Alagille Syndrome

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Nomenclature	
JAG1	Italicized gene
Notch	Signaling pathway
Notch	Receptor
NOTCH2, NOTCH3	Italicized gene

Introduction

Nearly 50 years ago, hepatologist Daniel Alagille recognized that a significant number of his patients with bile duct paucity also had abnormalities of the face, heart, eye, and spine [1]. He realized that this constellation was inherited in an autosomal dominant fashion in many families [2]. Alagille termed the disease "syndromic paucity of the interlobular ducts," in which the systemic manifestations were variable but included a particular facies, cholestasis, a heart murmur, posterior embryotoxon, and butterfly vertebrae. Watson and Miller reported patients with the same syndrome from the perspective of familial pulmonary artery stenoses with associated liver disease and termed the syndrome "arteriohepatic dysplasia" [3]. Alagille defined the diagnostic criteria for the syndrome as the combination of paucity and at least three of those five major criteria, providing a definition that proved useful and durable [4]. Over time, other organs have been recognized to be common or sometimes rare manifestations of the disease, and as more individuals were identified, the range of manifestations within an organ system was recognized to be as diverse as the number of organs potentially affected [5–9]. Alagille realized that the manifestations in the heart, liver, and spine were highly variable and furthermore that some family members had only minor involvement of a few organs. Over time, other names (including intrahepatic atresia, biliary hypoplasia, intrahepatic biliary dysgenesis, and Watson–Alagille syndrome) were used to describe the syndrome, but eventually the term Alagille syndrome (ALGS) became synonymous with this constellation of findings. Although the hepatic manifestations predominate in most patients, the term Alagille syndrome shifts the emphasis from a liver disease to a generalized developmental disorder.

While the genetic basis of ALGS was recognized very early on, it was not until the 1980s, when a number of patients with ALGS were noted to have deletions in chromosome 20p, that the site of the gene was proposed. In 1997, Li et al. [10] and Oda et al. [11] identified that mutations in *JAG1* cause Alagille syndrome. The discovery of this causative gene led to a

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tremendous increase in the knowledge about the manifestations of ALGS. *JAG1* is a ligand in the Notch signaling pathway, which is involved in the embryogenesis of many human organs, including those affected in ALGS. Mutations in *JAG1* are now found in nearly all patients with clinical ALGS [12]. A small number of patients with ALGS have been found to have mutations in *NOTCH2* (rather than *JAG1*) [13, 14], underscoring the importance of the Notch pathway in human development. With the current availability of clinical mutation and deletion analysis, genetic testing has supplanted the need for histologic documentation of paucity in many individuals. Furthermore, it has greatly expanded recognition of the number of minimally affected mutation carriers that have an "incomplete syndrome" yet have the risk of producing severely affected progeny. The spectrum of *JAG1*mutation carriers is now recognized to include individuals who have no apparent disease and others who have a major manifestation in other organs such as heart or vasculature, without clinically apparent liver disease or cholestasis. The prevalence of ALGS has been reported to be 1 in 70,000 births, but with the introduction of molecular testing and the identification of mildly affected individuals, this is likely to be a significant underestimate [15]. All of the early descriptive clinical studies focused only on patients who met the classical clinical definition of the syndrome. As a result, the reported clinical outcomes of morbidity and mortality were adversely biased toward the severest end of the spectrum. With the advent of genetic screening of the relatives of a proband, it has become apparent that many mutation carriers are minimally affected, and it is difficult to ascribe the term "syndrome" as it has been classically defined [15]. Some individuals with *JAG1* or *NOTCH2* mutations have manifestations that do not resemble ALGS [15–17]. Furthermore, a number of other diseases of Notch signaling have predominant manifestations in one organ system (bone, vasculature) that bear resemblance to the manifestations of ALGS in that organ, but without common effects on the other sites typically affected in ALGS [16, 18, 19].

Genetics

ALGS was previously reported to affect 1 in 70,000 newborns; however, this likely represents an underestimate of its true prevalence as molecular screening has identified mildly affected individuals with subtle or atypical ALGS manifestations [15]. The true prevalence is likely closer to 1 in 30,000. The majority of patients with ALGS (>90 %) carry a mutation in *JAG1*, located in the short arm of chromosome 20 [12]. To date more than 430 different mutations have been described. Sixty percent of ALGS individuals have sporadic mutations, and the remainder have inherited disease [20]. A few patients have a total gene deletion (3–7 %), and the rest have intragenic deletions, most of which are protein truncating. Nine percent of affected individuals have splicing mutations and 9 % have missense mutations [12]. A small percentage (approximately 1 %) of patients who fulfill the clinical criteria for ALGS, but do not carry a *JAG1* mutation, have been found to have a mutation in another gene, *NOTCH2* [14].

JAG1 encodes for a ligand in the Notch signaling pathway, which is a highly evolutionarily conserved intercellular signaling mechanism. There are five ligands (Dll1, Dll3, Dll4, *JAG1*, and 2) and four Notch [1–4] receptors known to date in mammals. *JAG1* is a single-pass type I membrane protein with an extracellular domain made of a N-terminal region, a Delta/Serrate/LAG2 (DSL) domain, 16 EGF tandem repeats, and a cysteine-rich region [21]. The Notch receptor consists of an extracellular segment, formed by multiple epidermal growth factor (EGF)-like repeats; a transmembrane part; and an intracellular domain. Once the receptor–ligand interaction has occurred, the intracellular domain is cleaved from the inner surface of the membrane and translocates into the nucleus where it regulates the transcription of different downstream genes, such as Hes1/Hey2 [21].

A small fraction (3–5 %) of ALGS individuals have deletions of chromosome 20p. Genome-wide SNP analysis of 25 patients with ALGS revealed 21 deletions ranging from 95 kb to 14.62 Mb [22]. Patients with deletions greater

than a critical 5.4 MB region had additional phenotypic features not usually associated with ALGS such as developmental delay and hearing loss. Interestingly deletions up to 5.4 MB did not confer additional clinical findings although there was haploinsufficiency for several genes other than *JAG1*.

The identification of individuals in the same family sharing the same *JAG1* mutation with different disease manifestations strongly supports the existence of genetic modifiers. Current research strategies are under way to identify these modifiers in different organ systems. Given the complexity and numerous members of the Notch signaling pathway, there are many candidate genes.

Clinical Manifestations and Management

Liver Disease in ALGS

Duct Paucity and Hepatic Histopathology

The hallmark of the liver disease in Alagille syndrome is bile duct paucity, along with the usual severe cholestasis that accompanies paucity (Fig. 11.1). Many patients, however, do not have paucity due to a variety of factors. The presence of paucity is no longer considered essential for diagnosis. Furthermore, bile duct paucity is not specific for ALGS. Paucity is seen as either a common or an occasional histologic feature of a highly diverse group of infectious, metabolic, immunologic, and genetic diseases. Paucity is also recognized to be a late histologic pattern of a number of diseases that have neonatal hepatitis or even bile duct proliferation on earlier biopsies. The progression to paucity is highly variable, from months to years depending on the etiology. Nevertheless, the finding of bile duct paucity in patients of any age should raise the possibility of ALGS, as it is the single most important and most common cause of paucity. It is an important caveat, however, that the presence of apparently normal duct number, or rarely even bile duct proliferation, can be seen in patients with ALGS and thus does not fully eliminate the syndrome from consideration.

The normal bile duct to portal tract ratio undergoes a developmental maturation, which is of particular consideration in a preterm infant, where the number is normally diminished [23]. In full-term infants and older children, the normal bile duct to portal tract ratio ranges between 0.9 and 1.8. The precise ascertainment of this ratio can be difficult in needle biopsies, particularly

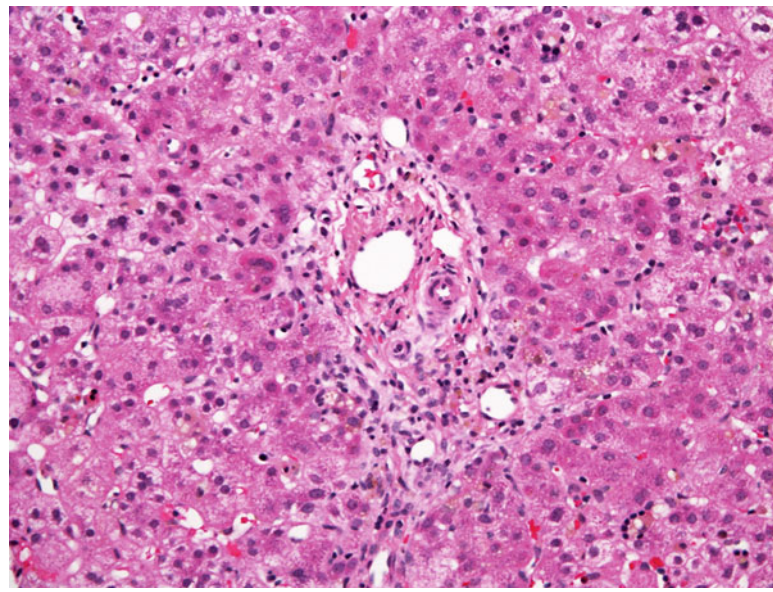

Fig. 11.1 Histology: Bile duct paucity in a large portal tract demonstrating several branches of portal vein and hepatic artery, from a 2-month-old infant with ALGS. Magnification × 200 (Courtesy of Dr. Pierre Russo, CHOP)

if the number of portal tracts is limited. It is important to only include ducts, but not ductules, in this ratio. The minimal number of portal tracts necessary for an accurate ratio can only reliably be achieved by a wedge biopsy. However, a reasonable assessment of the ratio can be obtained with most needle biopsy specimens containing at least six portal tracts [24]. A bile duct to portal tract ratio of less than 0.9 is suggestive of ALGS, but most older infants with ALGS have a ratio that is in the range of 0.5–0.75 [2]. In infants, the timing of the biopsy is commonly dictated by the need to discriminate biliary atresia from ALGS. Unfortunately, as many as 40 % of infants less than 6 months of age will not have established paucity recognized on early biopsy, although paucity is generally expected (up to 95 %) after that age in symptomatic children [7]. For diagnostic evaluations of neonatal cholestasis in the first 2 months of life, a biopsy is performed to identify bile duct proliferation, which suggests biliary atresia, other forms of obstruction, and certain metabolic liver diseases, but only very rarely ALGS. If proliferation is identified in an infant who has clinical features of Alagille syndrome, a careful and cautious evaluation should be undertaken, as a non-excreting DISIDA scan or a noncommunicating intrahepatic cholangiogram can each occur in ALGS, leading to an error in diagnosis and an unnecessary Kasai portoenterostomy. A number of ALGS infants have been misdiagnosed as biliary atresia [25, 26]. The overlap of ALGS and concomitant biliary atresia is controversial, but it appears to be, at the most, extraordinarily rare. More commonly, postoperative histologic assessment of the central and extrahepatic ducts demonstrates them to be patent yet extremely hypoplastic. The ultimate value of a needle biopsy at this age for ALGS infants, therefore, is to keep them out of the operating room, where a cholangiogram in the best of situations may be noncommunicating and misleading.

The importance of identifying paucity, and the role of the liver biopsy in establishing the diagnosis of ALGS, is diminishing as molecular diagnosis is increasingly available. While paucity was a required criterion for the diagnosis for many years, newer studies focusing on patients with *JAG1* mutations or with systemic disease identify paucity in approximately 80–85 % of patients. Many patients identified in this fashion have minimal clinical hepatic manifestations, and some have negligible biochemical disease. It is reasonable to assume that the hepatic manifestations of paucity and cholestasis as are as variable in incidence as cardiac, renal, ocular, or vascular findings. The role of biopsy in older children and adults may not only include the assessment of bile duct and portal tract number but also the identification of fibrosis and to rule out other concomitant disease.

The progression to cirrhosis in ALGS is not typical, although significant fibrosis or cirrhosis has been reported to occur in 10–50 % of patients in different series representing inclusion of the most severely affected individuals and also referral patterns to transplantation centers [6–9, 27] (see Fig. 11.2). The actual incidence of progressive liver failure or intractable portal hypertension has not been well characterized in the molecular era but is undoubtedly significantly lower.

Hepatic Clinical Disease

The vast majority of patients with symptomatic ALGS will present in the first 3 months of life, although the manifestations and severity of presentation can vary considerably. Presentation with severe cardiac disease such as tetralogy of Fallot or pulmonary atresia will be evident at birth, if not previously identified by fetal ultrasound. The presence of severe cardiac disease commonly obscures the importance of hyperbilirubinemia or aminotransferase elevations, thereby delaying diagnosis in some infants. Neonatal renal failure has occasionally been seen. Infants with ALGS may be small for gestational age. Facies, embryotoxon, and butterfly vertebrae are rarely sought until ALGS is already a major consideration.

Most symptomatic ALGS patients present with hepatic disease, frequently within the first month of life. ALGS is one of the more common etiologies of neonatal cholestasis and conjugated hyperbilirubinemia. At this age, it must be rapidly and correctly discriminated from biliary atresia

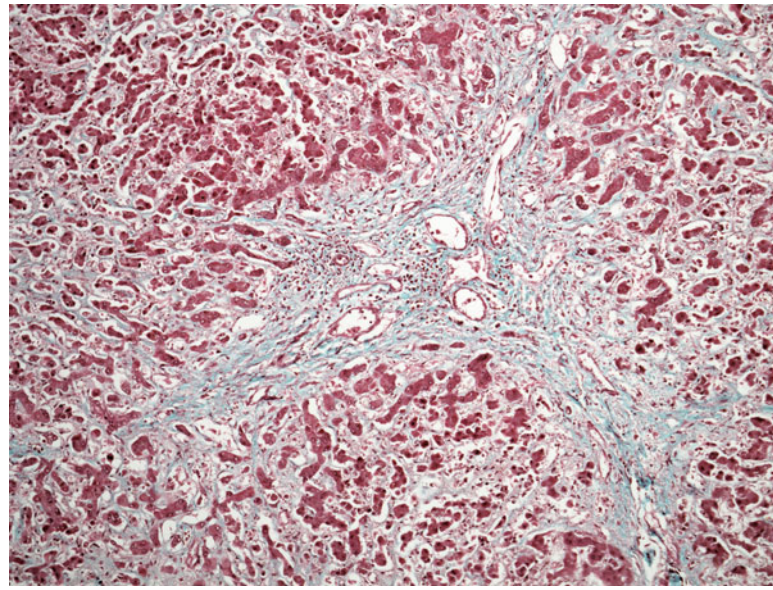

Fig. 11.2 Histology: Liver biopsy demonstrating portal fibrosis, with portal to portal bridging, focal nodule formation, and extensive sinusoidal fibrosis (Courtesy of Dr. Pierre Russo, CHOP)

and other treatable causes of neonatal cholestasis. ALGS, biliary atresia, other extrahepatic obstructions, cystic fibrosis, FIC3, and other disorders may present with conjugated hyperbilirubinemia and a rising GGTP level. A prompt assessment for other manifestations of the syndrome should be undertaken, usually culminating in a percutaneous liver biopsy to assess ductular histology. As stated above, extreme caution should be taken to differentiate ALGS from biliary atresia, as the therapy is different and surgical excision of the extrahepatic tree may worsen long-term hepatic outcome [25].

Essentially all patients with hepatic ALGS have some degree of conjugated hyperbilirubinemia. The bilirubin levels typically increase over the first months or years of life. In severely affected children, the bilirubin levels may be as high as 30-fold elevated. While modest bilirubin elevations may improve significantly in later childhood, extreme elevations generally portend the need for liver transplantation at some point, either for intractable pruritus, portal hypertension, or synthetic liver failure. The aminotransferases are commonly elevated, but to varying degrees that do not seem to correlate with excretory function or with outcome. Some patients have normal aminotransferase levels in infancy. More characteristically, the biochemical measures associated with cholestasis are significantly elevated. In addition to bilirubin, and sometimes even more striking, bile salt levels can be markedly elevated, reaching up to 100-fold elevations. Bile salt elevations can also be seen in older patients with normal total bilirubin. The gamma-glutamyl transpeptidase level is also commonly strikingly elevated, at times 50-fold or higher. Alkaline phosphatase is also quite elevated but can be also affected by vitamin D deficiency, bone disease, and the increased incidence of fractures in various stages of healing.

The apparent obstruction to bile flow and the resulting cholestasis progress over the first months to years and then in many patients improve thereafter. In addition to the jaundice, a striking and clinically disfiguring feature is the development of xanthomas (see Fig. 11.3). Hypercholesterolemia and hypertriglyceridemia are common features of ALGS. The elevations of cholesterol may exceed 1,000 mg/dl. In severely affected infants, xanthomas typically start to occur as the cholesterol level surpasses 500 mg/dl. As the level further rises, these xanthomas characteristically occur on the extensor surfaces of fingers and toes and in areas of minor trauma, including the abdomen, buttocks, inguinal area, and neck. In some patients, the face can be severely involved, particularly the ears, eyes, and nose. The xanthomas

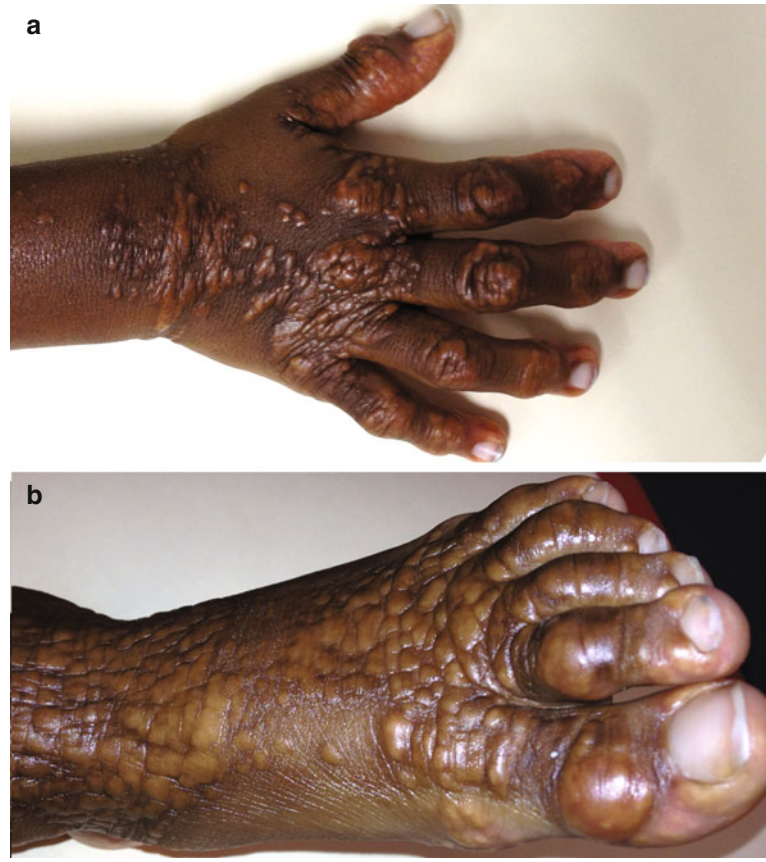

Fig. 11.3 (**a** and **b**) Profound disfiguring xanthomas of the hand and foot in a 4-year-old toddler with thousands of xanthomas and a serum cholesterol level of 825 mg/dl

can be particularly upsetting because they itch and are commonly traumatically injured, leading to bleeding, scabbing, and scarring. Patients can have hundreds, or even thousands of xanthomas, which can lead to disfigurement and emotional distress for patients and parents alike. As cholesterol levels decline, either with age progression, medical therapy, partial external biliary diversion, or liver transplantation, the xanthomas can resolve, although pigmentary abnormalities may mark the site of prior lesions (see Fig. 11.4).

Hepatic synthetic function is generally well preserved in patients with ALGS, despite profound deficits in excretion capability. Metabolic regulation is generally intact. Albumin and ammonia levels are generally normal. The prothrombin time is commonly elevated unless vitamin K supplementation is adequate, which in some very severely affected individuals requires repeated parenteral therapy. Worsening synthetic and metabolic functioning can occur, as can progression to cirrhosis and liver failure in approximately 10–20 % of patients whose onset of liver disease was in infancy.

A large number of *JAG1* mutation carriers have mild or even nonexistent liver disease. Some of these patients are identified because they have major manifestations in other organs (heart, moyamoya, kidney, etc.). Others identified by family screenings are the asymptomatic parents or siblings of a newly identified affected proband. For these individuals with minimal liver disease, progression to cirrhosis or liver failure (without another toxic or infectious event) is extraordinarily uncommon, and they should be reassured that their liver function will remain intact, even though other manifestations (kidney, vascular, etc.) should be considered.

Patients with severe cholestatic liver disease may have profound problems with growth, weight gain, and fat-soluble vitamin absorption. Diminished bile salt excretion and low

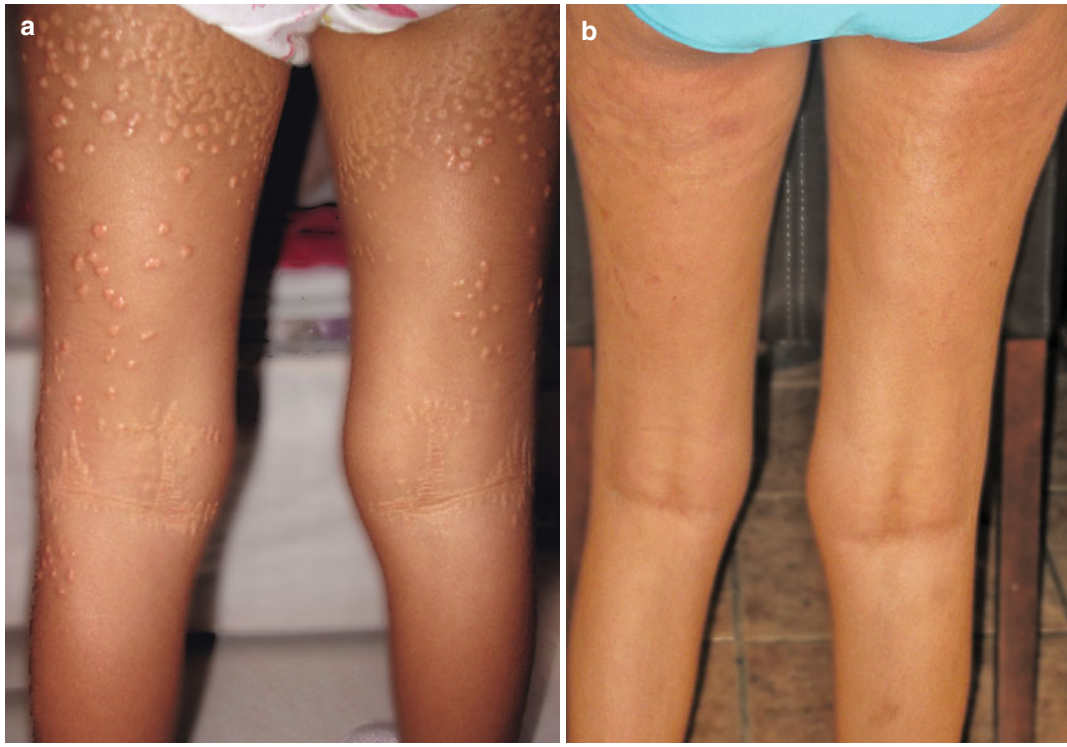

Fig. 11.4 Spontaneous resolution of severe xanthomas after a decade of medical therapy for hypercholesterolemia and pruritus. (**a**) Posterior thigh and popliteal fossa xanthomas, representative of thousands of large, elevated xanthomas at 3.5 years of age, with a serum cholesterol level of 1,260 mg/dl. (**b**) Spontaneous resolution of all xanthomas 11 years later, at age 14, with a serum cholesterol level of 200 mg/dl. Resolution occurred with medical therapy alone (Courtesy Dr. Joshua R. Friedman, CHOP)

intraluminal bile salt concentrations result in ineffective solubilization and absorption of dietary lipids, essential fatty acids, and the fat-soluble vitamins A, D, E, and K. Vitamin levels should be checked regularly, particularly in cholestatic infants. Excellent vitamin preparations are available to treat deficiencies, although sometimes the amount required and the uncovered cost of the supplements can be an impediment to successful therapy. Combination as well as individual vitamin preparations should be used in a customized fashion, as an individual patient's needs vary significantly. At times, therapy with preparations such are parenteral vitamin K or calcitriol is necessary to treat severe deficiencies. It should be recognized that other medications (e.g., cholestyramine) and special diets may contribute to refractory deficits. Essential fatty acid deficiency has occurred in ALGS. Many children do well with formula composed of or fortified with medium-chain triglycerides (MCT), which are better absorbed than long-chain fat. However, some children seem to do better with a low-fat diet, with an emphasis on supplementing essential fats. For severely affected infants, a gastrostomy tube is useful to augment intake and promote growth and development. This also has an advantage for infants and toddlers taking numerous doses of supplements, antipruritics, choleretics, sequestrants, and other medications.

Pruritus in ALGS

Pruritus is a particularly significant and commonly intractable feature of ALGS. Severe pruritus can occur in infancy and progress with age. For many patients, this is the single most

important and overarching feature of ALGS. The presence of pruritus generally trends with the levels of cholesterol and bile salts, and some antipruritic therapy is directed at those substances. Local cutaneous therapy has significant value. The avoidance of drying soaps, the copious administration of emollients and ointments, and careful attention to fingernail length are useful. Many medications have utility for the pruritus of ALGS. Antihistamines may decrease itching and if given only at night may help with sleep as well. Rifampin has been shown to decrease itching and in many cases is particularly effective [28, 29]. Ursodeoxycholic acid is a potent choleretic and can have a dramatic effect on reducing symptomatic cholestasis [30] although in some patients it may exacerbate pruritus. Bile acid-binding resins may increase the elimination of bile salts and secondarily decrease itching. Naltrexone, an opioid antagonist, can also be effective in some cases [31].

Refractory pruritus is an indication for liver transplantation consideration. Diversion surgeries have been developed to provide relief from intractable pruritus without the risks and mortality associated with liver transplantation, particularly for patients with preserved synthetic function and without cirrhosis. In partial external biliary diversion (PEBD), a biliary conduit is constructed from a segment of resected jejunum, and an anastomosis is made between the proximal portion of the conduit and the most dependent portion of the gallbladder. The distal roux limb is used to form an ostomy. For ALGS patients with severe, mutilating pruritus, the majority have a dramatic improvement in their pruritus score at 1-year post-diversion [32]. In addition, the subset of patients with extensive xanthomas had complete resolution within a year, concomitant with significant decreases in mean bile salt levels and mean cholesterol. In one patient who requested reversal, there was prompt recurrence of severe pruritus [32]. It has been estimated that PEBD diverts as much as 50 % of bile, whereas maximal therapy with resins such as cholestyramine can only divert about 5 % of the bile acid pool each day [33]. For many ALGS patients, PEBD has provided sustained and substantial improvement in quality of life and reduction of symptoms and serves an important role for patients who might otherwise require transplantation.

Some patients will decline PEBD since it requires the construction of a permanent draining ostomy. An alternative surgical approach is terminal ileal exclusion, which avoids the need for an external fistula. In this technique, approximately 15 % of the terminal ileum is bypassed via a direct anastomosis of the more proximal ileum to the ascending colon. This approach has been reported less frequently in ALGS [34, 35] but has been used successfully in familial intrahepatic cholestasis. In a small study on three ALGS patients, there was significant reduction of pruritus and improvement (but not resolution) of xanthomas, but the improvement in biochemical cholestasis was much less than PEBD, and some values worsened with follow-up [35]. Nevertheless, ileal exclusion may provide an alternative to PEBD for certain situations. The effect of diminished effective bowel surface area on nutrition and malabsorption has not been extensively studied.

Liver Transplantation in ALGS

Liver transplantation is a highly effective therapy for the hepatic disease of ALGS, and it is generally estimated to be necessary in approximately 20–30 % of patients with ALGS [7, 8], although some studies report much higher rates [9]. There are a number of reports from single centers mostly documenting 1-year survival rates in the 71–92 % range [7, 9, 27, 36–38], although some studies are limited by small patient numbers and relatively short mean follow-up.

Two recent large studies of transplant databases provide a broad overview of the outcomes and the complications of transplantation for ALGS, with a comparison to data for biliary atresia [39, 40]. Between 1987 and 2008, 461 ALGS patients (4 % of the total recipients) recorded in the UNOS database underwent hepatic transplantation. The 1-year ALGS patient survival rate was 82.9 %, and the 5-year survival rate was 78.4 %. The 1- and 5-year graft survival rates were 74.7

and 61.5 %. Each of these rates is lower than those seen for biliary atresia, where 1- and 5-year patient survival rates are 89.9 and 84 % [39]. There was a significantly increased rate of both graft loss and patient mortality in the first 30 days, compared to patients with BA. Early (<30 days) graft loss for ALGS was 11.7 % compared to 4.8 % for BA, and early mortality for ALGS was 9.6 % compared to 4.8 % for BA. In this series, graft failure and infection were the leading causes of death in ALGS transplants. Neurologic and cardiac complications also contributed to the excess mortality in ALGS [39]. Kaplan Meier analysis of 10-year patient survival was approximately 78 %, with contributions to mortality from pre-transplant creatinine elevations, extended cold ischemic time, and repeat transplantation. Although the data for survival was presented in aggregate, a sub-analysis demonstrated (for both ALGS and BA) that the percentage of deaths and the graft loss were significantly better in the newest quartile data reflecting a later era of transplantation care. This type of analysis has not been performed in most single-center studies, but it can be reasonably assumed that the data quoted above represents minimal estimates for patient and graft survival in the current era.

In a subsequent study utilizing the SPLIT (Studies on Pediatric Liver Transplantation) transplant database, 91 patients with ALGS (2.9 % of the total population of recipients) were compared to 236 age- and gender-matched BA patients transplanted between 1995 and 2009 [40]. The 1-year patient survival rate for ALGS patients was 87 % (compared to 96 % for matched biliary atresia patients). Nearly all of the excess mortality for ALGS patients occurred in the first 6 months after transplantation, and then subsequently the Kaplan–Meier curves are parallel. Early death in this cohort was associated with biliary, vascular, central nervous system (seizures, cerebral hemorrhage, edema), and renal (requiring dialysis, hemofiltration) complications. There is a high frequency of renal complications in ALGS both before and after transplantation. Posttransplant renal insufficiency worsened in many ALGS patients. At 1 year after transplant, 22 % had GFR less than 90 ml/min/1.73 m^2. In this study, ALGS patients were more growth-impaired overall than BA transplant patients, and although the height deficit persisted, they did demonstrate good catch-up growth (not seen in other smaller studies) [38, 40, 41]. Occasionally, concomitant liver–kidney transplantation or rarely liver–heart transplantation has been successfully performed.

The results of living-related transplants in ALGS are highly favorable. Studies of long-term 10-year survival after living-related transplantation for ALGS have results as high as 80 %. In one series, the survival rates were similar for living-related transplantations for ALGS and other pediatric liver diseases [41]. One caveat, however, is that ALGS is a dominantly inherited disease in which approximately 40 % of probands will have a *JAG1* mutation-carrying parent who may be minimally affected. It is imperative to fully assess living-related donor candidates for unapparent ALGS, because donors with unsuspected but severe biliary involvement have been identified only at the time of surgery [42]. In the era of molecular diagnosis, and with sufficient lead time, all potential living-related transplant recipients with ALGS should have exhaustive mutation analysis (including *NOTCH2* if *JAG1* is negative), which should identify a mutation in 95 % of individuals. All potential related donors should have targeted mutation analysis to supplement the other typical donor screening studies.

Indications for Liver Transplantation

The indications for transplantation, the timing of transplantation, and the assessment of associated risk factors are more complex compared to other pediatric liver diseases. Cholestasis and its complications are the most common indication for transplantation. In BA patients with a failed Kasai, the progression of liver disease is essentially inevitable, leading to worsening hepatic synthetic function, intractable portal hypertension, malnutrition, and worsening quality of life. For ALGS patients, the magnitude of the cholestasis is commonly much larger, but many ALGS infants have cholestasis that worsens and then improves over time (Fig. 11.5). The specific indications for transplant in ALGS include cholestasis, intractable pruritus, complications

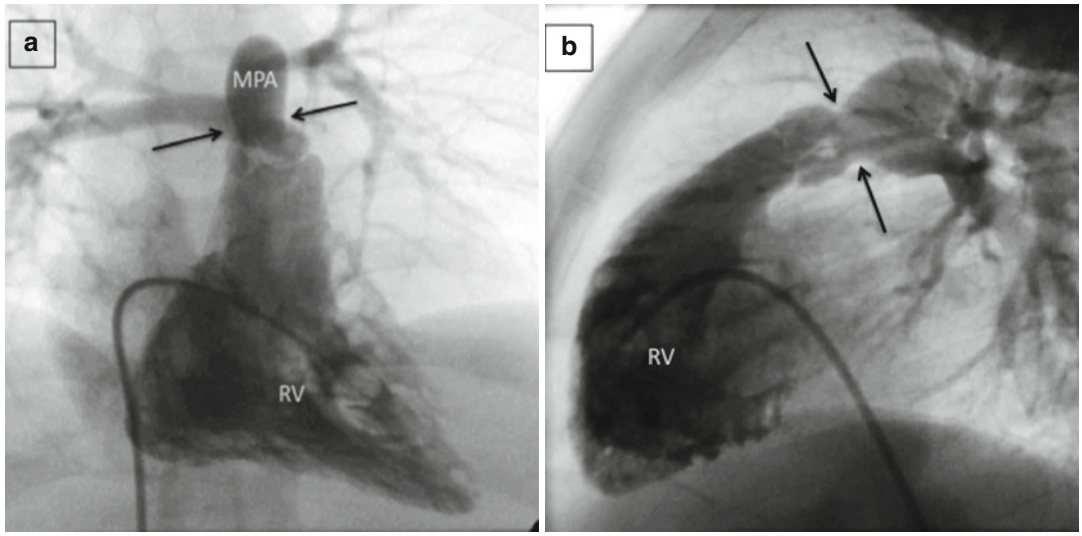

Fig. 11.5 Figure 1 is a right ventriculogram taken in anteroposterior (panel **a**) and lateral (panel **b**) views of a typical patient with Alagille syndrome. The *arrows* denote supravalvar pulmonary stenosis. There is post-stenotic dilation of the MPA, and the proximal branch pulmonary arteries are diffusely small. *RV* right ventricle, *MPA* main pulmonary artery

of portal hypertension, synthetic liver failure, growth failure from chronic malnutrition and vitamin deficiency, recurrent fractures and bone disease, and occasionally severe xanthomatosis [7, 9, 27, 39, 40]. Commonly, several of these are present concomitantly. Other therapies, however, can be implemented that can obviate or diminish the need for transplantation in some patients. For severe cholestasis with intractable pruritus and good synthetic function, ALGS patients may improve significantly with combination medical therapy, partial external biliary diversion, or ileal exclusion therapy. Surgical strategies to treat pruritus should generally be offered to patients who have a good chance of avoiding transplantation otherwise. If transplant is inevitable for other reasons, then a direct approach to transplant is generally advised.

Preoperative Assessment for Liver Transplantation

The progression to transplantation generally follows a long trajectory, with time to address and possibly treat many of the factors that could worsen outcome [43]. As a multisystem disease, ALGS presents many non-hepatic issues, which must be addressed. Malnutrition should be aggressively treated, with either nasogastric or gastrostomy supplemental nutrition if not already being administered. The most important part of the transplant assessment is the evaluation for cardiac and renal disease. A formal current cardiopulmonary assessment will help to predict challenges and identify problems that can be addressed preoperatively. For example, critical pulmonary artery stenoses can be addressed in the cardiac catheterization lab in order to decrease right heart pressures or to improve differential pulmonary perfusion. A protocol for cardiac assessment recommendations has been provided by the faculty at King's College [44]. The presence of pre-transplant renal disease should influence the posttransplant choice of immunosuppression. If significant renal impairment is present, renal-sparing immunosuppressive protocols with low target levels of calcineurin inhibitors and early introduction of other strategies should be considered. Dental disease, which is common in ALGS, should be addressed completely. Immunizations should be reviewed and updated. Because of the occasional vascular abnormalities seen in ALGS, patients should have careful imaging by CT or MR angiography to anticipate abnormalities. Although

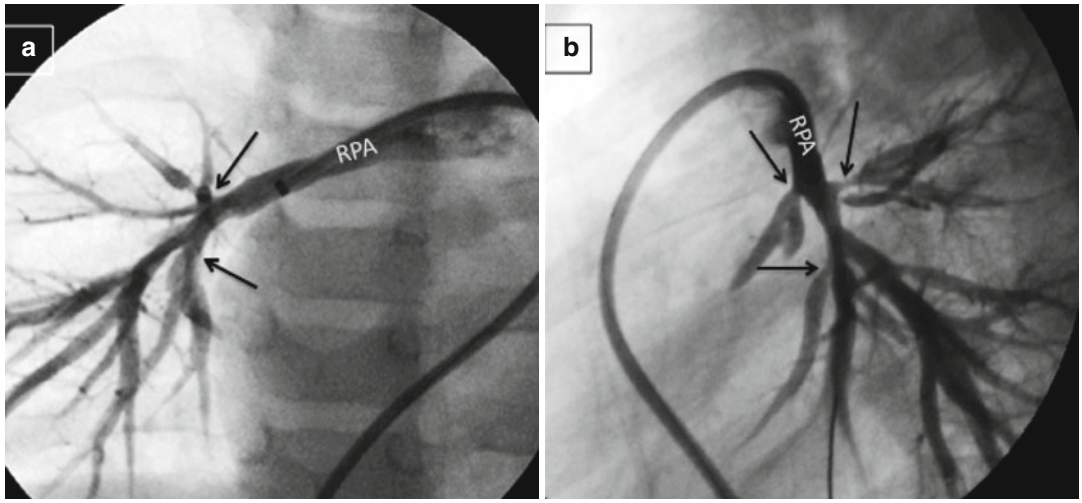

Fig. 11.6 Figure 6 is an angiogram of the right pulmonary artery (*RPA*) in anteroposterior (panel **a**) and lateral (panel **b**) views. The proximal RPA is diffusely small, and there are multiple focal areas of subsegmental arterial stenosis (*arrows*) (Courtesy, Matthew Gillespie, MD, CHOP)

there are no formal indications, brain and CNS vascular imaging seems prudent in children and adults who are being evaluated, as the presence of moyamoya, a vascular lesion, or a prior infarct may influence preoperative strategies.

Cardiac Involvement

In a comprehensive evaluation of 200 ALGS subjects, cardiovascular involvement was present in 94 % [45], with right-sided lesions being the most prevalent. Pulmonary artery anomalies are the most common abnormality identified (76 %) and may occur in isolation or in combination with structural intracardiac disease [45] (see Figs. 11.5 and 11.6). Intracardiac lesions were present in 24 % of 92 patients with ALGS [7]. The most common congenital defect is tetralogy of Fallot (TOF), which occurs in 7–12 % [7, 45]. It appears that severe forms of TOF (especially TOF with pulmonary atresia) occur with greater frequency in the ALGS population than in the general population of individuals with TOF. Approximately 40 % of patients with ALGS demonstrating TOF have pulmonary atresia. There is no correlation between the type of *JAG1* mutation and the nature of the cardiovascular involvement.

The management of cardiac anomalies in ALGS is largely driven by the same algorithms as in non-syndromic children; however, outcomes differ. In a large ALGS series, cardiac surgery was performed in infancy in 11 % [7]. The mortality rates were 33 % for those with TOF and 75 % for those with TOF with pulmonary atresia. The survival of patients with ALGS with these lesions is markedly lower than for patients (with these lesions) without ALGS. This may be due to concomitant significant peripheral pulmonary artery stenosis or other systemic manifestations of the syndrome. Asymmetric peripheral pulmonic stensoses can result in markedly asymmetric perfusion of the lungs (see Fig. 11.7). Nonsurgical invasive techniques have been used successfully for patients with ALGS, including valvuloplasty, balloon dilatation, and stent implantation. Heart–lung transplantation has also been performed in combination with liver transplantation in a child with ALGS, though this is a rare occurrence.

Cardiac disease accounts for nearly all of the early deaths in ALGS. Patients with intracardiac disease have an approximately 40 % rate of survival to 6 years of life, compared with a 95 % survival rate in patients with ALGS without intracardiac lesions [7].

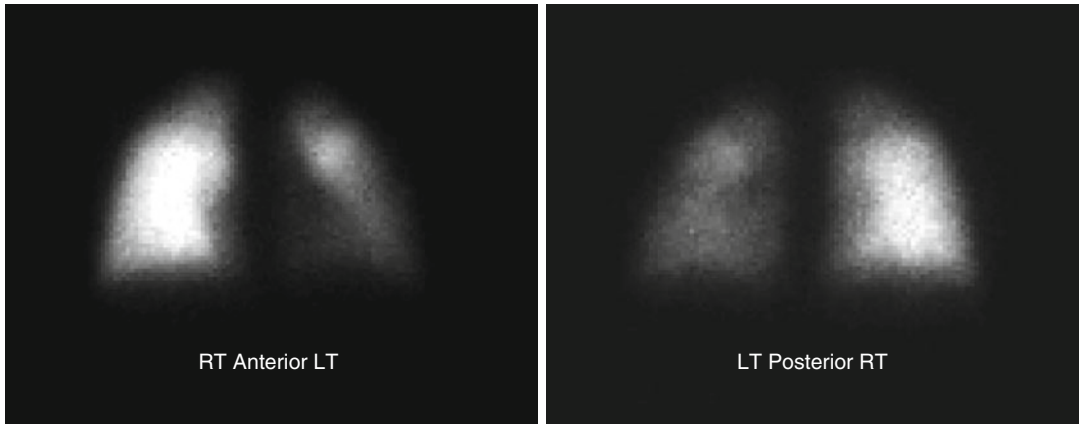

Fig. 11.7 Perfusion scan demonstrating markedly asymmetric flow to the lungs, with 75 % to the right and only 25 % to the left lung, due to asymmetric pulmonary arteriolar resistance

Renal Involvement

Renal involvement in ALGS has been widely reported, and the prevalence of renal involvement in larger series ranges from 40 to 70 % such that it has been proposed that renal anomalies now be considered a disease-defining criterion in ALGS. This clinical finding is supported by the known role of the Notch signaling pathway in nephron and glomerular development [46]. In a large retrospective study, there was a prevalence of 39 % of renal anomalies or disease, and the most common renal involvement was renal dysplasia (58.9 %), with renal tubular acidosis (9.5 %), vesicoureteric reflux (8.2 %), and urinary obstruction (8.2 %) following [47]. Hypertension in patients with ALGS could be of cardiac, vascular, or renal etiology, but the frequency of this has not been systematically studied.

The frequency of renal insufficiency in ALGS has also not been formally characterized though renal replacement therapy and transplantation are reported. In the study of liver transplantation outcomes in ALGS from the SPLIT cohort, a high frequency of renal complications in ALGS, both pre- and posttransplant, was noted [40]. Renal complications, GFR, and serum creatinine were worse in ALGS as compared to biliary at different time points. Most of the renal insufficiency in ALGS post-liver transplant was present at the time of transplant. ALGS children with preexisting renal insufficiency were less likely to have renal improvement, implicating intrinsic renal disease, which is not reversed by liver transplantation.

Functional and structural evaluation of the kidneys should be undertaken in all patients. The role of renal tubular acidosis in early growth failure is unclear, but administration of bicarbonate is necessary in some individuals. Renal function should clearly be reassessed during the hepatic transplant evaluation and calcineurin-sparing immunosuppressive regimes are recommended following liver transplantation.

Vascular Involvement

Unexplained intracranial bleeding is a devastating complication in ALGS. Intracranial bleeds occur in approximately 15 % of patients, and in 30–50 % of these events, the hemorrhage is fatal [7, 8]. There does not seem to be any pattern to the location or severity of intracranial events, which range from massive fatal bleeds to asymptomatic cerebral infarcts. Of note, the majority of intracerebral hemorrhagic episodes in ALGS occur in the absence of coagulopathy. Head trauma, typically of a minor degree, has been associated with the bleeding in a number of patients. The majority of cases of bleeding are spontaneous, however, with no clear risk factors.

Lykavieris studied a cohort of 174 individuals with ALGS and identified 38 patients (22 %) who had 49 bleeding episodes at multiple locations in the body [48]. All these hemorrhages occurred in the presence of normal platelet counts and prothrombin times, suggesting that ALGS patients may be at particular risk for bleeding.

Structural intracranial vascular abnormalities that could explain the occurrence of bleeding and stroke in ALGS have been described in some patients [8, 49, 50]. Aneurysms of the basilar and middle cerebral arteries and various internal carotid artery anomalies have been described. Moyamoya syndrome (progressive intracranial arterial occlusive disease) has also been reported in several children with ALGS (see Fig. 11.8). Emerick et al. prospectively studied 26 patients with ALGS using magnetic resonance angiography (MRA) of the head and identified cerebrovascular abnormalities in ten patients (38 %). This cohort consisted of asymptomatic and asymptomatic patients. One hundred percent of symptomatic patients had detected abnormalities, and, of note, 23 % of asymptomatic patients had anomalies detected [49]. These results suggest that MRA is useful in detecting these lesions and may have a valuable role in screening, although this remains somewhat controversial. The authors' current recommendation is for all asymptomatic ALGS patients to have a screening MRA as a baseline and for physicians to have a low threshold for reimaging ALGS patients in the event of any symptoms, head trauma, or suspicious neurologic signs.

Systemic vascular abnormalities have also been well documented in ALGS. Aortic aneurysms and coarctations and renal artery, celiac artery, superior mesenteric artery, and subclavian artery anomalies have all been described. In a large retrospective study, 9 % (25 of 268) of ALGS individuals had noncardiac vascular anomalies or events [50]. In addition, vascular accidents accounted for 34 % of the mortality in this cohort (surpassing mortality from hepatic or cardiac causes). These findings suggest that vascular abnormalities have been under-recognized as a potentially devastating complication of ALGS. The presence of vasculopathy in ALGS is consistent with the intricate role of the Notch signaling pathway in vascular development [21].

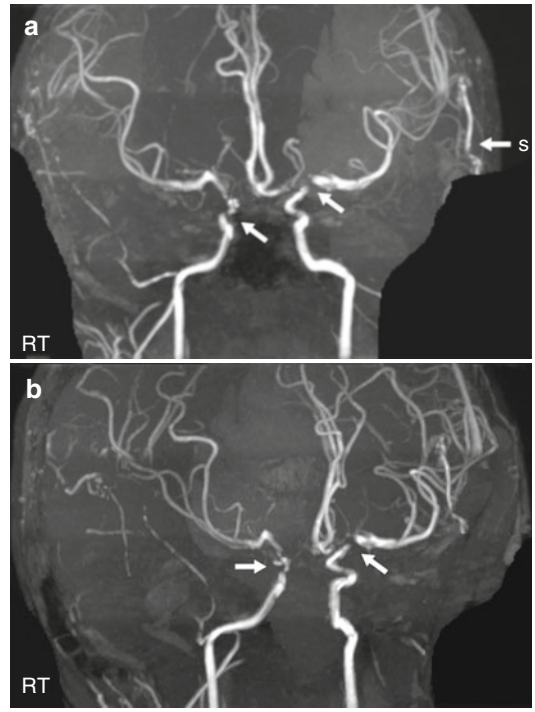

Fig. 11.8 Moyamoya **a** and **b**: MR angiogram demonstrating severe bilateral moyamoya in a 12-year-old boy with ALGS. (**a**) Severe stenoses of the left and the right internal carotid arteries (*arrows*). Anteroposterior view, also demonstrating successful reperfusion of the brain via a bitemporal synangiosis seen well on the left (*S*). (**b**) Oblique view better demonstrating multiple stenoses (*arrows*). The patient had previously suffered an ischemic infarct (Courtesy of Dr. Erin Simon, CHOP)

Facial Features and Skeletal and Ophthalmologic Involvement

These three features of ALGS are considered together since they rarely have clinical significance for the patient but are often important as diagnostic tools.

Facial Features

A characteristic facial appearance is probably one of the most penetrant features of the syndrome [51]. These features include a prominent forehead, deep-set eyes with moderate

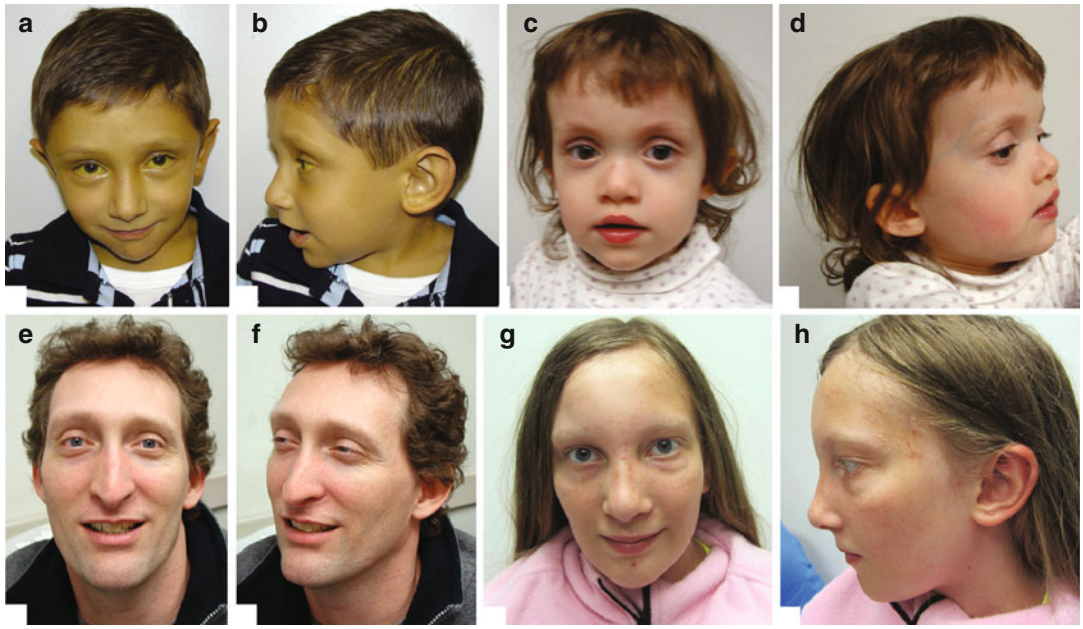

Fig. 11.9 Facies: Representative Alagille syndrome facial features. (**a**, **b**) A 5-year-old boy and (**c**, **d**) 2-year-old girl with typical features including broad and prominent forehead with pointed chin, rounded nasal tip, and seemingly wide-spaced eyes. (**e**, **f**) Father of child depicted in (c–d) with typical adult facial appearance with deep-set eyes, less prominence of forehead, and prognathism. (**g**, **h**) A 12-year-old girl showing facial features intermediate between those of childhood and adult (Courtesy of Dr. Ian Krantz, CHOP)

hypertelorism, a pointed chin, and a saddle or straight nose with a bulbous tip. The combination of these features gives the face an inverted triangular appearance. The facies are difficult to detect early in infancy but become more apparent with increasing age. In adults the facial characteristics of ALGS appear to change; the forehead is less prominent and the protruding chin is more noticeable (see Fig. 11.9). The correct identification of these adults, who commonly have minimal signs and symptoms of ALGS, would help physicians in the evaluation of adults with apparently idiopathic cardiac, hepatic, or renal disease. It should be noted that amongst the few patients reported to date, there appears to be a lower penetrance of characteristic facial features in ALGS patients with *NOTCH2* mutations [13].

Skeletal Involvement

The most characteristic skeletal finding in ALGS is the sagittal cleft or butterfly vertebrae, which is found in 33–87 % of patients [6–9] (see Fig. 11.10). This anomaly may occur in normal individuals and is also seen in other multisystem abnormalities, such as 22q deletion syndrome. The affected vertebral bodies are split sagittally

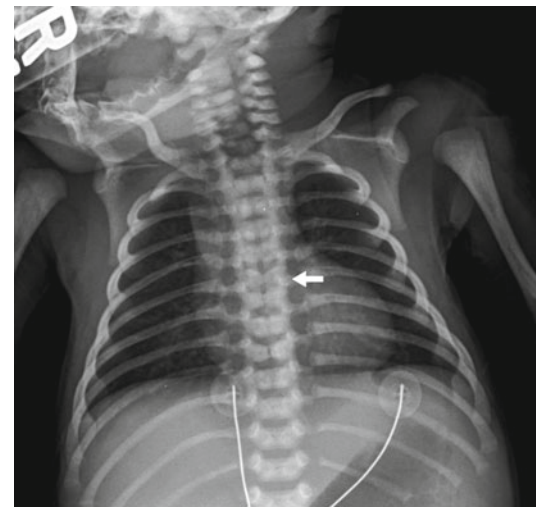

Fig. 11.10 Babygram in a neonate with congenital cardiac disease, demonstrating spinal abnormalities including a clear butterfly vertebra (*arrow*) (Courtesy of Dr. Sabah Servaes, CHOP)

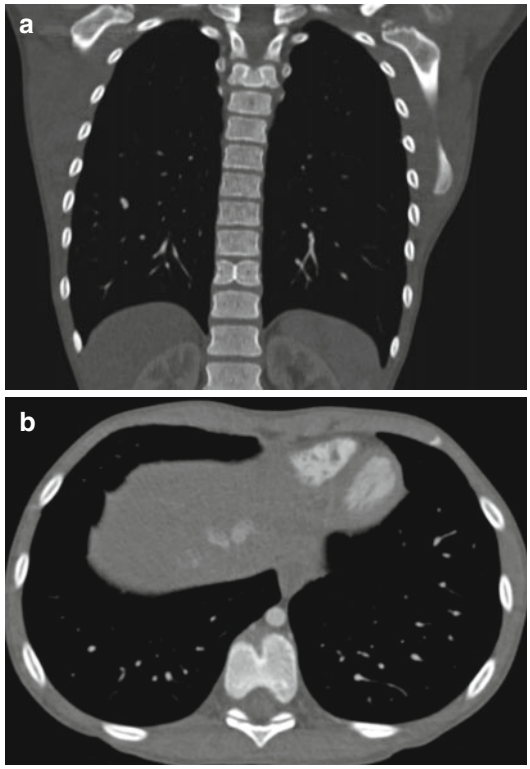

Fig. 11.11 CT scan with vertical reconstruction (**a**) and cross section (**b**) demonstrating butterfly vertebra at T10 (Courtesy of Dr. Sabah Servaes, CHOP)

into paired hemivertebrae because of a failure of the fusion of the anterior arches of the vertebrae (see Fig. 11.11). Generally, these are asymptomatic and of no structural significance. Other associated skeletal abnormalities include an abnormal narrowing of the interpedicular space in the lumbar spine, fusion of the adjacent vertebrae, hemivertebrae, the absence of the twelfth rib, and the presence of a bony connection between ribs. In addition, supernumerary digital flexion creases have been described in one-third of patients [52].

Severe metabolic bone disease with pathologic fractures is common in patients with ALGS. Recurrent fractures, particularly of the femur, have been cited as a major indication for hepatic transplantation. Preliminary survey data suggests that there is a propensity toward pathologic lower-extremity long-bone fractures in ALGS [53] (see Fig. 11.12). A number of factors may contribute to osteopenia and fractures, including severe chronic malnutrition and vitamin D and vitamin K deficiencies. There may also be an intrinsic defect in cortical or trabecular structure of the bones in patients with ALGS.

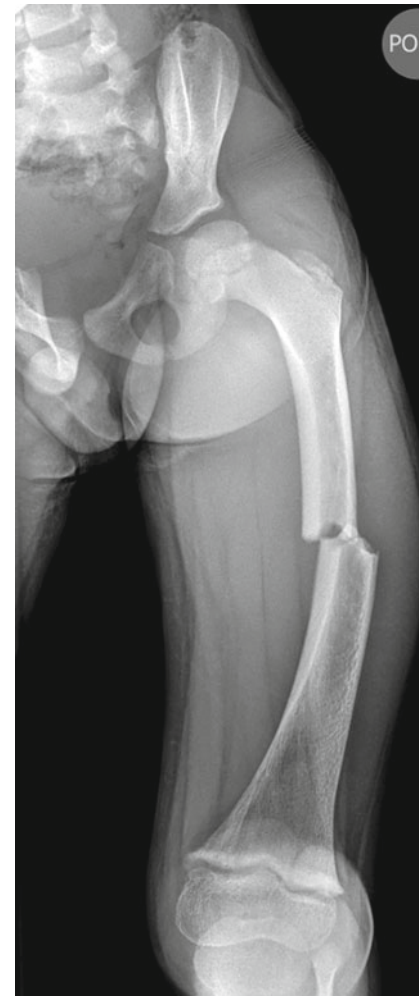

Fig. 11.12 A transverse fracture of the mid-shaft of the left femur, sustained atraumatically while running at play, in a 4-year-old with ALGS (Courtesy of Dr. Christina B. Bales, MD, CHOP)

ALGS patients frequently have short stature; and this is likely multifactorial in origin, resulting from cholestasis and malabsorption, congenital heart disease, and genetic predisposition.

Ocular Involvement

A large and varied number of ocular abnormalities have been described in ALGS, though posterior embryotoxon is the most common. Posterior embryotoxon is a prominent, centrally positioned

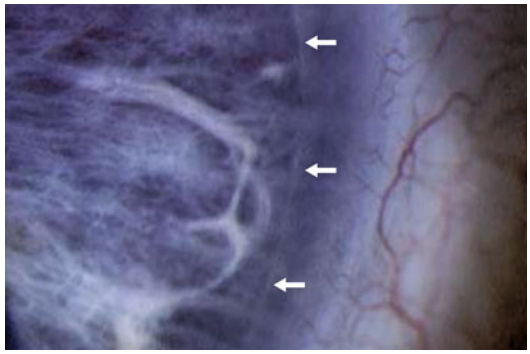

Fig. 11.13 Slit-lamp examination demonstrating posterior embryotoxon (*arrows*) in an infant with ALGS (Courtesy of Dr. William Anninger and Dr. Brian Forbes, CHOP)

Schwalbe's ring (or line) at the point at which the corneal endothelium and the uveal trabecular meshwork join and is most clearly identified during slit-lamp examination (see Fig. 11.13). Posterior embryotoxon occurs in 56–88 % of patients with ALGS but can also be detected in 22 % of children evaluated in a general ophthalmology clinic [54]. Posterior embryotoxon is also seen in other multisystem disorders such as chromosome 22q deletion. Other reported ALGS ocular anomalies include Axenfeld anomaly (seen in 13 % of ALGS patients), microcornea, keratoconus, congenital macular dystrophy, ectopic pupil, band keratopathy, cataract, iris hypoplasia, choroidal folds, and anomalous optic disks. In a large series of patients with ALGS studied systematically, Hingorani et al. identified posterior embryotoxon in 95 % of 22 patients, iris abnormalities in 45 %, diffuse fundic hypopigmentation in 57 %, speckling of the retinal pigment epithelium in 33 %, and optic disk abnormalities in 76 % [55]. Though not widely performed, ocular ultrasound can detect optic disk drusen in at least one eye in 95 % and bilateral disk drusen in 80 % of patients with ALGS [56].

Diagnostic Considerations

The diagnosis of ALGS has traditionally been based on clinical criteria defined as the presence of paucity of intrahepatic bile ducts in addition to three of the following major criteria: (1) cholestasis, (2) evidence of cardiac disease, (3) skeletal abnormalities, (4) ocular anomalies, and (5) characteristic facial features [2, 4]. The phenotype of ALGS has clearly broadened to include renal and vascular involvement, and these should now be included as disease-defining criteria.

The classical clinical diagnosis of ALGS rests on the presence of bile duct paucity and therefore requires a liver biopsy. However, a liver biopsy is no longer considered mandatory to make a diagnosis of ALGS. A liver biopsy is often necessary, however, to distinguish ALGS from biliary atresia in an infant with high-GGT cholestasis. When a liver biopsy is performed to differentiate between ALGS and BA, it should be noted that bile duct paucity is not always seen early on in life, whereas it is much more prevalent after 6 months of age [7]. Early biopsies of patients with ALGS may reveal bile duct proliferation or even giant cell hepatitis, thus further confusing the clinical picture with BA. Following a biopsy revealing bile duct proliferation and suggestive of obstruction, the interpretation of diisopropyl iminodiacetic acid (DISIDA) scans should be made with caution, as patients with ALGS can have non-draining scans. An intraoperative cholangiogram in the hands of an experienced surgeon remains the gold standard to differentiate BA from ALGS, since mutational analysis for ALGS cannot usually be performed rapidly.

It is now thought that three or four of the expanded clinical criteria are required to make the diagnosis of ALGS in children younger than 6 months of age. If there is a first-degree relative with a definitive diagnosis of ALGS, then only one or two criteria are needed. Of course the advent of molecular screening has clarified the diagnostic challenges in many subtle or atypical ALGS cases.

Molecular sequencing is now widely commercially available for *JAG1*. *NOTCH2* screening is more limited as a clinical test. A molecular diagnosis is not mandatory but can assist in an atypical ALGS case and is also useful for genetic counseling and prenatal diagnosis. *JAG1* sequencing identifies mutations in individuals with clinically defined ALGS in the majority

of cases (>90 %). Individuals that have clinical features of ALGS but are not found to be carrying *JAG1* mutations should have sequence analysis of *NOTCH2* [14].

If a mutation is identified in a child with ALGS, then the parents should be offered screening for the same mutation. *JAG1* mutations are inherited in 40 % of cases and are de novo in the remainder. If parents are found not to carry their child's mutation, the risk of disease recurrence in new offspring is very low. The only exception is in the case of germline mosaicism, which has been reported in patients with ALGS.

Prenatal genetic testing has been used to aid in the diagnosis of ALGS of a fetus. This requires amniocentesis or chorionic villous sampling and a known *JAG1* mutation in the family. Preimplantation genetic diagnosis has also been successfully performed in ALGS. It is imperative to carefully counsel parents undergoing any type of prenatal testing since there are no genotype–phenotype correlations in ALGS, so it is not possible to make predictions about a child's clinical course based on the type of mutation in the family or on the severity of disease in other family members.

Prognosis of Alagille Syndrome

The prognosis of Alagille syndrome varies tremendously depending on the extent and severity of the manifestations of the disease. The single most important predictive factor is complex congenital cardiac disease, which has been shown to contribute significantly to patient mortality in the first years of life [7]. The major long-term population studies of patient course and outcome have been performed at large hepatology centers, focusing mainly on patients with symptomatic liver disease diagnosed by having at least three of the major clinical criteria. In addition to this significant selection bias, a number of patients seen in these centers were referred expressly for transplantation, thus further artificially increasing the frequency of hepatic morbidity and mortality. Finally, most studies of outcomes include patients born decades ago, when cardiac, hepatic, nutritional, and transplant therapies were significantly less effective or not even available. During this time, it is also certain that many patients were not accurately diagnosed with Alagille syndrome, further complicating an accurate analysis. Since the advent of molecular diagnosis of mildly affected probands and less affected relatives, the denominator of Alagille "syndrome" *JAG1* mutation carriers has at least doubled, and the average outcome and severity have apparently proportionately improved, although this has not been formally reported. It is, however, this number that a *JAG1* mutation carrier should consider with a genetic counselor in order to make decisions about family planning.

In the largest series of long-term follow-up, Lykavieris et al. [27] report on their experience with symptomatic hepatic disease. For this study, all ALGS patients without hepatic disease were excluded. From 1960 to 2000, there were 163 patients with ALGS and liver disease, divided into two groups: 132 patients who presented with neonatal cholestatic jaundice and 31 who presented with hepatic disease later in life. Of the neonatal jaundice group, 102 remained jaundiced, 112 had poorly controlled pruritus, and 40 had xanthomas. Liver transplantation was performed on 33 %. Symptoms were considerably less in the 31 who presented at a later age, and none were referred for transplantation. For the total population of hepatic ALGS, the actuarial survival rates with native liver were 51 % at 10 years. and 38 % at 20 years. Overall survival rates were 68 % at 10 years and 62 % at 20 years. Surprisingly, the 10-year. survival rates for patients born before and after 1986 were the not different, at 67 versus 70 %, respectively. Of the 44 patients who underwent liver transplantation at a median age of 7 years old, indications were refractory pruritus in 36, xanthomas in 32, bone fractures in 15, and signs of end-stage liver disease in only five. The 10-year survival after liver transplantation in this study was 77 %. These results demonstrate that the liver disease in patients with ALGS, particularly those presenting in infancy, is severe in many instances, with significant morbidity and mortality.

In a report on 92 patients diagnosed with ALGS by classical clinical criteria between 1974 and 1997, Emerick et al. [7] found that the only early feature correlated with significant mortality was the presence of structural congenital cardiac disease. Patients and relatives who did not meet full clinical criteria were not included in this study, and essentially all patients had cholestatic liver disease. Liver transplantation for hepatic decompensation was necessary in 21 %, and 1-year posttransplant survival in this group was 79 %. The 20-year predicted life expectancy in this study was 75 % but only 60 % for the subset who required liver transplantation. Other significant factors contributing to mortality were intracranial bleeding and stroke [7].

Summary

In the nearly 50 years since Alagille described the syndrome, there have been significant advances in the diagnosis and treatments for Alagille syndrome. The finding that ALGS is caused by single-gene defects in the Notch signaling led to new insights into the role the pathway plays in controlling human embryogenesis. The easy and affordable availability of accurate mutation analysis has led to a more comprehensive understanding of the spectrum of Alagille "syndrome" and an expansion of the clinical presentations of *JAG1* mutation. Gene testing has provided clarity to patients who do not meet classical clinical criteria for the syndrome, and it has facilitated family planning and prenatal diagnosis. The role of Notch signaling is being extensively studied, and its role in bile duct development and tubulogenesis is predictable, given the manifestations of the disease. Vitamin and nutritional support greatly enhances the lives of children with ALGS, improving outcomes and preventing complications. Partial external biliary diversion can greatly alleviate pruritus in some patients. Advances in liver transplantation and immunosuppressive therapy have greatly improved the hepatic outcome, so that liver disease is a much less common cause of death and disability for patients.

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