1 Primary Prevention of Genetic Disorders and Place of Preimplantation Diagnosis

Gene therapy is still not a realistic option for genetic disorders, so prevention remains the main approach. Preventive measures may be applied at the community level by avoiding new mutations, protection from all possible environmental hazards, predictive testing for genetic and complex disorders, and prospective screening for common genetic disorders specific for each ethnic group. The optimal time for offering these preventive measures is the preconception or preimplantation stage (Fig. 1.1), as any detection afterward will involve the decision either to keep the pregnancy with the long-term social, familial, and financial consequences of a seriously affected child, or to terminate the planned and wanted pregnancy.

So the strategies for prevention range from preventing environmental hazards and vitamin supplementation programs to pre-pregnancy or prenatal diagnosis. The example of highly effective population-based preventive measures realized at the preconception stage has been prevention of neural-tube defects (NTD) and some other congenital abnormalities by folic acid or folic acid-containing multivitamins. On the other hand, preconception and preimplantation genetic diagnosis (PGD) has been established as a realistic option for primary prevention of genetic disorders as described in detail in this book.

The most relevant approaches for primary prevention of congenital disorders presently include (1) avoidance of new mutations through environmental programs, (2) reduction of pregnancy at advanced ages through community education and family planning, (3) periconceptional folic acid supplementation or multivitamin fortification of basic foodstuffs, (4) rubella vaccination, (5) avoidance of alcohol consumption and smoking during pregnancy, (6) prenatal diagnosis, and (7) pre-pregnancy (preimplantation) diagnosis. As seen in Fig. 1.1, the available actions could avoid congenital disorders of environmental origin, controllable by manipulating the environment, which can often be done by public health measures, and constitutional-remaining even when environmental causes are controlled, requiring more sophisticated approaches for detecting and managing risk. In addition, some conditions, such as NTD, have both

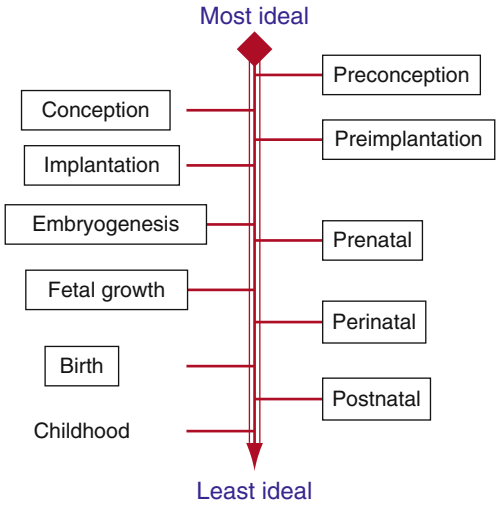

Fig. 1.1 Options offered for couples at genetic risk for diagnosis and prevention. Stages of development are shown on the *left*, and the points for application of preventive measure on the *right*

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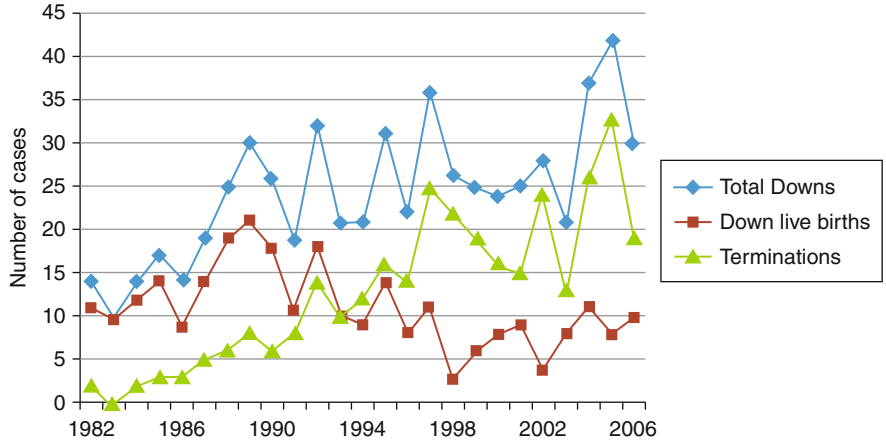

Fig. 1.2 Prevention of Down syndrome, 1979–1999, in Strasbourg, France, by Prenatal Diagnosis and termination of pregnancy [1]. The data are representative for all industrialized countries where population screening and prenatal diagnosis are operational for more than two decades, resulting in tremendous reduction of Down syndrome prevalence (*orange curve*) compared to the expected prevalence in the absence of preventive measures (*blew curve*), which is achieved by the increase in the number of pregnancy terminations (*green curve*)

genetic and environmental components, so the actions are first addressed to environmental causes and finding the key components to modify the realization of the congenital disorder, as described below. It is understood that most populations only get round to addressing the constitutional disorders when they have largely controlled environmental causes. The decision to adopt any of the available preventive programs depends on differences in health services development, ethnic distribution of congenital disease, and the local attitudes to genetic screening and termination of pregnancy. For example, induced abortions are still not permissible in many countries on religious grounds. On the other hand, the number of countries permitting prenatal diagnosis and termination of pregnancies for medical indications is steadily increasing.

The impact of community-based preventive approaches is obvious from the Down syndrome prevention program in the majority of industrialized countries of Europe and the USA. The programs are based on prenatal maternal serum screening for all pregnant women to detect pregnancies at increased risk, followed by the offer of definitive diagnosis and selective pregnancy termination, plus prenatal diagnosis offered to all women of advanced maternal age, which has resulted in the reduction of the birth prevalence of Down syndrome by 50% or more [1] in some regions (Fig. 1.2). However, this reduction is proportional to the number of pregnancy terminations, which changed annually with the success of the prevention program, as seen from Fig. 1.3. In some countries the effect of such programs is still growing (www.eurocat-network.eu), while in others it seems to be reaching a plateau (Figs. 1.4 and 1.5), reflecting differences in the development of the service as well as social and religious differences. However, the fact that this reduction is achieved through pregnancy terminations proportional to the number of Down syndrome prevented (Fig. 1.3) is a cause for serious concern [1, 2]. This is particularly relevant for high-income countries where women use family planning to postpone childbearing, leading to a rebound in the proportion of older mothers.

At present the most powerful approach for avoiding congenital disorders at the community level involves the expanded use of fetal ultrasound, which has improved the detection rate of affected pregnancies, and enables the choice of whether to terminate the pregnancy or to plan early treatment for the affected child. The effects may be evaluated by the community-based birth defect-monitoring systems available in an increasing number of countries. As termination is usually

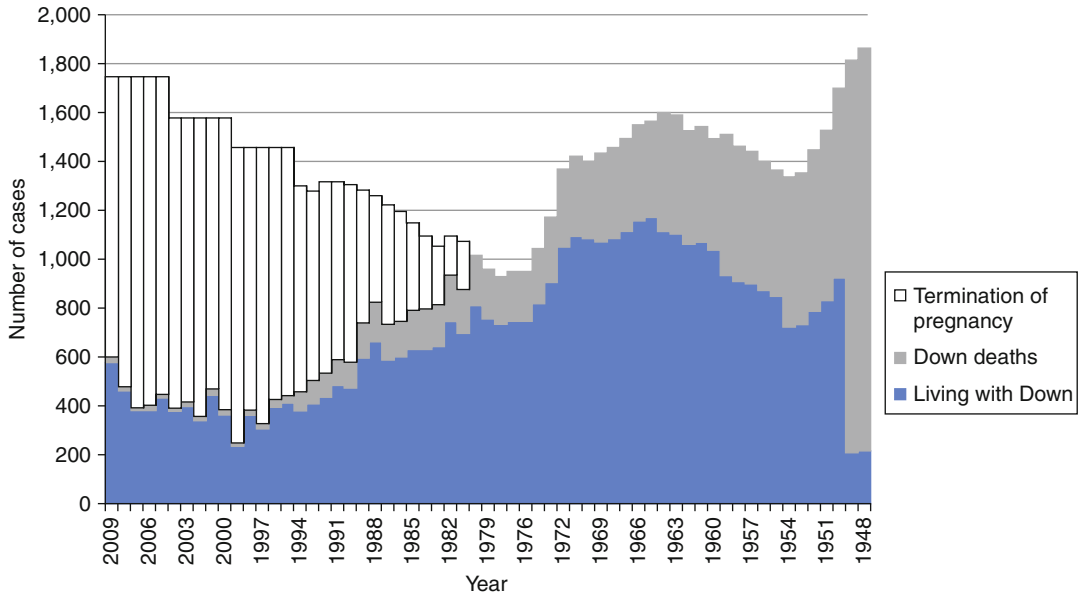

Fig. 1.3 Outcomes of pregnancies with Down syndrome fetus in France since 1948

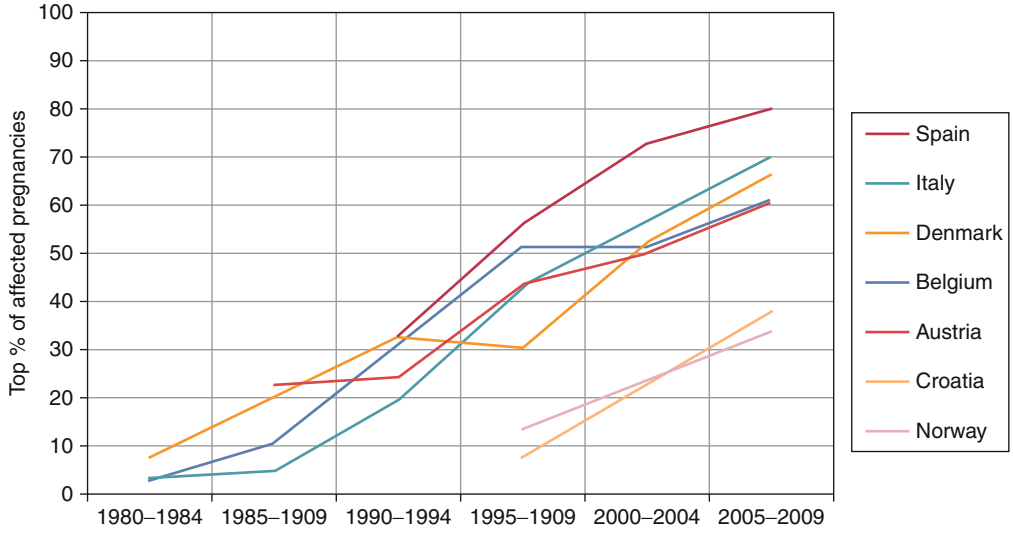

Fig. 1.4 Proportion of Down syndrome pregnancy termination following Prenatal Diagnosis in European countries (EUROCAT)

requested only for the most severe disorders, fetal anomaly scanning selectively reduces the proportion of children born with lethal or incurable conditions, including NTD (Fig. 1.6).

Despite the need for integrating programs, to combine all feasible approaches, maximizing the benefits and minimizing the negative aspects of preventive programs for congenital malformations, the emphasis is on the primary preventive measures, such as the pre-pregnancy vitamin supplementation, which has been shown to be one of the most efficient approaches for primary prevention of congenital disorders [3–12]. The effectiveness of this approach has been amply demonstrated for

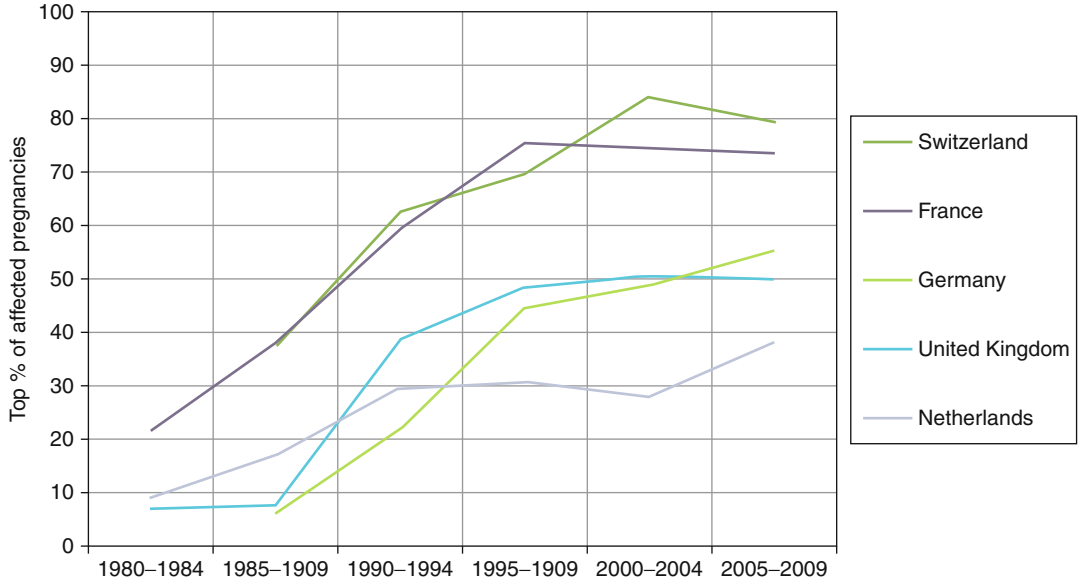

Fig. 1.5 Proportion of Down syndrome pregnancy termination following Prenatal Diagnosis in European countries – different tendency (EUROCAT)

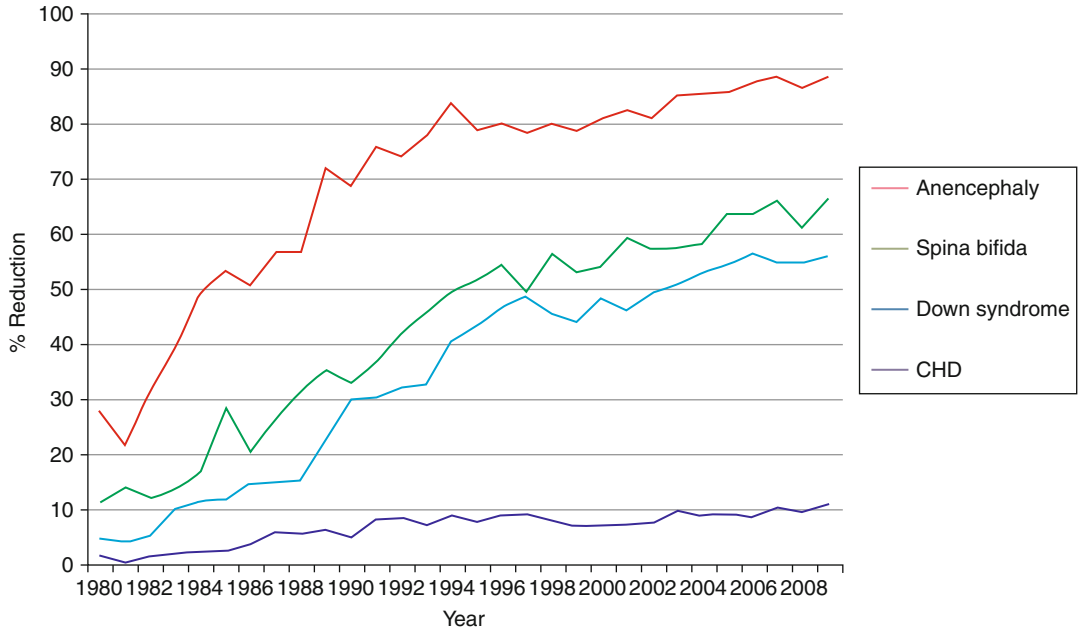

Fig. 1.6 Percent reduction of birth prevalence of some major congenital malformations by routine fetal anomaly scanning and termination of pregnancy. *CHD* congenital heart disease

NTD, leading to adoption of folic acid food fortification in many parts of the world (flour fortification initiative – FFI, www.Sph.emory.edu/wheatflour). In some populations, the application of this approach resulted in the overall reduction of the prevalence of congenital disorders by as much as half (from 40.6 per 1,000 to 20.6 per 1,000). This included the reduction of the prevalence of NTD and some other congenital abnormalities (see below).

It is obvious at present that routine fetal anomaly scanning is a very powerful intervention for detecting and avoiding congenital malformations. The downside is the number of terminations of wanted pregnancies: this could and should be minimized by multivitamin supplementation and folic acid food fortification. The benefit here is the replacement of terminations, stillbirths, and affected live births by wanted unaffected children. PGD has no part to play in this area, but is particularly useful for inherited (single-gene) disorders, as this is the area where prevention has so far had the least impact. This is mainly because risk detection is retrospective – after the birth of an affected child – and requires specialized diagnostic facilities and genetic counseling skills. Many parents with a child having a severe single-gene disorder use prenatal diagnosis or restrict further reproduction: this can have a significant effect when a large final family size is the population norm, but it can reduce affected birth prevalence by less than 10% when family size is small – increasingly the global norm. The exceptions are the hemoglobin disorders and Tay Sachs disease, where carriers can be diagnosed prospectively by population screening and conventional laboratory tests. In both cases major reductions in affected birth prevalence have been recorded.

The particularly keen interest in PGD for single-gene disorders is not only because of the high and recurrent risk of carrier couples. PGD offers the most interesting challenge for single-gene disorders also because:

They are the most intractable group of congenital disorders – i.e., a relatively low proportion yield to available therapeutic interventions.

At-risk couples face exceptionally high risks in each pregnancy.

Present approaches have been relatively ineffective in reducing affected birth prevalences (except for thalassemia and Tay–Sachs, and possibly cystic fibrosis in some restricted areas).

They require innovative methods for risk detection (not only population screening but also, e.g., extended family studies in populations where consanguineous marriage is common).

They require continual refinements of DNA-based diagnosis.

They cover a wide range of severity and age at onset: this makes for very difficult decision-making for people at risk for later-onset or ostensibly less severe disorders (e.g., family cancer syndromes): many who would find termination of pregnancy hard to accept and would gladly go for PGD if available and reasonably reliable.

So, if one looks to the future, PGD could provide the most important contribution to enabling people to make use of increasing genetic knowledge to preserve the health of their families.

The fact that the diet supplementation with folic acid or folic acid-containing multivitamins may substantially reduce the population prevalence of four groups of congenital disorders, including neural tube defects, cardiovascular, urinary tract, and limb deficiencies, represents an important breakthrough in prevention of congenital disorders. Although more data are needed to further confirm this and investigate the possibility of reduction of other birth defects, such as pyloric stenosis, the impact of folic acid on the prevalence of congenital disorders is in agreement with the fact that (1) mothers who give birth to a child with neural tube defects have mildly elevated blood and amniotic fluid levels of homocysteine; (2) hyperhomocysteinemia and/or lack of methionine can induce neural tube defects in animal experiments; (3) low maternal folate status appears to be a risk factor for neural tube defects; (4) vitamins of B group including folate/folic acid are important in homocysteine metabolism; and (5) vitamins B_6, B_{11}, and B_{12} are able to reduce hyperhomocysteinemia. It is known that homocysteine accumulates if conversion to methionine is slowed because of the shortage of folate or vitamin B12 or both, and a raised plasma homocysteine suggests suboptimal nucleic acid and amino acid metabolism. It also has direct harmful effects – e.g., it increases risk of cardiovascular disease through thickening the lining of blood vessels, and may also increase the risk of certain cancers and dementia [13–16].

It is also known that reactions catalyzed by tetrahydrofolate are crucial for cell growth and multiplication, making rapidly dividing cells particularly vulnerable to deficiency of either folate or vitamin B12. This may affect the embryo's morphogenetic movements, which

depend on focal rapid cell multiplication, increasing the risk of congenital malformation. Folate deficiency may be caused also by genetic factors, leading to rare variants of several enzymes involved in one-carbon transfer, which causes problems ranging from greatly increased plasma homocysteine levels, with very early onset cardiovascular disease, to developmental delay and neurological problems, with or without megaloblastic anemia. Variants of lesser effect in the same enzymes may contribute to genetic predisposition to cardiovascular disease and neural tube defects. For example, about 10% of many populations are homozygous for a common polymorphism of the enzyme methyl tetrahydrofolate reductase (MTHFR) (valine replaces alanine at codon 677). In homozygotes this reduces the enzyme activity by 50–70%, slows the regeneration of methionine leading to the raised plasma homocysteine, and increases risk of cardiovascular disease. So fetuses homozygous for the variant are at increased risk of neural tube defects. Variants of other enzymes, and other vitamins, may also influence homocysteine levels and the risk of neural tube defects. Accordingly, folic acid supplementation increases the supply of tetrahydrofolate, accelerates most folate-dependent metabolic reactions, and reduces plasma homocysteine levels [17–20].

The available data suggest that the cause of neural tube defects is not a primary lack of folate in the diet but an inborn error of vitamin B_{11} and/or homocysteine metabolism. An interaction between genetic predisposition and nutrition, therefore, may have a causal role in the development of neural tube defects, i.e., a dietary deficiency may trigger the genetic predisposition. The genetic-nutrient interaction through genetic predisposition and low folate status is associated with a greater risk for neural tube defects than either variable alone.

There are different options for ensuring appropriate multivitamin/vitamin B_{11} consumption in women of childbearing age, with each of them having their disadvantages and social feasibilities. The optimal daily intake of folate in the periconception period is 0.66 mg, whereas the usual intake per day is only about 0.18 mg. It seems also impossible to achieve a 3.7-fold increase in consumption through food intake alone, since this would require about 15 daily servings of broccoli or brussels sprouts. In addition, a large increase in the consumption of extra folate from natural foods is relatively ineffective at increasing folate levels. So the consumption of folate-rich foods may not be the most appropriate way to prevent the development of neural tube defects and other congenital abnormalities requiring the appropriate strategy for folic acid supplementation.

Over 90% of pregnancies where the fetus has a neural tube defect occur among women without any previous indication of increased risk. Identifiable risk groups include women with a prior affected pregnancy, who have a 3–4% recurrence risk, and women who are heterozygous for the MTHFR mutation. However, these groups account for only a small proportion of affected pregnancies. Trials of the effect of folic acid supplementation on the prevalence of neural tube defects provide conclusive scientific evidence for its preventive effect [3–12], suggesting that (1) dietary supplementation with folic acid or with multivitamin preparations containing folic acid, before and during early pregnancy (periconceptional supplementation), markedly reduces both the first occurrence of neural tube defects, and recurrence among women with a previously affected pregnancy, who have an increased (3–4%) risk; (2) the effect is greatest in areas with a high baseline prevalence of neural tube defects, but also applies in lower prevalence areas; (3) no harmful effects have ever been observed, with levels of supplementation ranging from 360 μg to 5 mg of folic acid daily.

Because of the expected strong impact on prevalence of congenital malformations, the current dietary folate intake for adults in the USA is recommended at a dose 400 μg, representing a daily intake of 200 μg folic acid equivalents, the recommended intake for pregnant women being 400 μg dietary folate plus 400 μg folic acid, representing a daily intake of 600 μg folic acid equivalents [21]. The UK Committee on Medical Aspects of Food and Nutrition Policy recommendation of 240 μg of folic acid per 100 g flour [22] is approximately equivalent to an additional folic acid intake of 200 μg a day. To obtain adequate protection against risk of neural tube defects the mean plasma folate should be about 10 ng/ml, while the mean plasma folate level in most populations is around 5 ng/ml [21]. There is

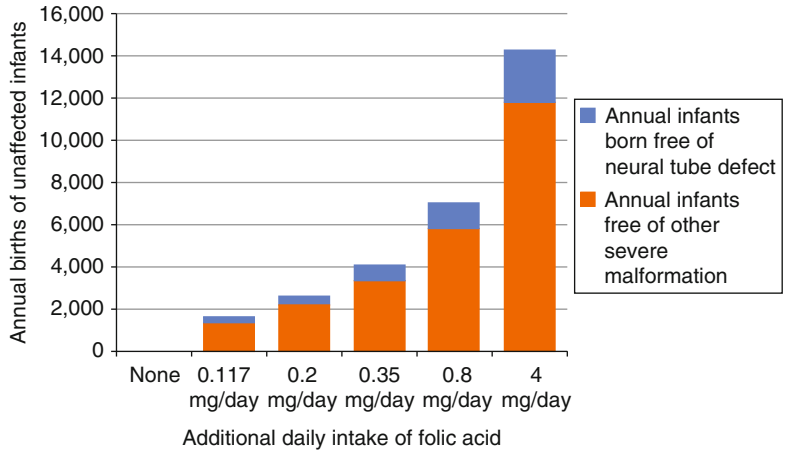

Fig. 1.7 Projected effect of folic acid fortification on annual gain of infants free of congenital malformations in North America. The estimates were prepared by Bernadette Modell for WHO/EURO meetings on Prevention of Congenital Disorders, Copenhagen 2001 and Rome 2002 [25, 26], providing the expected reduction of congenital malformations overall depending on the additional folic acid intake (*orange portion of the bars*) and the proportion of avoided neural tube defects (NTD) (*blue portion of the bars*)

Table 1.1 Global estimate of reduction of neural tube defects (NTD), congenital heart disease (CHD), and limb reduction defects (LRD) by folic acid (FA) food fortification

	All three conditions	NTDs	CHD/LRD	NTDs %
Potential annual affected births	1,035,604	388,442	647,162	37.5
Estimated annual affected births w FA	659,090	137,402	521,689	20.8
Born malformation-free with FA	376,513	251,040	125,473	66.7

Prepared by Bernadette Modell, personal communication

considerable variation within and between populations, but few have a mean plasma folate in the recommended range, and very few individuals could meet the above recommendations without food fortification or the use of folic acid supplements. The groups of greatest concern are those with folate intakes and plasma folate at the lower end of the range, for whom only food fortification is capable of bringing them into the recommended range.

Folic acid fortification of all cereal grain products at a level of 140 μg/100 g flour is mandatory in the USA and Canada, where the birth prevalence of neural tube defects has since fallen by about 20%, with no adverse effects reported [16, 23]. In Hungary, folic acid, vitamin B12, and vitamin B6 are being added to bread, with average daily intake of folic acid, vitamin B12, and B6 from this source at approximately 200, 1, and 1,080 μg, respectively [24]. The prevalence of neural tube defects has fallen by 41%. Based on this study and other relevant data on the potential reduction of congenital disorders through primary preventive measures, the expected overall reduction of the prevalence of congenital disorders in North America may be projected to be approximately 40,000 births of children with major congenital disorders [25] (Fig. 1.7). The potential global estimate of reduction of congenital disorders is presented in Table 1.1.

It is of importance to mention that folic acid fortification costs only about $1 per metric ton of flour. It is so minor that the extra cost is not sufficient even to change the price of a loaf of bread. However, the multivitamin food fortification does not substitute completely periconception supplementation, so the prospective pregnant

Table 1.2 Reported fall in prevalence of neural tube defects (NTD) following folic acid food fortification

Country/region	Year fortification started	NTD/1,000 pre-fortification	NTD/1,000 post fortification	% fall in NTD
Ontario Canada	1998	1.13	0.58	49
Nova Scotia Canada	1998	2.58	1.17	55
Chile	2000	1.64	0.8	51
South Africa (urban)	2003	1.41	0.98	31
Oman	1996	5.5	3.1	44

Prepared by Bernadette Modell, personal communication

women should be informed about the need for folic acid and multivitamin supplementation, in addition to the intake of the multivitamin-fortified food. Taking into consideration the estimated lifetime cost for a single patient with spina bifida, which is approximately $250,000, a complementary periconception supplementation would be highly cost-effective, although the major benefit is the birth of an unaffected child. This approach may be best realized by advising oral contraceptive users to start taking folic acid-containing multivitamins as soon as they stop using contraception, and using a multivitamin supplementation containing a physiological dose (0.4–0.8 mg) of folic acid, which contributes to prevention in addition to more efficient reduction of neural tube defects (about 90% versus 70%), and prevention of some other congenital disorders of public health importance. The available experience from those countries which implemented a national foodstuff fortification program is presented in Table 1.2.

However, prevention of congenital malformations is not actually the major objective of the above programs, as replacing an affected live birth by abortion, as shown in Figs. 1.2, 1.3, 1.4, and 1.5, does not solve the problem, as the abortion of a wanted pregnancy is also an unfavorable pregnancy outcome. This makes primary prevention by food fortification, preconception vitamin supplementation, or PGD much more attractive interventions not associated with distress, because, on the contrary, they increase the number of healthy wanted babies born and it has a highly positive effect on the quality of life of parents and children. In evaluating the effects of folic acid fortification the appropriate evaluation of the program is therefore gain in the number of unaffected pregnancies (Fig. 1.7), rather than reduction in the number of affected live births [26]. The same consideration applies to PGD, the main objective of which is to assist couples to have an unaffected child of their own.

Thus, one of the most exciting possibilities to avoid the genetic disease before pregnancy is preconception and preimplantation genetic diagnosis (PGD), which has recently become available worldwide. Instead of prenatal screening and termination of affected pregnancies, which is not tolerated in many communities and ethnic groups, the pre-pregnancy diagnosis provides an option for couples at risk to plan unaffected pregnancies from the onset (Fig. 1.1). This is the reason the preconception and pre-pregnancy diagnosis or PGD has already become an integral part of preventive services for congenital disorders, providing a choice for those couples who are unable to accept prenatal screening and termination of pregnancy. Together with other approaches for primary prevention, PGD may soon represent an important component of preconception clinics, which may soon shift their services from secondary preventive measures based on prospective carrier screening and prenatal diagnosis to preconception prevention and PGD, to ensure only unaffected pregnancy from the onset.

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