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Jagged1 Mutations in Patients Ascertained With Isolated Congenital Heart Defects

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Mutations in *Jagged1* cause Alagille syndrome (AGS), a pleiotropic disorder with involvement of the liver, heart, skeleton, eyes, and facial structures. Cardiac defects are seen in more than 95% of AGS patients. Most commonly these are right-sided defects ranging from mild peripheral pulmonic stenosis to severe forms of tetralogy of Fallot. AGS demonstrates highly variable expressivity with respect to all of the involved systems. This leads us to hypothesize that defects in *Jagged1* can be found in patients with presumably isolated heart defects, such as tetralogy of Fallot or pulmonic stenosis. Two patients with heart defects of the type seen in AGS and their relatives were investigated for alterations in the *Jagged1* gene. *Jagged1* was screened by a combination of cytogenetic and molecular techniques. Patient 1 was studied because of a four-generation history of pulmonic stenosis. Molecular analysis showed a point mutation in *Jagged1* in the patient and her mother. Patient 2 was investigated owing to the finding of tetralogy of Fallot and a "butterfly" vertebra on chest radiograph first noted at age 5 years. She was found to have a deletion of chromosome region 20p12 that encompassed the entire *Jagged1* gene. The identification of these two patients suggests that other patients with right-sided heart defects may have subtle findings of AGS and *Jagged1* mutations. Am. J. Med. Genet. 84: 56–60, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: tetralogy of Fallot; pulmonic stenosis; Alagille syndrome; *Jagged1*; congenital heart disease

INTRODUCTION

Congenital heart disease is the most common birth defect, affecting approximately 1% of infants; its incidence is estimated at close to ten times that level among stillbirths [Hoffman, 1995]. Heart defects are seen both as isolated findings and as components of syndromes. Studies of recurrence risks amongst sibs of index cases with isolated cardiac malformations have ranged from 2% to 5%, indicating a genetic contribution [Burn and Goodship, 1996]. Several syndromes include heart defects as consistent parts of the phenotype. Examples of such syndromes include Down syndrome (atrioventricular canal defects), DiGeorge/velocardiofacial syndromes (conotruncal defects), Williams syndrome (supravalvular aortic stenosis, pulmonary vascular involvement), and Alagille syndrome (pulmonary artery defects). Identifying the specific genes involved in these and other complex developmental disorders will contribute to our understanding of the molecular processes involved in cardiac development. *Jagged1* has been identified as the disease gene for Alagille syndrome (AGS) [Li et al., 1997; Oda et al., 1997]. *Jagged1* is a ligand in the Notch signaling pathway, a pathway that has been studied extensively in *Drosophila* and shown to be involved in early cell fate determination [Artavanis-Tsakonas et al., 1995].

AGS comprises bile duct paucity, cholestasis, heart defects (most commonly discrete branch pulmonic stenosis, peripheral pulmonary hypoplasia, pulmonic valve stenosis, and tetralogy of Fallot), eye involvement (anterior chamber defects, retinal pigmentary

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changes), vertebral defects (most commonly butterfly vertebrae), facial anomalies, and, less frequently, renal involvement [Alagille et al., 1987; Krantz et al., 1997]. AGS is an autosomal dominant trait with a wide range of phenotypic variability between and within families. Before the identification of *Jagged1* as the AGS syndrome disease gene, diagnosis was based on the clinical criteria set forward by Alagille et al. in 1987. In this article, Alagille et al. described the "complete" syndrome as encompassing bile duct paucity characteristic face, chronic cholestasis, posterior embryotoxon, "butterfly" vertebrae, and cardiac defects. The "partial" syndrome was described in the same article as consisting of bile duct paucity plus three or four of the other major criteria. Often a family is not brought to the attention of the geneticist or gastroenterologist unless a full picture of AGS is present. Several families that had been brought to our attention after the birth of a child with fully expressed Alagille syndrome (liver, cardiac, ocular, skeletal, and facial involvement), have had other first-degree relatives with apparently isolated heart defects (Fig. 1). In the absence of clinically evident liver involvement, the diagnosis of Alagille syndrome is not established. In 1973, Watson and Miller independently described a syndrome of familial pulmonary arterial stenosis with neonatal liver disease that they termed "arteriohepatic dysplasia." The association between liver dysfunction and heart disease is clearly the same as that earlier reported by Alagille et al. in 1969 and subsequently named Alagille syndrome. Four of the five families described in Watson and Miller's article had one or more members with apparently isolated cardiac lesions and no liver involvement.

The observation that relatives of AGS patients can have presumably isolated heart defects led us to hypothesize that some patients with apparently isolated cardiac defects would be found to have mutations in the *Jagged1* gene. We describe the cases of two patients found to have mutations in *Jagged1* on the basis of congenital heart defects.

MATERIALS AND METHODS

Two patients who were treated by the cardiology service, and their relatives, were investigated for alterations in the *Jagged1* gene. All individuals tested gave written informed consent approved by the Children's Hospital of Philadelphia Institutional Review Board. Chromosome analysis was carried out on peripheral blood lymphocytes at the 550- to 600-band level of resolution, using standard techniques.

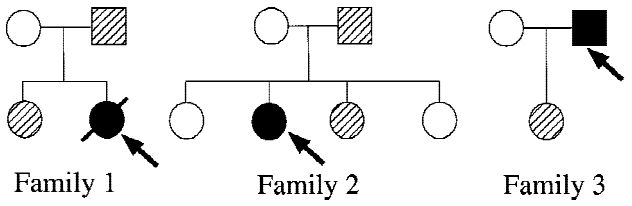

Fig. 1. Pedigrees of AGS families in which there are first-degree relatives of the patients with apparently isolated heart defects. The clinical diagnosis of AGS was established in the patients (indicated by the arrows) and confirmed by mutational analysis of *Jagged1*. Unshaded areas indicate unaffected relatives, hatched areas show those with heart disease, and black areas are those with AGS. In Family 1, the father of the patient has a heart murmur, and the sister has tetralogy of Fallot with small pulmonary arteries as well as posterior embryotoxon. In Family 2, the father of the patient has an atrial septal defect and was found to have mildly elevated liver enzymes later in life; the sister has pulmonic stenosis. In Family 3, the daughter of the patient has pulmonary artery stenosis.

Fluorescence in situ hybridization (FISH) studies were carried out using standard techniques, as described previously [Oda et al., 1997]. Yeast artificial chromosomes (YACs) containing portions of the short arm of chromosome 20 (955f7, 936f6, 809h11, 881h2, 940d11, 970f9, 914a4, and 954a5) were used as probes. The YACs were identified via the Whitehead Institute for Biomedical Research Database, and clones were obtained from the Centre d'Etude du Polymorphisme Humain (CEPH) YAC library. Briefly, total YAC DNA was extracted and labeled with biotin deoxyuridin triphosphate (dUTP) by nick translation using a commercially available kit (Oncor, Inc., Gaithersburg, Maryland). Labeled DNA was combined with Cot-1 and herring sperm DNA. Hybridization and washes were done using standard conditions.

DNA was extracted from lymphocytes (whole blood) or established lymphoblastoid cell lines of affected and unaffected members of each family and from unrelated normal control subjects using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The primers for polymerase chain reaction analysis were designed to cover all exons as well as the intron/exon boundaries of *Jagged1* [Li et al., 1997; Oda et al., 1997; Krantz et al., 1998]. SSCP was performed as previously described, and amplicons demonstrating band shifts were sequenced by the Nucleic Acid/Protein Core facility of the Children's Hospital of Philadelphia.

RESULTS

Patient 1 (M.E.) (Fig 2a,b) is a 3½-year-old girl diagnosed with peripheral pulmonic stenosis and no other medical problems. The initial echocardiogram at age 11 months showed normal intracardiac anatomy, with bilateral branch pulmonary artery hypoplasia and mild discrete proximal left-sided pulmonary artery stenosis. Subsequent cardiac catheterization at age 23 months demonstrated diffuse pulmonary hypoplasia with half-systemic right-sided ventricular pressures. The patient's mother had pulmonic stenosis, the maternal grandmother is reported to have a heart defect described as a "hole in her heart," and the maternal great-grandmother also had pulmonic stenosis. There was no history of jaundice or visual problems in the neonatal period. On examination by a clinical geneticist, the girl was found to have frontal bossing, deep-set eyes, a broad nasal bridge, and a pointed chin (consistent with the facial involvement in AGS). The same facial anomalies were also seen in the mother of the patient. These subtle anomalies in combination with the right-sided heart lesions led us to test for alterations in the *Jagged1* gene. Analysis of *Jagged1* by single strand confirmation polymorphism (SSCP) showed a shift in exon 2 (Fig. 3). Sequence analysis of this region uncovered a single base pair insertion (684insG), which resulted in an abnormal stop codon at amino acid 745 (M.E. was previously reported as pa-

Fig. 2. Patient 1 **(A,B)** has frontal bossing, deep-set eyes, and a pointed chin. Patient 2 **(C,D)** has frontal bossing.

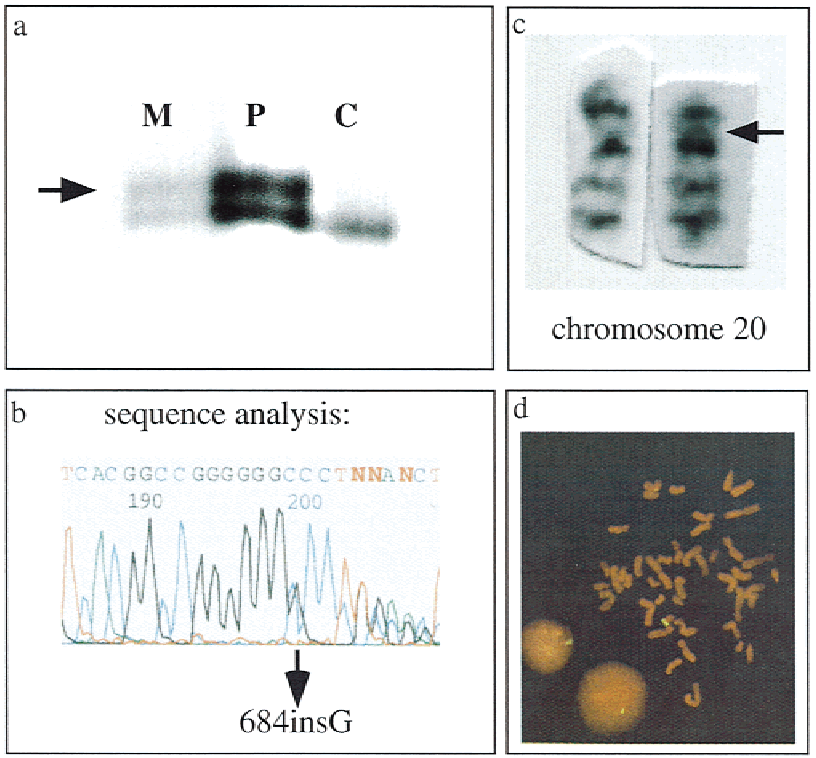

Fig. 3. **a:** SSCP analysis of exon 2 in patient 1 (P), her affected mother (M), and a control sample (C) demonstrates a shift in the patient and her mother and not in the control. **b:** Sequence analysis of exon 2 in patient 1 demonstrates an insertion of a 'G' at base 684, resulting in a frameshift. **c:** Partial karyotype of patient 2 shows her chromosomes 20 (arrow points to cytogenetically detectable deletion at band 20p12). **d:** FISH analysis on a metaphase spread from patient 2 shows a deletion of YAC 940d11 that maps within 20p12 and contains the *Jagged1* gene from one chromosome (signal seen only on one chromosome 20).

tient 19 in Krantz et al., 1998) (Fig. 4). An identical shift was also observed in the patient's mother.

After identification of the *Jagged1* mutation, the patient was reexamined for subclinical manifestations of AGS. She was found to have posterior embryotoxon (as did her mother) and moderately elevated liver enzyme levels: alkaline phosphorase (AP), 1,143 U/L (normal: 145–320); alanine aminotransferase 137 U/L (normal: 5–45); aspartate aminotransferase, 98 U/L (normal: 20–60); lactate dehydrogenase, 728 U/L (normal: 500–920); gamma glutamyl transpeptidase, 644 (normal: 6–19); total bilirubin, 0.4 mg/dl (normal: 0.6–1.4); cholesterol, 286 mg/dl (normal: 45–182); and triglycerides, 161 mg/dl (normal: 27–125). No vertebral defects were identified.

Patient 2 (L.C.) (Fig 2c,d) is a 5½-year-old girl with tetralogy of Fallot and hypoplastic pulmonary arteries. At 18 months she underwent surgical repair at an outside institution and was referred to our hospital at 5½ years for pulmonary artery angioplasties and stenting. Her height was less than the fifth centile, her head circumference was at the second centile, and she had relative hypertelorism, frontal bossing, and a high arched palate with a bifid uvula. On chest radiography an isolated "butterfly" vertebra was visible at T-11. The patient's development was appropriate for her age until approximately 1 year, after which there was evidence of mild developmental delays. Her speech was the most notably affected. The family history did not show any history of heart defects, liver abnormalities, eye abnormalities, or other birth defects. There was a history of consanguinity, in that the patient's maternal grandmother and paternal grandfather were first cousins. High-resolution chromosome analysis showed a deletion of the short arm of chromosome 20 (46,XX,del(20)(p11.23p12)) (Fig. 3).

To further characterize the deletion, FISH studies were carried out using a series of YAC clones derived from the short arm of chromosome 20. The patient was found to have deletions for five YACs (936f6, 809h11, 881h2, 940d11, and 970f9), encompassing a region of approximately six to eight megabases of DNA. The *Jagged1* gene is completely contained within YAC 940d11 [Li et al., 1997], and therefore this patient has a deletion of the entire gene, resulting in haploinsufficiency. Both parents have normal chromosomes, indicating that the 20p deletion in the patient arose de novo. After identification of the 20p12 deletion, the patient was reexamined for subtle signs of AGS. Results of liver function tests were normal, but she had bilateral posterior embryotoxon.

DISCUSSION

Mutation or deletion of *Jagged1* has been shown to cause AGS. Congenital heart defects are seen in more than 95% of AGS patients and vary in type and severity. The finding of relatives with more subtle signs of this disorder, and in some instances with apparently isolated heart defects, led us to hypothesize that some patients with apparently isolated heart defects of the types seen in AGS may also have subtle forms of AGS and mutations in *Jagged1,* the AGS disease gene.

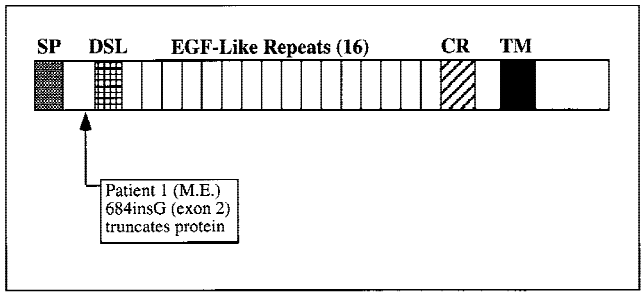

Fig. 4. Diagrammatic representation of the Jagged1 protein. Conserved regions of the protein are labeled as follows: SP, signal peptide; DSL, evolutionary conserved region (D, *Delta* from *Drosophila;* S, *Serrate* from *Drosophila;* L, *Lag-2* from *C. elegans*); EGF, epidermal growth factor; CR, cysteine-rich region; TM, transmembrane domain. The localization of the mutation of patient 1 is identified. Most of the functional domain of the protein would be lost as a result of the truncation caused by this mutation.

The majority of mutations in *Jagged1* identified to date in patients with clinically diagnosed AGS (meeting the diagnostic criteria of bile duct paucity plus at least three of five major clinical manifestations) have been in the conserved regions of the protein, and have led to a truncated protein product lacking at least the transmembrane domain (Fig. 4). Other patients with AGS have been found to have complete deletions of the *Jagged1* gene [Krantz et al., 1998]. The phenotypes of those patients with *Jagged1* mutations and those with complete absence of one copy of the gene have been indistinguishable. Therefore, we have hypothesized that a likely mechanism of action leading to the AGS phenotype is haploinsufficiency for the protein product. It is possible that the truncated forms of the protein that result from some of the identified mutations may, in fact, be secreted and act in a dominant negative fashion. There are no data to support this hypothesis in humans, however.

As is seen in AGS, our data suggest that both deletion and mutation of *Jagged1* can lead to apparently isolated right-sided heart defects. Patient 2 has a complete deletion of one copy of *Jagged1,* while patient 1 has a mutation that leads to a truncated and presumably inactive protein. While the cases of both patients described here were brought to light through a cardiology clinic and they were followed as nonsyndromic patients, once alterations in *Jagged1* were identified, these patients were more closely examined for signs of AGS. Although neither meets the diagnostic criteria for AGS, they can be classified as having a mild form of this disorder. Patient 1 comes from a family with four generations of congenital heart defects, and the diagnosis of a mild form of AGS was not considered until gene testing for *Jagged1* was available. The mutation in this family (a single nucleotide insertion in exon 2, resulting in a frameshift and subsequent stop codon) appears to cause heart malformations without significant liver dysfunction. The findings in patient 2 were not considered typical until age 5 years, when genetic, cytogenetic, and molecular evaluations were made. Patient 2 also does not meet the full diagnostic criteria for AGS and represents a "microform" of this disorder.

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