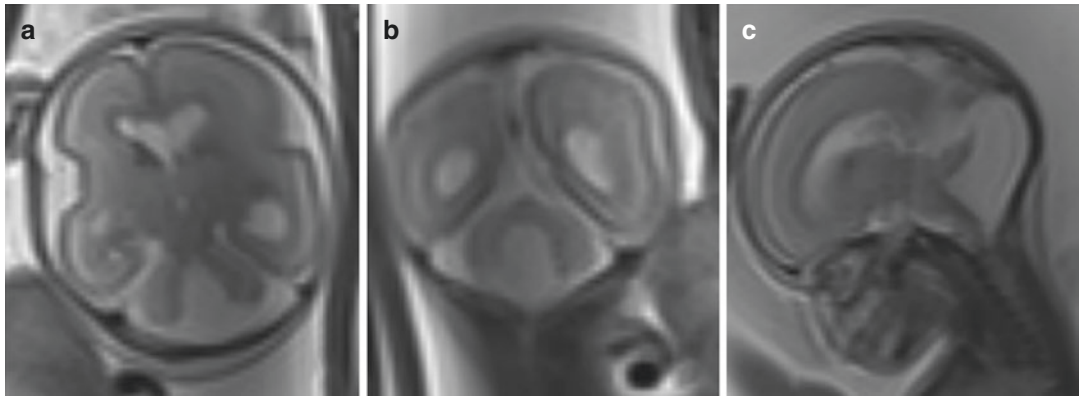

Fig. 15.5 (**a**, **b**) Axial and coronal views on T2WI showed cystic dilatation of the fetal fourth ventricle, communicating with the enlarged posterior cranial fossa cistern, unclear visualization of the cerebellar vermis, and outward displacement of both cerebellar hemispheres. (**c**) Sagittal view on T2WI showed that the cerebellar vermis was not present, the cerebellar structure was lifted and separated from the dorsal side of the brainstem, the angle between the two was significantly enlarged, the dilated fourth ventricle communicated with the enlarged posterior cranial fossa cistern, and the tentorium cerebelli was shifted upward

80 cm, uterine fundus 26 cm, intact fetal membranes.

MRI Findings and Analysis

Fetal MRI was performed with axial, coronal, and sagittal T2WI (Fig. 15.5a–c).

Genetic Testing and Prognosis

The pregnant woman underwent labor induction by amniocentesis with rivanol at 21 weeks of gestation. During the operation, 30 mL of amniotic fluid was drawn for whole external chromosome examination, and then a dead baby boy was delivered with a length of 22 cm, a sole of 2 cm, and a weight of 580 g. The operation went smoothly. Prenatal whole exome sequencing report shows that *TMEM138* gene is associated with *Joubert* syndrome type 16 (MIM: 614465), which is an autosomal recessive developmental disorder. The main manifestations of this disorder include Dandy-Walker malformation, encephalocele, ocular motor apraxia and retinal dystrophy, etc.

15.3 Research Progress

15.3.1 Prospects for the Fetal MRI

Currently, prenatal ultrasonography is often used as the primary screening method for diagnosing fetal disorders because of its low price, convenient examination and no radioactivity, but it still has limitations. Factors such as excessive amniotic fluid, obesity, and multiple pregnancies in pregnant women often influence the observation of other deformities such as structural abnormalities and are also greatly influenced by the operator's experience. Therefore, it should be supplemented by other imaging examinations. In 1983, MRI was first applied in fetal examination worldwide. In the 1990s, China began to use low-field MRI equipment for the diagnosis of fetal disorders. Subsequently, the continuous development of fetal MRI and the emergence of various new sequences of examination have continuously made up for the vacancy of prenatal diagnosis imaging data of various disorders. At present, it has become an important supplementary method for fetal imaging. Fetal MRI has high soft tissue resolution, high safety factor, no radiation damage, multiparameter imaging and multi-angle, large scanning field of view, all of which are helpful to provide rich and intuitive imaging information for a variety of emerging disciplines such as *in utero* pediatrics, intrapartum surgery, and neonatal surgery and provide important supporting evidence for clinical diagnosis and treatment decisions. At the same time, the emergence of a series of novel technologies and new methods has continuously improved the research

on fetal growth, development, and functions. Hence, it is believed that in the future, clinicians can make more accurate prognostic judgments and choose more favorable intervention timing and plans, thereby further reducing neonatal mortality and providing sufficient guarantees for fetal safety.

15.3.1.1 Progress in MRI Research on the Fetal Central Nervous System

At present, the value of fetal MRI is increasingly recognized as an important supplement to ultrasonography, and the central nervous system is accepted as one of the most appropriate systems for fetal MRI. Although ultrasonography can rule out most of the central nervous system disorders, it is affected by the volume of amniotic fluid, obesity of pregnant women, etc. The soft tissue resolution, the fetal brain structure, and lesion details are not clearly displayed, resulting in insufficient assessment of such disorders. Ultrasonography is yet still unable to display the germinal matrix of the fetal brain, and MRI is currently the only way to show this structure. For the judgment of brain development, although the exact mechanism of sulcus formation is not clear, fetal sulcus development often follows a certain sequence and pattern. Therefore, it is more accurate to determine the gestational age by sulcus development or to assess brain development based on gestational age. Ultrasonography cannot clearly visualize the fine structure in the brain, but fetal MRI can display multi-planar and multiparameter imaging with high soft tissue resolution. It can clearly show the brain and germinal matrix and has more prominent advantages in reflecting the etiology of ventriculomegaly, agenesis of corpus callosum, posterior cranial fossa malformation, and cerebral dysplasia. The indications of fetal MRI for the central nervous system mainly include: congenital brain malformations, such as ventricular enlargement, dysplasia of corpus callosum, posterior cranial fossa malformation, and cerebral cortical malformations; cerebrovascular lesions, such as cerebral infarction, vascular malformations, etc. [11].

Dysplasia of corpus callosum is one of the most common malformations of central nervous system development in fetuses. The risk stratification of agenesis of corpus callosum was divided based on the structural characteristics of fetal MRI, and the corresponding MRI scoring system was developed [12], which helps to improve the accuracy of prenatal diagnosis and prognosis of patients with agenesis of corpus callosum and provide more accurate preoperative counseling and decision-making. However, for the agenesis of corpus callosum difficult to be diagnosed in the early stage, a more comprehensive assessment system is still required. In addition to the analysis of fetal brain structure, some scholars have performed quantitative analysis of fetal brain MRI. For instance, quantitative study of fetal brain stem structure and cerebellar growth spatiotemporal development by MRI showed that the fetal pons increased relatively and the midbrain decreased relatively during 15–40 weeks of gestation, which was significantly different from the proportion of adults [13]; using the statistical data of fetal ultrasound with normal width of septum pellucidum, some scholars have made a relevant study to fill up the gap with MRI and obtained the normal values of fetal septum pellucidum for MRI images, thereby providing the MRI reference value. They also found that the width and height of fetal septum pellucidum had a downward trend from 27 weeks of pregnancy [14]. At present, deep learning, which has been widely studied and applied in adults, has also made some progress in fetal MRI. Some scholars have obtained a deep learning model for predicting the gestational age in early pregnancy by measuring the biparietal diameter on fetal MRI [15].

In addition, in fetal imaging, high-field MRI not only reflects the morphological changes of the brain but also uses functional MRI sequences to study fetal brain development [16]. Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) can noninvasively analyze the direction and extent of water molecules diffusion in three-dimensional space, reflecting the walking characteristics of nerve fibers in brain tissue. This

is of great significance in reshaping early fetal brain connectivity networks and revealing changes in brain networks under neuropathological conditions [17], providing a scientific basis for research on the etiology and pathogenesis of abnormal fetal white matter development. Some studies have found that the shape of projection fibers, association fibers, and brain stem fibers is relatively stable in early stage by using diffusion magnetic resonance imaging (dMRI) sequences, while other related fibers, such as the following inferior fronto-occipital fasciculus (IFOF) and superior longitudinal fasciculus (SLF), are more extended over time [18]. In some malformations that may affect fetal brain content before delivery, such as congenital heart disease (CHD), it is also possible to assess the neurodevelopmental impairment of the fetus using multimodal and functional MRI scan sequences. In recent years, some scholars have found in prenatal MRI studies that in some fetuses with CDH, compared with fetuses with near-normal brain content, CHD fetuses with severely reduced brain content have more significant reduction in brain volume [19]; diffusion tensor imaging has revealed that some fetuses with CDH have delayed white matter development in the early stage; MR spectroscopy has found that some fetuses with CDH have excessive cerebral lactate, suggesting possible hemodynamic abnormalities [20]. In summary, prenatal MRI is expected to provide the possibility for prenatal quantitative evaluation of fetal brain structure and function.

15.3.1.2 Progress in MRI Research on Fetal Respiratory System

In addition to the advantages of fetal MRI over ultrasound in the central nervous system, the advantages of diagnosing and evaluating the prognosis of fetal chest and other system lesions are gradually being recognized. Among them, congenital diaphragmatic hernia (CDH) and the resulting accompanying abnormalities are the focus of common fetal malformation research.

The mortality of congenital diaphragmatic hernia is high, which is mainly due to the blockage of lung development after the abdominal organs are herniated into the thoracic cavity, mainly manifested as reduced gas exchange between alveoli and pulmonary ventilation dysfunction; on the other hand, the compression of small vessels in the lung leads to vascular remodeling, resulting in increased pulmonary venous pressure leading to increased fetal left atrial pressure and hypofunction, further causing reduced lung mass and pulmonary dysplasia, finally making the condition of children with congenital diaphragmatic hernia aggravated. Children may have different degrees of cyanosis after birth and die in severe cases due to invalid rescue of circulatory failure of various systems. Therefore, early and accurate diagnosis and precise assessment of CDH are of great significance in guiding prenatal counseling, perinatal management, postpartum treatment, and selecting specific surgical timing and surgical plans [21].

Ultrasound is currently the preferred screening method for the diagnosis of congenital diaphragmatic hernia, and diagnosis can be made as early as 12 weeks of gestation. However, it is greatly affected by amniotic fluid volume and maternal position. The advantage of MRI in the diagnosis of congenital diaphragmatic hernia is that it is multiplanar with high soft tissue resolution, and different abdominal contents often present with different signals on MRI, for instance, the colon generally present with high signal intensity on T1WI due to containing more meconium, the small intestine with high signal intensity on T2WI due to containing more amniotic fluid, the liver with low signal intensity on both T1WI and T2WI, and the gastric bubble and gallbladder also with high signal intensity on T2WI. Fetal MRI can accurately distinguish various hernias, determine the presence or absence of diaphragmatic hernia sac [22], measure indicators that may be related to lung development and the severity of pulmonary hypertension, and perform qualitative and quantitative comprehensive risk assessment of fetal diaphragmatic hernia, thus assisting the prenatal counseling and the selection of perinatal surgical plan. In terms of assessing lung development, the actual/predicted lung volume ratio can be derived by delineating the lung margin on the fetal MR images to calculate the actual lung volume of the fetus.

Studies have found that this ratio has a significant correlation with fetal prognosis, with different cutoff values reported by domestic and foreign scholars [23, 24].

In assessing the severity of pulmonary hypertension, some scholars have implemented the concept of "mediastinal shift angle" in ultrasound to MRI, obtaining the relevant cutoff value, but the study scale is relatively small [25]. In addition, hepatic herniation can be used as an independent risk factor for congenital diaphragmatic hernia. By calculating the ratio of hepatic herniation volume to thoracic cavity volume in MR images, independent survival predictors can be obtained in addition to lung volume indicators [26]. At present, to optimize postpartum surgical planning, some studies also evaluate the feasibility of locating and quantifying diaphragmatic defects in congenital diaphragmatic hernia and analyze the necessity of prosthetic patch repair by fetal MRI 3D reconstruction, as well as analyze diaphragmatic growth dynamics by the repeated fetal MRI scanning and predict the necessity of patch placement based on MRI, along with personalized patch design study based on 3D printed template [27].

15.3.1.3 Progress in MRI Research on Fetal Cardiovascular System

Congenital heart disease (CHD) is the leading birth defect in China, and prenatal diagnosis is very important for timely treatment. Fetal echocardiography is the most important imaging method for evaluating cardiac anatomy and diagnosing cardiac malformations before delivery. However, the frequency of fetal echocardiography probe is relatively high. Moreover, during the third trimester, in case of oligohydramnios, maternal obesity, or anterior uterine fibroids and other conditions, more imaging studies are commonly required in the clinical context. MRI is absolutely unaffected by the above conditions; the later the week of pregnancy, the clearer the MRI shows. MRI has the advantages of good soft tissue resolution, spatial resolution, and large scanning field of view, which is a supplement to fetal echocardiography. Currently, fetal MRI can often provide additional valuable information beyond fetal echocardiography on fetal congenital heart malposition, whether the hearts of thoraco-abdominal conjoined infants are communicated, whether cardiac rhabdomyoma is complicated with tuberous sclerosis, extracardiac vascular malformation in fetal congenital heart disease, and fetal airway stenosis due to vascular rings. On the other hand, fetal MRI is also limited by the prohibited use of contrast agents and gating techniques. Thus, fetal heart imaging is also a challenging territory of MRI. As for the influence from the fast fetal heart rate and the inherent fetal movements, MRI scanning requires rapid imaging sequences to minimize motion artifacts. At present, the examination sequences mainly include the single shot fast spin-echo sequence and the steady-state free precession sequence. For addressing the gating issue, some scholars believe that autonomous gating is a feasible MRI technique for examining the embryonic cardiac structure. Through retrospective analysis of MRI data, the gating signal can be obtained from the data itself; at present, automatic fetal heart gating system devices are available in some American and European centers, which help to obtain high-quality MR images. In addition, there is a new gating method with non-gated artifacts by analyzing image units. In order to obtain appropriate reconstructed images, this technique requires phased imaging of the entire cardiac cycle. Although fetal cardiac MRI is challenging, it can still be complementary to ultrasound, providing more comprehensive imaging information.

15.3.1.4 Other Systems

Fetal sacrococcygeal teratoma is the most common tumor in fetuses and newborns and now can be diagnosed by ultrasonography and MRI. Ulm et al. followed 45 patients with teratoma discovered during the fetal period for up to 20 years and found that the proportion of survival with tumor and long-term disease is high, and MRI was helpful for teratoma risk stratification [28]. Chinese scholars have also pointed out that the MRI findings of fetal sacrococcygeal teratoma are characteristic, and prenatal MRI can definitely diagnose fetal sacrococcygeal teratoma and accurately

demonstrate the relationship between the lesion and the surrounding tissues such as pelvic cavity, abdominal cavity, and spine, which can be used as an important supplement to prenatal ultrasonography [29, 30].

In addition, MRI still plays a robust role in the diagnosis of fetal urinary system. Studies have shown that MRI can more comprehensively identify the causes of fetal urinary system abnormalities [31], such as obstruction, dilatation, and vesicoureteral dysplasia, so as to clearly define the location and nature of the lesions that cannot be clarified by ultrasound and provide a reference for clinical practice. This is of great significance for early fetal treatment.

15.3.2 MRI Study on the Relationship Between Placenta and Fetal Development

From the "Human Placenta Project" proposed by the National Institute of Child Health and Human Development, National Institutes of Health in 2014 to the "Early Life Plan" initiated by President and Dr. Kun Sun of the Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine in China in 2016, we realized that the placental functions play a key role in early life health. As the first organ to develop during pregnancy, the placenta is an important structure to ensure the normal pregnancy and the healthy development of the fetus. Since the fifth day after fertilization, the human placenta begins to develop from ectodermal trophoblast cells and goes through the pre-lacunar, lacunar, and villous stages. With the successful recasting of the uterine spiral arteries, the placenta is basically formed in the first trimester; during the second and third trimesters, the placenta becomes mature and performs nutritional, respiratory, secretory, and immunomodulatory functions throughout the process to ensure the healthy growth of the fetus. During the whole cycle of placental development and maturation, any factor leading to placental insufficiency will impair fetal development, manifested as intrauterine growth restriction, premature delivery, and even unexplained stillbirth [32].

Currently, there is no clear optimal time for placental MRI in the literature. MRI is usually required for confirmation after the abnormality is detected by ultrasonography. 1.5–3.0 T MRI with a multichannel body coil is recommended. The patient may be in the supine position and, if necessary, in the left lateral decubitus position to reduce patient discomfort. The bladder remains moderately full. When the parturient holds her breath, MR imaging will be performed on the transverse, sagittal, and coronal planes, respectively. Clinically, the placenta is primarily evaluated using structural MR imaging, including T1WI and T2WI (balanced steady-state free precession, bSSFP and single shot fast spin-echo, SSFSE) sequences. Since gadolinium-based contrast agents can penetrate the placenta and affect fetal development, the use of gadolinium-based contrast agents for dynamic contrast-enhanced MRI during pregnancy is not recommended in clinical practice. The experience of DWI in the evaluation of placental insufficiency is limited since this technique is occasionally included in placental imaging protocols in the clinical practice. The normal placenta is usually disc-shaped, and its size is determined by its medial thickness, usually between 2–4 cm, attached to the anterior, posterior, and lateral walls of the uterus. In the first and second trimesters, the placental signal is uniform, the surface is smooth, and the signal intensity was moderate on T2WI. With increasing gestational age, the placenta becomes progressively more mature, distinctly lobulated, with increased calcification and heterogeneous signals. In the third trimester, the placental lobules manifest as a round structure with slight hyperintensity while the normal subplacental musculature with slightly hypointensity on bSSFP. As pregnancy progresses, the normal muscular layer may appear as a thin and unstratified arc, especially in areas such as the maternal spine and aorta where the compression is evident. Blood vessels under the normal placenta and at the junction of the umbilical cord give rise to a flow-void hypointensity in the heavy T2WI sequence like SSFSE [33].

The clinical evaluation of placental MRI mainly focuses on diseases affecting maternal safety, such as placental accreta. With advancing

big data and computer science, machine learning has also begun to be used in placental MRI for the risk evaluation of such patients [34–37]. However, the placental MRI assessment associated with fetal development has occasionally been reported in clinical practice. When placental insufficiency is present, it may bring about reduction and heterogeneity in signal intensity on T2WI. Since conventional MR imaging, which can only provide morphological information, has a limited role in assessing placental function, functional assessment of placental tissue such as microperfusion, oxygenation levels, and metabolic status should be achieved by means of functional imaging [38]. At present, the most commonly utilized functional imaging technique is DWI. Studies have shown that compared with the normal placenta, the placenta of a growth restricted fetus is smaller. It's hyperintense on DWI with a lowered ADC value. Intravoxel incoherent motion diffusion-weighted imaging (IVIM-DWI) is an optimized DWI capable of measuring parameters of blood flow and perfusion-related tissue properties. With IVIM, diffusion coefficients, perfusion fractions, and pseudo-diffusion coefficients can be obtained, reflecting placental tissue perfusion in those with intrauterine growth restriction. Arterial spin labeling (ASL) MRI can label the H-protons in tissue blood by magnetization of water protons as an endogenous contrast agent and indirectly reflect the perfusion of placental tissue, mainly in the evaluation of placentas with intrauterine growth restriction and small for gestational age infants. Blood oxygenation level-dependent (BOLD) MRI and oxygen-enhanced (OE) MRI, which change the spatial magnetic field of voxels upon different hemoglobin concentrations in placental vessels and tissues and then affect T2/T2* transverse relaxation time or the reciprocal of its relaxation time (R2 or R2*), indirectly reflect the oxygenation status of placental tissues and can identify placental dysfunction associated with intrauterine growth restriction. Magnetic resonance spectroscopy (MRS) mainly including ^{31}P or ^{1}H spectroscopy can collect the distribution and content of phosphates or amino acids in the region of interest of placental tissue, which is also applicable for placental metabolism assessment in intrauterine growth restriction [39–42]. Since functional MRI (fMRI) has some defects in routine utility such as high scanning technology requirements, long scanning duration, and unstable evaluation parameters, it is currently not widely performed in clinical practice. Clinical implementation of the fMRI in this territory still warrants the collaboration among radiologists in the future.

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16 *In Utero* Genetic Screening and Diagnosis

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16.1 Introduction

Many countries and regions carry out prenatal screening for certain fatal and disabling serious genetic diseases with high incidence. Prenatal genetic screening is beneficial to the development of genetic counseling and prenatal diagnosis of genetic diseases. This section will focus on noninvasive prenatal screening based on high-throughput sequencing technology. Noninvasive prenatal testing (NIPT), based on cell-free fetal DNA (cffDNA) in maternal peripheral plasma, uses maternal peripheral blood as a sample to determine the extent to which the fetus is likely to have certain genetic diseases through high-throughput sequencing and bioinformatics analysis. NIPT was initially performed only for fetal trisomy 21. Later, it is extended to common aneuploidies, such as trisomy 18 and trisomy 13. Subsequently, the targets of NIPT screening are gradually extended to all aneuploidies and chromosomal structural abnormalities of the fetus thanks to the development of technology and the improvement of detection resolution. Currently, there are three sources of cffDNA: apoptosis of placental syncytiotrophoblast cells, which is the main source; apoptosis of fetal hematopoietic cells entering the maternal circulation; and direct transfer of fetal DNA molecules into maternal plasma through the placenta. cffDNA level from the placenta increases with the progression of pregnancy and the degree of placental apoptosis. cffDNA, as it is released by apoptotic cells, also has the characteristics of apoptotic cells, that is, fragmentation of genomic DNA. More than 99% of fetal DNA fragments are below 313 bp in length. For pregnant women in the first trimester, cffDNA can be detected in the peripheral blood, accounting for up to 20% of the total free DNA in maternal plasma. NIPT is generally performed after 12 weeks of gestation. In addition, cffDNA is rapidly degraded after birth and is not affected by the previous fetus.

The genetic diagnosis aims at individuals at risk (such as fetuses at high risk during genetic screening or with family history). Appropriate genetic testing methods can be used to determine whether they are patients with genetic diseases through genotype analysis to support subsequent disease management and fertility counseling. *In utero* treatment can be performed at appropriate times, and even gene therapy can be considered. *In utero* genetic diagnosis is also known as prenatal genetic diagnosis, using various diagnostic techniques for *in utero* diagnosis of fetal diseases. Prenatal diagnosis aims at congenital defects and genetic disorders in the fetus and is mainly for pregnant women who are at high risk or have a poor maternal or family history of pregnancy and childbirth. Prenatal diagnosis applies various targeted tests for different types of variants and is

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widely used, mainly including molecular genetics (genetic testing), biochemical genetic diagnosis (enzymatic diagnosis, genetic metabolite diagnosis), cytogenetic diagnosis (karyotyping, chromosome microarray chip, optical genome map counting), and invasive and noninvasive prenatal diagnostic techniques.

16.1.1 Prenatal Genetic Screening

16.1.1.1 Chromosome Aneuploidy

Chromosome aneuploidy refers to the gain (trisomy) or loss (monosomy) of any particular chromosome. Currently, routine NIPT test reports in China include fetal trisomy 21, trisomy 18, and trisomy 13. The main reasons are: (1) Among autosome aneuploidies, trisomy 21, trisomy 18, and trisomy 13 have the highest incidence in neonates, accounting for more than 80% of all chromosomal diseases. Other autosomal aneuploids generally stop developing in the first trimester of pregnancy (around 45 days of pregnancy), while NIPT is generally performedafter 12 weeks of pregnancy. Thus, the possibilities of detecting other aneuploid abnormalities are extremely low. (2) The positive predictive value of sex chromosome aneuploidy is limited.

Trisomy 21 is the most common chromosome aneuploidy, characterized by mental and growth retardation, with a fetal incidence of about 1 in 500 and a neonatal incidence of about 1 in 800. There is no effective treatment for the disease. Prenatal screening and diagnosis to confirm whether the fetus has the disease can help pregnant women make the best birth choices for themselves and their families. Sex chromosome aneuploidy (SCA) refers to numerical abnormalities of the X or Y chromosomes, commonly including 47, XXX; 47, XXY; 47, XYY, and 45, X. The incidence of SCA in human embryos is higher than that of trisomy 21 and correlates with maternal age. It has an incidence of about 1/400 in young pregnant women (≤35 years old) and can be as high as 1/210 in older pregnant women (>35 years old), with 45, X having the highest incidence of 1–1.5%. Compared to autosome aneuploidy, SCA rarely leads to severe malformations and generally has less impact on intelligence, with a low incidence affecting physical development. SCAis mainly manifested as abnormal development of secondary sexual characteristics, but the phenotypic variations are large. In addition to the overall appearance and intelligence of the fetus, pregnant women are also concerned of the future sexual development and fertility of the affected fetus. Genetic counselors should fully inform the relevant information about SCA, including phenotypic variations (mild, moderate, and severe symptoms and their incidence), progress in treating various symptoms, and adjuvant treatment for infertility. Of the SCA fetuses selected for termination, the vast majority are phenotypically normal, making it controversial whether prenatal screening and diagnosis of SCA should be routinely performed.

16.1.1.2 Chromosomal Microdeletion/ Microduplication Syndrome

Several studies showed that the positive rate of NIPT for fetal microdeletions/microduplications is about 0.13%, while the predictive positive rates range from 3.8% to 17%. The positive rate was as high as 48% in certain studies in high-risk pregnant women, but the clinical significance was very limited. It may be related to the maternal DNA background, sequencing depth, analysis method, and the nature of cffDNA. CNV is prevalent in humans and partially unrelated to phenotype, but all can affect NIPT analysis, while the sequencing depth is closely related to detection rate. In addition, cffDNA is not complete DNA; they are all small fragments. Errors can occur during assembling. Therefore, NIPT is currently only indicated for some well-studied chromosomal microdeletion/microduplication syndromes with severe clinical phenotypes, such as DiGeorge syndrome, 1p36 deletion syndrome, Angelman syndrome, Prader-Willi syndrome, and Cri-du-chat syndrome.

16.1.1.3 Limitations of the Method

Various factors impact the accuracy of NIPT, including maternal age and weight, duration of

pregnancy, twins or multiple births, egg or sperm donation, consanguineous marriage, placental chimerism, fetal or maternal chimerism, malignancy, and uniparental disomy. All these factors can lead to false positives (about 0.5%). Therefore, those who are positive still require amniocentesis for confirmation, while those who are negative for NIPT cannot be totally ruled out of fetal trisomy 21, trisomy 18, and trisomy 13, with a false-negative rate of about 0.05%. Occasionally, redrawing of blood is required for reanalysis. The causes of false-positive and false-negative results of NIPT are complex and include the following main reasons.

Confined placental mosaicism (CPM), in which the placenta contains two or more cell types with different genetic materials at the same time, is the main factor leading to inaccurate NIPT results. The fetus and placenta are derived from the same fertilized egg. It begins to develop by bifurcation and divides into approximately 10^4 cells around Day 10, which gradually and randomly differentiate into the placenta and fetus. Afterward, the placenta becomes a barrier between the mother and the fetus. The development of the placenta after formation is not completely synchronized with the development of the fetus, and genetic material mutations can also occur in the development of placental barrier, immunity, and other functions. cffDNA originates primarily from the placental trophoblast. The genetic materials of the placenta are not always identical to that of the fetus; therefore, NIPT is only a screening technique.

Maternal genome copy number variants (CNV) are common in humans. They are referred to as genomic rearrangements with a length of 1 KB or more, characterized by submicroscopic deletions and repetitions. Some CNVs do not cause any disease but may affect NIPT results. For positive NIPT results, the size of CNV fragments in fetuses and pregnant women should be comprehensively analyzed to recalculate Z values. The Decipher, DGV, and OMIM databases should be retrieved to exclude chromosomal diseases, so that NIPT false positives due to the mother's own CNV can be identified and unnecessary invasive diagnoses can be avoided.

Other causes, such as twin pregnancies, may also lead to false positives or false negatives. When the pregnant woman has a malignant tumor, tumor cells will continuously release free DNA into the maternal peripheral blood circulation. These free DNAs are more complex and cannot be differentiated from fetal-derived cffDNA by gene sequencing, resulting in inaccurate NIPT results. In addition, when the fetus itself is chimeric with a low proportion of abnormal cells, the fetal phenotype may not be significantly abnormal. However, NIPT can also detect these low proportions of abnormal free DNA and present positive results. In addition, it is difficult to detect a low proportion of abnormal cells by subsequent amniotic fluid karyotyping, which is one of the reasons for false positives. In addition, when a pregnant woman is a carrier of a balanced chromosomal translocation, maternal free DNA will contain DNA fragments from the breakpoints of the balanced translocation, and these fragment sizes have the potential to be consistent with fetal cffDNA, thus affecting NIPT detection and interpretation.

16.1.2 Prenatal Genetic Diagnosis

Prenatal diagnosis is usually an invasive procedure with certain risks, and the samples collected need to exclude maternal contamination. The most common prenatal sample collection and processing procedures include the following:

(1) Amniotic fluid: It is the most widely used amniocentesis in the second trimester (16–22 weeks of gestation) and can be used for karyotyping and detection of genetic and genomic diseases of fetuses.

(2) Villi: At 11–13 weeks of gestation, samples are collected by cervical or abdominal paracentesis according to the location of placenta, which is mainly used for cell and molecular biological detection in the first trimester, where there may be a karyotype discrepancy between the cells of the trophoblastic layer and the fetal cells.

(3) Umbilical cord blood: It is generally collected after 18 weeks of pregnancy for rapid karyotyping and diagnosis of fetal hematological

diseases and is technically difficult and has relatively high surgical complications.

16.1.2.1 Techniques of Molecular Genetic Diagnosis

1. Polymerase chain reaction (PCR). Currently, PCR techniques used in clinical testing are often combined with downstream analytical techniques, such as capillary electrophoresis or Sanger sequencing. A series of related techniques, such as reverse transcription-PCR, nested PCR, multiplex PCR, real-time fluorescence PCR, in situ PCR, asymmetric PCR, and digital PCR (dPCR), have been developed based on PCR.
2. Sanger sequencing is currently the "gold standard" for gene mutation detection. As a technique for clinical genetic disease detection, Sanger sequencing can be used in most of the gene sequence analysis. Sanger sequencing is mainly used in clinical genetic diagnosis to detect point variation and small deletion/insertion variants. As the gold standard for nucleic acid sequence analysis, Sanger sequencing can be used for genetic diagnosis and prenatal diagnosis of most genetic diseases. However, due to limitations in PCR technology or sampling, it is impossible to detect genetic diseases caused by low proportions of chimerism and germ cell chimerism.
3. Gel electrophoresis is one of the most commonly used techniques in molecular biology research. In clinical molecular diagnosis, separating the nucleic acids in the sample is often necessary. Gel electrophoresis can be used to separate, identify, and purify DNA or RNA fragments.
4. Multiplex ligation-dependent probe amplification (MLPA) is mainly used to detect large copy number variations above the exon level, such as DMD and SMA. In addition, MLPA can also be used for methylation detection (MS-MLPA), for example, for Prader-Willi syndrome or Angelman syndrome (PWS/AS).
5. High-throughput sequencing, also known as the next-generation sequencing (NGS), mainly includes whole exome sequencing (WES) and whole genome sequencing (WGS). The main purpose of WES is to find genetic disorders caused by substitutions or additions or deletions of a few bases and may also find copy number variants in large segments of the genome involving exonic regions. WGS is a NGS technique that does not require capture and covers all non-repetitive regions of the genome. Its average coverage is better than WES. WGS can detect noncoding regions and various base variants and the number of copies of genomic fragments more accurately.

Genetic testing can identify the etiology of the child, and the genetic risk of recurrence varies for different mechanisms. If the etiology is due to a deletion of a critical region or UPD, the risk of recurrence is usually less than 1%. If the etiology is due to imprinting defects, the risk of recurrence is as high as 50%. At the same time, it is necessary to be aware of the possibility that the parents of the affected child are carriers of balance transposition or have germ cell chimeric variants. The parents of the affected child are required to undergo relevant genetic counseling and prenatal diagnosis when they become pregnant again.

16.1.2.2 Biochemical Genetic Diagnosis

Biochemical genetic diagnosis is based on enzymatic analysis and metabolite determination techniques to diagnose diseases mainly characterized by abnormal biochemical metabolism. Inherited metabolic diseases are caused by defects in certain enzymes or receptors necessary to maintain the normal metabolism of the body. More than 900 such diseases are known, including metabolic diseases caused by metabolic disorders, such as amino acids, organic acids fatty acids, etc. They are mostly rare diseases. The diagnosis of inherited metabolic diseases mainly relies on laboratory tests. Almost half of the currently known inherited metabolic diseases are caused by the loss of enzyme activity. Loss of catalytic activity of enzymes can cause the accumulation of substrates, loss of metabolites, and other adverse biochemical reactions. Testing for

enzyme activity, substrates, or metabolites can help identify potential metabolic defects and causes of disease.

Enzymatic analysis is the most reliable means of diagnosing inherited metabolic diseases, provided that the enzyme defects that cause the disease can be determined and the corresponding determination methods can be established in the obtained specimens. At present, the activity of many enzymes is still difficult to detect, and fast and accurate biochemical determination and analysis techniques for metabolites are also very important. The combination of the two is the main testing means for prenatal biochemical genetic diagnosis.

The specimens for prenatal enzymatic examination are mainly placental villus tissue and amniotic fluid cells. Enzyme activity is determined by extracting the target proteins in the specimens. The common method is collecting samples from fetal tissues (such as exfoliated amniotic fluid cells or placental villous tissues, etc.), adding a trace amount of fluorescent or synthetic substrates, and testing with a fluorescence spectrophotometer or common spectrophotometer. The diseases targeted by enzyme activity determination include lysosomal storage disease and mucopolysaccharidosis. Enzyme activity determination can also be used to diagnose tetrahydrobiopterin reductase deficiency, copper oxidase deficiency, biotinidase deficiency, and other diseases. The lack of PCC enzyme activity in the amniotic fluid cells of fetuses with propionic acidemia (PA) was first confirmed by Gompertza et al. in 1975. The detection of PCC enzyme activity in amniotic fluid cells or chorionic villus cells has been gradually applied to the prenatal diagnosis of PA since then. However, due to the low levels of enzymes associated with inherited metabolic diseases in amniotic fluid and the complexity of the sample matrix, there is still a paucity of highly specific and accurate quantitative prenatal screening and diagnostic reagents for inherited metabolic diseases.

Prenatal metabolite determination is mainly aimed at amniotic fluid and fetal tissues. The metabolites are determined by tandem mass spectrometry, gas-phase inclusion/mass spectrometry, and high-performance liquid chromatography. The scope of application includes amino acid metabolism diseases, organic acidemia, fatty acid oxidation disorders, and other inherited metabolic diseases. Diseases that can be diagnosed by amniotic fluid mass spectrometry include methylmalonic acidemia, propionic acidemia, valeric acidemia-type I, beta-ketothiolase deficiency, isobutyryl-CoA dehydrogenase deficiency, total carboxylase synthase deficiency, and homocysteinemia. Diseases that may be diagnosed by amniotic fluid mass spectrometry include 3-methylcrotonyl-CoA carboxylase deficiency, 3-hydroxy-3-methylglutaric aciduria, biotinidase deficiency, ethylmalonic aciduria (ethylmalonic encephalopathy), and 2-methylbutyrylglycinuria.

In recent years, various new technologies have provided more possibilities for prenatal screening and diagnosis of genetic metabolic disorders. Reactive fluorescent probes, represented by molecular probes, are used for the qualitative and quantitative determination of various biomarkers in humans. With the development of mass spectrometry and its combination technology, more and more researchers use chromatography-mass spectrometry for prenatal detection. With the separation by chromatography and the identification by mass spectrometry, metabolites in amniotic fluid can be accurately quantified and qualitatively analyzed rapidly. Overall, there are still large gaps in the screening and diagnosis of *in utero* genetic metabolic disorders, and its clinical application is still limited. It is still in development, and its clinical effects have yet to be evaluated.

16.1.2.3 Cytogenetic Diagnosis

Cytogenetic diagnosis is the diagnosis of diseases caused by chromosomal aberrations by cytogenetic analysis of tissues or cell cultures. The main indications of cytogenetic diagnosis include: (1) chromosomal aberration or balanced translocation in one of the couples, (2) parents with normal karyotype but had a child with the chromosomal disorder, (3) parents with normal karyotype but had unexplained recurrent miscarriage and stillbirth, (4) pregnant women over 35

years of age, and (5) prenatal screening or preoperative imaging tests suggest a high risk of birth defects.

The main techniques for cytogenetic diagnosis include chromosome analysis, fluorescence in situ hybridization (FISH), chromosomal microarray (CMA) analysis, and optical genome mapping techniques.

16.1.2.3.1 Karyotyping Technique

Karyotyping is based on the characteristics of metaphase chromosomes, such as chromosome length, centromere position, the proportions of long and short arms, and the presence or absence of satellites; it analyzes, compares, ranks, and numbers chromosomes with the help of banding technology and makes a diagnosis according to the variation of chromosome structure and number. Karyotyping can provide an important basis for the determination of numerical and structural chromosomal variation. Chromosome examination during pregnancy can effectively screen fetal chromosomal abnormalities and reduce the birth rate of infants with genetic diseases. Karyotyping is the "gold standard" for traditional cytogenetic prenatal diagnosis. It can detect aneuploidy, structural rearrangements, and chromosomal aberrations with large fragments. The chromosomal aberrations that can be detected include trisomy 21, trisomy 13, trisomy 18, Turner syndrome, and Klinefelter syndrome.

16.1.2.3.2 Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) was once considered a hallmark technique in the development of molecular cytogenetics. With the development and clinical application of CMA technology, the application of FISH has been considerably reduced as prenatal diagnosis requires quick and accurate laboratory results. The application of FISH in prenatal diagnosis mainly uses α satellite probes to screen changes in the number of chromosomes 13, 18, 21, and X and Y. The greatest advantage is that it does not require cell culture but is performed directly on amniotic fluid and villous or cord blood interphase cells, and results can be obtained in only 1–2 days.

16.1.2.3.3 Chromosomal Microarray Technique

Chromosomal microarray (CMA) analysis, based on oligo-nucleotide synthetic probes, enables the simultaneous detection of thousands of loci throughout the genome in a single experiment. It targets at chromosomal microstructural abnormalities, that is, chromosomal aberrations that cannot be or are not easily detected by conventional chromosome banding methods. Since its first clinical application in 2007, CMA has shown great advantages over traditional methods such as karyotyping, BacArray, FISH, and MLPA. The continuous discovery of many new disease-related genomic imbalances through CMA testing has made it an indispensable tool for cytogenomic testing and molecular diagnosis. Its applications include postnatal diagnosis of neonatal and childhood genetic diseases, as well as prenatal diagnosis of fetal genetic diseases and diagnosis or screening of genetic diseases before implantation of embryos. CMA is currently the core technology in cytogenomic detection methods and is a first-line detection method for prenatal chromosomal aberrations. In addition to detectable numerical chromosomal abnormalities, microstructural abnormality-related diseases that can be detected include William's syndrome, 22q11 microdeletion syndrome, Wolf-Hirschhorn syndrome, and cri du chat syndrome.

16.1.2.3.4 Optical Genome Mapping Technique

Optical genome mapping technique can simultaneously detect structural variants and copy number variants and comprehensively identify large balanced and unbalanced SVs (currently the advantage of karyotyping and metaphase FISH), CNV (by CMA), repeat contraction disorder (by Southern blot), and multiple repeated amplification disorder (traditional PCR-based method or Southern blot). Its advantage is that more accurate breakpoint regions can be obtained for translocation and chromosome rearrangement. In

addition, it has high sensitivity and specificity for diseases detected by traditional Southern methods, such as multiple repeated amplification-associated facioscapulohumeral muscular dystrophy (FSHD).

16.2 Clinical Practice

16.2.1 Case 1: Fetal Chromosome Abnormality Due to Paternal Nucleolus Organizer Region (NOR) Translocation

Present Medical History The first child of this family presented with intellectual disability, and chromosome microarray (CMA) found a novel 4.86-Mb deletion at 10q26.3: 46, XY, del (10) (q26.3), array hg19 (chr10: 130, 665, 570-135, 524, 747). The couple choose prenatal diagnosis at 16 weeks of their second pregancy.

Prenatal Diagnosis CMA + karyotyping of amniotic fluid cells.

Genetic Testing Results The fetal CMA test revealed the same deletion as the proband (Fig. 16.1a), and the karyotyping of cultured amniotic fluid revealed a very small fragment of unknown origin at 10q terminal (Fig. 16.1b).

Analysis of Results The proband had a novel deletion at the end of the chromosome 10q. The recurrent risk was usually considered low, but the results of amniotic fluid sample test showed that the fetus had the same deletion at 10q terminal as the proband, suggesting possible underlying structural abnormalities leading to the same genetic changes. Combined with karyotyping of the cultured amniotic fluid, it is revealed that there were very small fragments of unknown origin at 10q terminal (Fig. 16.1). To clarify its origin, peripheral blood karyotyping of parents as well as the proband was recommended.

Further Testing Plan Karyotyping (G-banding + NOR banding) + fluorescence in situ hybridization (FISH) for the proband and his parents.

Testing Results The peripheral blood karyotyping of parents and proband suggested that small fragments at the end of 10q of unknown origin were present in the father and proband (Fig. 16.2), suggesting probability of a reciprocal translocation of 10q terminal with NOR. NOR banding

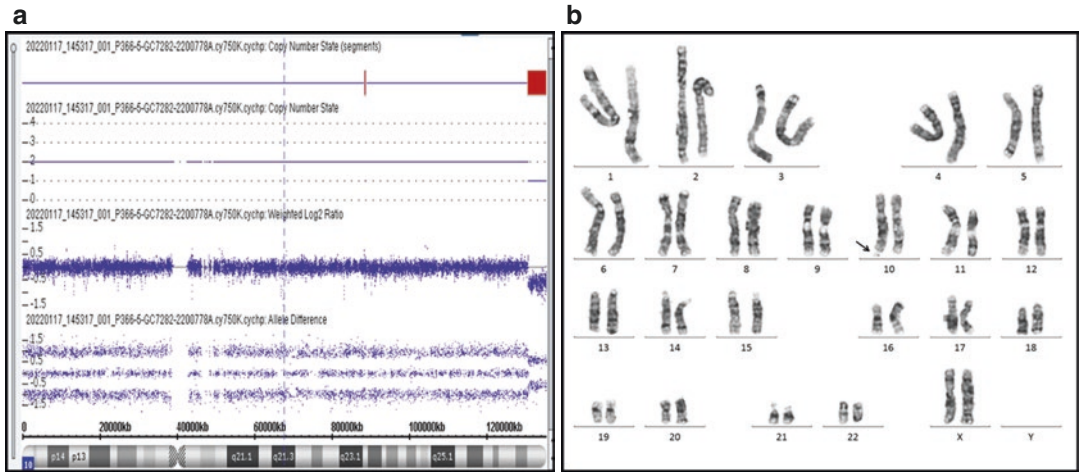

Fig. 16.1 (**a**) CMA results of amniotic fluid cells; (**b**) karyotyping results of amniotic fluid cells

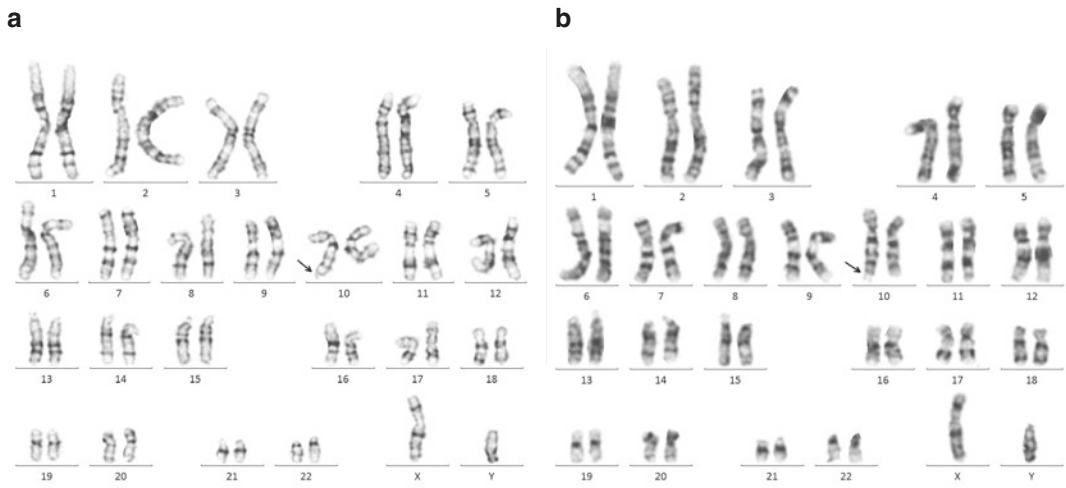

Fig. 16.2 (**a**) Peripheral blood G-banding karyotyping of the father; (**b**) peripheral blood G-banding karyotyping of the proband

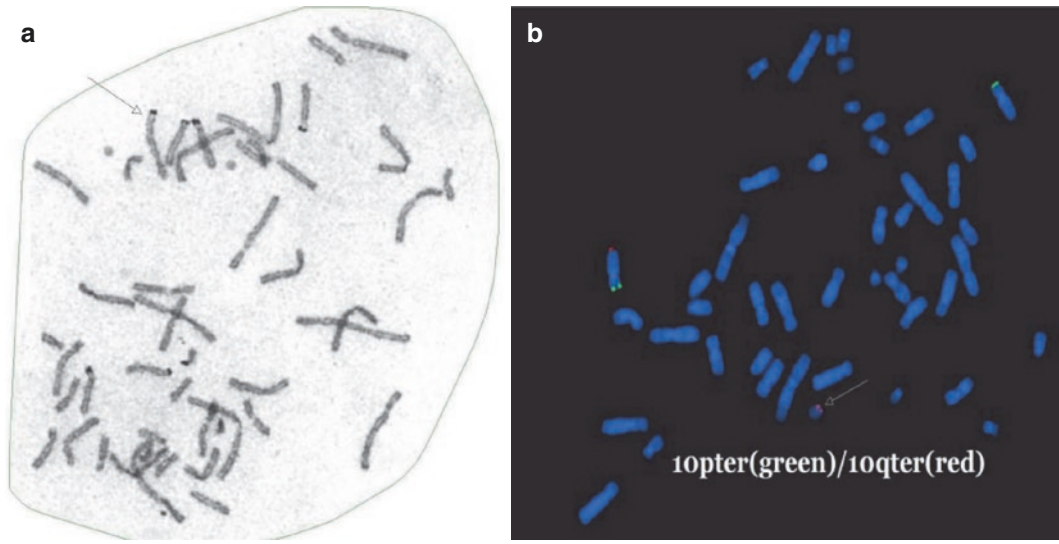

Fig. 16.3 (**a**) Peripheral blood NOR banding karyotyping of the father; (**b**) Peripheral blood FISH results of the father

results confirmed the presence of nucleolus organizer region at 10q terminal of the father (Fig. 16.3a), and FISH results confirmed that 10qter signal -translocates to the end of the short arm of Chromosome 22 (Fig. 16.3b). Conclusively, the above results confirmed that the father had a reciprocal translocation between the 10q terminal and the nucleolar organizer region of the short arm of Chromosome 22, which resulted in the proband and fetus inheriting the derived Chromosome 10 with the 10q terminal deletion.

Discussion The nucleolus organizer region (NOR) contains ribosomal RNA cluster on the short arms of acrocentric chromosomes. Localization of NORs in positions of other than the short arms of acrocentric chromosomes through translocations or insertion is rare. Translocation of NORs to the terminal region of another chromosome is relatively frequent. The ps or qs indicates a NOR at the tip of the arm of a chromosome. The phenotypic effect of carrier with ps or qs depends on whether the translocation process led to a deletion at the tip of the recipient chromosome.

Ps or qs have been reported in several chromosomes with normal or abnormal phenotype. Usually, the NOR translocation is harmless, while in some instances, it is pathogenic. [1] reported that a male who had a satellited X chromosome manifested developmental delay, mental retardation, hypertelorism, ptosis of one eye, low-set ears, and hearing disturbance. The molecular test revealed a microdeletion at Xq28. [2] reported a child of 4qs with cerebellar ataxia and mental retardation. No loss of genetic material revealed at the molecular level indicated the disruption of a causative locus at the site of translocation. We have identified a patient carrying de novo 10qs, Ag-positive NOR on the distal long arm of one Chromosome 10, and reciprocal acrocentric Chromosome 22 was observed indicated a reciprocal translocation involving the short arm of acrocentric Chromosome 22. The microdeletion at distal 10q revealed by CMA contributed for the abnormal phenotypes for our patient. Thus, the application of molecular cytogenetic methods (such as FISH and CMA) in the evaluation of NOR translocation is required.

The genetic diagnosis of this family is based on the combination of multiple techniques to clarify the nature and source of abnormalities. Finally, it is confirmed that multiple abnormalities were originated from reciprocal translocations of the father, providing an accurate basis for the risk assessment of recurrence in the family, while guiding the development of the reproduction plan.

16.2.2 Case 2: The Results of Noninvasive Screening Suggested that Fetal Abnormality was Originated from the Reciprocal Chromosomal Translocation in the Mother

16.2.2.1 Case History

A couple presented for prenatal genetic counseling for advanced maternal age. They chose a noninvasive prenatal diagnosis for fetal common aneuploid screening. After the informed consent was signed, blood sample from the pregnant woman was collected into EDTA-containing tubes.

16.2.2.2 Lab Examination

NIPT: All standard procedures, including cell-free DNA isolation, library construction, and sequencing, were performed as reported before. Whole genome sequencing (WGS) was used in this test. For aneuploidy detection, we used an advanced bioinformatics method combining a locally weighted polynomial regression to eliminate GC-bias and a binary hypothesis to get a higher accuracy for aneuploidy detection. This test now contains risk assessment for trisomy 21, trisomy 18, and trisomy 13. Microdeletion and microduplication have great effect on the accuracy measurement of DNA representation for a whole chromosome so that we designed FCAPS pipeline to detect microdeletion and microduplication. The FCAPS through maternal plasma has shown great importance in clinical application since it was routinely performed from July 2012. Counseling clinicians were notified if any of these additional abnormalities were suspected.

Karyotype analysis: Routine karyotyping analysis of the parent was performed on GTG-banded metaphases from cultures of PHA-stimulated peripheral blood lymphocytes according to standard procedures.

Fluorescence in situ hybridization: FISH analysis was performed on metaphase prepared from peripheral blood lymphocytes obtained by standard protocols. TelVysion 5p SpectrumGreen, 5q SpectrumOrange, TelVysion 7p SpectrumGreen, and TelVysion 7q SpectrumOrange (Vysis, Richmond, UK) were used to confirm the translocation between chromosome 5p and 7p.

16.2.2.3 Results

NIPT detected a suspected 5p terminal deletion under a genomic coverage of current standard aneuploidy detection (Fig. 16.4). Regrettably, we did not get the sample of fetus to confirm the existence of the 5p deletion as amnio centesis failed twice, and the couple chose induced labor for fetal ultrasound anomies.

Followed karyotype and FISH analysis of the mother revealed the reciprocal translocation between chromosome 5p and 7p (Fig. 16.5). According to the karyotype analysis of the mother, the fetus might be affected due to inherited unbalanced subtelomeric translocation

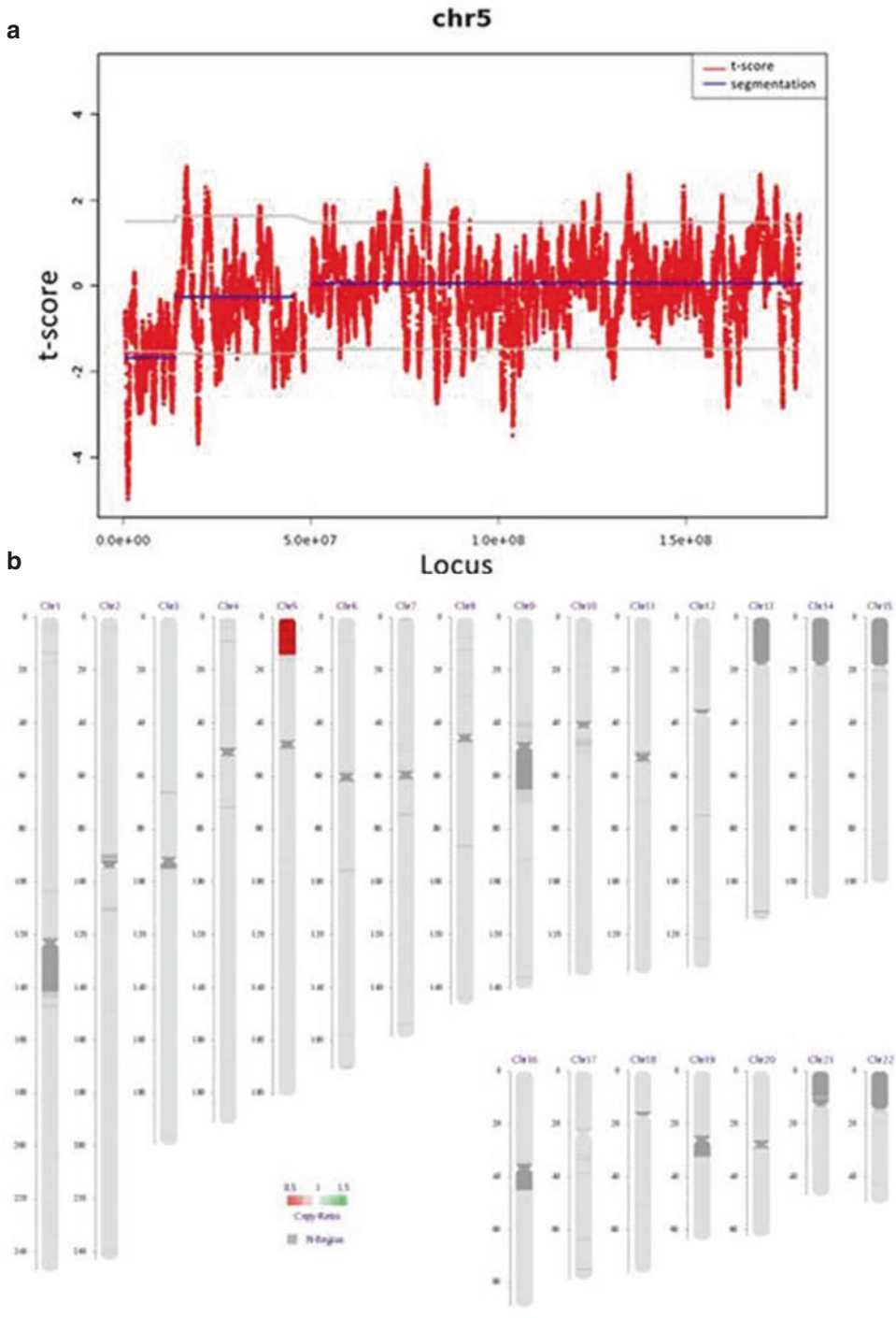

Fig. 16.4 FCAPS analysis of maternal plasma. (**a**) For the region affected on chromosome 5p, T-score is lower than the rest part of chromosomal 5, illustrating a suspicious 13.45Mb deletion on short arm of chromosomal 5 (chr5:569019-14014775). (**b**) Digital karyotyping of the sample with partial deletion on chromosome 5. Red indicated deletion, green indicated duplication, and gray indicated normal region

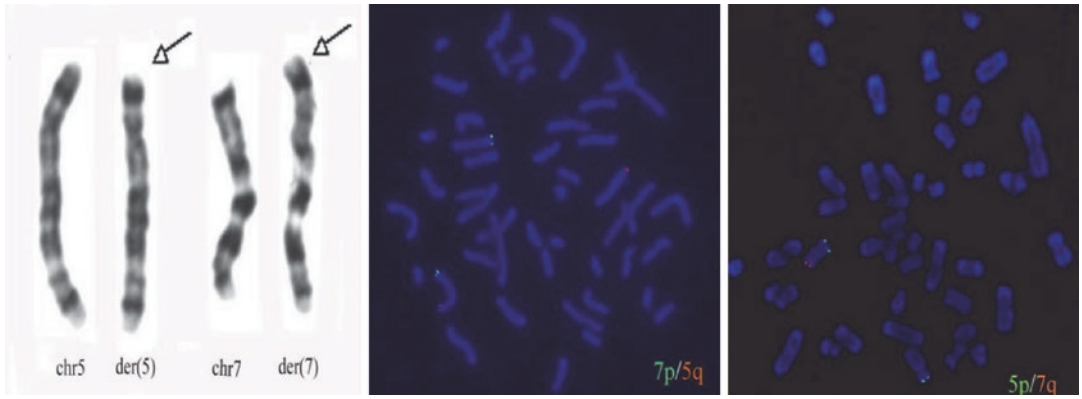

Fig. 16.5 Left panel: Partial karyotype of the mother (GTG-banding), arrow indicates the derivative chromosome 5 and chromosome 7. Middle panel: FISH analysis shows a normal TelVysion 7p signal (spectrum green) and another signal on 5p terminal, as well as two normal TelVysion 5q (spectrum orange) signals. Right panel: Fluorescence in situ hybridization analysis shows a normal TelVysion 5p signal (spectrum green) and another signal on 7p terminal, as well as two normal TelVysion 7q (spectrum orange) signals

(karyotype 46, N, der(5)t(5;7) (p15.2; p22.1) mat resulting in a partial 5p monosomy and 7p trisomy, the deletion size of 5p was estimated to be more than 10~15Mb, and the duplication segment was estimated to be less than 10 Mb.

16.2.2.4 Discussion and Clinical Significance

In this case, massively parallel sequencing blindly detected a suspected 5p terminal deletion under a genomic coverage of current standard aneuploidy detection. Regrettably, we did not get the fetal sample to confirm the existence of the 5p deletion asamniocentesis failed twice, and the couple chose induced labor for fetal ultrasound anomies, while followed karyotype and FISH analysis of the mother revealed the reciprocal translocation between chromosome 5p and 7p. According to the karyotype analysis of the mother, the fetus might be affected due to inherited unbalanced subtelomeric translocation (karyotype 46, N, der(5)t(5;7) (p15.2; p22.1) mat resulting in a partial 5p monosomy and 7p trisomy, the deletion size of 5p was estimated to be more than 10–15 Mb, and the duplication segment was estimated to be less than 10 Mb. The massive sequencing showed the deletion on 5p was about 13.45 Mb, which is in accordance with karyotype analysis, whereas the duplication on 7p was not found by sequencing. The coverage of our patient is 0.1-fold. In other aneuploidy detection studies using massively parallel sequencing, a 25-Mb deletion in chromosome 11q21-23 and a duplication of 6q, which was 37.5-Mb in size, were identified. These data suggested under the standard genomic coverage for aneuploidy detection, the results of noninvasive sequencing of the corresponding cffDNA in maternal plasma could blindly detect chromosome aberrations more than 10–15 Mb.

By massively parallel sequencing of cffDNA isolated from maternal plasma, a recent study detected a confirmed 3-Mb deletion on chromosome 22q11.2. To detect this 3-Mb fetal deletion, they used a genomic coverage of fourfold—an increase in coverage of approximately 20-fold over that of current standard aneuploidy detection. Another study detected a known 4.2-Mb deletion on chromosome 12. A recent blind study showed that genetic changes in all seven cases were detected by massively parallel sequencing, including a 300kb microdeletion. The coverage was about 40- to 90-fold. Although rare, these studies have shown the feasibility of this method for detecting subchromosome-level CNVs, and the detected resolution could be increased with

adequate genomic coverage. However, a major limitation of deep sequencing with higher coverage is the relatively high cost right now. Consider higher cost of deep sequencing, now targeted methodology would be more cost-effective, especially for family with inherited chromosome aberrations, or having a proband with known chromosome changes. Genome-wide analysis of the sequencing data for detecting relatively large genetic aberrations would be more reliable and usable for clinical tests now.

In this case, we found a suspected 5p terminal deletion under a genomic coverage of current standard aneuploidy detection. The chromosome changes of the fetus inferred indirectly from the mother's karyotype. Large genetic aberrations could be blindly detected by massively parallel sequencing of cffDNA under a genomic coverage of current standard aneuploidy detection. Further studies are warranted to evaluate the performance of massively parallel sequencing in detecting partial chromosome changes. With improved data analysis ability and adequate *genome coverage*, massively parallel sequencing of cffDNA in maternal plasma would be another option for detecting molecular karyotype with advantage of fine resolution and safety for noninvasive procedures.

16.2.3 Case 3: Multiple Cases of Angelman Syndrome in Fetus and Family Caused by Maternal Imprinting Center Deletion

16.2.3.1 Present Medical History

The pedigree is shown in Fig. 16.6. The woman had a history of giving births to three children, all of whom presented with developmental delay/intellectual disability. The father and mother were non-consanguineous, and both were phenotypically normal. The woman was now at 28 weeks of the fourth gestation and sought the diagnosis to clarify the genetic etiology of her family.

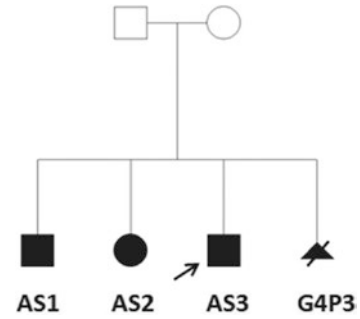

Fig. 16.6 Pedigree map of AS1 patient

16.2.3.2 Probands in the Family

Patient AS3, the proband, presented with developmental delay at the age of 9 years and 8 months old. The patient was the third child born at full term by vaginal delivery with no history of asphyxia or hypoxia, and the parents were healthy. The birth weight was 3.28 kg. The development was delayed since childhood. The child could walk independently at the age of 3–4 years old and could only say simple words, such as "dad" and "mom." The patient has a happy, excitable demeanor with frequent smiling, laughter, and hand-flapping movements and showed ataxia when walking and unstable gait, with arms lifting and bending posture, especially when walking. The neurological examination revealed high muscle tone and deep hyperreflexia of the lower limbs. No sleep disorders, seizures, and hair or skin abnormalities were found. Cranial MRI and metabolic screening results were normal.

Patient AS1 was the first child born at full term by vaginal delivery, with no history of asphyxia or hypoxia, and the weight at birth was unknown. The patient had development delay since childhood, could walk independently at the age of 4 years, and had significantly language development delay. The patient is currently 16 years old and has no active language expression. The patient had microcephaly, happy demeanor, ataxia, abnormal walking posture, unstable gait, and thumb adduction. The neurological examination revealed high muscle tone and deep hyperreflexia of the lower extremities. No sleep

disorders, epilepsy, and no abnormalities in hair and skin color were found. Cranial MRI results and metabolic screening were normal, karyotyping was 46, XY, and electroencephalogram showed abnormalities (increased diffuse slow waves).

Patient AS2, the old sister of proband AS3, was the second child born at full term by vaginal delivery, with asphyxia after birth, oxygen inhalation for half an hour, and the weight at birth was unknown. The patient had delayed development since childhood, could walk independently at the age of 4 years, had significantly language development delay, and lacked speech expression. The patient had a microcephaly, strabismus, happy demeanor, ataxia, abnormal walking posture, and unstable gait. Cranial MRI and metabolic screening were normal, and electroencephalography showed no abnormalities.

16.2.3.3 Laboratory Tests and Results

Four to five milliliters of EDTA-anticoagulated peripheral blood were drawn from the proband and parents, and genomic DNA was extracted with QIAamp DNA (Qiagen) extraction kit. Whole exome sequencing and CNV-seq detection of the proband were performed according to standard procedures, and no clear pathogenic CNVs and pathogenic SNV and Indel variants were detected.

The proband underwent MS-MLPA. MRC-holland MS-MLPA kit (ME028) was used; hybridization, ligation, PCR amplification, and capillary electrophoresis were performed according to standard steps for fragment separation; and a coffalyser was used for data analysis. The MLPA analysis results of the proband showed that the signal intensity of the SNRPN exon U5 probe region, that is, the central region of the AS blot (AS-IC), was reduced by 50%, showing the presence of heterozygous deletions. After methylation-sensitive enzyme digestion, five methylation-specific probes from the PWS/AS region (four probes located in the SNURF-SNRPN exon 1/promoter region and intron 1, as well as one probe located in MAGEL2) were shown to be completely unmethylated, indicating that the IC-deletion was on the maternal allele (Fig. 16.7). Later, MS-MLPA was performed for the parents, which showed that the same AS-IC deletion and five methylation-specific probes were completely unmethylated in the proband. The mother only showed heterozygous deletion of AS-IC, while the degree of methylation was normal (Fig. 16.7). The MLPA results of the father showed no AS-IC deletion, and the degree of methylation was normal.

Six STR loci (D15S1035, D15S817, D15S128, D15S210, D15S986, and D15S1364) in the 15q11-13 region were selected for linkage analysis of this region in pedigree samples, including the proband, parents, and grandparents. The linkage analysis showed that the proband inherited the mother's alleles derived from their grandfather.

16.2.3.4 Treatment, Follow-up, and Outcome

For patients in the family with confirmed diagnoses, it is recommended to continue rehabilitation training and symptomatic treatment. For the fetus of this pregnancy, umbilical cord blood puncture, MS-MLPA test, and STR linkage analysis were performed for prenatal diagnosis. After the diagnosis of the proband was confirmed, prenatal diagnosis by umbilical cord blood puncture was performed in the current pregnancy. MS-MLPA test of umbilical cord blood samples indicated that the fetus had AS-IC deletion as the proband, and the five methylation-specific probe regions were completely unmethylated, confirming the diagnosis of AS. The results are shown in Fig. 16.7. According to the genetic testing results, the fetus had AS. The parents decided to perform induction of labor. The results of methylation MLPA in fetal tissue after abortion were the same as those in umbilical cord blood. Genetic counseling was provided for the couple to inform them that there would be a 50% risk of the fetus becoming an AS patient for all subsequent birth and that prenatal diagnosis may be sought. MS-MLPA testing was recommended for the proband's grandfather and grandmother to determine whether the grandfather was a carrier. If so,

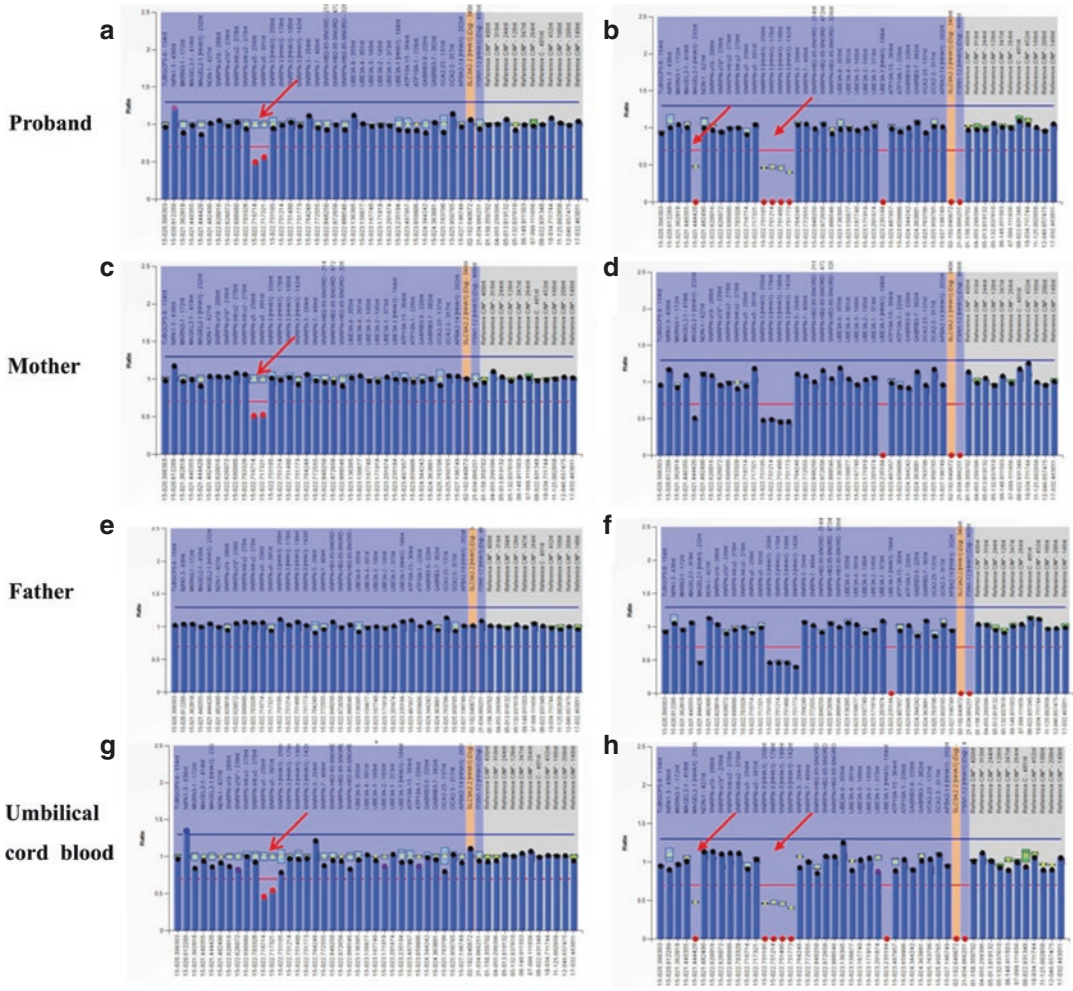

Fig. 16.7 Methylation MLPA test results. (**a**, **c**, **g**) All gene copy number test results in the 15q11-13 region of the proband, mother, and umbilical cord blood, all of which showed heterozygous deletion (red arrow) in SNRPN exon U5 probe region (AS—imprinting center region). (**e**) The copy number results in this region in the paternal sample were normal. (**b**, **d**, **h**) Copy number detection results after methylation-sensitive enzyme digestion, which reflected the degree of methylation of genes in the 15q11-13 region. In maternal samples, five methylation-specific probes from the PWS/AS region (four for SNURF-SNRPN exon 1/promoter region and intron 1, and one probe located in MAGEL2) showed a normal degree of methylation, and the proband and cord blood samples showed no methylation at all in these five methylation-specific probe regions (red arrows). (**f**) Degree of methylation in this region in the paternal sample

the possibility of cryptic transmission of the causative variant in the woman's family was not excluded, and relevant family members could seek genetic testing and genetic counseling.

16.2.3.5 Discussion

Angelman syndrome (AS, OMIM#105830) is a neurological developmental disorder caused by abnormal function of the maternal imprinting gene *UBE3A*. The clinical manifestations of the disease include severe intellectual disability, significant language impairment, no language or very little language expression, happy behavior, hyperactivity, paroxysmal unexplained laughter, frequent hand clapping, ataxia, microcephaly, epilepsy, sleep disorders, and hypopigmentation [3–5]. The clinical incidence is about 1/10,000–1/20,000 [3]. Here, we reported the process from etiological diagnosis to prenatal diagnosis in

multiple familial AS patients caused by imprinting center (IC) deletion.

This pedigree is a classic AS syndrome with clinical manifestations caused by AS-IC deletion. Among the many molecular causes of AS, IC deletion is relatively rare and often easily neglected. Although some AS-IC deletions occur newly on the maternal chromosome and have a very low risk of recurrence, in most cases, IC deletion is inherited from phenotypically normal mothers, resulting in multiple AS patients in the family. Therefore, when there are many AS patients in the family, it is easily mistaken for other autosomal recessive monogenic diseases or X-linked recessive diseases (when the affected offspring are all males). Such abnormalities only result in incorrect maternal imprinting when transmitted through females, resulting in abnormal offsprings. There are many patients in this pedigree, including male and female patients, with normal parental phenotypes, and, therefore, tend to be presumed to have abnormal chromosomal structure, autosomal recessive disorders, and X-linked genetic disorders in terms of presumed mode of inheritance without fully realizing the possibility of imprinting defect-related disorders.

IC plays a regulatory role in imprinting, resetting, and maintenance. 15q11q13 contains a set of imprinting genes that are expressed under the control of IC, some of which are expressed by paternal alleles and others by maternal alleles only. UBE3A gene is located in the region 15q11-13 and is specifically expressed in the hippocampus and cerebellum of the human brain only by the maternal allele. AS is mainly caused by functional inactivation of maternally expressed UBE3A in neural cells [6, 7]. The main molecular etiologies include (1) maternal deletion of key region of 15q11-q13 (75%), (2) paternal uniparental diploidy (1–2%), (3) imprinting center deletion (10–15%), (4) imprinting defects (3%) (excluding imprinting center deletions), and maternal UBE3A gene mutations (5–10%) [8]. Among them, maternal deletions of a key region of 15q11-q13 and paternal uniparental diploidy are often caused by errors in germ cell meiosis, usually with a low risk of recurrence; relatively speaking, imprinting defects or maternal UBE3A gene mutations may be inherited through phenotypically normal mothers, with a risk of recurrence of up to 50%, often resulting in many patients in the family. In patients with high clinical suspicion of AS, there are many molecular tests to confirm this, but a relatively comprehensive test is the methylation method, preferably MS-MLPA, which can detect 80% of AS cases. However, about 5–10% of patients with mutations in the UBE3A gene cannot be detected by this method. In conclusion, methylation testing is still recommended first and foremost in the clinical pathway of AS molecular diagnosis, regardless of the presence of a single patient in a family or multiple patients, and it is recommended to follow the EMQN/ACGS guideline pathway (Fig. 16.8) [9].

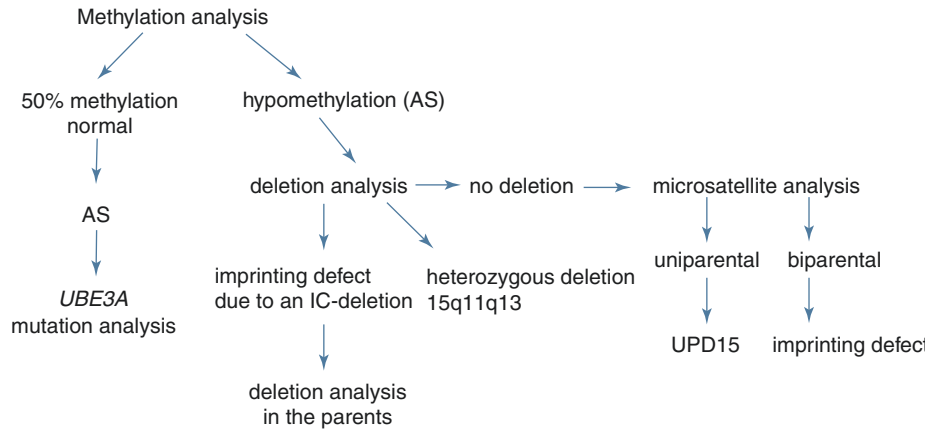

Fig. 16.8 Detection strategy for the molecular analysis of AS using a MS-MLPA

In conclusion, patients in this family had methylation abnormalities inherited from the mother due to lack of IC, which led to AS; the identification of causative genes helps the family to implement prenatal diagnosis and avoid the birth of the child with AS, while making effective guidance for accurate recurrence risk assessment and reproduction of this family; MS-MLPA is recommended as the first choice for patients with clinically suspected AS.

16.3 Research Progress

16.3.1 Noninvasive Screening for Monogenic Diseases

Monogenic diseases are Mendelian genetic diseases controlled by a pair of alleles and are caused by dominant gene and recessive gene mutations. Although uncommon, they account for about 20% of infant mortality and about 10% of child hospitalization, posing a greater threat to human health. Prenatal genetic diagnosis of pregnancies at risk for monogenic diseases still requires invasive techniques such as amniotic villus biopsy sampling, amniocentesis, and umbilical cord blood sampling, which may lead to miscarriage and cause complications such as infection and teratogenicity. Fetal and placenta-derived circulating cell-free DNA (cfDNA) from maternal plasma has been reported, allowing the development of noninvasive tools for the detection of fetal genetic abnormalities from maternal blood draws. Currently, the wide application of noninvasive prenatal screening for monogenic diseases is still difficult due to the low prevalence of each specific mutation in the general population (which hinders positive predictive values). For noninvasive pre-invasive DNA detection technologies for monogenic genetic diseases, the currently used methods include droplet digital PCR, targeted next-generation sequencing (NGS), COLD-PCR and microarray, allele-specific real-time PCR, circulating single-molecule amplification and resequencing technology (cSMART) and micro-sequencing, and single-cell sequencing.

16.3.1.1 Droplet Digital PCR (ddPCR)

Droplet digital PCR (ddPCR) is a novel molecular diagnostic technique. The sample is divided into many independent chambers through limited dilution, and each chamber contains or does not contain a copy of the target gene. Multiple chambers are simultaneously amplified independently in parallel. After amplification, the number of negative and positive chambers is calculated through the presence or absence of a fluorescence signal, and absolute quantification can be achieved. Compared with PCR, this segmentation improves detection sensitivity and specificity due to reduced competitive effects of target molecules, and mutations such as CF (CFTR gene), neonatal diabetes (KCNJ11), and achondroplasia (FGFR3) can be detected by droplet digital PCR (ddPCR). At the same time, spinal muscular atrophy and the number of SMN1 gene and the SMN2 gene copies can also be accurately detected. In 2018, Joan Camunas-Soler et al. reported a study on prenatal diagnosis of monogenic diseases using ddPCR technology.

16.3.1.2 Targeted Next-Generation Sequencing

Bell et al. reported a screening study of genes associated with 448 severe recessive monogenic genetic diseases. A total of 7717 regions of 437 target genes were enriched through hybrid capture or microdroplet polymerase chain reaction, and high-depth sequencing was performed by next-generation sequencing (NGS) and evaluated using strict bioinformatics filters. With an average target coverage of 160 times, 93% of nucleotides have at least 20 times coverage, and mutation detection/genotyping has ~95% sensitivity and ~100% specificity for substitutions, insertions/deletions, splicing, large deletion mutations, and single nucleotide polymorphisms. The results demonstrated the promise of next-generation sequencing technology as a comprehensive screening method to replace existing technologies for single screening of a few genetic disorders in clinical practice. The use of targeted next-generation sequencing in the noninvasive

prenatal diagnosis of bone dysplasia disorders has also been reported in the literature.

16.3.1.3 Circulating Single-Molecule Amplification and Resequencing Technology (cSMART)

The application of circulating single-molecule amplification and resequencing technology (cSMART) in diseases, such as favism, Wilson's disease, hereditary deafness, and phenylketonuria, has been greatly successful. Some experts have proposed the further application of cSMART in the detection of monogenic genetic diseases in twin pregnancies with a normal fetus and a fetus as a carrier.

16.3.1.4 NIPD-Single Cell Sequencing

Single-cell sequencing is performed on circulating fetal cells isolated from maternal blood or trophoblasts extracted from the exterior of the cervix. The use of fetal cells avoids the problem of detecting fetal variants in the maternal context and provides non-fragmented DNA, which is more suitable for sequencing analysis. However, the number of fetal cells in the maternal circulation is so low that it is difficult to isolate them. Another major issue when considering the use of DNA isolated from these cells for WES or WGS is the allelic failure rate after whole genome amplification. There are also concerns about sampling deviation associated with single-cell analysis. Although fetal single-cell sequencing is promising, it still requires a great deal of work to validate the application of this technology in clinical diagnostic settings.

16.3.2 In Utero Gene Therapy

With the advancement of human prenatal diagnosis technology and molecular genetics research, various genetic diseases can be diagnosed in early pregnancy, making it possible to perform gene therapy *in utero* before the onset of clinical symptoms. *In utero* gene therapy (IUGT) refers to the introduction of normal genes into target cells before the birth of the fetus to replace genetically defective genes or to inhibit and repair abnormally expressed genes by gene editing to prevent and treat hereditary diseases and minimize damages caused by genetic diseases.

IUGT is divided into in vivo and ex vivo gene therapies. The former injects the vector carrying exogenous target gene directly into the specific tissues and organs of the fetus; the latter injects the target cells obtained from the fetus into the fetus after being genetically engineered in vitro. IUGT has several potential advantages: (1) The fetal size is small, (2) the development of fetal immune system is not yet mature, (3) there are abundant stem/progenitor cell populations in the fetus, (4) the unique fetal anatomy facilitates gene delivery, and (5) the fetal blood-brain barrier is permeable. However, IUGT also has its risks: (1) damage to normal fetal development, (2) increased risk of germline transfer of genetic modifications, (3) induction of genotoxicity and/or neoplasia, and (4) harm to mother. Therefore, IUGT is still in the stage of animal experiments and scientific research, and there are still many problems to be solved before its formal clinical application.

16.3.2.1 Gene Vectors and Delivery Methods

Vectors are tools for introducing target genes into fetal target cells and are broadly divided into viral vectors and nonviral vectors. The former is derived from modifying naturally occurring viruses, including lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses (AAV). The latter includes physical methods, chemical methods, and molecules with special structures, for example: (1) direct injection of "naked" DNA, (2) electroporation, (3) gene gun, (4) ultrasonic pulse method, (5) hydrodynamic delivery, (6) polymer, and (7) liposome fusion carrier. The modified virus is used as the viral vector, which has greater packaging capacity and higher transfection efficiency. The transgene is more effective but has the risks of immunogenicity and tumorigenesis. Nonviral vectors are safer but have lower transfection rates and lower expression levels of transferred genes and disappear with cell division and are unsatisfactory with regard to targeted specificity and directed

insertion. The carrier selection depends on the target tissue, packaging capabilities, immunogenicity, environment, and goals of prenatal treatment. The carrier selected must be safe for both the fetus and mother and must not induce an immune response.

16.3.2.2 Gene Editing

IUGT can be broadly divided into gene replacement and gene editing strategies. Gene replacement is the replacement of a defective or missing copy of a gene by a vector carrying the target gene. Gene editing is the repair of an incorrect copy of a gene by providing the correct DNA template and using endogenous or exogenous cellular systems to restore function. The CRISPR/Cas9 system is a powerful gene editing tool in which Cas9 targets genomic regions complementary to sgRNAs and generates double-stranded/single-stranded DNA breaks that are then repaired by nonhomologous end-joining or homologous recombination mechanisms. CRISPR/Cas9 enables therapeutic genome editing and facilitates the development of newer, more complex single-base editors. Base editors can generate point mutations directly in cellular DNA without causing double-stranded DNA breaks, thereby reducing potential chromosomal translocations and genomic rearrangements. Targeted specificity and efficient delivery of gene editing technology is essential for fetal applications, and delivery vectors for gene therapy must be optimized to maximize gene persistence and minimize the risk of off-target. The delivery vectors currently used for *in utero* gene editing include adenoviruses and AAV, or less immunogenic and safer nonviral vectors, such as electroporation, lipid nanoparticles (LNPs), and gold nanoparticles (AuNPs).

16.3.2.3 Research Status

With the continuous progress of genetic diagnosis and treatment technology, studies based on mouse, sheep, and nonhuman primate models have demonstrated the safety and efficacy of IUGT in a variety of genetic diseases, including hereditary hematological diseases, neurological diseases, metabolic system diseases, monogenic lung diseases, sensory organ diseases, and X-linked hypohidrotic ectodermal dysplasia, etc.

Hemophilia is a group of bleeding disorders due to the deficiency of certain coagulation factors in the blood, which can lead to life-threatening neonatal central nervous system bleeding. Using lentivirally transduced c-Kit + placental cells carrying the FVIII gene transplanted into the peritoneal cavity of fetal sheep, plasma coagulation factor VIII activity was significantly increased at 4 months (1 month of age) after transplantation, indicating the feasibility of *in utero* gene therapy for hemophilia A. Plasma coagulation factor IX in the fetal macaque model of hemophilia B reach the after-curement level through viral vector transduction, and no long-term side effects of viral vector or transgene expression are observed after four years. β-mediterranean anemia refers to a group of hemoglobinopathies in which the synthesis of the β-chain is partially or completely inhibited. It occurs more often in children than in infancy, leading to severe chronic progressive anemia, which requires blood transfusions to sustain life. Lentiviral vectors with β-globin expression were injected into the uterus of a humanized β-mediterranean anemia mouse model, and blood hemoglobin levels remained normal after birth. The applicability of lentiviral vectors for fetal use has been demonstrated, but researchers have discovered unexpected integration sites, including tumor genes. Thus, while lentiviral vectors appear promising for *in utero* gene therapy, further studies targeting tissue-restricted promoters, targeting specificity, and overall safety are necessary prior to use in fetuses. Another study packaged peptide nucleic acid/DNA into biodegradable polymer nanoparticles and delivered systemically to β-mediterranean anemia fetuses. Mice treated *in utero* had hemoglobin levels restored to the normal range, reduced splenomegaly, and increased survival, indicating that nanoparticles are an alternative gene delivery platform that could be generalizable.

Spinal muscular atrophy (SMA) is a disease characterized by degeneration of alpha motor neurons, leading to progressive weakness, paral-

ysis, and ultimately death due to respiratory failure. Injection of an AAV treatment vector carrying *SMN* gene into the lateral ventricles of fetal mice (E14.5-E15) resulted in a significant increase in survival life span in the treated mice. Gauchers disease (GD) is a neurodegenerative disease that may be fatal to newborns, and children have shown significant irreversible neuropathological features *in utero*. Injection of the AAV vector carrying *GBA* gene into the cranium of fetal mice restored neuronal glucocerebrosidase expression, eliminated neurodegeneration and neuroinflammation, and allowed the mice to survive for at least 18 weeks with normal activity. However, it is technically difficult to use minimally invasive injection techniques to introduce vectors directly into the fetal brain or ventricle through the skull for gene therapy. Therefore, exploring vectors that can cross the blood-brain barrier is necessary. Studies have shown that after intravenous injection of single-stranded or self-complementary forms of AAV2/9 vectors into the fetus, comprehensive transduction of the central nervous system (including all regions of the brain and retina) and peripheral nervous system (including the myenteric plexus) is detected, indicating that the AAV2/9 carrier has a strong neurological transduction ability and can be used for *in utero* treatment of subsequent neurological diseases.

Hereditary tyrosinemia type 1 (HT1) is an autosomal recessive disorder that mainly affects the liver and kidneys and can be life-threatening in severe cases. The team used the base editor to effectively introduce nonsense mutations in *Hpd* gene in hepatocytes from the tyrosinemia mouse model. Silencing of *Hpd* gene improved liver function in mice and prevented neonatal mice from dying by blocking the accumulation of toxic metabolites in the tyrosine catabolic pathway. Another study utilized posttranslational endopeptide-mediated fusion, delivered CRISPR base-editing genes using dual AAV to correct pathogenic variants in *Idua* gene in fetuses with Huler syndrome (a group of inherited disorders of mucopolysaccharide metabolism caused by lysosomal abnormalities), and improved case phenotypes in the skeletal muscles and heart of mice and survival of mice. Neither study found off-target CRISPR, nor gene editing in germline or maternal tissues.

Mutations in the gene encoding pulmonary surfactant protein C, *SFTPC*, may lead to neonatal respiratory failure or idiopathic pulmonary fibrosis. Delivery of the CRISPR gene editing system to surfactant protein C-deficient mouse fetuses using an adenoviral delivery method can kill mutant genes that lead to surfactant protein C deletion and improve mouse survival. At the same time, studies have shown that direct injection of CRISPR vectors into amniotic fluid can more effectively and accurately edit lung epithelial cells in the respiratory tract than intravenous injection. As an ideal target for IUGT, the fetal lung is fluid-filled to facilitate effective gene transduction, while the air-tissue interface after birth and lung injury and inflammation will hinder effective gene delivery.

In an animal model of Leber congenital amaurosis, a serious inherited retinal disease that causes congenital blindness in infants and young children, fetal gene therapy using AAV or lentiviral vectors can lead to effective treatment of gene transduction and restoration of visual function in the retinal pigment epithelium. Similarly, AAV was also able to efficiently transduce therapeutic genes into the developing cochlea of embryonic mice. *MsrB3*, a key gene involved in auditory function, was delivered to the inner ear sac of *MsrB3*-knockout mice with congenital hearing loss on Day 12.5 of the embryonic period, and hearing recovery was observed at 28 days after birth. However, *in utero* treatment of both eye and ear diseases relies on injecting gene vectors into the developing embryonic sensory organs, which is difficult to achieve in clinical practice.

X-linked hypohidrotic ectodermal dysplasia (XLHED) is caused by mutations in the gene encoding ectodysplasin A (EDA). Lack of EDA during fetal development can permanently damage sweat glands and lead to fatal hyperthermia. In a groundbreaking clinical study, recombinant EDA protein was administered intra-amniotically to three affected fetuses at the end of the second month of pregnancy. The infants were able to sweat normally, and there were no XLHED-

related disease phenotypes in the 14–22 months follow-up.

16.3.2.4 Ethical Issues

IUGT offers the possibility of curing or lowering the severity of various genetic diseases for which no effective therapy is currently available. However, IUGT still has ethical and practical issues. Firstly, the diseases of the fetus must be accurately diagnosed. Currently, there is no effective postpartum cure or remission method that is safe for the fetus and with minimal or at least treatable risk to pregnant women with the diseases. Secondly, it must be conducted in an experienced fetal medicine center. After ethical discussion, the family members must be fully informed of the advantages and disadvantages of IUGT and the risks to the mother and fetus; the patient's (and family members') will must be respected, and an informed consent must be obtained from the patient (and family members). Finally, scientific researchers of IUGT technology must be rigorous and serious and conduct clinical experiments after the animal models are confirmed feasible and can improve adverse outcomes, provide the information on side effects found in the study, and take corresponding measures as far as possible.

16.3.2.5 Outlook

IUGT is still in the early stage of research. It is successful in rodents and large animals, but safety issues, such as insertion mutations, impacts on organ development, and germ cell transmission, still require in-depth study of animal models in preclinical practice. In addition, there are ethical and potential problems in changing the human genome. However, tissue specificity and safety of genetically modified genes may be solved by applying tissue-specific promoters or regulating gene expression and developing safer transgenic technologies. The development of IUGT depends on the progress of prenatal diagnosis technology and the continuous improvement of gene therapy technology. If the safety and ethical issues can be solved, IUGT will be suitable for the treatment of more and more monogenic and polygenic diseases.

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Pediatric Rehabilitation

17

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17.1 Introduction

17.1.1 Pediatric Rehabilitation for Diseases Originated *In Utero*

As the development of modern medicine continues to improve the survival rate of infants at birth, there is an increasing demand for rehabilitation. The causes of several common diseases in pediatric rehabilitation can be traced back to the pregnancy and perinatal period. Common diseases originated *in utero* and requiring rehabilitation in childhood include cerebral palsy, congenital muscular torticollis, congenital heart disease, congenital talipes equinovarus, and congenital scoliosis. Cerebral palsy (CP), also known as brain paralysis describes a group of permanent disorders in the development of posture and central motor function accompanied by activity limitation. It results from nonprogressive damage to the brain of the developing fetus *in utero* or infants after birth. Congenital muscular torticollis (CMT) is caused by the unilateral thickening or shortening of the sternocleidomastoid muscle, which is associated with the *in utero* development of sternocleidomastoid muscle and intrauterine fetal posture. Preoperative and postoperative rehabilitation of congenital heart disease plays an important role in improving prognosis and promoting normal physical and mental development in children. Congenital talipes equinovarus may be attributed to the genetic or chromosomal abnormalities or compression *in utero*. Congenital scoliosis is usually caused by abnormal formation and segmentation of the spine and ribs *in utero*. Most children with these conditions can manifest as growth retardation, immature brain development, poor physical fitness, susceptibility to infection, and motor, cognitive, speech, and social development disorders, which require early rehabilitation to promote recovery, improve function, facilitate motor, cognitive and speech development, prevent complications, and eventually improve their long-term quality of life as far as possible. The rehabilitation and follow-up process should continue throughout the infancy or even to the early childhood.

17.1.2 Rehabilitation Assessments

Rehabilitation assessment serves as the basis of rehabilitation, including pain assessment, feeding assessment, reflex assessment, and development assessment. The purpose of rehabilitation assessment is to determine the necessity for rehabilitation and the presence of dysfunction in order to predict the potential development disorders.

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17.1.2.1 Pain Assessment

There are more than 40 infant pain assessment scales, among which neonatal infant pain scale (NIPS) and comfort scale are commonly used for pain assessment.

NIPS can be used for the assessment of full-term and preterm infants. The items include the facial expression, crying, breathing pattern, arms, legs, and state of arousal, with the observation period of about 1 min. Each item is assigned a score of 0 or 1, except for crying that is scored 0, 1, or 2, with a total score ranging from 0 to 7. A total score of 0–2 is interpreted as mild to no pain, 3–4 as mild to moderate pain, and 4 or more as severe pain.

The comfort scale involves the assessment of six behavioral and two physiological functions of the newborns, including alertness, calmness/agitation, respiratory response, physical movement, blood pressure, heart rate, muscle tone, and facial tension; the observation period is about 3 min. These items require only observation except for muscle tone assessment, which requires a contact with the infant. The assessment begins with calculating the upper and lower limits of heart rate and mean arterial pressure at baseline. The rater will observe the body and face of the infant as well as the vital signs monitor over 2 min to evaluate movement, body position, facial expression, and response to environmental stimuli. During the assessment, heart rate and mean arterial pressure should be observed every 15–20 s to ensure that these parameters are within 15% of the baseline. Muscle tone should be rated 10 s before the end of the observation, with individual item scores recorded to calculate the total score. The score of each item ranges from 1 to 5, and the total score is the sum of the individual item scores, with a full score of 40. Higher scores indicate higher stress.

17.1.2.2 Feeding Assessment

Feeding assessment includes recording of the milk volume and observation of the infant's condition during feeding. The latter facilitates the evaluation of oral motor function. The infants should be observed for whether their lips are well attached to the nipple during breastfeeding and whether there are regular breaks during sucking, in addition to record the occurrence of choking and nausea.

17.1.2.3 Reflex Assessment

Reflex assessment includes rooting reflex, sucking reflex, Moro reflex, tonic neck reflex, grasp reflex, Babinski reflex, and stepping reflex. Rooting reflex and sucking reflex appear at 28 weeks of gestation and persist into 3 months after birth. If these reflexes are not properly integrated after birth, the infants may have a weak sucking and difficulty in feeding, which require assisted feeding. Moro reflex can be observed within 4–6 months after birth. Infants with retained Moro reflex demonstrate a decreased coordination of head, trunk, and limbs. Tonic neck reflex begins at 1–2 months after birth and disappears after 6 months of age, which helps to integrate all motor skills. Grasp reflex appears at 30 weeks of gestation until 3–4 months after birth, and infants with abnormal grasp reflex will show a delay in voluntary grasping.

17.1.2.4 Developmental Assessment

Alberta Infant Motor Assessment (AIMS) can be used to assess the development of infants. This sensitive tool is suitable for evaluating the maturation of motor development in infants aged 0–18 months or from birth to independent walking, especially in preterm or high-risk infants. AIMS consists of 58 items evaluated at four positions, including prone (21 items), supine (9 items), sitting (12 items), and standing (16 items). During the assessment, the rater identifies the least and most advanced mature items he/she observed in a given position, which are designated as the "motor window" for that position. Items prior to the "motor window" are regarded as acquired skills for the infant. One point is given for each item observed within the "motor window." Add up the scores from each position to yield the total raw score of AIMS. Percentiles can be derived from the AIMS percentile graph, and infants below the fifth or tenth percentile are considered as suspected developmental delay. AIMS allows the healthcare providers to: (1) assess the motor skills that the infants have acquired, especially

the quality of movement; (2) identify infants with immature motor development or abnormal movement patterns at early stage; (3) understand the motor ability of the infants aged 0–18 months as well as the subtle changes in motor ability during development, along with changes in the quality of each movement in detail; and (4) screen for deficiencies in fetal motor development, therefore facilitating early detection of motor development abnormalities and establishing intervention plans as early as possible.

17.1.3 Rehabilitation Approaches

17.1.3.1 Rehabilitation During Pregnancy

17.1.3.1.1 Physical Activity

Appropriate physical activity during pregnancy is beneficial to fetal development. There is evidence that low to moderate physical activity during pregnancy has no adverse effect on the pregnant women and actually demonstrates certain benefits for fetal development during normal pregnancy [1]. Earlier research suggested that moderate weight-bearing exercise in the first trimester of pregnancy promotes placental development [2]. In pregnant women with normal body weight before pregnancy, low-intensity physical activity reduces the risk of excessive gestational weight gain and gestational hypertension, thereby reducing fetal complications such as intrauterine growth restriction and macrosomia [3]. Prenatal depression is an important risk factor for excessive activity and growth restriction in fetus as well as prematurity, which may lead to a range of emotional and behavioral problems in newborns [4]. Aerobic exercise of moderate-intensity during pregnancy is effective in reducing the occurrence of prenatal depression [5, 6].

17.1.3.1.2 Hydrotherapy

Hydrotherapy is one of the rehabilitation approaches commonly used for pregnant women. Warm water bath (32–34°C) combined with aerobic exercise in water can promote blood circulation, relieve adverse emotions, improve arterial elasticity, and reduce the risk of gestational hypertension in pregnant women [7]. Prenatal hydrotherapy is associated with pain palliation, reduced use of analgesics, accelerated labor process, and decreased fetal malformations [8].

17.1.3.1.3 Music Therapy

Music therapy can alleviate the adverse emotions of pregnant women and have a positive effect on the fetus. There are marked changes in physical condition and endocrine level during pregnancy, which may cause anxiety, depression, and other adverse emotions. Cortisol increases in response to stress and anxiety, which can cross the placenta and has a negative effect on fetal development. The fetal brain is highly sensitive during early pregnancy, and brain development is particularly susceptible to small changes in the physiological environment *in utero*, which in turn affects the motor development, learning behavior, sleep patterns, and emotional control of infants after birth [9]. Studies have shown that listening to soothing music for 40 min every day over 2 weeks significantly relieved maternal anxiety during pregnancy, accompanied by a significant increase in the birth weight and chest circumference of newborns [10].

17.1.3.1.4 Nutritional Intervention

Appropriate nutritional intervention during pregnancy is beneficial to the growth of the fetus. A randomized controlled trial in China demonstrated that adequate caloric intake, ingestion of high-quality protein, and supplement with unsaturated fatty acids and vitamins in pregnant women significantly increased the birth length and weight of newborns [11]. Taurine can be administered during pregnancy if fetuses are at risk for intrauterine growth restriction [12]. Animal studies have shown taurine supplementation during pregnancy can promote neurological development, improve the weight and ultrastructure of the brain, reduce neuronal apoptosis, promote neuronal cell proliferation, increase glial cell-derived neurotrophic factors, and improve early neurological function in fetal rats [13]. Supplement with exogenous melatonin in pregnant women is associated with reduced oxidative

stress, normalized nerve myelination, and significant brain function improvement in fetuses [14].

17.1.3.2 Ultra-Early Rehabilitation for Infants

Neonates demonstrate slow and generalized responses to external stimuli, which can be attributed to their immature cerebral cortex and corticospinal tract as well as the incomplete nerve fiber formation. Infancy is characterized by rapid brain development with high plasticity, and earlier rehabilitation is associated with better outcomes. At the age of 0–4 months, brain development mainly involves dendrite growth together with neural myelination and development, while abundant motor and sensory stimuli contribute to myelination and development of brain cells. Ultra-early rehabilitation can promote the recovery and regeneration of the injured brains during the critical period of brain development, which reduce the permanent damage caused by brain injury with potent rehabilitation or compensation ability. Ultra-early rehabilitation for infants includes positioning, exercise training, feeding training, pulmonary rehabilitation, touch therapy, and home-based rehabilitation guidance, which have demonstrated the following effects: (1) provide effective pain palliation, reduce the environmental stress that perceived, pacify emotions, and promote sleep; (2) improve motor function and reduce the incidence of cerebral palsy; (3) contribute to the development of normal posture; (4) promote intelligence and growth; (5) attenuate the impact of brain injury on long-term nervous system development; (6) facilitate the maturation of digestive and respiratory function; and (7) shorten the duration of hospitalization.

17.1.3.2.1 Positioning

Positioning is an integral part of ultra-early rehabilitation. Proper positioning is crucial for the growth and development of infants. Positioning and posture management can address the infant's sensory needs of being wrapped, which have been proven to (1) effectively improve the stress tolerance and self-soothing ability, store energy, and contribute to recovery from disease; (2) promote brain development and the establishment of correct movement patterns and prevent the occurrence of abnormal muscle tension; and (3) prevent skull asymmetry. Premature infants are recommended to have their neck, trunk, and limbs in flexed posture to promote flexor muscle development. Infants with gastroesophageal reflux should be placed in a prone position to reduce reflux. Positioning can help the infants to maintain proper position with the aid of specially shaped cushions or rehabilitation assistant devices for positioning. Common assistant devices include snuggle up positioning aids, frog-style positioning aids, prone positioning aids, bendable positioning aids, multi-posture positioning aids, sleeping bags, and head positioning aids. In addition, parents should be instructed on how to properly position their infants.

17.1.3.2.2 Exercise Therapy

Exercise is useful for preventing and treating the potential muscle tone and posture abnormalities of the infants, enhancing muscle strength, facilitating the establishment of correct movement patterns, and promoting the overall improvement in physical development. Antigravity training can be feasible, such as head-control training when sitting up with support.

17.1.3.2.3 Feeding Training

Infants with feeding disorders require a feeding training. Infants feeding with nasogastric tubes may have sucking training while pacified by their parents; if there is an improvement in sucking ability, the infants can practice sucking with mother's nipple or pacifier to gradually increase the volume of milk they sucked. Infants with hypertonia should avoid overstretch of their heads backward during feeding, whereas their heads and jaws should be retracted forward to improve their sucking ability. Infants with hypotonia should maintain their jaws retracted forward and their cheeks gently pressed to create a sealed environment in their mouths, which allows them for sucking effectively.

17.1.3.2.4 Pulmonary Rehabilitation

Pulmonary rehabilitation includes postural drainage, chest clapping, vibration, and respiratory function training, with the aim to promote mucus excretion, reduce the occurrence of atelectasis, and maintain airway patency. Placing the infant in drainage position facilitates the expulsion of mucus from the alveolar region to the central bronchus, thus promoting expectoration. Chest clapping is usually performed by the rehabilitation therapists, nurses, or parents using a tight cup-shaped hand or baby mask, with the amount of force applied depending on the infant's condition. Proper chest clapping can facilitate mucus discharge without any discomfort. If the infant is too weak to tolerate chest clapping, treatment with vibrator may be considered. Respiratory function training is typically administered by rehabilitation therapists, which can also be performed by parents after discharge. Patients with pulmonary hemorrhage, pulmonary embolism, acute pulmonary edema, pulmonary abscess, intracranial hypertension, severe osteoporosis, severe bronchospasm, asthma attack, bronchiolitis, heart failure, severe cardiac insufficiency, pneumothorax without chest tube insertion, coagulation disorders, and dysphoria should not receive pulmonary rehabilitation.

17.1.3.2.5 Touch Therapy

Touch therapy is a scientific, progressive, and skillful massage of the baby's skin with the hands of the practitioner, which provides gentle and appropriate stimulation to the central nervous system through the skin receptors, regulating the function of the endocrine and immune systems and producing positive physiological effects on the body. Touch therapy is effective in alleviating adverse emotions, relaxing tense muscles, enhancing mobility and immune function, promoting growth and development, and improving social adaptation.

17.1.3.2.6 Home-Based Rehabilitation Guidance

Home-based rehabilitation guidance is an important part of rehabilitation. Parents should be instructed to master the proper holding positions and feeding methods and help their infants with appropriate exercise and breathing training, therefore promoting growth and development.

17.2 Clinical Practice

17.2.1 Ultra-Early Rehabilitation for Infants at High Risk of Cerebral Palsy

17.2.1.1 Case

The patient, female, G2P1, was born prematurely by caesarean section at 32 weeks + 2 days of gestation, with the birth weight of 2020 g and the Apgar score of 2–5–9. After delivery, the infant was given warming, mucus suction, oxygen by nasal cannula, umbilical vein bolus injection of 5% GS and dicynone, and intramuscular injection of Vitamin K_1 to prevent bleeding. The mother had a history of gestational diabetes and took ceftriaxone sodium 1 week before delivery due to fever and right hydronephrosis, without history of gestational hypertension, hypothyroidism, smoking or alcohol, radiation exposure, or other drugs/toxicants exposure. The mother had no medical history of familial or metabolic diseases. Physical examination revealed slightly higher muscular tone of extremities, Moro reflex (−), rooting reflex (−), sucking reflex (−), and grasp reflex (−). Two weeks after birth, the patient experienced continuous crying, difficulty sleeping, and anorexia; cranial ultrasound examination showed bilateral subependymal hemorrhage and possible bilateral choroid plexus hemorrhage; Alhberta infant motor assessment indicated that the infant was below the fifth percentile, suggesting abnormal motor development. The patient was considered at "high risk of cerebral palsy" according to her medical history, symptoms, signs, and auxiliary examinations. The infant was admitted to NICU for clinical symptomatic and supportive treatments, where she started to receive ultra-early rehabilitation. Rehabilitation interventions include position management, touch therapy, feeding training, and passive movement training of the extremities. Parents were given rehabilitation guidance at

discharge, which included daily position management and home-based exercise training. At the follow-up after 3 months, the re-evaluation of Alberta infant motor assessment showed a percentile of 25–50%, suggesting an improvement in motor development.

17.2.1.2 Positioning

Preterm infants are recommended to maintain the position similar to that *in utero* with the aid of supports, without any restrictions on the free movement of their limbs, which is useful for improving posture and the collaborative development of flexor-extensor muscles. In addition, preterm infants demonstrate improved gas exchange capacity and chest wall synchronization in prone position; for preterm infants requiring supplemental oxygen, it is recommended to adopt the prone position to improve the oxygen saturation under continuous ECG monitoring.

17.2.1.3 Exercise Therapy

The commonly used exercise therapies include Bobath and Vojta treatment, which administer rehabilitation training based on the characteristics of nerve growth and development in children to reduce the muscle tone, improve the limb spasticity, stimulate the compensation of the body and brain, and contribute to establishing a normal movement pattern. Generally, the Bobath method allows for suppressing abnormal postures and promoting normal postural development and recovery through key points control and reflex inhibition [15]. The Vojta method is a treatment that stimulates specific parts of the body by compression and promotes normal movements and reflexes via repeated stimuli, thus inducing two locomotion patterns, namely reflex rolling and reflex crawling [16].

17.2.1.4 Feeding Training

Non-nutritive sucking (NNS) training, such as manual stimulation and pacifiers, can reduce the pain in preterm infants and accelerate the transition from tube feeding to oral feeding.

17.2.1.5 Touch Therapy

Early physical touch is beneficial to the physical development and cognitive function of preterm infants, which can increase gastrointestinal motility, reduce the occurrence of respiratory diseases, and promote parent-child relationship.

17.2.1.6 Hydrotherapy

Hydrotherapy is effective in improving gross motor function, relieving pain, and improving sleep quality, which has a positive effect on weight gain and feeding tolerance in high-risk infants.

17.2.1.7 Early Sensory Intervention

Daily administration of tactile, auditory, visual, and vestibular multisensory combined interventions (e.g., touch, listening to music, circulating light environment, and shaking the crib) can improve early neurodevelopment of the newborns and has a positive effect on the long-term neurological outcomes. Mothers are instructed to provide tactile stimulation to their newborns using kangaroo mother care, which can improve neonatal comfort, reduce pain stimulation, and promote the establishment of parent-child relationship [17–20].

17.2.1.8 Follow-Up Management and Home-Based Rehabilitation Education

Long-term, comprehensive, and standardized follow-up management is recommended for all infants at high risk. Monthly follow-up is recommended up to 6 months of age, every 2 months from >6 months to 1 year, every 3–6 months from >1 to 3 years, and every 12 months from >3 to 6 years. During the follow-up period, healthcare professionals should give prompt instructions to parents on the targeted rearing methods. The follow-up involves assessment of growth and development, neurological examinations, early screening scales, and diagnostic assessment scales related to movement, language, and cognition.

17.2.2 Ultra-Early Rehabilitation for Congenital Muscular Torticollis

17.2.2.1 Case

The patient, male, G1P1, was born prematurely by caesarean section at 33 weeks + 2 days of gestation due to "threatened preterm labor, premature rupture of fetal membrane for 10 days, fetal malposition, and intrauterine distress," with the birth weight of 1800 g and Apgar score of 9–9–9. The infant was given symptomatic and supportive treatments after birth, such as warming, monitoring, hydration, and Vitamin K1 for hemostasis. The mother had no threatened abortion or medication history during pregnancy, and there is no medical history of familial or metabolic diseases. Two weeks after birth, the patient was found to have a mass in the right neck, without fever, jaundice, or limb twitching. Physical examination showed normal muscle strength and muscle tone of the limbs. The right side of his face is smaller than the left side, with head tilting to the right and jaw turning to the left. A palpable mass was identified in the right sternocleidomastoid muscle, which was hard, well defined, and no tenderness (size: 2 cm * 2 cm). Neck flexion to the left and rotation to the right were restricted. Ultrasonography of the neck revealed a mass in the right inferior sternocleidomastoid muscle. According to the medical history, symptoms, signs, and auxiliary examinations, the right neck mass was primarily considered to be "right congenital muscular torticollis," and ultra-early rehabilitation was administered immediately. Rehabilitation interventions include massage, stretching therapy, and active neck movement training. Parents were given home-based rehabilitation guidance at discharge, including home environment change, position management, and home-based exercise training. After discharge, the patient was referred to outpatient rehabilitation. Following 3 months of treatment, the right neck mass subsided and the cervical range of motion improved. Ultrasonography of the neck showed a slightly thickened inferior segment of the right sternocleidomastoid muscle.

17.2.2.2 Ultra-Early Rehabilitation

Clinical evidence suggests that rehabilitation for congenital muscular torticollis (CMT) should be administered as early as possible. Normalization of neck mobility can be observed in 98% of the CMT patients within 1.5 months if the treatment is started before 1 month of age [21]. For infants at 1 month of age, the treatment duration should be extended to 6 months. Patients who initiate treatment after 6 months of age require at least 9–10 months of intervention. A longer delay in the start of intervention is associated with a lower possibility of achieving the normal cervical range of motion [22].

17.2.2.2.1 Passive Neck Movement

Passive neck movement is the most common intervention for CMT patients, among which the low-intensity, continuous, painless passive stretching is recommended to avoid minor damage to muscle tissue. Passive stretching treatment is effective in 90% of the CMT cases [23]. According to a recent study, the stretching treatment of 100 times per day are recommended for infants with CMT [24].

17.2.2.2.2 Active Neck Movement

Active exercise training for the neck and trunk is recommended during treatment and home-based care to promote bilateral symmetry in neutral position through exercising the weak muscles. For example, prone position activities are recommended. Placing a baby in prone position can promote neck muscle stretching on both sides, in addition to enhancing neck and spine extensor strength. Besides, guiding the baby to turn its head toward the affected side by visual and audible stimuli also contributes to enhancing cervical spine rotation. It is recommended to change the location of the crib, ensuring that the side with light or with more activity of the caregiver is on the affected side of the infant; therefore, the infant is more likely to turn its head toward the affected side.

17.2.2.2.3 Symmetrical Trunk Movement

Since up to 25% of the CMT infants may have transient motor asymmetry [25], developing

symmetric movements should be incorporated into the rehabilitation interventions and family health programs to prevent asymmetric movement patterns in prone, sitting, crawling, and walking postures.

17.2.2.2.4 Home-Based Rehabilitation Guidance

Parents or other caregivers are instructed to integrate the concepts of active neck movements and symmetrical trunk movements into their daily life, to improve the recovery rate, and to shorten the intervention duration. For example, the infant should keep its chin rotated toward the affected side of the neck during feeding. Playing in prone position should be encouraged when the baby is awake to develop symmetrical motor skills in this position [26].

17.2.2.2.5 Massage Therapy

Massage therapy can be administered to the sternocleidomastoid muscle on the affected side. Specifically, a slow and gentle massage is applied along the sternocleidomastoid muscle on the affected side including the mass from the top down to the bottom by repeated pushing, pinching, kneading, and grasping [27].

17.2.3 Ultra-Early Rehabilitation for Congenital Heart Disease

17.2.3.1 Case

The patient, female, G1P1, was delivered by caesarean section at 40 weeks of gestation due to premature rupture of fetal membrane, with the birth weight of 3360 g and Apgar score of 9–9–9. After delivery, the infant was given warming, mucus suction, and umbilical vein bolus injection of 5% GS for hemostasis. Fetal echocardiography at 23 weeks of gestation revealed possible cardiac malformation and severe pulmonary valve stenosis, and pulmonary atresia to be ruled out. Reexamination of fetal echocardiography at 35 weeks of gestation suggested pulmonary atresia and moderate tricuspid regurgitation. The mother had no history of gestational hypertension, gestational diabetes, pharmacotherapy, or exposure to radiation, cat or dog. Postnatal cardiac ultrasonography showed pulmonary valve stenosis (atresia), patent ductus arteriosus, patent foramen ovale, and moderate-to-severe tricuspid regurgitation. The infant received ultra-early rehabilitation after birth, including positioning, passive movement training, feeding training, and pulmonary rehabilitation. Balloon dilatation for pulmonary valve stenosis was performed 5 days after birth. Postoperative rehabilitation was administered when the vital signs became stable after surgery, including positioning, passive movement training, feeding training, pulmonary rehabilitation, and touch therapy. At discharge, the parents were given instructions on home-based exercise training rehabilitation. Reexamination at 3 months after surgery indicated that the growth and development of the patient were good, no remarkable findings in cardiac function was noted, and the Alberta infant motor assessment showed a percentile of 50–75%, suggesting a normal motor development.

17.2.3.2 Preoperative Rehabilitation

17.2.3.2.1 Positioning

It can reduce the stress of the infants, improve their self-quieting ability, store energy, address the infant's sensory needs of being wrapped, promote brain development and respiratory system development, alleviate the impact of gravity on the lungs, improve muscle strength, contribute to the establishment of correct movement patterns, and prevent abnormal muscle tone. The infant is maintained in flexion posture, with head and hands in midline position, neck in neutral position, shoulders extended forward, pelvis tilted backward, and hips bent to the midline. There should be adequate space for free movement within the boundary to increase tactile input and allow for different positioning.

17.2.3.2.2 Touch Therapy

It can promote the development of nervous system as well as normal growth and development of the infant, enhance immune response, reduce the stress response to stimuli, relieve tension and anxiety, and improve sleep and self-cognition. A full-body massage including the head,

chest, abdomen, limbs, palms, fingers, soles, toes, and back was given to the infant in a quiet and comfortable environment. Touch therapy should be performed 24 h after birth and 1 h after feeding, twice a day for 15 min each session.

17.2.3.2.3 Feeding Intervention

Eighty-four percent of the infants with severe congenital heart disease, especially those requiring early surgery, have feeding difficulties, characterized by poor sucking, salivation, choking, long feeding time, rapid breathing, and cyanosis. Infants with feeding difficulties should receive feeding intervention as early as possible, and breastfeeding is recommended. For those who cannot suck from common feeding bottles, medical feeding trainers or special feeding bottles with controlled milk flow and consistency can be used. Caregivers should pay attention to the feeding position and perform oral motor exercise, so as to improve the immunity and swallowing function of the infants.

17.2.3.2.4 Passive Joint Movement

It can improve the physical activity level, promote metabolism, enhance external stimulation, and prevent joint stiffness. Caregivers are required to move the large joints of the infants in combination with appropriate stretching exercises and chest expansion exercises, provided that the infants show no signs of resistance.

17.2.3.3 Postoperative Rehabilitation

17.2.3.3.1 Exercise Therapy

Exercise therapy can be initiated on limbs and trunk 5 days after surgery, which is effective in improving the gross motor function, physical activity, exercise tolerance, and quality of life for the infants. Low-intensity exercise can be performed for 10 min each session, increasing in increments of 3–5 min per week and up to 30 min over 6–8 weeks. Heart rate, respiration rate, oxygen saturation, subjective feeling, lip color, and subjective comfortableness will be monitored during this process, and the exercise should be stopped immediately in case of any discomfort.

17.2.3.3.2 Sensory Integration

Increasing the input of various sensory stimuli contributes to the development of sensory perception and cognitive function, along with an increased brain metabolism. Visual training: cards are used to improve the visual tracking of the infants; hearing stimulation: light music or mother's voice allows the infant to perceive the presence of sound and look for the sound source, thus improving the sensitivity and discrimination to the sound; tactile training: brush and tactile ball can be used to strengthen the perception of hands and feet, with the stimulus intensity increased progressively; vestibular function training: the infant maintains a prone or sitting position on the Bobath ball, followed by shaking the ball from side to side for vestibular stimulation; motor stimulation: appropriate vertical head-up, raising head in prone position, and passive turning movement.

17.2.3.4 Home-Based Rehabilitation

17.2.3.4.1 Developmental Training

It can promote the motor development of infants through a series of play activities based on the developmental milestones. Professional therapists should provide instructions on specific operating skills.

17.2.3.4.2 Physical Activity

Infants should actively participate in age-appropriate, growth-promoting, enjoyable and safe physical activities, with no limit to the duration of activity. Parents and other infants are also recommended to participate in physical activities.

17.3 Research Progress

17.3.1 Risk Factors for Common Diseases Originated *In Utero* and Requiring Pediatric Rehabilitation

17.3.1.1 Risk Factors for Cerebral Palsy

The main risk factors for cerebral palsy during pregnancy are premature delivery, intrauterine growth restriction, infection, and multiple preg-

nancy. Premature delivery is the most significant risk factor for cerebral palsy. Fetal brain development peaks after 28 weeks of gestation, and premature infants have immature organ function and unstable physiological environment, making them susceptible to complications such as ventricular hemorrhage and periventricular leukomalacia [28, 29]. The risk of cerebral palsy in fetuses born before 32 weeks of gestation can be dozens of times higher than that in full-term infants [30, 31]. Genetic defects and intrauterine factors are the primary causes of intrauterine growth restriction, and the latter include chorioamnionitis, preeclampsia, and placenta previa [32]. These prenatal factors increase the risk of thrombosis in fetal circulation, which may lead to perinatal stroke and postnatal hemiplegia, and also cause an overall developmental delay by impairing placental function [33]. The additional risk of cerebral palsy in multiple pregnancy comes mainly from the risk of premature birth and low birth weight associated with multiple pregnancy. Some pathogens can be transmitted from mother to fetus through the placental barrier, threatening the normal development of the fetus. Viral infections (e.g., cytomegalovirus, Zika virus, and rubella virus) during pregnancy may cause damage to the fetal brain and are associated with the development of cerebral palsy [34, 35]. Available evidence shows that prophylactic use of macrolide antibiotics during pregnancy is associated with an increased incidence of cerebral palsy in the offspring, which has not been observed in other classes of antibiotics [36].

Perinatal risk factors for cerebral palsy mainly involve breech delivery and the use of mechanical ventilation. Vaginal breech birth is associated with higher risk of delivery complications, such as shoulder dystocia, umbilical cord prolapse, and uterine inertia. Therefore, persistent breech presentation is regarded as a relative indication for caesarean section in clinical practice. Available studies have demonstrated that planned caesarean section fails to improve the long-term neurodevelopment of newborns delivered in breech presentation, suggesting that intrapartum injury may not be the dominant risk factor for cerebral palsy in these fetuses [37]. It is believed that in maternal factors such as fetal growth restriction, oligohydramnios, and gestational diabetes may affect the postnatal brain development of infants with breech presentation at term [38]. Mechanical ventilation in neonates, especially premature infants, may contribute to brain injury. This can be typically explained by the cerebral vasoconstriction resulting from carbohemia and hyperoxemia in the brain tissues of premature infants, leading to subsequent white matter ischemia and softening, which is directly associated with the development of cerebral palsy [32].

17.3.1.2 Risk Factors for Congenital Muscular Torticollis

First pregnancy, multiple pregnancy, reduced amniotic fluid volume, and uterine compression syndrome are identified as the most common prenatal risk factors for congenital muscular torticollis [39–42]. This is attributed to fetal head descent in late pregnancy or abnormal fetal position *in utero*, resulting in trauma to the sternocleidomastoid muscle. The most common perinatal risk factors for congenital muscular torticollis are prolonged labor, macrosomia, perinatal compartment syndrome, breech presentation, footling presentation, and dystocia delivery (forceps or vacuum-assisted vaginal delivery) [41, 43–45]. This is due to the venous occlusion caused by persistent medial cervical flexion and rotation *in utero* or trauma to the sternocleidomastoid muscle during delivery. Roemer [46] reviewed 44 children who had undergone surgical procedure for torticollis and found that 27 (60%) of these children were delivered by breech, footling, or podalic version. During the process of delivery, the fetuses are susceptible to birth injuries when the delivered legs and trunk are lifted upward to rotate their faces from the pelvis toward the vaginal orifice, or when their heads are clamped with the obstetric forceps. Therefore, Roemer concluded that the physical birth injury would contribute to the development of congenital torticollis, and the sternocleidomastoid mass was consistent with the hematoma secondary to muscle or fascial sheath tears [46]. If this conclusion holds true, it will provide supportive evidence for the theory of birth injury pathogenesis of con-

genital torticollis [46]. Ho et al. also noted that caesarean section, assisted breech delivery, and instrumental delivery were associated with higher risk for torticollis in the infants [47]. However, a recent study suggested that complicated birth or birth trauma was not the cause of congenital torticollis, since most children with congenital torticollis were delivered at term without birth trauma or moderate to severe asphyxia [48]. At present, a number of risk factors have been identified for congenital muscular torticollis, but the exact etiology remains controversial.

17.3.1.3 Risk Factors Affecting the Outcome of Congenital Heart Disease

Over the past few decades, novel surgical techniques along with the advances in cardiopulmonary bypass, intensive care, cardiac catheterization, noninvasive imaging, and medical therapy have significantly reduced the mortality in children and adolescents with complex congenital heart disease [49, 50]. However, survivors are at risk of developmental diseases caused by biological and environmental risk factors, and the prevalence and severity of developmental delay (DD) or other dysfunction increase with the increasing complexity of congenital heart disease. Recent studies have shown that children with complex congenital heart disease were diagnosed with dysfunction in intelligence, language, visual perception, attention, executive function, and motor skills [51–53]. There are many risk factors for DD or other dysfunction in children with congenital heart disease. Studies have shown that the main risk factors for congenital heart disease complicated with dysfunction include underlying syndromes, circulatory abnormalities specific to heart defects, the medical and surgical therapies required, and genetic or developmental disorders [54, 55]. More specifically, since the fetal and neonatal periods are critical for brain growth and maturation, myelination, and neural network development, for neonates with complex congenital heart disease who undergo cardiac surgery during this period, interventions such as drugs and operations that alter cerebral blood flow and impair cerebral oxygen delivery may affect subsequent brain development [56]. For children with cyanotic congenital heart disease who have not underwent surgery during neonatal or infant period, although they avoid the inherent risks associated with heart surgery, these children still have a higher risk of DD as their underlying congenital heart disease and the palliative operation or surgical repair they may have undergone later in childhood can lead to chronic hypoxemia [57]. Children with congenital heart disease and certain comorbidities have demonstrated an increased risk of DD. Congenital heart disease with preterm delivery (<37W), microcephaly, other congenital abnormalities (especially suspected genetic syndromes associated with developmental disorders), genetic polymorphisms, developmental delay in infancy, multiple cardiac interventions, history of mechanical support (ECMO or VAD), history of heart transplantation, history of cardiopulmonary resuscitation, prolonged postoperative hospitalization, and perioperative seizures are the risk factors for DD or dysfunction in children with congenital heart disease [54]. Additional risk factors include parent's guilt about the cause of birth defects, attachment problems, fear of the child's death, stress related to surgery, and parental competence in feeding issues. With the improved survival rate of children with complex congenital heart disease and the increased number of children and adults with congenital heart disease, intensive and ongoing surveillance and screening for the risk factors in children with congenital heart disease have become increasingly important.

17.3.2 Prevention and Early Rehabilitation for Common Diseases Originated *In Utero* and Requiring Pediatric Rehabilitation

17.3.2.1 Rehabilitation Prevention for Cerebral Palsy

Primary prevention is the focus for the prevention of cerebral palsy. According to the latest guidelines, the high-risk factors for cerebral palsy are related to multiple aspects involving pregnancy,

delivery, and postnatal period. Prenatal rehabilitation preventions include medical therapy such as magnesium sulfate, progesterone and antenatal steroid, dietary supplementation with zinc and creatine, music therapy, appropriate physical activity, and mild hypothermia treatment. Since premature delivery is the most important risk factor for cerebral palsy, interventions that provide protection against premature delivery during pregnancy can prevent the development of cerebral palsy. Continuous cardiotocography can be used for fetal assessment and intermittent auscultation during labor to evaluate intrauterine hypoxia, thus guiding the selection and adjustment for the mode of delivery. In addition, studies have shown that maintaining the body temperature at 33–34°C within 6 h after birth for a period of 48–72 h can effectively reduce the neonatal mortality and the incidence of cerebral palsy [58, 59]. Mild hypothermia is recommended for neonates with moderate to severe encephalopathy born after 36 weeks of gestation.

The secondary prevention for cerebral palsy includes early detection of abnormalities and early intervention or rehabilitation for children who have shown brain injuries or suspicious motor development patterns, aiming to minimize the functional impairments and prevent or treat the complications and secondary conditions in children with cerebral palsy. The diagnosis and severity assessment of cerebral palsy are more challenging during infancy. Studies have demonstrated that the MRI and Hammersmith Infant Neurological Examination are more sensitive in the prediction of cerebral palsy at this stage. Institution-based rehabilitation for dysfunctions associated with cerebral palsy should be initiated as early as possible, which can improve the short-term performance and long-term outcome of the children through altering their function, neuroplasticity, and biomechanics during the central nervous system development stage. Furthermore, as a permanent movement disorder, children with cerebral palsy require home-based rehabilitation for supportive care. For pediatric rehabilitation, the parent-therapist partnership has the potential to improve the treatment compliance and therefore the efficacy of rehabilitation.

For children with cerebral palsy who have presented with activity limitation or disability, rehabilitation prevention should be administered to minimize the impact of disability or incapacity. Virtual reality-assisted rehabilitation training enables children to perform different functional activities in sports games through providing visual and auditory information to the user. Actions such as lower limb weightlifting, jumping, and throwing can exercise the coordination, and balance ability of the children, squat, and hip extension may help to relieve muscle spasms, while walking and running simulations provide users with gravity shift training. Virtual reality technology enables children to perform exercise training in different scenarios, which improves patient's motivation, trunk stability, walking function, and aerobic exercise capacity. The lower-limb exoskeleton rehabilitation robot can judge the movement intention of the patients based on normal gait and directly control the movement of multiple joints, thus facilitating the patients to walk in a more normal posture and gait while reducing the load on their lower limbs. Robot-assisted gait training (RAGT) is designed to provide high-intensity repetitive exercise, which can increase the walking distance, endurance and stability of the children with spastic cerebral palsy, improve their functional status and the quality of life, reduce the use of walking aids, and ease the burden on caregivers. Adult patients with cerebral palsy may still have abnormal gait due to their abnormal muscle strength and muscle tone, limited joint range of motion, or poor balance and coordination, while RAGT can improve their walking function, activities of daily living, and social participation. Currently, there are few studies on the use of RAGT in children, and further research is deemed necessary to investigate the quantitative measures such as energy consumption, balance, and posture control in children with cerebral palsy.

17.3.2.2 Rehabilitation Prevention for Congenital Muscular Torticollis

Prevention for the high-risk factors such as fetal malposition *in utero* or birth injuries due to diffi-

cult labor is the focus of CMT prevention, and the specific measures include: pregnant women should sleep on a firm mattress after approximately 5 months of gestation with alternating positions between left and right sides and avoid sitting for long period, so as to prevent the fetus tilting its head to one side with subsequent ischemic changes in the sternocleidomastoid muscle on that side driven by inadequate blood supply to the muscle, which may eventually cause torticollis. Enhanced prenatal examinations should be performed before delivery and choose the proper delivery mode to avoid birth injury. In case of dystocia, caesarean section can be considered to avoid congenital torticollis due to birth injury in the infants; in case of fetal malposition noted during prenatal examination, aerobic exercise such as yoga can be performed under the direction of a physician to avoid congenital torticollis associated with fetal malposition during delivery. The efficacy of these interventions depends on patient age and disease severity, as younger age and lower severity are associated with superior treatment effect and shorter treatment course. Therefore, early diagnosis and treatment is the key to the prevention and treatment of congenital muscular torticollis.

17.3.2.3 Rehabilitation Prevention for Congenital Heart Disease

Prenatal care is the essential rehabilitation prevention for congenital heart disease. Medical conditions of the pregnant women may have an impact on their fetuses, especially rubella and influenza. If this occurs, pregnant women should seek medical attention immediately rather than taking medications without permission, which will increase the risk of congenital heart disease [60]. Unhealthy lifestyle of the pregnant women is associated with an increased incidence of congenital heart disease, which should be corrected whenever possible. Among them, smoking and alcohol consumption are identified as the risk factors for congenital heart disease. Thus, women should abstain from these behaviors as much as possible during preparation for pregnancy [61]. In addition, folic acid supplementation with daily intake of 400–800 mg for 3 months before pregnancy is effective in preventing fetal neural tube defects and congenital heart disease [62, 63].

Secondary rehabilitation prevention for congenital heart disease highlights the early diagnosis and treatment of the disease through prenatal screening, aiming to reduce the harmful effect of congenital heart disease and improve the treatment effect in these patients. Fetal echocardiography is one of the most important prenatal screenings for pregnant women at high risk. Color Doppler flow imaging provides precise information on the location and size of cardiac defects, which measures the pulmonary arterial pressure based on tricuspid regurgitation [64].

The Pediatric Cardiac Fitness Program includes multidisciplinary cardiac risk assessment, psychosocial assessment, exercise program consisting of aerobic exercise, strength training and stretching training, nutrient recommendation, and positive psychological intervention, which is a significant part of the tertiary rehabilitation prevention for congenital heart disease. The program aims to achieve optimal fitness by improving the motor ability and self-confidence of children with congenital heart disease in combination with promoting social participation, rather than focusing solely on disease control [65].

For children with common diseases originated *in utero* and requiring rehabilitation, the interventions should vary depending on the general condition of the disease and the clinical feature of dysfunction. Rehabilitation contributes to the development of normal posture as well as the motor, cognition, social, and physical development of the children. In addition, long-term follow-up on neuromotor development in this pediatric patient population is also of great importance.

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18 Multi-Disciplinary Team in In Utero Pediatrics and Case Management

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18.1 Multi-Disciplinary Team (MDT) in Fetal Medicine and in Utero Pediatrics

Since the 1980s, the introduction of a new concept (i.e., fetus as a patient) along with the development of medical technologies in the screening, diagnosis and treatment of birth defects have contributed to the advent of fetal medicine and in utero pediatrics. Unlike the era of perinatal medicine, maternal-fetal medicine considers the mother and fetus as individual subjects to provide personalized, comprehensive, one-stop healthcare management throughout their lifetime, rather than focuses exclusively on reducing the maternal and perinatal mortality [1].

18.1.1 MDT

MDT is a group of healthcare professionals from two or more relevant disciplines, including but not limited to surgery, internal medicine, radiotherapy, imaging, pathology, intervention, professional nursing, and psychotherapy. The members of MDT will attend clinical seminars regularly to propose systematic diagnosis and treatment plans, allowing for accurate diagnosis and proper treatment while avoiding overdiagnosis, overtreatment, misdiagnosis and mistreatment [2].

At present, the MDT concept has been widely recognized by the medical community, and has become an important diagnosis and treatment model in many large healthcare institutions for the treatment of tumors, diabetes, and other diseases. The collaborative diagnosis and treatment model involving multi-disciplinary experts can provide patients with standardized, comprehensive and individualized diagnosis and treatment plans, thus saving the medical resources and improving the efficiency and patient satisfaction of these plans.

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18.1.2 MDT in Fetal Medicine and In Utero Pediatrics

The diagnosis and treatment of fetal diseases usually involve multiple disciplines, including imaging, clinical genetics, neonatology, pediatric subspecialty, rehabilitation as well as adult internal medicine and surgery, in addition to obstetrics. Accurate diagnosis and health assessment should be provided by an experienced fetal medicine specialist to the affected fetus with focus on the fetal and maternal conditions. The well organized MDT would followed based on specific diseases for the outcome of the fetus. For families who decide to continue pregnancy, standardized, personalized and continuous evaluation and treatment plans should be established to reduce the perinatal mortality and morbidity of the affected fetuses and improve their long-term outcomes, while taking into account the safety of the pregnant women.

Since structural abnormalities are the most common fetal diseases, prenatal consultation, diagnosis, treatment and prognosis of such abnormalities as well as the involvement of pediatric subspecialists are of great importance. The optimal timing for diagnosis and treatment should be moved from "neonatal period" to "fetal period", which also contributes to the development of MDT diagnosis and treatment model in the in utero pediatric diseases.

MDTs of fetal medicine and in utero pediatric diseases should be considered as a whole, as they not only work in a cooperative and parallel manner, but also provide diagnosis and treatment in a sequential and successive pattern. Their integration and development would result in a secure and "closed loop" system for the diagnosis and treatment of maternal and fetal diseases [1].

18.1.3 Role and Function of In Utero Pediatrics in MDT

The specialists in the MDT of fetal medicine and in utero pediatrics are expected to undertake their own responsibilities while cooperate with each other.

Fetal abnormalities are initially diagnosed by the *experts in maternal-fetal medicine or fetal medicine*, who will provide comprehensive medical examinations involving imaging, genetics and etiology to the affected fetuses based on the reproductive history, family history and medical history of their parents, and make clinical diagnosis according to his/her medical judgment. These physicians are responsible for transferring the "bad news" directly to the families and providing reasonable advices, but they are not involved in the postnatal treatment and long-term follow-up of the infants. It is difficult to provide the precise information for the pregnant women and their families, such as whether the fetus should receive surgery or drug therapy after birth, the efficacy and cost of the treatment, as well as the short-term or long-term outcomes.

In utero pediatric subspecialists, who are primarily responsible for the postnatal diagnosis and treatment, can provide evidence-based medical advice to the families, making them the most appropriate healthcare professionals to participate in the multi-disciplinary consultation of fetal diseases. Although the "unborn patients" cannot receive physical examinations, MDT may contribute to the diagnosis and treatment in the following aspects:

1. According to the diagnostic criteria of postnatal diseases, to assist fetal medicine specialists for further interpretation of the results from prenatal imaging and genetic tests, thus increasing the accuracy of prenatal diagnosis.
2. Aid the fetal medicine specialists to accumulate their knowledge on prenatal prognostic indicators. For example, prenatal assessment of the condition of pulmonary artery in the tetralogy of Fallot, can help to provide more accurate judgement for prognosis, regarding the difficulties in postnatal treatment as well as the expected morbidity and mortality of the newborns.
3. Support fetal medicine specialists to evaluate the indications for intrauterine intervention (e.g., prenatal assessment for the severity of severe aortic/pulmonary stenosis or atresia, diaphragmatic hernia and meningomyelocele), balance the potential benefit and risk, and contribute to the management of intrauterine treatment depending on the type and ease of the treatment.

4. Help to create an integrated management plan covering antepartum, intrapartum and postpartum for the affected fetuses, and establish a green channel for in utero transfer, neonatal rescue/transfer as well as pediatric diagnosis and treatment according to the disease severity, so as to improve the survival rate and quality of life for the newborns.
5. Provide long-term follow-up and medical care to the children until their adulthood.

The involvement of in utero pediatric subspecialists in the MDT consultation may contribute to the consistency of prenatal and postnatal information and the communication with the family members, which may include:

1. Interpreting the significance of available results from imaging and genetic tests. The medical judgment of the fetal conditions based on the information derived from available test results will be communicated to the families. Specifically, pediatric subspecialists should provide advices on the severity of the disease, the indication, timing and plan of intrauterine or postnatal intervention, along with the complications and effectiveness of the intervention, provided that they have a clear understanding of the natural history and outcome of the prenatal disease.
2. Pediatric subspecialists play a critical role in the follow-up of long-term outcome of fetal diseases such as neurological development and vital organ functions after birth. Families should be provided with professional instructions to make them aware of the potential long-term outcome.
3. Provide personalized consultations in certain cases of fetal disease, such as multiple pregnancy, pregnancy complications or concomitant conditions that may increase the risk of iatrogenic preterm delivery, to ensure that the families are adequately informed about the risk and outcome of the fetal diseases, therefore making pregnancy-related decision based on thorough understanding of all available information. (The role of the pediatricians in the diagnosis and treatment of fetal diseases)

The number of prenatal MDT consultations with a pediatrician in attendance has shown a negative correlation with the anxiety level of patients [2]. According to the data from the Diagnosis and Treatment Center for In Utero Pediatric Diseases of Xinhua Hospital, compared with 2018, the mid-term induced abortions due to fetal factors has decreased by 57.43% in all induced abortions in 2021, which seems to be benefitting from the MDT consultation for in utero pediatrics established in 2019.

18.1.4 Model and Process of MDT in In Utero Pediatrics

The diagnosis and treatment of fetal abnormalities requires the participation of a multidisciplinary team, for the purposes of: (1) providing patients with optimal diagnosis and treatment plan according to the evidence based medicine (EBM) based supporting evidences; (2) more importantly, breaking the inter-hospital barriers, promoting the inter-disciplinary cooperation, and providing "one-stop" services in a patient-centered manner, thus the patients can avoid the frequent visits to multiple medical departments. Currently, there are two primary ways for in utero pediatric subspecialists to play a role in the multi-disciplinary diagnosis and treatment of fetal abnormalities:

18.1.4.1 MDT Led by Fetal Medicine Specialists and Supported by In Utero Pediatric Subspecialists

The MDT model led by fetal medicine specialists and oriented by fetal diseases develops a seamless connection with the subspecialty teams of in utero pediatrics (Fig. 18.1). As the leader of the multi-disciplinary consultation, fetal medicine specialists are "generalists" who have received subspecialty training in maternal-fetal medicine, and are required to have a variety of professional skills, such as prenatal imaging diagnosis, interventional prenatal diagnosis and genetic consultation, intrauterine intervention, management of high-risk pregnancy and maternal pregnancy

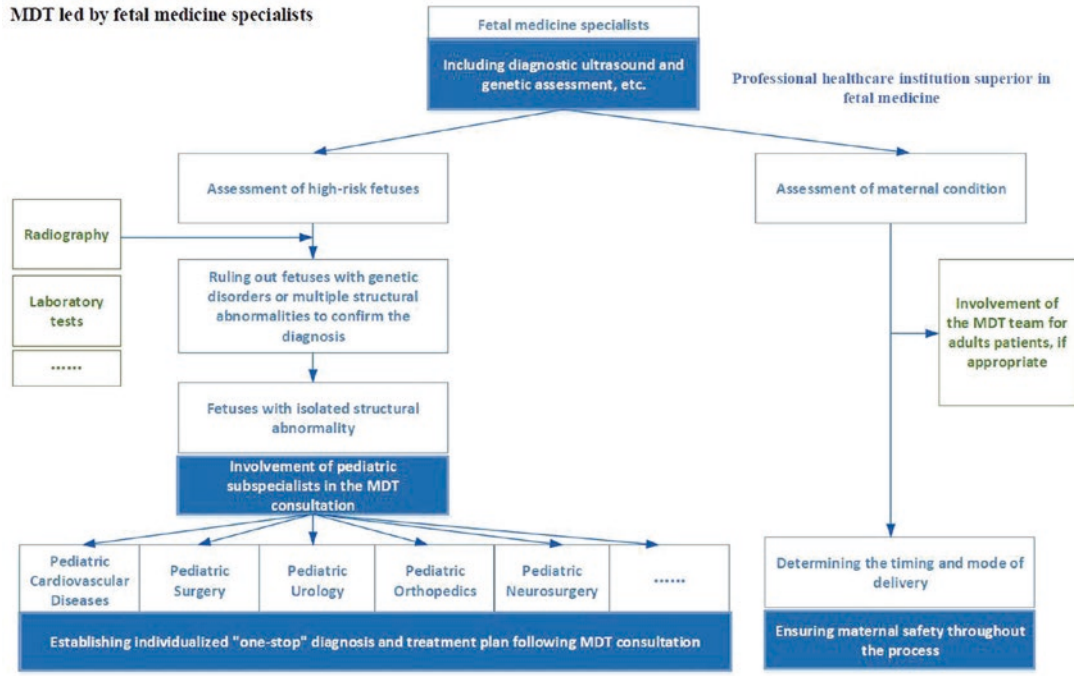

Fig. 18.1 MDT led by fetal medicine specialists (reproduced with permission from [1])

complications, as well as intrauterine monitoring and delivery of the affected fetuses.

In the multi-disciplinary consultation, the primary roles of fetal medicine specialists include: (a) to ensure the safety of the mother throughout the process. It should be noted that mother is considered as an important participant, decision-maker, recipient and even a patient (e.g., some pregnant women may have genetic diseases with milder phenotypes) in the intrauterine diagnosis and treatment of fetal diseases; therefore, it is necessary to evaluate fetal diseases in the context of the mother's condition. As the guardian for the safety of pregnant women, fetal medicine specialists should keep in mind that protecting the safety and health of the mother is the first priority of all the practices in intrauterine diagnosis and treatment [3]. (b) to provide a comprehensive assessment for high-risk fetuses, such as ruling out other concurrent structural or genetic abnormalities of the fetus by prenatal imaging and genetic diagnosis. Subsequently, according to the type of fetal abnormalities, a disease-oriented multi-disciplinary consultation should be initiated, with pediatric subspecialists in attendance to support of the prognostic assessment and the sequential treatment plan covering antepartum, intrapartum and postpartum periods. (c) to provide prenatal monitoring and perinatal management for the affected fetus. Fetal medicine specialists are expected to perform individualized prenatal monitoring and reasonable intrauterine interventions, and to determine the timing, mode and place of delivery based on the severity of fetal abnormalities.

The above-mentioned model requires the healthcare institution involved in the MDT to have a well-established maternal-fetal medicine or fetal medicine center as well as experienced staff in these areas; currently, this model has been adopted by the fetal medicine department of the First Maternity and Infant Hospital Affiliated to Tongji University. However, since the concept of fetal medicine has been introduced recently into China, only few healthcare institutions have set with well-established fetal medicine subspecialties. Moreover, given the absence of discipline construction standards and training systems,

most healthcare institutions are not qualified to organize such consultation to provide "one-stop" management for the fetuses and their mothers [1].

18.1.4.2 MDT Led by In Utero Pediatric Subspecialists and Supported by Fetal Medicine Specialists

With the widespread use of prenatal ultrasound screening, more structural abnormalities can be identified in prenatal period. Therefore, in utero pediatric subspecialists have become the attending physicians of the affected fetuses. In this case, the MDT consultation should be led by in utero pediatric subspecialists with experts in prenatal imaging, genetics and perinatal management in attendance, so as to perform comprehensive assessments for the fetuses and provide solutions regarding the delivery of the pregnant women (Fig. 18.2).

The advantage of this model is that the in utero pediatric subspecialists, as the attending physician of the fetus after birth, can participate in the whole process of prenatal and postnatal management. However, this model has the drawback, that is the pediatric subspecialists only focus primarily on the diseases in their own specialties, which might neglect the maternal conditions while the fetus is being managed. Thus, the assistance and involvement of fetal medicine specialists are necessary to ensure the adequate assessment of maternal safety at the same time.

18.1.5 Form of MDT in In Utero Pediatrics

MDT consultations can be implemented in flexible and diverse forms, including offline "face-to-face" communications or online video consultations via the Internet. However, most MDT consultations are carried out by a professional core team with fixed members on a regular basis. The purpose of MDT consultation, regardless of the formats, is to make the family fully informed of the diagnosis and treatment plan along with the outcome of the fetal disease in

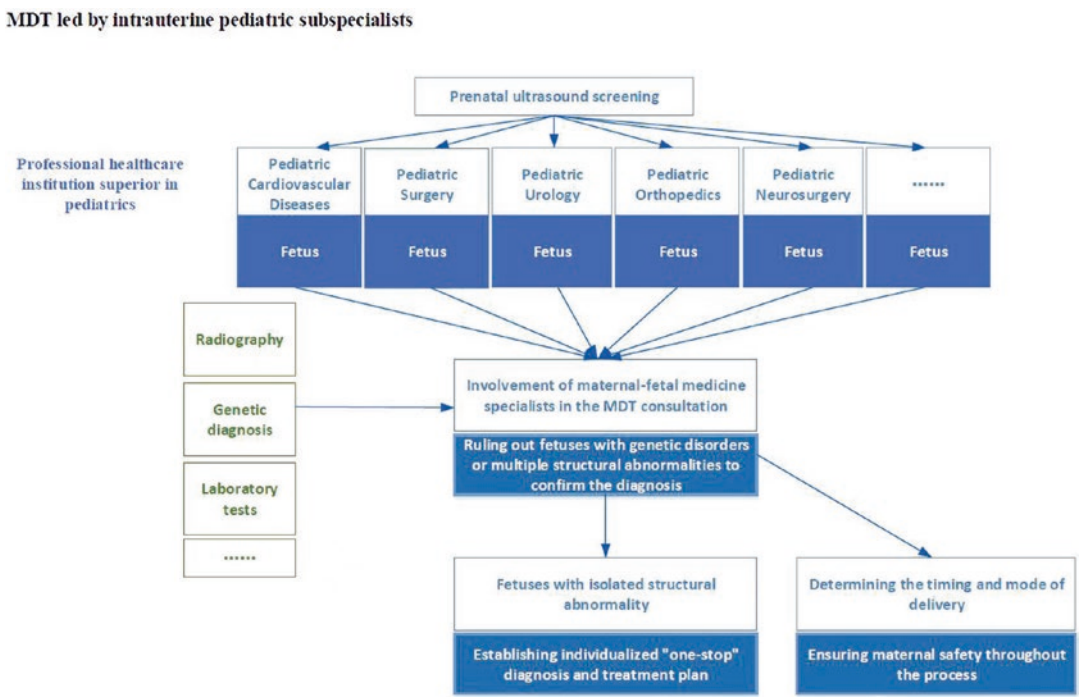

Fig. 18.2 MDT led by in utero pediatric subspecialists (reproduced with permission from [1])

prenatal and postnatal periods through effective communication, and facilitate the pregnancy-related decision and perinatal management. The frequency of MDT consultation should also be determined on a case-by-case basis, and multiple consultations may be feasible if changes have been noted in fetal disease.

The clinicians involved in MDT consultation include neonatologists, radiologists, geneticists and assistant physicians, in addition to in utero pediatric subspecialists. The assistant physician in MDT consultation is responsible for collecting the medical history, preparing the consultation records, and compiling and archiving the files from individual specialties. The geneticists and radiologists with relevant expertise can facilitate the accurate diagnosis of complex fetal diseases and provide guidance on disease management [4]. The neonatologist and the nursing team also play a crucial role in the successful delivery of the fetus, the medical management during the neonatal period, and the referrals to the corresponding pediatric subspecialties.

18.2 Case Management Model in the Diagnosis and Treatment of In Utero Pediatric Diseases

The diagnosis and treatment of a fetal patient is quite challenging, since the maternal safety should also be taken into consideration rather than focusing only on the fetal disease itself. This involves not only the treatment of relevant diseases, but also the long-term pregnancy management as well as the growth and development through infancy, which requires the assistance from a comprehensive medical service support system. The introduction of case management achieves full management of diagnosis and treatment for the pregnant women and their fetuses, as well as providing psychological supports to the family members of the patient. Case management allows for the family to receive continuous, comprehensive, efficient, high-quality and considerate services. It plays a special and important role in the standardized management of intrauterine pediatric diseases and the improvement of medical management services. The case manager in in utero pediatrics is also known as the disease manager of the family.

18.2.1 Case Management

The concept of case management (CM) was proposed in the mid-nineteenth century. At that time, case manager was referred to as the plan coordinator who provided medical care to the vulnerable groups such as the poor, the sick or the elderly. Since 1980, the US government has undertaken a reform of the national health system to control the sharp increase of medical costs and improve the medical resource utilization. In this context, case management and clinical pathway were implemented in public health system, aiming to reduce the length of hospital stay and the medical costs while maintaining the quality of medical service. In 1985, hospitals in Taiwan introduced the case management process to the medical care of diabetic patients, and demonstrated promising results. At present, case management is receiving increasing awareness in China, which has been applied to the diagnosis and treatment of tumors as well as the management of chronic diseases. It has shown favorable results.

Case management takes a case-centered approach to provide integrated and comprehensive medical services, which focuses on the cooperation among multiple medical teams. It has apparent advantages in improving the management efficiency and reducing the waste of medical resources [5].

As multiple disciplines are involved in the diagnosis and treatment of individual case, case managers should coordinate and integrate the opinions of the healthcare professionals from different specialties to ensure that the patients receive integrated and continuous medical services at appropriate time and place, allowing for a balance between cost-effectiveness and quality. Case management plays an important role in the standardization of hospital management, improvement of medical service process, integration of community resources, patient and disease

management, and continuity of the medical care, thus achieving a triple-win situation for patients, medical team and nursing team. Case management model is mainly developed for patients with specific diseases. The role of therapeutic alliance (TA) is similar to a "disease manager" for patients; as for the physicians, TA acts as a "secretary to patients with specific diseases".

18.2.2 Case Management Team in In Utero Pediatrics

The case management team in in utero pediatrics consists of the following members: MDT physicians, case managers, specialists, nurses, rehabilitation therapists, and social workers. These members take a holistic approach to provide seamless, comprehensive, integrated healthcare management services to the mothers, fetuses and their families. Among them, the case manager will participate in and manage the diagnosis, treatment and follow-up of the patient throughout the process.

1. Case manager/case management leader: the case manager of in utero pediatrics is the core personnel in the case management team who is responsible for developing specific plans for case management and healthcare services, aiming to provide optimal care to mothers and fetuses/newborns through integration, coordination and optimization of the process. A case manager must have extensive experience as a senior professional nurse in obstetrics or pediatrics, or have served as head nurse in these departments.
2. Multi-disciplinary support team: as a fragment-integrated care model, case management does not rely exclusively on the case manager; on the contrary, multi-disciplinary and cross-institutional collaboration led by the case manager has become the mainstream trend, which involves hospitals at all levels, communities and other institutions. The case management team can be comprised of the case managers, specialists, nurses, healthcare technicians, pharmacists, dietitians, psychologists, rehabilitation therapists, community nurses and social workers. Each member in the team will take his/her own responsibility according to the overall arrangement by the case manager. The case manager who acts as the organizer, director and communicator of the team is expected to conduct effective coordination, allowing for the complete and ongoing implementation of the case management [6]. For fetuses with poor outcomes, symptomatic treatments should be administered; while the familial physical, psychological and social problems should be addressed carefully. The medical care and guidance for the future pregnancy would be provided, which is an important part of maternal-fetal comprehensive care.
3. Responsibilities of the case manager: to coordinate the communication between the family and the hospital around the treatment and nursing services, make an appointment with the physicians, arrange for hospital admission, contact the patient for follow-up, and provide additional medical services associated with the diagnosis and treatment. More specifically, the responsibilities include: (a) to coordinate relevant medical resources for MDT consultation as per the diagnosis and treatment plans; (b) to communicate with the nursing teams of the outpatient and ward departments to arrange for a hospital visit on behalf of the patient; (c) to answer the questions raised by the patient during diagnosis and treatment and provide reasonable solutions; (d) to follow the patient after discharge, maintain a record for the growth and development of the child, and prompt the family to practice health-related self-management until the end of diagnosis and treatment.
4. Case management team of in utero pediatrics: at least one head nurse or a nurse with experience as head nurse should be staffed, who would provide overall tracking of patient management, such as coordinating and arranging laboratory tests, participating in the disease assessments, addressing the needs of the patient, organizing multi-disciplinary consultation, as well as monitoring and managing

the implementation of the treatment plan in accordance with the clinical pathway of the disease. The case manager is also responsible for the medical services after discharge, including patient follow-up and supervision of the return visits. There should also be a customer service team in place to provide information regarding the facilities around the hospital, including the catering, accommodation and transportation, and is responsible for the communication and interview with patients and their families according to the workflow. Understanding the needs of medical team, nursing team and patients in a timely manner, enables the case management team to establish a communication platform and a maintenance mechanism for providing "one-stop" services to patients and their families.

18.2.3 Process of Case Management in In Utero Pediatrics

The case management team focuses on the needs throughout the maternal-fetal or maternal-infant diagnosis and treatment, and provides appropriate, timely and adequate support for communication. The communication channels would be established for follow-up. Via Internet and telephone, the team can contact the patients both in normal and emergency conditions, and then address the needs of the patients and their families in a timely manner (Fig. 18.3).

The implementation of case management in in utero pediatrics mainly involves three steps, namely "case registration, case management and case closure", while each step involves five key aspects, including "assessment, plan, implementation, evaluation and feedback".

1. Case Registration Process:
   (a) Explaining the management plan: explain the purpose of the plan to the family before registration along with the routine outpatient procedures that require their cooperation;
   (b) Establishing case management file: enter the information and establish contacts with the family;
   (c) Making an initial assessment: assess the education level, economic condition, family support system, awareness of the diseases, previous healthcare compliance, diet and exercise habits, and nutritional status of the family;
   (d) Developing plan: develop health management plan following discussion with the family, especially the pregnant women, such as self-monitoring of blood glucose, diet prescription, and exercise prescription;
   (e) Making an appointment for an outpatient visit or hospitalization: explain the precautions for the next examination and provide support for appointment service.
2. Operation Process of Case Management for Subsequent Visits
   (a) Remind the patients of the time for outpatient or inpatient treatment;
   (b) Re-assessment: assess the physical and mental state of the mother, fetus and the family;
   (c) Physician-nurse communication: coordinate and communicate with physicians and nurses promptly for the problems in individual cases to share the information;
   (d) Providing consultation and question answering services on an ongoing basis: provide health education in a timely manner regarding the problems involved in individual cases and give further explanation for their consultations;
   (e) Records Registration.
3. Operation Procedures for Case Management:
   (a) Establishing a relationship: establish a trusting relationship with the patient;
   (b) Assessment: assess the disease awareness (e.g., current medical history, prior medical history, high risk factors) of the patient, economic condition (medical insurance, family support system, finance) of the family, psychological needs, resource problems and other personal needs from the views of patients,

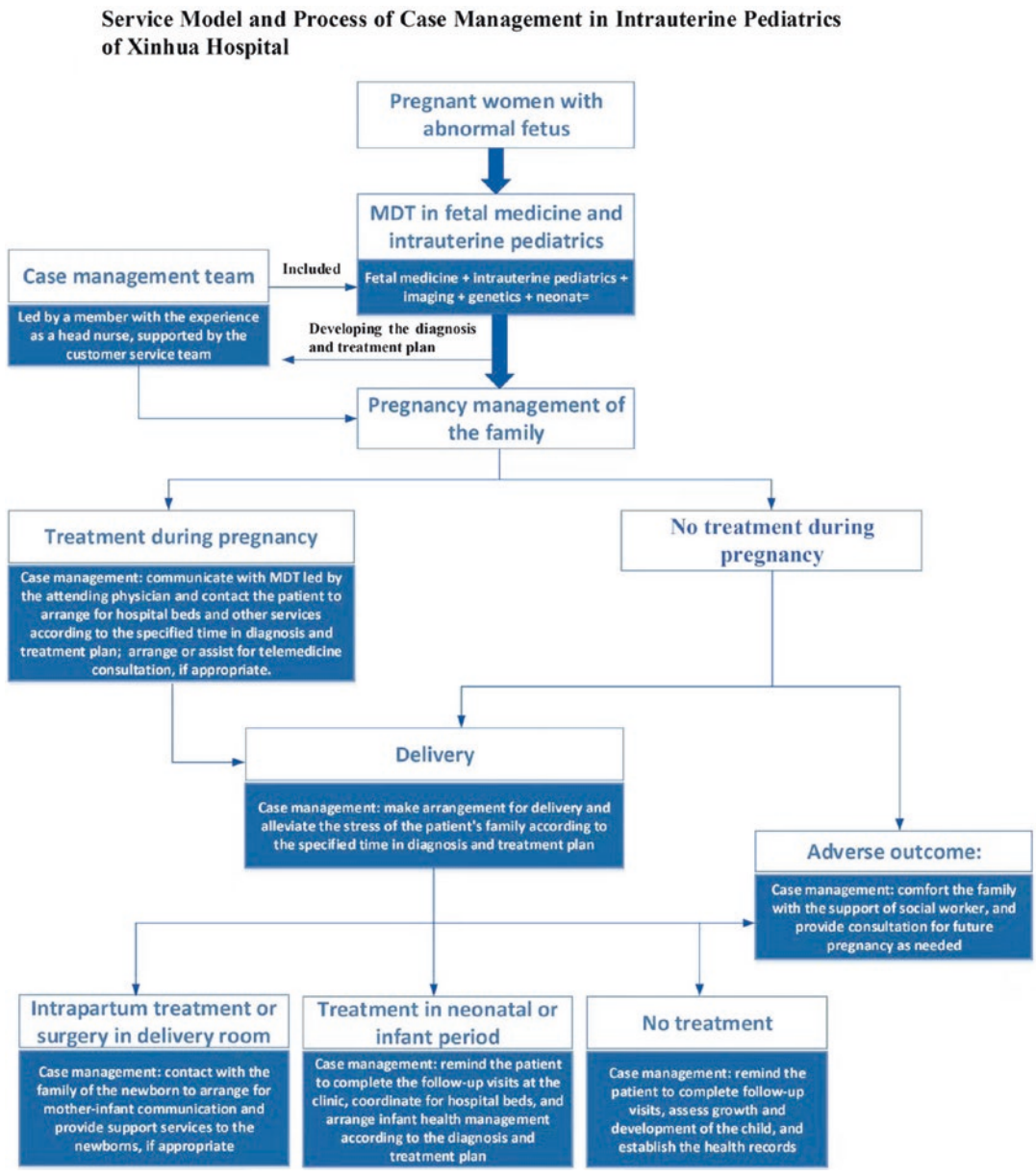

Fig. 18.3 Service Model and Process of Case Management in Intrauterine Pediatrics of Xinhua Hospital

family members and healthcare providers.

(c) Planning: the patient and his/her primary caregiver should also contribute to the development of an individualized plan. Case manager will make decisions on the accessibility, feasibility, appropriateness and priority of the patient's needs, with full respect for the patient's own wishes.

(d) Resources access: integrate the resources within the hospital, including the social workers, as well as the information about the hospital and its surrounding facilities for the patients' conveniences.

(e) Integration and supervision: according to the monitoring indicators and integrated data, the team can supervise and monitor the plan progress toward completion. The case manager should analyze and evaluate each patient, identify the special circumstances that occur during the implementation of the plan and the reasons for failure to fulfill the task as planned, followed by reanalysis, revisions and re-evaluations.

(f) Case closure and feedback: terminate the relationship with the patient, make summary and evaluation, provide feedback on the medical management mechanism, and optimize the process; provide collaborative supports for long-term care of patients and primary caregivers, and establish long-term operation mechanism.

18.3 Other Supports for MDT in In Utero Pediatrics and Case Management

1. At present, there is no industrial standard for the charging and quality control of MDT consultation in China. Therefore, a unified standard should be established to ensure the sound and sustainable development of the MDT consultation model. Given the impact of COVID-19 pandemic in recent years, it is quite challenging to conduct inter-hospital consultation and cross-region referral. Fortunately, the introduction of "5G+ artificial intelligence" cloud service platform for the diagnosis and treatment of in utero pediatric diseases, which has the potential to achieve the ultimate goal of MDT consultation. Moreover, the techniques might bring maximum benefit to the mother and fetus with the optimal medical resources at the minimum costs, so as to avoid the waste of medical resources and reduce patients' traveling and anxiety arousing by the repeated hospital visits.
2. Establish a Customer Relationship Management (CRM) system to support the case manager's work. The user-friendly CRM system allows to manage cases in batch, which brings convenience for the long-term follow-up. Meanwhile, the CRM system facilitates the establishment of individual health records for the long-term health management, involving the postnatal growth and development, and thus can contribute to conduct the clinical studies.
3. The development of fetal disease specialty has received increasing awareness in recent years. To establish a medical service model based on the needs of the fetuses and their families, can promote the continuous development of this discipline, and also helps the further interdisciplinary integration. Breaking down the boundaries between disciplines and hospitals is a must for the development of fetal medicine and in utero pediatrics, while more EBM based experience should be accumulated. Meanwhile, the core concept of "fetus as a patient" should be practiced in clinical work, and direct to a "one-stop" closed-loop for fetal diagnosis and treatment, which would develop a high-quality diagnosis and treatment system for the fetal diseases in China. These efforts would bring "new hopes" for the fetus and its family, and finally improve the postnatal life after birth.

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