Am. J. Hum. Genet. 62:1361–1369, 1998

Spectrum and Frequency of *Jagged1* (*JAG1*) Mutations in Alagille Syndrome Patients and Their Families

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Summary

Alagille syndrome (AGS) is a dominantly inherited disorder characterized by liver disease in combination with heart, skeletal, ocular, facial, renal, and pancreatic abnormalities. We have recently demonstrated that *Jagged1* (*JAG1*) is the AGS gene. *JAG1* encodes a ligand in the Notch intercellular signaling pathway. AGS is the first developmental disorder to be associated with this pathway and the first human disorder caused by a Notch ligand. We have screened 54 AGS probands and family members to determine the frequency of mutations in *JAG1*. Three patients (6%) had deletions of the entire gene. Of the remaining 51 patients, 35 (69%) had mutations within *JAG1*, identified by SSCP analysis. Of the 35 identified intragenic mutations, all were unique, with the exceptions of a 5-bp deletion in exon 16, seen in two unrelated patients, and a C insertion at base 1618 in exon 9, also seen in two unrelated patients. The 35 intragenic mutations included 9 nonsense mutations (26%); 2 missense mutations (6%); 11 small deletions (31%), 8 small insertions (23%), and 1 complex rearrangement (3%), all leading to frameshifts; and 4 splice-site mutations (11%). The mutations are spread across the coding sequence of the gene within the evolutionarily conserved motifs of the JAG1 protein. There is no phenotypic difference between patients with deletions of the entire *JAG1* gene and those with intragenic mutations, which suggests that one mechanism involved in AGS is haploinsufficiency. The two missense mutations occur at the same amino acid residue. The mechanism by which these missense mutations lead to the disease is not yet understood; however, they suggest that mechanisms other than haploinsufficiency may result in the AGS phenotype.

Received January 9, 1998; accepted for publication April 3, 1998; electronically published May 8, 1998.

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0002-9297/98/6206-0013$02.00

Introduction

Alagille syndrome (AGS [MIM 118450]), also known as arteriohepatic dysplasia, is a multisystem developmental disorder demonstrating autosomal dominant inheritance (Alagille et al. 1975; Alagille et al. 1987; Krantz et al. 1997*a*). It is one of the most common genetic causes of chronic liver disease in childhood, with a minimal estimated frequency of 1/70,000 live births (Danks et al. 1977). Affected individuals display abnormalities of the liver, heart, eyes, skeletal system, facial features, kidneys, and pancreas, with variable expression. The criteria for diagnosis, as outlined by Alagille et al. (1987), include the histologic finding of bile duct paucity on liver biopsy, in association with three of five major clinical findings. These major diagnostic criteria include cholestasis, heart defects (most commonly, involvement of the pulmonary artery and its branches), posterior embryotoxon (a defect of the anterior chamber of the eye), vertebral abnormalities (classically, butterfly vertebrae), and characteristic facial features (a high, broad forehead; widely spaced, deep-set eyes; and a prominent, pointed chin, all of which give the face a "triangular" appearance). Although these criteria are required to classify an individual as having AGS, it has long been recognized that family members of AGS patients may have isolated individual features and that they most likely represent more mildly expressed forms (microforms) of the same disorder (Shulman et al. 1984; Dhorne-Pollet et al. 1994; Elmslie et al. 1995; Krantz et al. 1997*a*).

Recently, the human *Jagged1* gene (*JAG1*) on chromosome 20p12 was identified as the AGS disease gene (Li et al. 1997; Oda et al. 1997). *JAG1* encodes a ligand in the Notch signaling pathway. This pathway has been extensively studied in *Drosophila* and *Caenorhabditis elegans* and has been shown to be of importance in cell-fate determination (Kopan and Weintraub 1993; Artavanis-Tsakonas et al. 1995; Robey et al. 1996). The JAG1 protein is ~1,220 amino acids in length and is encoded by a gene that spans 36 kb and is composed of 26 exons, encoding a 5.5-kb message (Li et al. 1997).

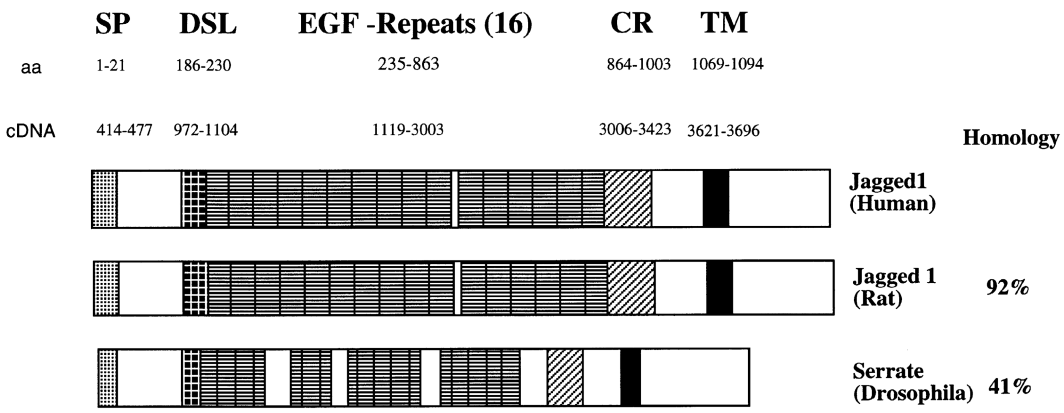


Figure 1 Structure and evolutionary conservation of the JAG1 protein. Conserved regions include the signal peptide (SP), the delta-serrate-lin12–like region (DSL), EGF-like repeats, the CR region, and the transmembrane (TM) domain. The amino acid (aa) residues and cDNA base pairs correlating to the regions in the human protein and cDNA are indicated above the protein diagrams. Homology to the human protein is indicated on the right.

The JAG1 protein has several domains that show high interspecies conservation (Lindsell et al. 1995). These include the DSL domain (an acronym for conserved regions seen in the *Drosophila delta*, *serrate*, and *C. elegans lag 2* genes; Lindsell et al. 1995), the epidermal growth factor (EGF)–like repeats, and the cysteine-rich (CR) region (fig. 1). These regions are thought to play critical roles in ligand-receptor interactions (Muskavitch 1994). Initial studies in multiple families showed that mutations in this gene segregated with the disease phenotype (Li et al. 1997; Oda et al. 1997). All mutations identified at that time were unique and were predicted to lead to a truncated protein product that would be missing its transmembrane domain and would be presumed to be nonfunctional. The phenotypes of patients with mutations in *JAG1* were indistinguishable from those of patients with deletions of chromosome 20p12 that encompassed the entire *JAG1* gene. These findings led to the proposal of haploinsufficiency as a mechanism leading to the disease phenotype.

This study describes the screening of a cohort of AGS patients and their families, performed to identify the spectrum of mutations in *JAG1* in this population and to determine whether any correlations between genotype and phenotype could be established.

Material and Methods

Patients

Fifty-four unrelated patients meeting the diagnostic criteria for AGS (Alagille et al. 1987) and their family members were included in the study. Forty-five of the patients studied here were previously investigated by microsatellite analysis and FISH for deletions of 20p12 (Krantz et al. 1997*b*). All patients were referred from within the United States, with the exception of patient 65, who was from Guatemala. The ethnic backgrounds of the patients were as follows: Caucasian, 33/54 (61%); African American, 12/54 (22%); Hispanic, 6/54 (11%); and Asian, 3/54 (6%). Informed consent was obtained from all individuals in the study. Lymphoblastoid cell lines were established and DNA prepared by use of standard procedures.

FISH

FISH was performed as described elsewhere (Krantz et al. 1997*b*), with YACs 940d11 and 881h2.

Microsatellite Analysis

Microsatellite markers from across the previously defined AGS critical region (Krantz et al. 1997*b*), within which *JAG1* is localized, were used to identify those patients with deletions encompassing this gene.

SSCP Analysis

SSCP was carried out as described elsewhere (Li et al. 1997). All 26 exons were screened on all probands. Larger exons (exons 2, 4, 25, and 26) were subdivided to allow for optimal shift detection on electrophoresis. All relevant and available family members of probands with detectable shifts were also analyzed for the specific exons involved. Primers and PCR conditions are listed in table 1.

Table 1

Primers and PCR Conditions Used for SSCP Analysis of the *JAG1* Gene

Exon	Sense (5′–3′)	Antisense (5′–3′)	Length (bp)
1[a]	TCCAATCGGCGGAGTATATTAGAG	AGAGGACGGCTGGGAGGGA	251
2A[a]	GCGCTGACCTACCTCCTTCCCT	CACGATGCGGTTGCGGTC	313
2B[d]	GCTTCGGCTCAGGGTCCA	CCAGGCGCGGGTGTGAG	132
3[c]	GTACAATGTTCGTCTGTT	GTCCACAGGAGCGATACT	100
4A[a]	CATGATTCACCACTTTGT	GCATAGTGTCCAAAGAAG	190
4B[a]	CTGTAATAAGTTCTGCCG	TCCCACCCCACCTGAGATA	195
5[a]	GGAATTTGCAGACTTTAATGCAA	CCTGCAGTCCCTGGGAGT	160
6[d]	AAGGCTAACCTGGAGGTGTGCTGC	TCCCACCCTGGGTCTCATCC	198
7[a]	GTCTCTTAGTGACTCCA	GCTTATAGAATGGTTGGGA	270
8[d]	CATCCCTCTCTGACTGCCATCC	ACCTCTCCCCAACGTGGTATCTT	217
9[a]	GGATCTAATTGTCAGATGGAT	GTCACAGGGATGTTCTTA	190
10[a]	TTCCAAGGTGGGGGAGAT	TGGAAACAAAGTCACTCAC	195
11[a]	CACCCAGCGTAATAACCTT	CTAGTGTCGCACAAATCT	175
12[d]	TGAAGCCCTGTGTTTGTGGAATAC	GAAAAGTAAAGGGAAGCGGAGGAG	318
13[c]	GTTTTACTCTGATCCCTC	CAAGGGGCAGTGGTAGTAAGT	260
14[d]	GAATGCCGCATCTGTGGGTG	AGGCTGGGGAGCACTGGTC	166
15[a]	AGGAGGGAGCCATGAAAACTGC	CAACATGACCCATACATCCCAGAG	251
16[a]	GCCTGTCGTGAATGGTCCTGGA	CAAGGCCAACTACCACTT	190
17[b]	GCTATCTCTGGGACCCTT	CCACGTGGGGCATAAAGTT	200
18[a]	CCTCCAGACTGTTTCTGGATA	GCAAGTCCCCAAGGGTGTCA	250
19[c]	GTTGAAGTCCTCACTATT	GTGGATGAGTGCTGGCTT	105
20[a]	GTGTGGTCTTTGCTTTCT	CCAGACGGAGACAGTCCTT	130
21[a]	CATCAGTCCCTAAACTTGAACTCCATT	CGCTCACCCCAGAAGACCCAT	196
22[a]	GCTCACCCGTCTCCACCTGT	GAACTGCGGCAGCCATCATGT	160
23[a]	ATGGCTGCCGCAGTTCA	CAAGCAGACATCCACCAT	350
24[a]	CTTACACGTGTGTGGGTTTCTC	GCCATCGAATAATGAGGT	230
25A[d]	GCAGACACAGATTGTCGA	CGCCTCTGAACTCTTACT	190
25B[a]	AGTAAACGTGATGGAAAC	CCCTCGACCTGATGGCTTTA	200
26A[a]	TCTTGGAGAGTTAATTGGTTTTGTGC	CCTTGATGGGGACCGTGTTG	250
26B[a]	GCTGAACCAGATCAAAAACCCCA	TTGTCCAGTTTGGGTGTTTTGTCG	239
26C[a]	GCGTATACGCTGGTAGACAGAGAAGA	GACAGTTTAAAGAACTACAAGCCCTCAGA	196

[a] PCR: 35 cycles of 30 s at 94°C, 1 min at 50°C, and 30 s at 72°C.
[b] PCR: 35 cycles of 30 s at 94°C, 1 min at 52°C, and 30 s at 72°C.
[c] PCR: 35 cycles of 30 s at 94°C, 1 min at 55°C, and 30 s at 72°C.
[d] PCR: 35 cycles of 20 s at 94°C, 20 s at 60°C, and 20 s at 72°C.

Sequence Analysis

All confirmed shifts detected by SSCP analysis were subsequently sequenced on an ABI 377 sequencer in the core facility at The Children's Hospital of Philadelphia.

Results

Deletion Detection

Fifty-four unrelated patients with clinically diagnosed AGS were screened with microsatellite markers spanning an ~1.5-mB region encompassing *JAG1* to determine whether larger deletions were present, resulting in loss of an allele and causing haploinsufficiency for the *JAG1* gene product. Forty-five patients were reported by Krantz et al. (1997*b*); the remaining patients were screened as part of this study. Three patients (6%) were found to have deletions ranging 1.5–6.5 mB in size. One of these patients had a cytogenetically detectable deletion (Krantz et al. 1997*b*). A second patient had an apparently balanced translocation, involving chromosomes 2 and 20, that segregated with the disease phenotype in her family and was subsequently found to have a molecularly detectable deletion within chromosome 20p12 (Spinner et al. 1994; Krantz et al. 1997*b*). The karyotype of the third patient was normal at a resolution of 550 bands, with a deletion detected by molecular analysis (Rand et al. 1995). All three patients' deletions encompassed the entire *JAG1* gene, resulting in haploinsufficiency for the gene product. The phenotypes of these patients have been reported elsewhere (Spinner et al. 1994; Rand et al. 1995; Krantz et al. 1997*b*) and did not show any difference from those of patients with point mutations in the gene.

Mutation Detection

The 51 patients clinically diagnosed with AGS that remained after screening for deletions of the entire gene

Table 2

Summary of Mutations in *JAG1* Identified in AGS Probands

Mutation[a,b]	Patient Number	Parental Origin of Mutation[c]	Exon	Protein Region	Effect on Protein Structure
Deletion:					
2692delTG	4	Paternal	18	EGF-like repeats	Truncates protein
2531del4	6	De novo	17	EGF-like repeats	Truncates protein
2531delCA	8	De novo	17	EGF-like repeats	Truncates protein
2504del5	21	ND	16	EGF-like repeats	Truncates protein
1104delAG	23	Maternal	4B	DSL	Truncates protein
2535del4	24	Maternal	17	EGF-like repeats	Truncates protein
2066delC	29	Paternal	13	EGF-like repeats	Truncates protein
1874delCA	32	ND	12	EGF-like repeats	Truncates protein
3019delTG	36	De novo	22	CR region	Truncates protein
3052delGT	60	Maternal	22	CR region	Truncates protein
2504del5	65	De novo	16	EGF-like repeats	Truncates protein
Insertion:					
3102ins5	10	Paternal	23	CR region	Truncates protein
2472insT	17	ND	16	EGF-like repeats	Truncates protein
684insG	19	Maternal	2A	5′ of DSL	Truncates protein
2587insG	20	ND	17	EGF-like repeats	Truncates protein
1708ins4	47	De novo	10	EGF-like repeats	Truncates protein
1618insC	50	ND	9	EGF-like repeats	Truncates protein
2271insG	62	ND	14	EGF-like repeats	Truncates protein
1618insC	66	ND	9	EGF-like repeats	Truncates protein
Missense:					
R184C	15	De novo	4A	5′ of DSL	?
R184H	56	ND	4A	5′ of DSL	?
Nonsense:					
R235X	28	ND	5	EGF-like repeats	Truncates protein
K392X	30	ND	9	EGF-like repeats	Truncates protein
C306X	31	ND	7	EGF-like repeats	Truncates protein
R739X	39	De novo	18	EGF-like repeats	Truncates protein
Q398X	41	ND	9	EGF-like repeats	Truncates protein
R866X	46[d]	De novo	22	CR region	Truncates protein
C892X	52	ND	23	CR region	Truncates protein
Q820X	57	De novo	21	EGF-like repeats	Truncates protein
S888X	59	ND	22	CR region	Truncates protein
Splice site:					
1329+2T→G	13	De novo	6	Splice-donor site	?
1761→12T→G	25	Paternal	11	Splice-acceptor site	?
1808+1G→T	44	ND	10	Splice-donor site	?
2871+1G→T	63	ND	20	Splice-donor site	?
Complex:					
1072GCA→TG	55	ND	4B	DSL	Truncates protein

[a] Numbering is that of Li et al. 1996, GenBank accession number U73936.
[b] Mutation nomenclature is that of Beaudet et al. 1993.
[c] ND indicates that it was not possible to confirm the parental origin of the mutation.
[d] Patient 46 has a paternally inherited P/R866 polymorphism and a 3024C→T change leading to a nonsense mutation.

were screened for mutations in *JAG1* by SSCP analysis. All band shifts identified after electrophoresis were sequenced.

Mutations were identified in 35 (69%) of 51 patients screened. With the exceptions of a 5-bp deletion in exon 16, seen in two unrelated patients, and a C insertion at base 1618 of exon 9, in another two unrelated patients, all mutations were unique (seen in only one family; table 2). Eleven (31%) of the 30 mutations were small deletions: 1 of these involved a single base pair, 6 involved two bp, 2 involved 4 bp, and the recurrent mutation seen in two patients involved a 5-bp deletion. This recurrent deletion occurs at a site in the gene where a 5-bp GAAAG repeat sequence exists, with one of the repeats deleted in both patients. All deletions led to frameshifts that resulted in downstream stop codons and truncated protein products (table 2). Eight (23%) of the mutations were insertions. Of these, six were sin-

gle–base-pair insertions (including the C insertion at base 1618 of exon 9, seen in two unrelated patients), one patient had a 4-bp insertion, and one patient had a 5-bp insertion. All led to frameshifts resulting in a prematurely truncated protein product. A single patient (3%) had a complex rearrangement that involved an insertion and a deletion, leading to a frameshift and a truncated protein product. Nine patients (26%) had single–base-pair changes that led to immediate stop codons (nonsense mutations).

Four patients had mutations predicted to lead to alterations in splicing. Patients 13, 44, and 63 had mutations in the consensus splice donor sites of exons 6, 10, and 20, respectively. Patient 25 had a T→G mutation 12 bp from the splice donor site of exon 13. The mutation segregated with the AGS phenotype in this family (found in the proband, her affected father, and her affected sister) and was not seen in over 100 ethnically matched controls. Heteroduplex mobility analysis was performed on cDNA from the three affected patients in this family with presumed splice-site alterations, and, in all cases, an electrophoretic band shift was identified that was not seen in control samples, consistent with an alternatively spliced RNA product (data not shown).

Two patients (6%) with missense mutations have been identified. The two missense mutations occurred at the same amino acid residue (184), in one case changing the normal arginine to a cysteine, and in the second case changing it to a histidine. With the exception of the two missense mutations, all other mutations identified in this screening presumably result in truncated proteins minimally missing the transmembrane and intracellular domains.

Several polymorphisms in *JAG1* were also identified through this screening (table 3). With the exception of the P/R866 change, none of the polymorphisms led to an altered amino acid residue. All polymorphisms were seen in unaffected family members as well as in control samples. The proline-to-arginine change at position 866 (P/R866) was seen in proband 46 and her unaffected father. In addition, the proband has a de novo 3024C→T change in the same codon that results in a stop codon (table 2). The putative P/R866 polymorphism was also present in a homozygous state in the unaffected mother of another patient.

In those patients where both parental samples were available (18/35), 10 mutations (56%) arose de novo, and 8 (44%) were inherited. Parental origin was maternal for four of the inherited mutations and paternal for the other four. Of the three patients with complete gene deletions, one patient inherited the deleted chromosome from her mildly affected father (Spinner et al. 1994), with the other two deletions appearing de novo. In all cases, parents with *JAG1* mutations had clinical features consistent with a diagnosis of AGS or of an AGS microform (having some of the physical findings of AGS, but not enough to fit the criteria to establish a full clinical diagnosis of AGS as set forth by Alagille et al. 1987).

Table 3

Polymorphisms Identified in *JAG1* in AGS Probands

Polymorphism[a]	Codon Change	Amino Acid(s)	Exon
1001C/T	TGC→TGT	Cys	4A
1991C/T	ATC→ATT	Ile	13
2627A/C	ACA→ACC	Thr	17
2795C/T	AGC→AGT	Ser	20
P/R866	CCA→CGA	Pro→Arg	22
3830C/T	TAC→TAT	Tyr	26B
3941T/C	TAT→TAC	Tyr	26B

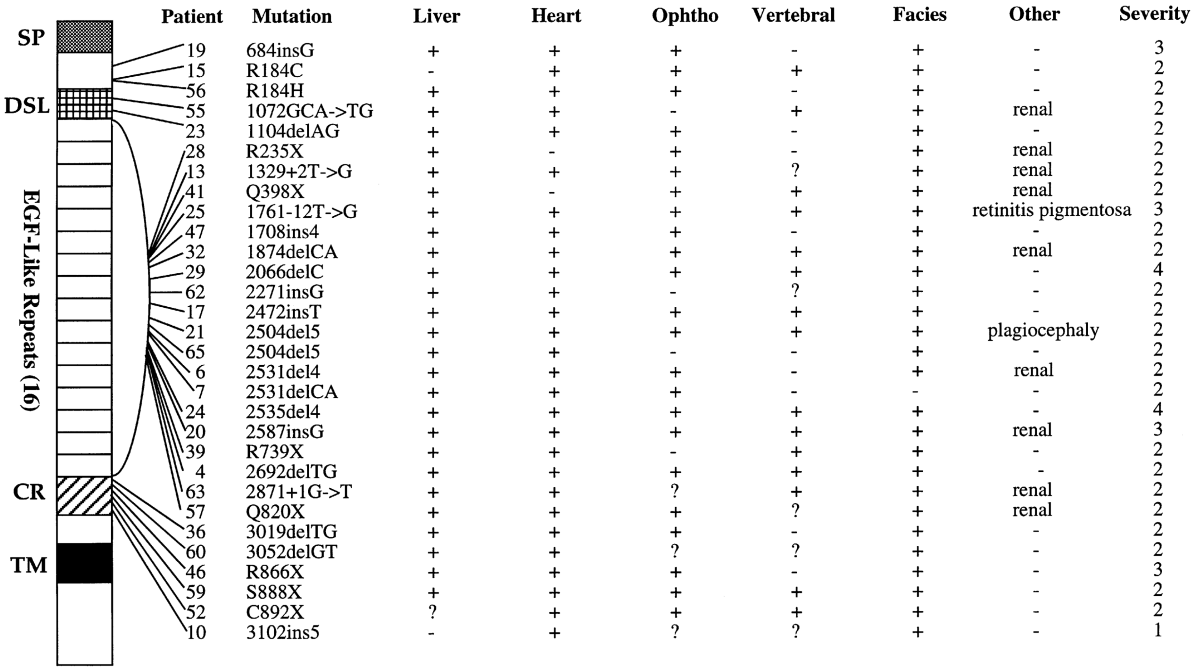
[a] Numbering is according to Li et al. 1996, GenBank accession number U73936.

Genotype-Phenotype Correlation

To evaluate the functional significance of the mutations identified in *JAG1*, we undertook a careful evaluation of phenotypes of patients with intragenic mutations and of patients with larger deletions encompassing the entire gene. Patients were evaluated for severity of disease as defined by morbidity (heart surgery, liver transplant) and mortality, as well as by the number of clinical features met in establishing the diagnosis of AGS. Although the number of patients with deletion of the entire gene was small, there was no appreciable difference noted in the phenotypes of these patients (fig. 2). This is in keeping with an earlier study that looked at a greater number of patients with deletions reported in the literature, prior to the identification of *JAG1* as the AGS disease gene (Krantz et al. 1997*b*). There was no difference in severity or phenotypes among patients with identified mutations and those in whom no mutations in *JAG1* have been found. No significant differences were appreciated in phenotypes based on type (missense vs. truncating) or localization of mutations. No renal manifestations were seen in the two patients with missense mutations or in patients with disruption of the JAG1 protein distal to the EGF-like repeats; however, the numbers of patients are small, and the results are not significant.

Discussion

In our analysis of 54 patients meeting the clinical criteria for AGS, we were able to identify 3 patients (6%) with deletions encompassing the entire *JAG1* gene and 35 patients (65%) with intragenic mutations. Using the techniques outlined in this article, we were able to identify a total of 38 (70%) of 54 unrelated AGS patients



Patient	Mutation	Liver	Heart	Ophtho	Vertebral	Facies	Other	Severity
19	684insG	+	+	+	-	+	-	3
15	R184C	-	+	+	+	+	-	2
56	R184H	+	+	+	-	+	-	2
55	1072GCA->TG	+	+	-	+	+	renal	2
23	1104delAG	+	+	+	-	+	-	2
28	R235X	+	-	+	-	+	renal	2
13	1329+2T->G	+	+	+	?	+	renal	2
41	Q398X	+	-	+	+	+	renal	2
25	1761-12T->G	+	+	+	+	+	retinitis pigmentosa	3
47	1708ins4	+	+	+	-	+	-	2
32	1874delCA	+	+	+	+	+	renal	2
29	2066delC	+	+	+	+	+	-	4
62	2271insG	+	+	-	?	+	-	2
17	2472insT	+	+	+	+	+	-	2
21	2504del5	+	+	+	+	+	plagiocephaly	2
65	2504del5	+	+	-	-	+	-	2
6	2531del4	+	+	+	-	+	renal	2
7	2531delCA	+	+	+	-	-	-	2
24	2535del4	+	+	+	+	+	-	4
20	2587insG	+	+	+	+	+	renal	3
39	R739X	+	+	-	+	+	-	2
4	2692delTG	+	+	+	+	+	-	2
63	2871+1G->T	+	+	?	+	+	renal	2
57	Q820X	+	+	+	?	+	renal	2
36	3019delTG	+	+	+	-	+	-	2
60	3052delGT	+	+	?	?	+	-	2
46	R866X	+	+	+	-	+	-	3
59	S888X	+	+	+	+	+	-	2
52	C892X	?	+	+	+	+	-	2
10	3102ins5	-	+	?	?	+	-	1

Figure 2 Phenotype-genotype correlations in AGS patients with identifiable mutations in *JAG1*. The JAG1 protein is depicted on the left (SP, signal peptide; DSL, delta-serrate-lin12 conserved region; TM, transmembrane region). Localization of identified mutations is indicated by the lines connecting the patient number to the protein diagram. A plus sign (+) indicates presence of feature, a minus sign (−) indicates absence of feature, and a question mark (?) indicates unknown. Severity scale: (1) = subclinically affected; (2) = clinically affected, mild; (3) = clinically affected, severe (requiring surgical intervention or transplant), (4) = deceased.

with alterations in *JAG1*. All of the identified mutations were unique, with the exceptions of patients 50 and 66, who had a C insertion at base 1618 of exon 9, and patients 21 and 65, who had the same 5-bp deletion in exon 16. A 5-bp GAAAG repeat exists at this site in exon 16, with both patients having deletions of one of these repeats. The existence of this small repeated sequence may predispose it to recombination errors and may account for the recurrent deletion seen in these two unrelated patients.

With the exception of the two missense mutations, all other mutations identified in this study are predicted to result in a truncated protein. In all cases, these mutations are predicted to lead to the deletion of at least the transmembrane and intracellular domains. The truncating mutations all occur within the protein motifs that show interspecies conservation and are thought to be important in ligand-receptor interaction (Lindsell et al. 1995; fig. 1). This report describes the first missense mutations identified in *JAG1*. The two missense mutations occur in the same amino acid residue (an arginine) at position 184. This position is upstream of the truncating mutations that are spread throughout the DSL, EGF-like repeats, and CR region (fig. 3). No mutational hot spots were found.

The identification of only two missense mutations among the 38 changes identified is of interest. Several other single–base-pair substitutions, leading to immediate stop codons or resulting in polymorphisms that either did not change the coded amino acid or led to a conservative amino acid change, were identified (table 3). Since all of the truncating mutations identified in *JAG1* are predicted to result in proteins missing the sequence necessary to anchor them to the cell membrane, we hypothesize that a mechanism for the expression of the AGS phenotype is that of haploinsufficiency for the *JAG1* protein product. In *Drosophila*, mutant delta and serrate (Notch ligands that are highly homologous to *JAG1*) have been studied. These mutant proteins are similar to the proteins we predict in some AGS patients on the basis of our mutation analysis (i.e., truncated proteins lacking the transmembrane and intracellular domains). Studies in *Drosophila* have shown that these mutant proteins are secreted and appear to have a dominant negative effect on Notch signaling (Sun and Artavanis-Tsakonas 1996). Although we do not have direct

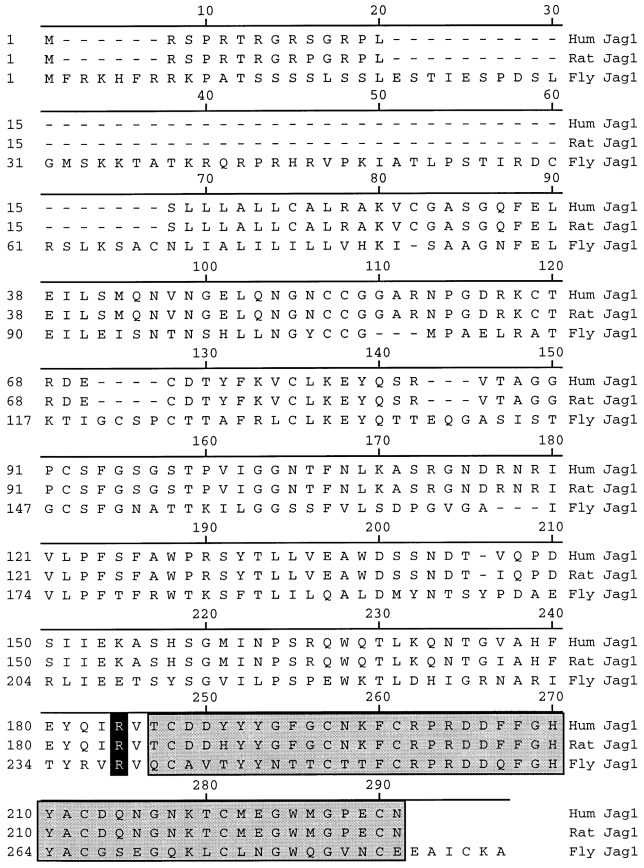


Figure 3 Amino acid sequence of the 5′ end of the JAG1 protein, up to and including the DSL domain (indicated by shaded box). Amino acid 184 (arginine), the amino acid residue involved in the only two missense mutations identified, to date, in *JAG1,* is denoted by a blackened box and demonstrates conservation from *Drosophila* to humans.

evidence that this is not occurring in some of our patients, we have shown that those patients with deletions of the entire *JAG1* gene display the same phenotype as those with intragenic mutations leading to a truncated protein. In addition, at least three of our patients with intragenic alterations of *JAG1* (patients 19, 23, and 55) have mutations that would severely truncate the protein product, leading to loss of the domains that interact with the Notch receptor. These findings are consistent with haploinsufficiency for *JAG1* causing AGS.

The two missense mutations in *JAG1* at amino acid residue 184 (arginine) are the only point mutations identified that do not result in a truncated protein product. Arginine 184 lies two amino acids upstream of the DSL domain and other conserved structures thought to be involved in ligand-receptor interactions. This residue shows high interspecies conservation (fig. 3) and may be important in these interactions, either directly or through its effect on secondary or tertiary protein structure. These mutations may result in haploinsufficiency but more likely result in an ineffective JAG1 protein and may in fact have a dominant negative effect. Further studies in lower organisms and in in vitro cellular systems will have to be undertaken to fully understand the role these mutations play in the pathogenesis of AGS.

We were able to identify mutations in 38/54 patients (70%) who met the diagnostic criteria for AGS, leaving 30% of patients with no detectable alteration in *JAG1*. Explanations that could account for this include the inability of the screening technique to detect all mutations, or, alternatively, there may be another locus responsible for AGS. SSCP has been shown to detect ~80% of mutations within coding regions and exon/intron boundaries (Orita et al. 1989). Partial gene deletions that include complete exons, conserving the remaining exons and exon-intron boundaries, would potentially not be detected by SSCP analysis. Alterations in the intronic sequences that were not screened may lead to new splicing events that could disrupt the protein as well. Protein truncation and heteroduplex mobility-analysis assays may be able to detect some of these types of changes. Alterations in 5′ upstream sequences as well as 3′ downstream sequences involved in regulation of transcription and processing would also not be detected by the present methodology. Although no data have suggested another locus responsible for AGS, the data mapping AGS to 20p12 are based on reports of patients with cytogenetically detectable deletions of this region (Byrne et al. 1986; Schnittger et al. 1989; Anad et al. 1990; Leguis et al. 1990; Zhang et al. 1990; Teebi et al. 1992; Spinner et al. 1994; Rand et al. 1995; Krantz et al. 1997*b*) and one isolated linkage study in a large three-generation family (Hol et al. 1995). The Notch signaling pathway, of which *JAG1* is a component, has at least seven other members identified in humans (Artavanis-Tsakonas et al. 1995; Ellisen et al. 1991; Joutel et al. 1996; Li et al. 1997; Oda et al. 1997) and at least twice that many identified in lower organisms (Muskavitch 1994; Artavanis-Tsakonas et al. 1995). It is possible that mutations in one or several of these other components or in still other unidentified genes could also result in the AGS phenotype.

The ability to provide molecular confirmation for AGS will be of great benefit to families with an affected child. The expression of AGS is extremely variable both between and within families. A molecular assay will permit identification of family members with microforms of this disorder, greatly improving our ability to appropriately counsel affected families. Mutation identification in *JAG1* in any given AGS family will also allow for the possibility of prenatal diagnosis.

Screening for mutations in *JAG1* by SSCP analysis is a daunting task because of the presence of almost ex-

clusively private mutations across a large coding region. Since the majority of mutations detected to date lead to a prematurely terminated protein, the use of the protein-truncation test (Roest et al. 1993; Becker et al. 1996) may result in a simpler diagnostic assay.

Acknowledgments

As always, we are grateful for the support of the families of the AGS patients we have studied. We are grateful to Dr. Rebecca Oakey for helpful discussions, as well as to the numerous clinicians who have submitted samples for analysis, including Drs. B. Blumberg, B. Crandall, L. Pasztor, M.E. Pierpont, E. Spence, P. Whitington, M. Bialer, M. Bocian, J. Kabori, M. Golabi, T. Costa, J. Graham, M. Jones, S. Bhatt, and G. Kupchik, and genetic counselors K. Armfield, M. Fox, J. Derstine, C. Brasington, and M. Huggins. This work was supported by Clinical Research Grant FY-97-0565 from the March of Dimes Birth Defects Foundation (to N.B.S.), and by NIH grant 1R01DK53104-01 (to N.B.S.). This investigation was also supported in part by Public Health Service (NIH) research grant 3M01RR00240-32S7CRC (to I.D.K.), by the McCabe Fund Pilot Project Award (to I.D.K.), by funds from the "Newell DeValpine Foundation" (to E.B.R.), and by NIH grant DK02338 (to E.B.R.).

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for Alagille syndrome gene, *Jagged1* [U73936])

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim (for Alagille syndrome [MIM 118450])

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