

Segregation analysis of Alagille syndrome

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Abstract

Alagille syndrome (AGS) is a well defined genetic disorder characterised by five major features. An autosomal dominant mode of transmission with reduced penetrance has been suggested by the analysis of a limited number of families. However there has been no statistical analysis. We report here the first segregation analysis of AGS, using 33 families collected through 43 probands. Segregation analysis of these families allowed us to conclude that AGS is transmitted as a dominant disorder with 94% penetrance and 15% of cases are sporadic. The expressivity of the phenotype was variable and 26 persons (15 parents and 11 sibs) were identified as presenting minor forms of the disease. These results are valuable for genetic counselling.

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Alagille syndrome (AGS, MIM 118450) or syndromic paucity of interlobular bile ducts is a well defined genetic entity characterised by five major features¹⁻³: chronic intrahepatic cholestasis owing to paucity of interlobular bile ducts; peripheral pulmonary artery hypoplasia or stenosis; butterfly-like vertebral arch defects; posterior ocular embryotoxon and peculiar facies. Various minor or additional signs (including growth and mental retardation and renal disorders)⁴⁻⁶ may also be observed. The association of the five main features constitutes the complete form of the syndrome. However, it is accepted that diagnosis can be established if at least three of the five main features are present.²

AGS represents the second most frequent cause of intrahepatic cholestasis in infancy with a prevalence of one in 70 000 live births.⁷ The evolution of the disease is variable: only 12% of patients develop cirrhosis⁸ but approximately 20% require liver transplantation either for hepatic failure or refractory pruritus and severe malnutrition.⁹

In 1986 Byrne *et al*¹⁰ reported for the first time the association of AGS with an interstitial deletion of the short arm of chromosome 20 in an isolated case. At present, only 18 other cases of microdeletion of the short arm of chromosome 20 have been described. Nine were clearly associated with AGS, the remaining nine exhibiting some of the major features of AGS.¹¹⁻¹⁷ Both chromosome 20 abnormalities and AGS are rare and therefore this disease can be assigned to the short arm of chromosome 20.^{10 12-14}

An autosomal dominant mode of transmission with reduced penetrance and variable expressivity was first suggested in 1978. The analysis of a small number of exceptional families has led to this view being widely accepted. However, there has been no statistical analysis of a large number of families to determine the mode of transmission.¹⁸⁻²¹

We report here the largest sample of AGS families yet described, allowing us to perform a segregation analysis. Thirty-three informative nuclear families which had been ascertained through an affected child were studied to test the hypothesis of an autosomal dominant mode of transmission of the disease and to estimate the penetrance (probability of a gene carrier being affected) and the proportion of sporadic cases (new mutations or phenocopies).

Materials and methods

DATA

The data used in this analysis were from studies of families of children with AGS diagnosed in the paediatric hepatology unit of Hôpital de Bicêtre over the last 30 years. The initial sample was composed of 79 probands followed in this hospital. This sample is different from that published in the previous review of 80 cases in 1987 by Alagille *et al*.² Eighteen patients were lost to follow up and parts of their medical records were not available, and 17 patients born since 1987 were included.

The results of previous family investigations (parents and sibs) were collected from the medical records of the 79 probands. When data were missing, family members were asked by mail to attend an outpatient appointment.

FAMILY INVESTIGATIONS

The affected status of the parents and sibs was difficult to determine because of the variable expressivity of AGS. The affected phenotype was assessed by several investigations of the members of nuclear families.

The clinical examinations performed were as described by Alagille *et al*¹ and comprised: ophthalmic examination using a slit lamp or gonioscopic examination to detect posterior embryotoxon; chest x ray to identify the butterfly-like vertebral arch defects which are mainly present at the dorsal level; physical examination of the heart to identify cardiac murmur; presence of cholestasis was only assessed by clinical investigations (jaundice, pruritus, xanthomas). Peculiar facies (prominent forehead, deep set eyes, mild hypertelorism, straight nose, and small pointed chin) was not included among the features to be

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studied in families because of its subjectiveness.

Only 33 families were retained for segregation analysis: 23 families were excluded because of the absence of sibs, three because of paternity exclusion detected by marker typing (data not shown), and 20 because not all the data were available. The study group of 33 families can be divided into three groups: families with parents examined, families with parents partially examined, and families with parents not examined but obligate carriers because of an affected collateral relative.

ESTIMATION OF ASCERTAINMENT PROBABILITY

The ascertainment probability π is the probability that an affected child is a proband. Assuming that it is independent of the number of affected sibs and the severity of the condition, this parameter may be estimated from the distribution of probands among affected cases in sibships with at least two affected cases (multiplex sibships) by the maximum likelihood method, using the formula:

$$P_{(a,r)} = \frac{C_a^r \pi^a (1-\pi)^{r-a}}{1 - (1-\pi)^r}$$

where r is the number of persons affected, and a the number of probands among persons affected in the sibship.²²

SEGREGATION ANALYSIS

Segregation analysis was performed using the 33 nuclear families of the study group by the scoring procedure developed by Fisher and adapted for segregation analysis by Morton.²³ The model is characterised by a proportion, x , of sporadic cases (not inherited) and a proportion, $1-x$, of genetically inherited cases with a segregation frequency, p . The segregation frequency is the probability that a child be affected in multiplex and simplex families of the same origin where AGS segregates. This frequency is presumed to be the same in all families and is characteristic of the transmission mode of the disease.

It is possible by extension of existing formulae²⁴⁻²⁶ and by use of maximum likelihood scores²⁷ to estimate the segregation frequency, p , and the proportion of sporadic cases, x , using the Newton-Raphson iteration method. Let: p = the segregation frequency, x = the proportion of sporadic cases (recent mutations or phenocopies), s = the size of the sibship, r = the number of affected children.

Families with an affected or obligate carrier parent

Families with an affected patient or with an obligate carrier parent (because of an affected collateral relative) are genetic cases. The likelihood, $L_{1(p)}$, of sibships containing at least one proband has to take into account the probability of ascertainment π as follows:

$$L_{1(p)} = \frac{C_s^r p^r (1-p)^{s-r} (1-(1-\pi)^r)}{1 - (1-p\pi)^s}$$

Families without an affected or carrier parent

The cases in these families are genetic if there is more than one affected child. If there is only one affected child (simplex families), this isolated case may be either a true sporadic case or a chance isolated case. Therefore, the probability that a sibship be simplex is given by:

$$L_{2(p,x)} = \frac{sp\pi(x + (1-x)(1-p)^{s-1})}{xsp\pi + (1-x)(1-(1-p\pi)^s)}$$

and the probability that a sibship be multiplex by:

$$L_{3(p,x)} = 1 - L_{2(p,x)} \\ = \frac{(1-x)(1-(1-p\pi)^s - \pi sp(1-p)^{s-1})}{xsp\pi + (1-x)(1-(1-p\pi)^s)}$$

The distribution of affected children in multiplex sibships ($r \geq 2$) gives information about p . $L_{4(p)}$ is the likelihood for a sibship of size s with r affected children:

$$L_{4(p)} = \frac{C_s^r p^r (1-p)^{s-r} (1-(1-\pi)^r)}{1 - (1-p\pi)^s - \pi sp(1-p)^{s-1}}$$

The likelihood for the whole sample is computed iteratively, using a priori p and x values, until the maximum likelihood is reached, providing the estimates of p and x .

Results

DETERMINATION OF THE PHENOTYPE IN MEMBERS OF NUCLEAR FAMILIES

The frequencies of the five major features in the sample of 43 probands in the 33 different families of the study group were similar to those in the whole sample of 79 patients and those described by Alagille *et al*² in 1987 in a review of 80 cases (table 1).

Among the 66 parents and 64 sibs, 45 and 24 respectively underwent at least one of the three following clinical investigations; ophthalmic examination, chest x ray, and physical examination of the heart. No subject exhibited clinical signs of cholestasis. The 21 parents and 40 sibs that were not examined were considered to

Table 1 Frequencies of major abnormalities in Alagille syndrome

	Frequency (%) of major abnormalities among:		
	Previously described 80 cases ²	Whole sample of 79 probands	43 probands of 33 families used for segregation analysis
Chronic cholestasis	91	98.7	100
Peripheral pulmonary artery stenosis	85	100	100
Vertebral abnormalities	87	69.7	84.3
Posterior embryotoxon	88	86.6	87.5
Characteristic facies	95	91	87.5

Table 2 Number of parents (A) and sibs (B) with one or more features of Alagille syndrome with respect to the number of investigations performed

A					
No of investigations performed in parents	No of major features				No of parents
	3	2	1	0	
3	2	2	2	12	18
2		2	3	9	14
1			4	9	13
Total	2	4	9	30	45

B					
No of investigations performed in sibs	No. of major features				No of sibs
	3	2	1	0	
3	1	2	3	3	9
2		0	0	2	2
1			5	8	13
Total	1	2	8	13	24

Table 3 Frequencies of major features of Alagille syndrome among parents and sibs of the probands

	Systolic murmur		Butterfly-like vertebral arch defects		Posterior embryotoxon	
	Parents	Sibs	Parents	Sibs	Parents	Sibs
Number examined	39	23	32	10	32	12
Frequency of feature (%)	28.2	21.8	12.5	0	25	50

Table 4 Number of affected parents and sibs with respect to total number examined

	Total No	No examined	No affected
Parents	66	45	15
Sibs	64	24	11

be negative for these investigations. The distribution of the positive findings according to the number of investigations performed is given in table 2A and B respectively for parents and sibs. The frequencies of each symptom observed (cardiac murmur, butterfly-like vertebral arch defect, and posterior embryotoxon) are given in table 3. The frequencies of these symptoms in the relatives of the probands are higher than in the general population (see below). We defined the affected phenotype in members of nuclear families by the presence of at least one of the four major features investigated (table 4).

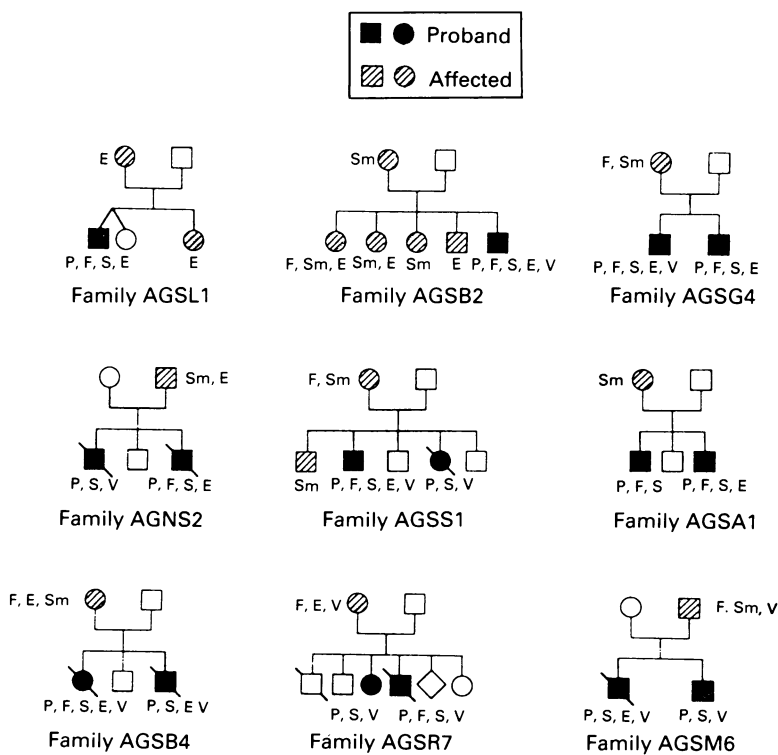
The families were divided into three categories: 14 simplex families without affected parents, four multiplex families with obligate carrier parents, and 15 simplex or multiplex families with affected parents. The pedigrees of the nine multiplex families with affected parents are given in the figure.

ASCERTAINMENT PROBABILITY

Among the 33 sibships of the affected children, 13 were multiplex. Using the distribution of probands among affected cases in these sibships, we estimated the probability of ascertainment, π , to be 0.66 (SE 0.10).

ESTIMATION OF p AND x

The segregation ratio p for the whole sample was estimated to be 0.47 (SE 0.19), leading to a 94% estimate for penetrance, under a dominant mode of transmission. This penetrance is not significantly different from 1. Among families without affected parents the estimated proportion of sporadic cases is 0.45 (SE 0.07). In the whole sample, the corresponding proportion is 0.15. These results allowed us to conclude that AGS has an autosomal dominant



Pedigrees of the multiplex families with affected parents. P=paucity of interlobular bile ducts, S=pulmonary stenosis, Sm=systolic murmur, E=embryotoxon, V=butterfly vertebrae, F=peculiar facies.

mode of transmission with complete or almost complete penetrance and 15% sporadic cases.

Discussion

This analysis of 33 nuclear families shows that AGS has an autosomal dominant mode of transmission with a nearly complete (94%) penetrance. We also report the first estimation of the proportion of sporadic cases which was 15%. This study thus confirms the dominant mode of transmission, first proposed in 1978, based on observation of several generations of isolated families²⁰ and a limited number of pedigrees.^{10,12,21} The penetrance appears to be higher than previously described.

The major problem of the analysis is that not all parents and sibs of the probands underwent complete clinical examination, resulting in an underestimation of the frequency of the symptoms. This leads to an underestimation of the penetrance and to an overestimation of the proportion of sporadic cases. These possible errors do not affect our conclusion that AGS is a dominant disease with close to complete penetrance.

We postulate that the presence of only one feature (the facies being excluded) is sufficient for considering a family member to be affected with AGS. The justification for this is that these symptoms are rare in the general population. The incidence of congenital cardiopathies²⁸ in the general population^{29,30} is 0.2 to 0.8%, of which 3 to 6% are stenosis of the pulmonary artery and its branches. No statistics are available for the frequency of butterfly-like vertebrae, either among vertebral anomalies or in the general population. There are very few published reports describing this anomaly, reflecting its rarity,^{31,32} and it is a rare condition especially at the dorsal level (D Pariente, personal communication). Embryotoxon is the most frequent symptom of AGS in the general population (affecting about 8 to 10%).³³ However, the frequency of 31.8% observed in parents and sibs examined makes it likely that the relatives of a proband with only this feature have a minor form of AGS. We excluded peculiar facies as a criterion for AGS in sibs and parents of an affected child because it cannot be objectively measured.³⁴ The inclusion of this marker would lead to a higher estimation of the frequency of the carrier state.

The 45% proportion of sporadic cases in families without affected parents indicates that parents without major features of AGS and with an isolated affected child have a 55% (1-0.45) probability of being a gene carrier. This result provides information for genetic counselling.

The expressivity of the phenotype in probands' relatives is variable: parents and sibs with only one or two major features are considered as presenting minor forms of the disease. The previous suggestion that a maternal factor increases the severity of the clinical expression in affected offspring²¹ is not supported by our observations. Nevertheless, in our sample of 33 families, mothers were affected

in 12 families whereas fathers were only affected in three families. This difference is significant ($p=0.02$). Therefore, our data are consistent with an excess of transmitting females. The reasons for this excess are unclear.

The characteristics of Alagille syndrome, which include an autosomal dominant mode of transmission, small chromosomal deletions detected cytogenetically in some patients, familial aggregation, sporadic cases, and variability in phenotype, suggest that Alagille syndrome belongs to the family of contiguous gene syndromes.^{35,36}

One of our patients has a microdeletion of the 20p11.23-12.2 region. There have been previous similar reports.^{10,12-14} The locus involved in Alagille syndrome probably therefore lies in this region. This can be tested by further linkage analysis in familial cases without this microdeletion. The segregation analysis reported here provides the parameters necessary for such a linkage analysis.

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