Localization of Alagille syndrome to 20p11.2–p12 by linkage analysis of a three-generation family

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Abstract Alagille syndrome (AGS) or arteriohepatic dysplasia is a rare but well-defined clinical entity that is usually inherited as an autosomal dominant trait. A limited number of patients carry a deletion in chromosome 20p, with 20p11.23–p12.2 as the area of minimal overlap. Recently, a family has been identified in which a balanced translocation with a breakpoint in 20p12 co-segregates with the AGS phenotype. Here, we report a three-generation family with AGS and in which the affected members have a normal karyotype. Linkage analysis was performed with markers from the 20p candidate region. A lod score of Z=2.96 was obtained with D20S27 at no recombination. Combining D20S27 and D20S61 to a single highly informative locus resulted in a maximum lod score of Z=3.56 at θ=0.0. Haplotype analysis positioned AGS between D20S59 and D20S65, markers that define an interval of about 40 cM. Allelic loss was not observed for the tested markers and no abnormalities in the PAX1 candidate gene were detected. These findings demonstrate that the locus on chromosome 20p could be responsible for AGS in cytogenetically normal patients and argues for a general role of this locus in the aetiology of AGS.

Introduction

Alagille syndrome (AGS; MIM 118450) is a rare (1:70000 births) but well-defined condition with the following major characteristics: interhepatic biliary hypoplasia, peripheral pulmonary artery stenosis, posterior embryotoxon, typical facies and butterfly vertebrae (Watson and Miller 1973; Alagille et al. 1975, 1987; Mueller 1987). It is usually inherited as an autosomal dominant trait with almost complete penetrance (Dhome-Poliet et al. 1994), whereas the severity of the disorder can vary considerably among affected relatives (Shulman et al. 1984). The aetiology of AGS is largely unknown. An interstitial deletion at chromosome 20p11.2–p12 has been detected in a limited number of patients indicating that this chromosomal region encompasses a locus for AGS (Byrne et al. 1986; Schnittger et al. 1989; Zhang et al. 1990; Legius et al. 1990; Anad et al. 1990; Teebi et al. 1992; Desmaze et al. 1992). This has been substantiated by the detection of a family in which a balanced translocation t(2;20) (q21.3;p12) co-segregates with the AGS phenotype (Spinner et al. 1994). However, the relevance of this locus for patients with no cytogenetic abnormalities remains to be demonstrated. Here, we report the results of linkage analysis in a three-generation family with an autosomal, dominantly inherited AGS using markers from the 20p11.2–p12 candidate region.

Materials and methods

Genotyping

Venous blood was sampled from the relevant family members of the present family (Fig. 1), and genomic DNA was isolated by the procedure of Miller et al. (1988). The following highly polymorphic microsatellite markers located at chromosome 20p11.2–p12 were employed in the analysis: D20S27, D20S41, D20S48, D20S50, D20S58, D20S59, D20S61, D20S65 and D20S66. Information concerning the relative order of these markers and the genetic distances (Fig. 2) was obtained from the literature (Hazan et al. 1992; Keith et al. 1992) and from the Genome Database. Genotyping involved polymerase chain reaction (PCR) amplification of 50 ng genomic DNA in a total volume of 15 μl with 0.3 μM of each locus-specific primer, 200 μM of each dATP, dGTP, dTTP and 2.5 μM dCTP, 0.6 μCi α32P-dCTP (10 mCi/mmol, 3000 Ci/mmol; Amersham) in PCR buffer [50 mM TRIS-HCl pH 9.0, 50 mM KCl, 7 mM MgCl2, 16 mM (NH4)2SO4] with 0.06 U SuperTaq DNA poly-

Original Investigation

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Results

Clinical report

The family (Fig. 1) reported here is of mixed Dutch-Indonesian origin and was ascertained when the proband (III-1) was referred for genetic counselling to our department. Incorrectly, the referral diagnosis was autosomal recessive congenital liver fibrosis. Analysis of the pedigree and medical information from affected family members revealed the correct diagnosis, viz. AGS or arteriohepatic dysplasia. The diagnostic criteria as formulated by Mueller (1987) were applied to all studied family members, except to some individuals of generations I and II (Fig. 1) who were either living in Indonesia or who refused an examination. With the exception of female II.6, they were all obligate carriers, as revealed by their position in the pedigree. When considered necessary for the diagnosis, additional clinical investigations (echocardiography, ophthalmologic examination, radiography of spinal column, liver function test and physical examination for facial characteristics, viz. prominent forehead, deep set eyes, long nose with flattened tip and prominent chin) were performed. Cardiovascular abnormalities were detected in six out of eight patients, embryotoxon in two out of four, abnormal liver functioning in five out of seven, vertebral abnormalities in three out of four, and six out of six patients had facial characteristics of AGS. Cytogenetic analysis of III-6 (Fig. 1) revealed a normal karyotype.

Mapping of the AGS locus

Linkage analysis was performed with markers from the 20p candidate region. Positive lod scores were obtained with most of these markers (Table 1), whereas two markers, D20S48 and D20S50, were not informative. With D20S27, the maximum lod score was Z=+2.96 at 0.0 recombination. When D20S27 and D20S61 were regarded as a single locus to optimize the informativity, linkage analysis resulted in a lod score of Z=+3.56 at 0.0 recom-

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<th>Locus</th>
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<tr>
<td>D20S59</td>
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<td>D20S65</td>
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Table 1: Results of two-point linkage analysis.

Fig. 1: Linkage analysis of a family with autosomal, dominantly inherited AGS. Individuals who were not available for extensive clinical testing but who could be regarded as gene carriers from their position in the pedigree have been depicted by an open carrier symbol. The question mark in the symbol of female II.6 refers to her unknown clinical status, because she is not an obligate carrier and she also refused clinical examination. Haplotypes were constructed from seven informative markers. The haplotype that co-segregates with the disorder is shown by a hatched bar.
bination. Recent physical mapping studies by one of us (I.H.) indicate that D20S41 is located between D20S27 and D20S61. This alternative order of markers would not interfere with the present results, because a combination of D20S27, D20S41 and D20S61 into a single informative locus results in the same lod score at \( \Theta = 0.0 \). To define the AGS locus more accurately, haplotypes were constructed with most of the markers employed for linkage analysis (Fig. 1). This places the disorder between D20S59 and D20S65, a region of approximately 40 cM.

The gene for PAX1, which has been mapped by fluorescence in situ hybridization at 20p11.2 (Schnittger et al. 1992), has been proposed as a factor contributing to the AGS phenotype. To screen the DNA of patients from the present family for abnormalities of the PAX1 gene region, a probe for Southern blot hybridization was produced by PCR amplification using the published sequence (Burri et al. 1989). On Southern analysis, the normal band pattern and signal intensities indicate that the patients carry the two normal copies of the PAX1 gene (not shown).

**Discussion**

Linkage analysis of a large family with AGS has allowed us to map the disorder between D20S59 and D20S65 at 20p11.2–p12, a region of the genome that carries a deletion in some patients with AGS (Schnittger et al. 1989; Zhang et al. 1990; Legius et al. 1990; Anad et al. 1990; Teebi et al. 1992; Desmaze et al. 1992) and that harbours the breakpoint of a balanced translocation in patients from a single family (Spinner et al. 1994). At present, our results do not permit a further refinement of the mapping of the AGS locus, because the segment of 20p that co-segregates with the disorder spans the minimal area of overlap of deletions and of the translocation breakpoint (Fig. 2). Recently, additional markers have been reported that may be of use in obtaining a more concise location of AGS (Gyapay et al. 1994). However, the main conclusion of the present study remains that the candidate locus on 20p could be responsible for AGS in karyotypically normal patients.

Studies in the mouse suggest that the gene for PAX1, located at 20p11.2, could play a role in the aetiology of AGS. The expression pattern of the murine homologue indicates that the PAX1 protein is involved in the formation of the vertebral column (Deutsch et al. 1988, Wallin et al. 1994), whereas mutations in the gene give rise to vertebral abnormalities as observed for the mouse strain "undulated" (Balling et al. 1988). However, in the present family, no abnormalities of this gene were detected by Southern analysis. Moreover, this gene and two other candidate genes coding for hepatic nuclear factor 3B and cystatin C, respectively, have recently been excluded from the area of minimal overlap of deletions associated with AGS (Deleuze et al. 1994b). Apparently, hemizygosity of these genes is not involved in AGS. However, it cannot be excluded that an altered expression of those genes, as caused by positional effects, could influence the AGS phenotype in patients with a chromosomal abnormality.

In summary, although the segregation of a microdeletion or any other small chromosomal abnormality in the present family cannot be excluded, our findings show that the locus on chromosome 20p could underlie AGS in karyotypically normal patients, arguing for a general involvement of this locus in AGS. However, definitive evidence of a major locus for AGS on 20p has to await the identification of the gene(s).

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**References**


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