Alagille syndrome: family studies

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Abstract

Alagille syndrome (AGS) is one of the major forms of chronic liver disease in childhood with severe morbidity and a mortality of 10 to 20%. It is characterised by cholestasis of variable severity with paucity of interlobular bile ducts and anomalies of the cardiovascular system, skeleton, eyes, and face. Previous studies suggest a wide variation in the expression of the disease and a high incidence of new mutations. To determine more accurately the rate of new mutations and to develop criteria for detecting the disorder in parents we systematically investigated parents in 14 families with an affected child. Clinical examination was supplemented by liver function tests, echocardiography, radiographic examination of the spine and forearm, ophthalmological assessment, and chromosome analysis. Six parents had typical anomalies in two or more systems pointing to the presence of autosomal dominant inheritance. Systematic screening of parents for the features defined in this study should improve the accuracy of genetic counselling.

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Alagille syndrome is a common cause of cholestatic liver disease in childhood, with an estimated incidence of 1 in 70 000 live births.¹ The association between intrahepatic cholestasis, a characteristic face, and a cardiac murmur was described in 1969 by Alagille *et al*² as a new and distinct form of cholestasis in infancy. Further reports by Watson and Miller³ and Alagille *et al*⁴ provided more evidence for a new syndrome. Liver disease occurs in association with paucity of interlobular bile ducts (intrahepatic biliary hypoplasia), detectable on liver biopsy. It is accompanied by cardiovascular abnormalities, in particular peripheral pulmonary stenosis, skeletal anomalies, and ophthalmological defects. The characteristic skeletal abnormality is butterfly vertebrae caused by a persistent sagittal cleft through the vertebral body; these may fuse with time and therefore may not be present in older patients with AGS. Other skeletal abnormalities such as shortening of the bones of the forearm and hands and narrowed interpedicular distance of the vertebrae may be present.^{5,6} The eye abnormality usually seen is posterior embryotoxon, an abnormal prominence of Schwalbe's line.⁷ Posterior embryotoxon is known to occur in 8 to 15% of the normal population.⁸

In a review of 80 cases in 1987, Alagille

*et al*⁹ suggested that there were five cardinal features of the syndrome: paucity of intrahepatic bile ducts, cardiovascular abnormalities, vertebral arch defects, posterior embryotoxon, and a characteristic face. In the same year, Mueller¹⁰ suggested that the diagnosis could be made in the presence of any three of six features: intrahepatic biliary hypoplasia, peripheral pulmonary stenosis, posterior embryotoxon, butterfly vertebrae, a characteristic face, and a first degree relative with AGS. In 1986, Byrne *et al*¹¹ found a deletion of the short arm of chromosome 20 in a baby with intrauterine growth retardation, jejunal stenosis and dysmorphic facial features associated with peripheral pulmonary stenosis, vertebral abnormalities, and cholestasis secondary to paucity of interlobular bile ducts. Reviewing previous reports of monosomy 20p they found that all had some features of Alagille syndrome. A further 13 cases of deletions of 20p associated with AGS have been described¹² including one case in which the deletion had been transmitted from an affected mother to her daughter. This points to the existence of a locus or loci on chromosome 20p that are responsible for producing AGS.

AGS is now established as being inherited in an autosomal dominant fashion,¹³⁻¹⁶ but with extreme variability of expression and a high rate of new mutation. Several families, including Watson and Miller's original families,³ have been described in which Alagille syndrome has been transmitted from one generation to the next with variation in the phenotype. In the cases so far published, a mild phenotype in the parent has led to a more severe phenotype in the offspring, leading to the suggestion that anticipation may occur in this disorder. In addition, Shulman *et al*¹⁷ suggested that inheritance from the mother resulted in a more severe phenotype leading this author to suggest similarities with the inheritance of myotonic dystrophy. However, there is little information to date about the proportion of affected children who inherit the disorder from a parent. Nor is there published information on the systematic evaluation of parents with regard to the abnormalities present in AGS. Knowledge of the minimal expression of the disease would enable more accurate counselling of the families of children with AGS.

The aim of the study, therefore, was to develop criteria to aid diagnosis in an affected parent. In addition we wished to determine in what proportion of affected children there was evidence of autosomal dominant inheritance of the disease and whether maternal or paternal transmission of the disease had an effect on the severity of the phenotype of the offspring.

Figure 1 Pedigrees of families in which there was evidence of autosomal dominant inheritance.

Patients and methods

The patients studied were ascertained through the Children's Liver Unit of King's College Hospital and The Hospital for Sick Children, Great Ormond Street, London. Index cases were included in the study if they had a definite diagnosis of Alagille syndrome based on the criteria of Mueller.¹⁰ All 14 index cases, 24 parents, and four sibs were examined at The Hospital for Sick Children. In four families only one parent was examined, the remainder being unavailable for study. All index cases and their parents had the following investigations performed: liver function tests (LFTs) (serum bilirubin, alkaline phosphatase, glutamyl transpeptidase, aspartate transaminase), serum cholesterol, blood chromosomes, radiographs of the spine, forearm, and hand, echocardiography, anterior and posterior segment ophthalmic examination, and ocular electrophysiology. The bones of the hand and interpedicular distances of the spine were measured. The results were analysed using an

unpaired *t* test to search for minor differences between the two parent groups, affected and unaffected.

Results

We found definite evidence for autosomal dominant inheritance of the syndrome in six of the 14 families studied. The pedigrees of the families displaying dominant inheritance are shown in fig 1. Subject II·7 in family 7 had an interesting family history. Two sisters died in infancy and another in childhood following repeated hospital admissions. All were known to have had heart murmurs but no other details were available. It is possible that they were also affected.

In three cases the father was the affected parent and in three the mother. In only one case had the affected parent previously suspected that he was affected. Only three sibs (family 1 III·3, family 7 III·5, family 12 III·4) were examined and none of them underwent

blood tests or radiography. However, on clinical examination, there was no evidence of the disease in any of the three.

In four of these six families there was a history of miscarriage, all occurring at over 10 weeks' gestation. Two mothers had two miscarriages, and a further two had one each, out of a total of 22 pregnancies. In the non-familial group of eight families there were two miscarriages out of a total of 22 pregnancies. Although there superficially appears to be an excess of miscarriages in the familial group, this did not reach statistical significance using χ^2 with Fisher's exact test.

The clinical findings and results of investigations in the affected parents are presented in table 1. All affected parents had posterior embryotoxon and at least one other major syndromic feature. Five had abnormalities of the spine and eye. In three, midline notches on the vertebral end plates were present representing fused butterfly vertebrae. Four also had a short ulna. Two had anomalous optic discs and a pigmentary retinopathy. Electrophysiology of the eye was normal in all cases, including the parent with pigmentary retinopathy. Three had pulmonary murmurs on clinical examination but in the two who underwent echocardiography no abnormality was detected. In only two was there any abnormality of the liver function tests, in one a mildly raised bilirubin, and in the other a mild rise of alkaline phosphatase. The mother in family 14 and the father in family 6 had a history of jaundice in infancy which was unexplained and recovered spontaneously. In all parents blood chromosomes were normal. Using an unpaired *t* test, no significant difference was found between affected and unaffected parents in any of the following parameters: lengths of the bones in the hand, lumbar interpedicular distance, aspartate transaminase, albumin, bilirubin, and triglycerides. However, the alkaline phosphatase levels were significantly higher in affected parents with a *p* value of <0.05.

The severity of the disease in the affected parent varied and did not correlate with the severity in the child. Neither did the sex of the

parent influence the severity of disease in the child. For example, in family 1, the affected mother (II.3) gave no history of jaundice in childhood and had no biochemical evidence of liver disease. She did have skeletal abnormalities and posterior embryotoxon (table 1). She had a daughter (III.2) with severe liver disease, requiring liver transplantation. III.2 also required surgery for an anomalous left coronary artery at the age of 1 year and had peripheral pulmonary stenosis. In contrast, both the mother of family 12 (II.2) and her daughter (III.3) had abnormalities in all four systems.

Discussion

Evidence for autosomal dominant inheritance was present in six of the 14 families studied, representing 43% of those studied. The majority of children therefore represented possible new mutations. We found no evidence for anticipation as was suggested by Shulman *et al.*¹⁷ There was no significant difference in the phenotype of children who had inherited the disorder from their mother as compared with those who inherited from their father, suggesting that comparisons with myotonic dystrophy are not valid.

Although previous studies have suggested that the new mutation rate is high in this disorder, the number of children with the syndrome attributable to a new mutation has been far from clear. Few family studies have been published and the numerous case reports of the syndrome may be biased towards reporting of those in which there is a family history. It has therefore been difficult to elucidate the proportion of affected children who represent new mutations, a figure that would aid genetic counselling. Our data suggest that at least 50% of those with AGS represent new mutations, deduced from the fact that investigation of their parents was normal. These results concur with segregation analysis performed by Dhome-Pollet *et al.*¹⁶

AGS has frequently been described as a disease displaying variable penetrance. Strictly, penetrance means the frequency with which

Table 1 Characteristics of affected parents

	Family					
	1	6	7	8	12	14
Parent affected	Mother	Father	Father	Father	Mother	Mother
Jaundice in infancy	No	Yes	No	No	No	No
LFTs	Normal	Raised alkaline phosphatase (131 U/l)	Normal	Normal	Bilirubin 23 µmol/l (ref range <12 µmol/l)	Not done
Cholesterol (mmol/l)	Not done	8.1	5.1	Not done	4.8	Not done
Echocardiography	Normal	Normal but pulmonary murmur present	Normal but pulmonary murmur	Normal	Not done, pulmonary murmur	Not done
Radiography	Midline notches D6-D8, short ulna	Scoliosis, short ulna	Tight scoliosis, short ulna	Midline notches on vertebral end plates	Midline notches on vertebral end plates D7, D8, D10, short ulna	Normal spine, short 4th metacarpal
Ophthalmic examination	Posterior embryotoxon	Posterior embryotoxon, divergent strabismus, anomalous discs	Posterior embryotoxon	Posterior embryotoxon	Posterior embryotoxon, pigmentary retinopathy, anomalous discs	Posterior embryotoxon

those carrying the gene express it. Complete penetrance implies that all who have the gene express it, variable penetrance or incomplete penetrance implies that there are some people who carry the gene but do not express it. The only possible historical evidence for incomplete penetrance in Alagille syndrome is the family described by Mueller *et al*¹³ in which two apparently normal parents had two affected children. In no published case has the disease been seen to skip a generation. We did not examine any of the grandparents but there was no evidence from the family histories of the unaffected parents of non-penetrance of the gene. It is possible that the expression of the disease was very mild in the family of Mueller *et al*¹³ and went undetected, but an alternative explanation would be that of gonadal mosaicism. One mechanism known for non-penetrance is imprinting. Reviewing published family data for evidence of imprinting we found no evidence for its presence in AGS. In our group three children inherited Alagille syndrome from their fathers and three from their mothers; there was no difference in phenotype according to the parent of the origin. Our findings do not support imprinting as an important mechanism in

AGS. Only one sib in our group appeared to be affected, out of eight, which is at odds with autosomal dominant inheritance with complete penetrance. However only three sibs were examined, and none underwent investigation. It is therefore possible that some of them may have been mildly affected and went undetected.

It has been postulated that AGS may represent a contiguous gene syndrome.¹⁸ None of the probands had dysmorphic features additional to those seen in AGS. Three of the 14 probands had significant learning difficulties or motor developmental delay. Of these, one had ataxic cerebral palsy in addition to AGS attributed to hypoxic-ischaemic encephalopathy, and another had severe liver disease requiring transplantation at the age of 3 years. Post-transplantation he began to make rapid developmental progress. We found no firm evidence to suggest that AGS is a contiguous gene syndrome.

In addition, we found no evidence for the existence of anticipation in this disorder. In three families the affected child appeared to have similar disease severity to the parents. If subjects II-3, II-4, and II-5 in family 7 were affected, their early deaths imply that their disease was more severe than that of both II-7 and III-1. It is more likely that those that are mildly affected survive and reproduce and those that have more severe disease die or are unable to reproduce, resulting in the superficial appearance of anticipation. In addition, there was no evidence for a "maternal factor" resulting in increased severity of disease when the disease was transmitted from an affected mother to her offspring.

There is increasing evidence that AGS is not as benign as was originally thought, and there are reports of long term complications (notably, hepatocellular carcinoma and late onset liver failure) occurring in AGS.¹⁹ It is not known whether those parents retrospectively ascertained run a risk of developing the complications associated with the syndrome, although there is no documented case of these complications occurring in a patient with no clinical or biochemical evidence of liver disease. Until more is understood about the long term natural history of this disease it will be difficult to be reassuring when counselling these families.

In order to provide accurate genetic counselling to the families of children with Alagille syndrome it is important to be able to distinguish between those that represent new mutations and those that have inherited AGS from an affected parent. The extreme variability of expression of the syndrome has made this a difficult task. Based on this small study a set of major and minor criteria was developed for aiding diagnosis in a family which presents with an affected child. Major criteria are established for the diagnosis of children with Alagille syndrome,^{9,10} but similar criteria do not apply to adults with the disorder. The major and minor criteria are shown in table 2. All parents of children with AGS will fulfil at least one of the major criteria, that of having a first degree relative with Alagille syndrome. In addition, all

Figure 2 (Above) Proband and affected father from family 7. (Below) Proband from family 8 with affected father and unaffected mother.

Table 2 Major and minor criteria for use in diagnosis of parents

Major criteria	Minor criteria
History of prolonged jaundice in infancy requiring investigation	Alkaline phosphatase >103 U/l
Pulmonary murmur	Short ulna
Posterior embryotoxon	Pigmentary retinopathy
Vertebral end plate notches	Anomalous optic discs
First degree relative with Alagille syndrome (omit if proband)	

Table 3 Number of major and minor criteria present in affected parents

Parent	Major	Minor
1	3	1
6	4	3
7	3	1
8	3	0
12	4	3
14	3	0

our affected parents had at least two major criteria, and in those that had been completely investigated, between one and three minor criteria (table 3). In our group of six affected adults, this represented the minimal expression of the syndrome.

The variability of expression can make it difficult to be categorical about whether a parent is affected or not. It is clear from our evaluation of 14 families that a detailed history, examination, and investigation are required to distinguish the affected group from the unaffected group, and the development of major and minor criteria for diagnosis may help in the future evaluation of families.

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