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MUTATION UPDATE

Jagged1 Mutations in Alagille Syndrome

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We have summarized data on 233 Alagille syndrome patients reported with mutations in Jagged1 (JAG1). This data has been published by seven different laboratories in Europe, the United States, Australia, and Japan. Mutations have been demonstrated in 60-75% of patients with a clinically confirmed diagnosis of Alagille syndrome. Total gene deletions have been reported in 3–7% of patients, and the remainder have intragenic mutations. Seventy two percent (168/233) of the reported mutations lead to frameshifts that cause a premature termination codon. These mutations will either lead to a prematurely truncated protein, or alternatively, nonsense mediated decay might lead to lack of a product from that allele. Twenty three unique missense mutations were identified (13% of mutations). These were clustered in conserved regions at the 5′ end of the gene, or in the EGF repeats. Splicing consensus sequence changes were identified in 15% of patients. A high frequency of de novo mutations (60-70%) has been reported. The spectrum of mutations identified is consistent with haploinsufficiency for JAG1 being a mechanism for Alagille syndrome. Hum Mutat 17:18–33, 2001.

KEY WORDS: Alagille syndrome; AGS; Jagged1; JAG1; notch signaling pathway; CADASIL; developmental disorder

DATABASES:
JAG1 – OMIM: 601920, 118450 (AGS); GDB:6175920; GenBank: NM_000214, AL035456, U73936; HGMD: JAG1

BACKGROUND

Alagille syndrome (AGS, MIM# 118450) is a multi-system, dominantly inherited, developmental disorder. The disease is characterized by reduction in the number of bile ducts seen on liver biopsy, in association with cardiac (most commonly pulmonary vascular involvement), skeletal, ocular, facial, and less frequently, renal, neurovascular and pancreatic abnormalities [Emerick et al., 1999; Crosnier et al., 2000a]. The disorder was originally described in the early 1970s [Watson and Miller 1973; Alagille et al., 1975] and in 1986 the first report of a patient with a cytogenetically visible deletion of chromosome band 20p12 lead to the physical mapping of the disease locus [Byrne et al., 1986]. Further reports of deletions and translocations involving 20p12 [Spinner et al., 1994; Krantz et al., 1997] lead to the physical mapping and the positional cloning of the disease gene [Pollet et al., 1997]. In 1997,

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mutations in *JAGGED1* (*JAG1*; MIM# 601920) were shown to segregate with AGS [Li et al., 1997; Oda et al., 1997]. Since that time over 300 Alagille syndrome patients have been studied and mutations in *JAG1* have been demonstrated in approximately 70% [Krantz et al., 1998; Yuan et al., 1998; Crosnier et al., 1999; Onouchi et al., 1999; Pilia et al., 1999; Colliton et al., 2000; Heritage et al., 2000; Crosnier et al., 2000c]. Transmission analysis showed a high frequency of sporadic cases (70%) [Crosnier et al., 1999]. JAG1 is a cell surface protein that functions as a ligand in the Notch pathway, an evolutionarily conserved, fundamental signaling pathway first studied in *Drosophila melanogaster* [Artavanis-Tsakonas et al., 1999]. The name "Notch" derives from the characteristic notched wing found in flies carrying only one functioning copy of the gene. Homozygous mutations in Notch are lethal, and affected flies show hypertrophy of the nervous system, indicating the inability for appropriate cells to adopt an alternative cell fate. The gene has been shown to be expressed in a wide variety of mammalian tissues, and the finding that mutations in *JAG1* cause Alagille syndrome demonstrates the key role this gene plays in normal development of the heart, liver, skeleton, eye, face, and pancreas.

JAG1 is one of two Jagged genes in humans (*JAG1* and *JAG2*; MIM# 602570). These are cell surface proteins, with a single pass transmembrane domain. The JAG proteins share structural similarity with all of the Notch ligands identified to date (Delta and Serrate in *D. melanogaster*, LAG-2 in *C. elegans*, Delta-like 1, Delta-like 3, and Delta-like 4 in vertebrates). All ligands contain a large extracellular domain, a transmembrane domain, and a small intracellular portion. The extracellular domain of the protein includes a 21 amino acid signal peptide, a 40 amino acid DSL region that is highly conserved among all Notch ligands (DSL is named for Delta, Serrate, and Lag-2), 16 epidermal growth factor (EGF)-like regions, and a cysteine rich region [Lindsell et al., 1995] (Fig. 1B). The EGF-like repeats consist of 40–50 amino acids and are found in a large number of extracellular proteins with diverse functions. They invariably contain six conserved cysteine residues that form three disulfide bonds, which are believed to be important for protein stabilization and protein-protein interaction [Campbell and Bork, 1993]. A subset of the EGF-like repeats in human JAG1 and JAG2 are calcium-binding and the presence of calcium has been shown to be essential for mouse Jagged1-Notch interaction [Shimizu et al., 1999]. Both JAG1 and JAG2 contain a 24 amino acid insertion that interrupts the 10th EGF repeat.

MUTATIONS AND POLYMORPHISMS

The *JAG1* mutations identified in Alagille syndrome patients to date are distributed in the part of the gene encoding the extracellular and transmembrane domains of the protein. While there were no examples of specific mutations that occurred at a very high frequency, there was an uneven distribution of the mutations across the exons. The JAG1 gene is composed of 26 exons, which code for a 5.5 kb message. Forty percent of the mutations occurred in exons 2, 4, 9, 17, and 23 (90/233), which accounts for 28% of the coding region (Table 1 and Fig. 1A). An additional 25% occurred in exons 1, 5, 6, 12, 20, and 24 so that over 65% of the mutations could be found in these 11 exons. Overall, 3–7% of AGS patients tested have been found to have a deletion of the entire *JAG1* gene [Krantz et al., 1998; Crosnier et al., 1999; Colliton et al., 2000]. The remainder of the mutations are intragenic, and in this paper we present 233 mutations. The majority of these lead to premature termination codons (nonsense mutations, or small deletions/insertions that lead to frameshifts) (168/233 or 72%). There were 31/233 (13%) missense mutations, and 34/233 occurred in splicing consensus sequences and are therefore predicted to lead to abnormal splicing patterns.

The fact that total gene deletions cause Alagille syndrome signifies that haploinsufficiency for *JAG1* is a disease causing mechanism. However, it is possible that some of the mutations identified in AGS patients (e.g. protein truncating and/or missense mutations) may act via a dominant negative mechanism. All of the mutations that lead to the formation of a premature termination codon will either produce a truncated protein or will be degraded secondary to nonsense mediated

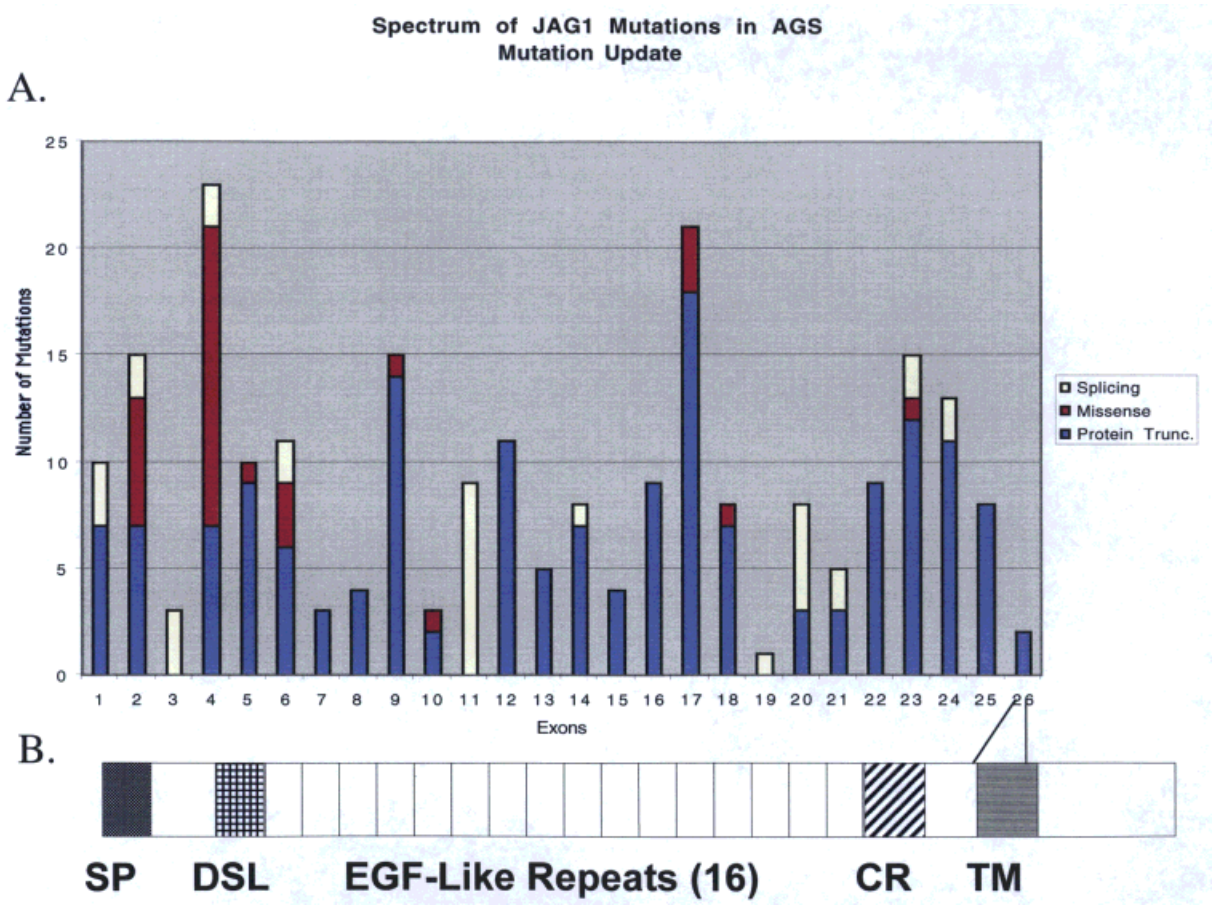

FIGURE 1. **A: Graphic representation of the 26 exons of JAG1 demonstrating the distribution of mutations identified. Splicing, missense, and premature termination codon mutations are indicated. B: Drawing of the primary motifs that are present in the JAG1 protein, aligned with the exons. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

decay. Either way these molecules will not be present at the cell surface. Interestingly, it has been demonstrated that a truncated, soluble form of mouse Jag1 demonstrates biological activity. NIH 3T3 cells transfected with this soluble, truncated Jag1 demonstrated an altered pattern of gene expression, and the soluble Jag1 was efficient in modifying differentiation of these cells [Wong et al., 2000].

Twenty-three unique missense mutations have been identified. Ten of these occur in the 5′ region of the protein either within the conserved DSL region (three mutations) or 5′ to the DSL (10 mutations). The fact that over half of the missense mutations cluster in this region is consistent with its having a crucial role in JAG1 function. Experiments in the mouse have demonstrated that the DSL region is crucial for Jagged1 binding to the Notch2 receptor [Shimizu et al., 1999]. Mutation of amino acid 184, which lies immediately adjacent to the DSL domain, is the most common missense mutation, now reported in at least seven cases (Table 1). Preliminary studies of this mutant have demonstrated that the R184H protein is abnormally glycosylated, resulting in incorrect intracellular trafficking, with failure of this JAG1 molecule to reach the cell membrane [Dowhanick-Morrissette et al., 1999; Dowhanick-Morrissette and Spinner, in preparation]. Nine of the missense mutations were in the EGF repeats, and one in the cysteine rich region. Seven of the 10 mutations in these regions lead to loss of conserved cysteine residues, and one lead to the creation of a cysteine from a

tryptophan residue. This is similar to the type of mutation seen in the *NOTCH3* gene in patients with CADASIL (see below).

Eighteen polymorphisms in the *JAG1* gene have been identified and these are presented in Table 2. Two of the polymorphisms lead to the production of an altered amino acid residue (R/Q 744 and P/R 871). Polymorphisms were seen in controls, as well as in unaffected family members, and as a second change in individuals with another mutation.

BIOLOGICAL RELEVANCE

The Notch signaling pathway is a complex developmental pathway that has been demonstrated to regulate cell fate determination in *Drosophila melanogaster* and *Caenorhabditis elegans*. Jagged1 is a cell surface protein that interacts directly with the Notch transmembrane receptors. *Jagged1* is present in the rat, mouse, and human, and is highly homologous to other cell surface ligands for Notch including the *Delta* and *Serrate* genes in *Drosophila*, and *Lag-2* in *C. elegans*. Heterozygous mutations of mouse *Jag1* cause an isolated iris coloboma, but homozygous mutations result in embryonic demise with defects in vascular remodeling in both the embryo and the yolk sac [Xue et al., 1999]. There is a single *Notch* locus in *Drosophila*, and four (*NOTCH* 1, 2, 3, and 4) in humans. *JAG1* has been shown to be expressed widely throughout development and specifically during embryogenesis in structures that are destined to contribute to the cardiovascular system and the bile ducts [Mitsiadis et al., 1997; Loomes et al., 1999; Louis et al., 1999; Crosnier et al., 2000b]. Recently, in situ hybridization on human embryos showed that *JAGGED1* was mainly expressed in the cardiovascular system, but also in organs/tissues affected by major and minor features of AGS (Crosnier et al., submitted). Alagille syndrome was the first developmental disorder found to be caused by a genetic abnormality in the Notch signaling pathway. More recently, mutations in the Notch ligand Delta-like 3 have been shown to cause axial skeletal defects in patients with a recessive form of spondylocostal dysostosis [Bulman et al., 2000]. The *DLL3* mutations in the three families studied were either protein truncating (two mutations) or missense (one family). The missense mutation identified is in a highly conserved glycine residue within the fifth EGF repeat (G385D).

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), is an adult onset neurological disorder characterized by recurrent strokes and dementia, which has been shown to be caused by mutations in Notch3, located on chromosome 19. Like the Notch ligands, the Notch proteins themselves are single pass transmembrane proteins with a large extracellular domain containing 34 EGF-like repeats. Most CADASIL patients have a missense mutation in *NOTCH3* leading to addition or loss of a cysteine residue within one of the EGF-like repeats [Joutel et al., 1997]. The mutations in CADASIL patients all lead to an odd number of cysteine residues in the affected EGF domain which would be predicted to disrupt the canonical disulphide pairing [Joutel et al., 2000].

DIAGNOSTIC RELEVANCE

The diagnosis of Alagille syndrome (AGS) is primarily based on the presence of the characteristic clinical findings. Studies of *JAG1* in individuals with a clinically confirmed diagnosis of AGS have demonstrated mutations in 60 to 75% of patients. Moreover, for genetic counseling, one should keep in mind that 70% of mutations are de novo [Crosnier et al., 1999]. Therefore, the laborious screening of *JAG1* is not recommended for confirmation of the diagnosis in individuals who meet the clinical criteria but should be performed before prenatal diagnosis.

CLINICAL RELEVANCE

The clinical manifestations of AGS and of *JAG1* mutations have been shown to be highly variable ranging from subtle, sub-clinical findings to life threatening liver and/or heart disease. This was recognized originally through family studies [Watson and Miller, 1973; Shulman et al., 1984], and more recently by molecular testing [Li et al., 1997; Krantz et al., 1998; Krantz et al., 1999; Crosnier et al., 1999; Meunier-Rotival et al., 1999]. No genotype-phe-

TABLE 1. *JAGGED1* Mutations and Inheritance in AGS Patients

Exon or intron	Amino acid change[a] or other effect	Protein domain[b]	Predicted consequence	Mutation position[a]	Mutation type	Origin	Reference
EX1	No start codon	SP	No protein	3GC>TT	Deletion-insertion	de novo	Colliton et al. [2000]
EX1	Stop at nt 213	SP	Premature termination codon	30CGGGCGC>ACTCGGGA	Deletion-insertion	de novo	Crosnier et al. [2000]
EX1	L14X	SP	Premature termination codon	40delC	Deletion	ND	Crosnier et al. [2000]
EX1	Stop at nt 134	SP	Premature termination codon	41delT	Deletion	ND	Crosnier et al. [1999]
EX1	LLALLCA17-23del	SP	7 aa in-frame deletion	48-68del21	Deletion	de novo	Colliton et al. [2000]
EX1	Stop at nt 213	(1)	Premature termination codon	66-67delTG	Deletion	ND	Colliton et al. [2000]
EX1	Stop at nt 134	(1)	Premature termination codon	68delC	Deletion	de novo	Colliton et al. [2000]
EX1	R25X	(1)	Premature termination codon	73C>T	Transition	ND	Crosnier et al. [1999]
EX1	Splice site	(1)	Aberrant splicing	73del12	Deletion	de novo	Heritage et al. [2000]
IVS1	Splice site	(1)	Aberrant splicing	82-7del22	Deletion	de novo	Crosnier et al. [2000]
EX2	G33D	(1)	Amino acid substitution	98G>A	Transition	ND	Colliton et al. [2000]
EX2	L37S	(1)	Amino acid substitution	110T>C	Transition	de novo	Colliton et al. [2000]
EX2	Q43X	(1)	Premature termination codon	127C>T	Transition	ND	Colliton et al. [2000]
EX2	E48X	(1)	Premature termination codon	142G>T	Transversion	ND	Crosnier et al. [2000]
EX2	Stop at nt 479	(1)	Premature termination codon	146delT	Deletion	ND	Crosnier et al. [1999]
EX2	Q50X	(1)	Premature termination codon	148C>T	Transition	ND	Colliton et al. [2000]
EX2	CDTYF71-75del	(1)	5 aa in-frame deletion	211-225del15	Deletion	de novo	Colliton et al. [2000]
EX2	C78S	(1)	Amino acid substitution	233G>C	Transversion	ND	Colliton et al. [2000]
EX2	L79H	(1)	Amino acid substitution	236T>A	Transversion	Maternal	Crosnier et al. [1999]
EX2	Stop at nt 426	(1)	Premature termination codon	270-271insG	Insertion	ND	Crosnier et al. [2000]
EX2	Stop at nt 426	(1)	Premature termination codon	270-271insG	Insertion	Maternal	Krantz et al. [1998]
EX2	A127T	(1)	Amino acid substitution	379G>A	Transition	Maternal	Crosnier et al. [1999]
EX2	P129R	(1)	Amino acid substitution	386C>G	Transversion	Paternal	Crosnier et al. [1999]
IVS2	Splice site	(1)	Aberrant splicing	388-5T>A	Trnasversion	de novo	Crosnier et al. [1999]
IVS2	Acceptor splice site	(1)	Aberrant splicing	388-18del21	Deletion	Maternal	Colliton et al. [2000]
EX3	Donor splice site	(1)	Aberrant splicing	437-439del3,439+1del11	Deletion	Maternal	Colliton et al. [2000]
IVS3	Donor splice site	(1)	Aberrant splicing	439+1G>A	Transition	Maternal	Crosnier et al. [2000]
IVS3	Donor splice site	(1)	Aberrant splicing	439+1G>A	Transition	ND	Colliton et al. [2000]
EX4	I152T	(1)	Amino acid substitution	455T>C	Transition	ND	Pilia et al. [1999]
EX4	Stop at nt 537	(1)	Premature termination codon	468-469insCT	Insertion	ND	Heritage et al. [2000]

EX4	P163L	(1)	Amino acid substitution	488C>T	Transition	de novo	Crosnier et al. [1999]
EX4	Q166X	(1)	Premature termination codon	496C>T	Transition	de novo	Crosnier et al. [1999]
EX4	Q172X	(1)	Premature termination codon	514C>T	Transition	ND	Colliton et al. [2000]
EX4	Stop at nt 537	(1)	Premature termination codon	517-518insACAC	Insertion	de novo	Colliton et al. [2000]
EX4	Y181N	(1)	Amino acid substitution	541T>A	Transversion	ND	Colliton et al. [2000]
EX4	R184G	(1)	Amino acid substitution	550C>G	Transversion	Maternal	Crosnier et al. [1999]
EX4	R184C	(1)	Amino acid substitution	550C>T	Transition	de novo	Krantz et al. [1998]
EX4	R184C	(1)	Amino acid substitution	550C>T	Transition	ND	Heritage et al. [2000]
EX4	R184H	(1)	Amino acid substitution	551G>A	Transition	ND	Krantz et al. [1998]
EX4	R184H	(1)	Amino acid substitution	551G>A	Transition	Paternal	Crosnier et al. [1999]
EX4	R184H	(1)	Amino acid substitution	551G>A	Transition	Maternal	Colliton et al. [2000]
EX4	R184L	(1)	Amino acid substitution	551G>T	Transversion	ND	Ppilia et al. [1999]
EX4	C187S	DSL	Amino acid substitution	560G>C	Transversion	de novo	Crosnier et al. [1999]
EX4	Y191X	DLS	Premature termination codon	573C>G	Transversion	de novo[e]	Crosnier et al. [1999]
EX4	C220F	DSL	Amino acid substitution	659G>T	Transversion	de novo	Crosnier et al. [2000]
EX4	Stop at nt 1232	DSL	Premature termination codon	659GCA>TG	Deletion-insertion	ND	Krantz et al. [1998]
EX4	C229G	DSL	Amino acid substitution	685T>G	Transversion	de novo	Crosnier et al. [1999]
EX4	C229Y	DSL	Amino acid substitution	686G>A	Transition	ND	Heritage et al. [2000]
EX4	Stop at nt 720	DSL	Premature termination codon	693-694delAG	Deletion	Maternal	Li et al. [1997]
IVS4	Donor splice site	DSL	Aberrant splicing	694+2T>A	Transversion	ND	Onouchi et al. [1999]
IVS4	Acceptor splice site	DSL	Aberrant splicing	695-2A>G	Transition	de novo	Yuan et al. [1998]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	de novo	Crosnier et al. [1999]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	de novo	Crosnier et al. [1999]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	ND	Crosnier et al. [2000]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	Paternal	Yuan et al. [1998]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	de novo	Yuan et al. [1998]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	ND	Colliton et al. [2000]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	de novo	Colliton et al. [2000]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	Maternal	Colliton et al. [2000]
EX5	R235X	EGF1	Premature termination codon	703C>T	Transition	ND	Krantz et al. [1998]
EX5	R252K	EGF1	Amino acid substitution	755G>A	Transition	ND	Onouchi et al. [1999]
EX6	Stop at nt 1232	EGF1	Premature termination codon	756-762del7	Deletion	ND	Colliton et al. [2000]
EX6	Stop at nt 786	EGF1	Premature termination	757-758insT	Insertion	de novo	Crosnier et al. [1999]

(*continued*)

TABLE 1. (*Continued*).

Exon or intron	Amino acid change[a] or other effect	Protein domain[b]	Predicted consequence	Mutation position[a]	Mutation type	Origin	Reference
			codon				
EX6	Y255X	EGF1	Premature termination codon	765T>A	Transversion	ND	Crosnier et al. [1999]
EX6	Q258X	EGF1	Premature termination codon	772C>T	Transition	ND	Crosnier et al. [1999]
EX6	C284F	EGF2	Amino acid substitution	851G>T	Transition	Paternal	Crosnier et al. [1999]
EX6	C284X	EGF2	Premature termination codon	852-853delTG	Deletion	ND	Crosnier et al. [1999]
EX6	C284X	EGF2	Premature termination codon	852-853delTG	Deletion	de novo	Pilia et al. [1999]
EX6	W288C	EGF2	Amino acid substitution	864G>C	Transversion	Maternal	Crosnier et al. [1999]
EX6	W288C	EGF2	Amino acid substitution	864G>C	Transversion	Maternal	Crosnier et al. [2000]
IVS6	Donor splice site	EGF2	Aberrant splicing	886+2T>G	Transversion	de novo[f]	Oda et al. [1997]
IVS6	Donor splice site	EGF2	Aberrant splicing	886+2T>G	Transversion	de novo[f]	Krantz et al. [1998]
EX7	C306X	EGF3	Premature termination codon	918T>A	Transversion	ND	Krantz et al. [1998]
EX7	E326X	EGF3	Premature termination codon	976G>T	Transversion	de novo	Crosnier et al. [2000]
EX7	Stop at nt 999	EGF3	Premature termination codon	981-982insG	Insertion	Maternal	Crosnier et al. [1999]
EX8	E337X	EGF4	Premature termination codon	1009G>T	Transversion	Paternal	Pilia et al. [1999]
EX8	E353X	EGF4	Premature termination codon	1057G>T	Transversion	Maternal	Colliton et al. [2000]
EX8	Stop at nt 1232	EGF4	Premature termination codon	1066delC	Deletion	Paternal	Crosnier et al. [2000]
EX8	Stop at nt 1125	EGF4	Premature termination codon	1079-1080delGT	Deletion	ND	Colliton et al. [2000]
EX9	Stop at nt 1125	EGF4	Premature termination codon	1121-1122insT	Insertion	de novo	Crosnier et al. [1999]
EX9	Stop at nt 1140	EGF5	Premature termination codon	1135-1136insT	Insertion	ND	Pilia et al. [1999]
EX9	G386R	EGF5	Amino acid substitution	1156G>A	Transition	de novo	Heritage et al. [2000]
EX9	Q390X	EGF5	Premature termination codon	1168C>T	Transition	Paternal	Crosnier et al. [1999]
EX9	Stop at nt 1179	EGF5	Premature termination codon	1168-1169insC	Insertion	ND	Heritage et al. [2000]
EX9	K397X	EGF5	Premature termination codon	1189A>T	Transversion	ND	Krantz et al. [1998]
EX9	Stop at nt 1232	EGF5	Premature termination codon	1191delG	Deletion	de novo	Colliton et al. [2000]
EX9	Stop at nt 1242	EGF5	Premature termination codon	1205-1206insC	Insertion	ND	Colliton et al. [2000]
EX9	Stop at nt 1242	EGF5	Premature termination	1205-1206insC	Deletion	Maternal	Colliton et al. [2000]

			codon				
EX9	Stop at nt 1242	EGF5	Premature termination codon	1205-1206insC	Insertion	ND	Krantz et al. [1998]
EX9	Stop at nt 1242	EGF5	Premature termination codon	1205-1206insC	Insertion	ND	Krantz et al. [1998]
EX9	Stop at nt 1242	EGF5	Premature termination codon	1205-1207insC	Insertion	Paternal	Yuan et al. [1998]
EX9	Q403X	EGF5	Premature termination codon	1207C>T	Transition	ND	Krantz et al. [1998]
EX9	Q403X	EGF5	Premature termination codon	1207C>T	Transition	ND	Colliton et al. [2000]
EX9	W404X	EGF5	Premature termination codon	1212delG	Deletion	Maternal	Crosnier et al. [1999]
EX10	Stop at nt 1356	EGF6	Premature termination codon	1294-1295insCAGC	Insertion	de novo	Krantz et al. [1998]
EX10	Y435X	EGF6	Premature termination codon	1305C>G	Transversion	Paternal	Colliton et al. [2000]
EX10	C438F	EGF6	Amino acid substitution	1313G>T	Transversion	de novo	Crosnier et al. [1999]
IVS10	Cryptic splice site	EGF6	Aberrant splicing	1349-12T>G	Transversion	Paternal	Krantz et al. [1998]
EX11	Splice site	EGF7	Aberrant splicing	1395G>T	Transversion	ND	Oda et al. [1997]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395_1G>T	Transversion	ND	Krantz et al. [1998]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395+3A>G	Transition	de novo	Crosnier et al. [1999]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395+3A>G	Transition	Paternal	Crosnier et al. [1999]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395+3A>G	Transition	Maternal	Heritage et al. [2000]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395+3A>G	Transition	Paternal	Pilia et al. [1999]
IVS11	Donor splice site	EGF7	Aberrant splicing	1395+4del8	Deletion	ND	Heritage et al. [2000]
IVS11	Acceptor splice site	EGF7	Aberrant splicing	1396-2 delCTCTTCTA	Deletion	de novo	Crosnier et al. [1999]
EX12	Stop at nt 1467	EGF7	Premature termination codon	1459-1460delGA	Deletion	de novo	Crosnier et al. [1999]
EX12	Stop at nt 1467	EGF7	Premature termination codon	1461-1462delCA	Deletion	ND	Krantz et al. [1998]
EX12	Stop at nt 1490	EGF8	Premature termination codon	1463delT	Deletion	de novo	Crosnier et al. [1999]
EX12	Stop at nt 1502	EGF8	Premature termination codon	1485-1486delCT	Deletion	de novo	Crosnier et al. [1999]
EX12	Stop at nt 1502	EGF8	Premature termination codon	1485-1486delCT	Deletion	ND	Heritage et al. [2000]
EX12	Stop at nt 1502	EGF8	Premature termination codon	1485-1486delCT	Deletion	ND	Heritage et al. [2000]
EX12	Stop at nt 1502	EGF8	Premature termination codon	1485-1486delCT	Deletion	de novo	Colliton et al. [2000]
EX12	Stop at nt 1688	EGF8	Premature termination codon	1491-1494delGAAT	Deletion	ND	Oda et al. [1997]
EX12	Stop at nt 1587	EGF8	Premature termination codon	1522-1523insA	Inserton	de novo	Colliton et al. [2000]
EX12	Stop at nt 1587	EGF8	Premature termination codon	1538-1539delGT	Deletion	ND	Colliton et al. [2000]
EX12	Q523X	EGF8	Premature termination	1567C>T	Transition	ND	Colliton et al. [2000]

(*continued*)

TABLE 1. (*Continued*).

Exon or intron	Amino acid change[a] or other effect	Protein domain[b]	Predicted consequence	Mutation position[a]	Mutation type	Origin	Reference
			codon				
EX13	Y547X	EGF9	Premature termination codon	1641T>A	Transversion	ND	Colliton et al. [2000]
EX13	Stop at nt 1688	EGF9	Premature termination codon	1656delC	Deletion	Paternal	Li et al. [1997]
EX13	E553X	EGF9	Premature termination codon	1658G>T	Transversion	de novo	Colliton et al. [2000]
EX13	Stop at nt 1716	EGF10	Premature termination codon	1713-1714delCT	Deletion	ND	Crosnier et al. [1999]
IVS13	Acceptor splice site	EGF10	Aberrant splicing	1721-1G>C	Transversion	de novo	Heritage et al. [2000]
EX14	Stop at nt 2225	EGF10	Premature termination codon	1747delG	Deletion	de novo	Colliton et al. [2000]
EX14	Stop at nt 1845	EGF10	Premature termination codon	1767-1768insA	Insertion	de novo	Crosnier et al. [2000]
EX14	Stop at nt 2225	EGF10	Premature termination codon	1801delC	Deletion	de novo	Colliton et al. [2000]
EX14	Stop at nt 2225	EGF10	Premature termination codon	1807delG	Deletion	ND	Colliton et al. [2000]
EX14	Stop at nt 2225	EGF10	Premature termination codon	1810-1811insGA	Insertion	Paternal	Crosnier et al. [1999]
EX14	Stop at nt 2225	EGF10	Premature termination codon	1852-1858del7	Deletion	ND	Colliton et al. [2000]
EX14	Stop at nt 1881	EGF10	Premature termination codon	1859-1860insG	Insertion	ND	Krantz et al. [1998]
EX14	Y625X	EGF10	Premature termination codon	1875C>G	Transversion	de novo	Crosnier et al. [1999]
EX15	C633X	EGF11	Premature termination codon	1899-1900delTG	Deletion	de novo	Crosnier et al. [1999]
EX15	C633X	EGF11	Premature termination codon	1899-1900delTG	Deletion	de novo	Colliton et al. [2000]
EX15	Stop at nt 1992	EGF11	Premature termination codon	1986-1987insC	Insertion	de novo	Crosnier et al. [2000]
EX15	Stop at nt 2004	EGF11	Premature termination codon	1998-1999insC	Insertion	Maternal	Crosnier et al. [1999]
EX16	Stop at nt 2061	EGF12	Premature termination codon	2039-2040insG	Insertion	de novo	Crosnier et al. [1999]
EX16	N687X	EGF12	Premature termination codon	2058-2059insT	Insertion	ND	Krantz et al. [1998]
EX16	D692X	EGF12	Premature termination codon	2073-2074insT	Insertion	Paternal	Crosnier et al. [1999]
EX16	Stop at nt 2225	EGF12	Premature termination codon	2094delA	Deletion	ND	Onouchi et al. [1999]
EX16	Stop at nt 2225	EGF12	Premature termination	2096-2100del5	Deletion	ND	Krantz et al. [1998]

			codon				
EX16	Stop at nt 2225	EGF12	Premature termination codon	2096-2100del5	Deletion	de novo	Krantz et al. [1998]
EX16	Stop at nt 2225	EGF12	Premature termination codon	2103delC	Deletion	ND	Onouchi et al. [1999]
EX16	Stop at nt 2225	EGF12	Premature termination codon	2110delT	Deletion	ND	Crosnier et al. [1999]
EX16	S704X	EGF12	Premature termination codon	2111C>G	Transversion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2127	EGF13	Premature termination codon	2118-2119delCA	Deletion	de novo	Krantz et al. [1998]
EX17	Stop at nt 2127	EGF13	Premature termination codon	2120-2121insG	Insertion	ND	Colliton et al. [2000]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2121-2124delTCAG	Deletion	ND	Colliton et al. [2000]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Krantz et al. [1998]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	Maternal	Li et al. [1997]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Crosnier et al. [2000]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	ND	Crosnier et al. [2000]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	de novo	Pilia et al. [1999]
EX17	Stop at nt 2225	EGF13	Premature termination codon	2122-2125delCAGT	Deletion	ND	Onouchi et al. [1999]
EX17	C714Y	EGF13	Amino acid substitution	2141G>A	Transition	Maternal	Colliton et al. [2000]
EX17	Stop at nt 2181	EGF13	Premature termination codon	2173-2174insG	Insertion	ND	Krantz et al. [1998]
EX17	Stop at nt 2181	EGF13	Premature termination codon	2173-2174insG	Insertion	de novo	Pilia et al. [1999]
EX17	Stop at nt 2181	EGF13	Premature termination codon	2174-2175insA	Insertion	de novo	Crosnier et al. [1999]
EX17	C731S	EGF13	Amino acid substitution	2191T>A	Transversion	Maternal	Crosnier et al. [1999]
EX17	W735X	EGF13	Premature termination codon	2204G>A	Transition	ND	Pilia et al. [1999]
EX17	C740R	EGF13	Amino acid substitution	2218T>C	Transition	de novo	Crosnier et al. [1999]
EX18	R744X	EGF14	Premature termination	2230C>T	Transition	de novo	Krantz et al. [1998]

(*continued*)

TABLE 1. (*Continued*).

Exon or intron	Amino acid change[a] or other effect	Protein domain[b]	Predicted consequence	Mutation position[a]	Mutation type	Origin	Reference
			codon				
EX18	R744X	EGF14	Premature termination codon	2230C>T	Transition	ND	Colliton et al. [2000]
EX18	R744X	EGF14	Premature termination codon	2230C>T	Transition	ND	Colliton et al. [2000]
EX18	R744X	EGF14	Premature termination codon	2230C>T	Transition	ND	Onouchi et al. [1999]
EX18	C753R	EGF14	Amino acid substitution	2257T>C	Transition	ND	Crosnier et al. [2000]
EX18	Stop at nt 2352	EGF14	Premature termination codon	2277-2278insA	Insertion	Paternal	Colliton et al. [2000]
EX18	Stop at nt 2352	EGF14	Premature termination codon	2279-2280delTG	Deletion	Paternal	Krantz et al. [1998]
EX18	Stop at nt 2456	EGF14	Premature termination codon	2338-2339insTG	Insertion	ND	Crosnier et al. [2000]
IVS19	Splice site	EGF15	Aberrant splicing	2372+3delAAGT	Deletion	ND	Crosnier et al. [1999]
EX20	C806X	EGF15	Premature termination codon	2418C>A	Transversion	de novo	Colliton et al. [2000]
EX20	Stop at nt 2456	EGF15	Premature termination codon	2429delC	Deletion	Maternal	Crosnier et al. [2000]
EX20	Stop at nt 2466	EGF15	Premature termination codon	2442-2443insG	Insertion	de novo	Colliton et al. [2000]
IVS20	Donor splice site	EGF15	Aberrant splicing	2458+1G>T	Transversion	ND	Krantz et al. [1998]
IVS20	Donor splice site	EGF15	Aberrant splicing	2458+2delTAAG	Deletion	de novo	Crosnier et al. [1999]
IVS20	Donor splice site	EGF15	Aberrant splicing	2458+2delTAAG	Deletion	de novo	Crosnier et al. [1999]
IVS20	Donor splice site	EGF15	Aberrant splicing	2458+2delTAAG	Deletion	Paternal	Crosnier et al. [1999]
IVS20	Donor splice site	EGF15	Aberrant splicing	2458+2delTAAG	Deletion	de novo	Crosnier et al. [1999]
IVS20	Acceptor splice site	EGF16	Aberrant splicing	2459-1G>C	Transversion	ND	Pilia et al. [1999]
IVS20	Acceptor splice site	EGF16	Aberrant splicing	2459-1G>A	Transition	Paternal	Colliton et al. [2000]
EX21	Q825X	EGF16	Premature termination codon	2473C>T	Transition	de novo	Krantz et al. [1998]
EX21	VDEI836-839del	EGF16	4 aa in-frame deletion	2505-2516del12	Deletion	ND	Colliton et al. [2000]
EX21	Y842X	EGF16	Premature termination codon	2526C>A	Transversion	ND	Pilia et al. [1999]
EX22	Stop at nt 2606	CR	Premature termination codon	2601-2602insGG	Insertion	ND	Colliton et al. [2000]
EX22	Stop at nt 2631	CR	Premature termination codon	2601-2602insG	Insertion	Paternal	Oda et al. [1997]
EX22	Stop at nt 2631	CR	Premature termination codon	2606-2607delTG	Deletion	de novo	Krantz et al. [1998]
EX22	Stop at nt 2631	CR	Premature termination codon	2607-2608insT	Insertion	Paternal	Yuan et al. [1998]
EX22	P/R871X[c]	CR	Premature termination codon	2611C>T	Transition	de novo	Krantz et al. [1998]
EX22	C880X	CR	Premature termination	2639-2640delGT	Deletion	Maternal	Krantz et al. [1998]

			codon				
EX22	C880X	CR	Premature termination codon	2639-2640delGT	Deletion	Maternal	Oda et al. [1997]
EX22	C892X	CR	Premature termination codon	2676C>A	Transversion	ND	Krantz et al. [1998]
EX22	S893X	CR	Premature termination codon	2678C>A	Transversion	ND	Krantz et al. [1998]
EX23	W896X	CR	Premature termination codon	2688G>A	Transition	de novo	Heritage et al. [2000]
EX23	Stop at nt 2831	CR	Premature termination codon	2694-2695insGTGGC	Insertion	Paternal	Li et al. [1997]
EX23	R900X	CR	premature termination codon	2698C>T	Transition	de novo	Crosnier et al. [1999]
EX23	R900X	CR	premature termination codon	2698C>T	Transition	ND	Crosnier et al. [1999]
EX23	R900X	CR	premature termination codon	2698C>T	Transition	de novo	Heritage et al. [2000]
EX23	C902S	CR	Amino acid substitution	2705G>C	Transversion	Maternal	Colliton et al. [2000]
EX23	Stop at nt 2850	CR	Premature termination codon	2725-2726insC	Insertion	de novo	Pilia et al. [1999]
EX23	Q924X	CR	Premature termination codon	2770C>T	Transition	ND	Crosnier et al. [2000]
EX23	Stop at nt 2831	CR	Premature termination codon	2774-2775insTG	Insertion	ND	Colliton et al. [2000]
EX23	Stop at nt 2831	CR	Premature termination codon	2817-2818insGGTCTTCC	Insertion	de novo	Crosnier et al. [1999]
EX23	Stop at nt 2906	CR	Premature termination codon	2836delA	Deletion	ND	Crosnier et al. [2000]
EX23	Stop at nt 2895	CR	Premature termination codon	2874-2875delTG	Deletion	ND	Crosnier et al. [2000]
EX23	Stop at nt 2895	CR	Premature termination codon	2874-2875delTG	Deletion	de novo	Colliton et al. [2000]
IVS23	Donor splice site	CR	Aberrant splicing	2916+1G>C	Transversion	de novo	Crosnier et al. [1999]
IVS23	Donor splice site	CR	Aberrant splicing	2916+1G>C	Transversion	de novo	Oda et al. [1997]
IVS23	Donor splice site	CR	Aberrant splicing	2917-1G>C	Transversion	de novo	Crosnier et al. [1999]
EX24	Splice site[d]	CR	Aberrant splicing	2918-2919insTTTTAGGG	Insertion	de novo	Pilia et al. [1999]
EX24	Stop at nt 2943	CR	Premature termination codon	2926-2927insA	Insertion	Paternal	Crosnier et al. [1999]
EX24	Stop at nt 2982	CR	Premature termination codon	2943-2944insC	Insertion	ND	Colliton et al. [2000]
EX24	Stop at nt 2966	CR	Premature termination codon	2960delA	Deletion	ND	Colliton et al. [2000]
EX24	Stop at nt 3030	CR	Premature termination codon	3000-3001ins19	Insertion	de novo	Colliton et al. [2000]
EX24	Stop at nt 3104	CR	Premature termination codon	3001-3004delGCTT	Deletion	Paternal	Colliton et al. [2000]
Ex24	Stop at nt 3030	CR	Premature termination codon	3001-3020del20	Deletion	de novo	Crosnier et al. [1999]
EX24	C1002X	CR	Premature termination	3006C>A	Transversion	Paternal	Crosnier et al. [1999]

(*continued*)

TABLE 1. (*Continued*).

Exon or intron	Amino acid change[a] or other effect	Protein domain[b]	Predicted consequence	Mutation position[a]	Mutation type	Origin	Reference
			codon				
EX24	E1003X	(2)	Premature termination codon	3007G>T	Transversion	de novo	Crosnier et al. [1999]
EX24	Stop at nt 3104	(2)	Premature termination codon	3013-3014insTT	Insertion	de novo	Colliton et al. [2000]
EX24	Stop at nt 3104	(2)	Premature termination codon	3016-3017insTTCCC	Insertion	de novo	Crosnier et al. [1999]
EX24	Stop at nt 3104	(2)	Premature termination codon	3027delC	Deletion	de novo	Crosnier et al. [1999]
EX25	Stop at aa1036	(2)	Premature termination codon	3035-3036insTA	Insertion	Maternal	Yuan et al. [1998]
EX25	Stop at nt 3096	(2)	Premature termination codon	3070-3071insAAGATAT	Insertion	ND	Pilia et al. [1999]
EX25	Stop at nt 3104	(2)	Premature termination codon	3099delC	Deletion	ND	Colliton et al. [2000]
EX25	Stop at nt 3143	(2)	Premature termination codon	3123-3124insAA	Insertion	ND	Crosnier et al. [2000]
EX25	Stop at nt 3164	(2)	Premature termination codon	3161-3164delAAGT	Deletion	de novo	Crosnier et al. [1999]
EX25	Stop at nt 3185	(2)	Premature termination codon	3164-3167delTAAG	Deletion	de novo	Crosnier et al. [1999]
EX25	Stop at nt 3185	(2)	Premature termination codon	3164-3167delTAAG	Deletion	de novo	Crosnier et al. [2000]
EX25	Stop at nt 3321	(2)	Premature termination codon	3196-3197insA	Insertion	de novo	Crosnier et al. [1999]
EX26	Stop at nt 3321	TM	Premature termination codon	3221insTGAG	Insertion	ND	Colliton et al. [2000]
EX26	Stop at nt 3321	TM	Premature termination codon	3224-3225delCT	Deletion	Paternal[a]	Yuan et al. [1998]

[a]The *JAGGED1* sequence is that of the cDNA of the GenBank accession no. HSU73936; the nucelotide (nt) positions are based on those of the reference minus 413, i.e., the A of the ATG of the Met codon is denoted nucleotide +1.
[b]SP, signal peptide, (1) between signal peptide and DSL domain (named for Delta/Serrate/Lag-2, a region conserved between the Drosophila Delta and Serrate and the *C. Elegans* Lag-2 gene); EGF, epidermal growth factor-like repeats; CR, cysteine-rich region, (2) between cysteine-rich region and transmembrane domain; TM, transmembrane domain. ND, not determined.
[c]The allele with the arginine residue of the P/R871 polymorphism is mutated in a stop codon.
[d]An 8-bp duplication at the junction between intron 23 and exon 24 could create an alternative splice site or a premature stop codon.
[e]Mutation is found in two brothers, but in neither of their unaffected parents (germinal mosaicism).
[f]Mutation is found in both of male affected identical twins, but not in either of the unaffected parents.
[g]Mutation is found in both of fraternal twin boys, who inherited it from their father.

TABLE 2. Polymorphisms in the *JAGGED1* Coding Sequence

Exon	Nucleotide polymorphism[a]	Amino acid
2	105C/T[b]	F35
2	267G/A[b]	G89
2	270G/T[b]	G90
2	294C/T[b]	S98
4	588C/T[c]	C196
5	744A/G[b]	P248
6	765T/C[b]	Y255
7	924C/T[b]	N308
11	1389C/T[b]	S463
13	1578C/T[c]	I526
13	1707G/A[d]	T569
17	2214A/C[c]	T738
18	2231G/A[b]	R/Q744
20	2382C/T[c]	S794
22	2612C/G[c]	P/R871
23	2766C/T[b]	D922
26	3417C/T[c]	Y1139
26	3528T/C[c]	Y1176

[a]The *JAGGED1* nucleotide sequence is that of the cDNA of the GenBank accession no. HSU73936; the nucleotide (nt) positions are based on those of the reference minus 413, i.e., the A of the ATG of the Methionine codon is denoted nt +1.
[b]From Crosnier et al. [1999].
[c]From Krantz et al. [1998].
[d]From Pilia et al. [1999].

notype correlation has been observed in any of the reported studies and as stated, the same mutation can be associated with a range of clinical findings, even within families.

FUTURE PROSPECTS

The finding that mutations in *JAG1* cause Alagille syndrome has improved diagnosis in clinically uncertain cases, allows carrier detection and prenatal testing if a mutation can be identified, and provides information regarding the fundamental abnormality in this developmental disorder. However, many questions remained unanswered. Do the 30% of patients in whom no mutations have been identified have as yet undiscovered mutations in *JAG1* or is there another locus for AGS? An approach to answering this is to perform linkage analysis on families with multiple affected members, in whom no mutation has been identified. These studies are currently in progress in our laboratories. Development of a useful molecular diagnostic test will be greatly aided by the ability to identify disease-causing mutations in a higher percentage of patients. How can the variable expressivity be explained? Are there genetic or environmental modifiers or is the variation reflective of random fluctuations of Jagged-Notch signaling in the presence of a single functioning *JAG1* allele?

The identification of *JAGGED1* as the disease gene for AGS links a critical developmental pathway to a multisystem congenital disorder in humans. The findings that mutations in another human developmental disorder, spondylocostal dysostosis, are caused by mutations in the Notch ligand Delta-like 3 underscores the crucial role of this signaling pathway in human development. It is anticipated that other human disorders will be found to be caused by defects in members of the Notch signaling pathway, contributing further insight into the molecular events of both human disease and normal and abnormal development.

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