

Identification and Cloning of the Human Homolog (*JAG1*) of the Rat *Jagged1* Gene from the Alagille Syndrome Critical Region at 20p12

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Notch proteins are a family of closely related transmembrane receptors proven to be instrumental in cell fate decisions. Recently, Notch ligands Delta and Jagged have been identified in *Drosophila* and rat, respectively. We have isolated the human homolog of the rat *Jagged1* gene, *JAG1*, from a CpG island in a YAC clone covering the Alagille syndrome critical region at chromosome 20p12 (tel-*SNAP*-*D20S186*-cen). Alagille syndrome is an autosomal dominant disorder characterized by neonatal jaundice, paucity of intrahepatic bile ducts, and abnormalities of the heart, skeleton, and eyes. The human *Jagged1* (*JAG1*), therefore, appears to be a strong candidate gene for this disease. Here we describe the identification, full-length cDNA cloning, expression patterns, and precise physical location of this gene within the Alagille syndrome critical region.

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Alagille syndrome (AGS; MIM No. 118450) is an autosomal dominant disorder characterized by neonatal jaundice and paucity of intrahepatic bile ducts on liver histology, first described in 1975 (2). Features that often accompany this syndrome include congenital heart defects; abnormal vertebrae and decreased interpediculate distance in the lumbar spine; retinal pigmentary changes and posterior embryotoxon; variable degrees of mental retardation; and a typical facies consisting of a broad forehead, pointed mandible, deep-set eyes, and a bulbous tip of the nose (1, 2). The disease has a wide range of expression, varying from an apparently normal phenotype to severe cases where cirrhosis and liver failure require liver transplantation early in childhood.

The locus for the AGS has been mapped to 20p12 based on multiple cases of interstitial deletions (5–7) and on linkage analysis in a three-generation family to markers from this region (8, 9). Molecular analysis of a t(2;20)(q21.3;p12) translocation/deletion in an Alagille patient identified *D20S186* as the centromeric

boundary (15, 16). From the telomeric end, *D20S162* defined the breakpoint for the hemizyosity region in a patient with a submicroscopic deletion (14). However, recently, a closer marker, *SNAP*, was reported to be the distal boundary by FISH analysis of chromosomes from a deletion patient (10, 11). The critical region for the AGS gene, therefore, is defined by *SNAP* and *D20S186* and represents a 1.1- to 1.4-Mb interval with two CpG islands (Fig. 1A) within a 3.7-Mb YAC clone contig reported for this region (13).

YAC 755E10, a 1-Mb clone from the CEPH mega YAC library (3), extends over more than half of the AGS region and contains a CpG island with *SacII*, *MluI*, and *NotI* sites, (the only *NotI* site in the entire 3.7-Mb region) (Fig. 1A). The region flanking the *NotI* site was isolated from the YAC DNA by subcloning a *NotI*-*EcoRI* fragment into a plasmid vector. Essentially, YAC DNA from YAC 755E10 was purified by pulsed-field gel electrophoresis, digested with *NotI* and *EcoRI*, and ligated with a *NotI/EcoRI*-digested Bluescript cloning vector (pBSII; Stratagene). A clone with a 2.9-kb insert was identified by screening colonies with labeled human genomic DNA as a probe. The sequence of this clone from the *NotI* site showed high homology with rat *Jagged1* cDNA sequence (12) (GenBank Accession No. L38483). The full-length human homolog of the rat *Jagged1* gene was assembled starting from the 2.9-kb genomic fragment as shown in Fig. 1B. A human cDNA clone (IMAGE: <http://www-bio.llnl.gov/bbrp/image/image.html>; Clone ID. 117734) (Fig. 1B, b) was identified from the EST database when the rat *Jagged1* cDNA sequence was subjected to BLAST analysis (4). This human cDNA clone was confirmed to originate from the 20p12 region by hybridization to a Southern blot containing digests from YAC 755E10 and other overlapping YAC clones from the AGS critical region as well as a chromosome 20-only somatic cell hybrid (data not shown). Primers designed from this 1-kb human cDNA sequence were used to test its expression by PCR in several Superscript cDNA libraries (GIBCO BRL). Both liver and brain cDNA libraries were screened by the Genetrapp system (GIBCO BRL), and several cDNA clones were isolated. The largest clone was a 4.3-kb cDNA clone (Fig. 1B, c) isolated from a human liver

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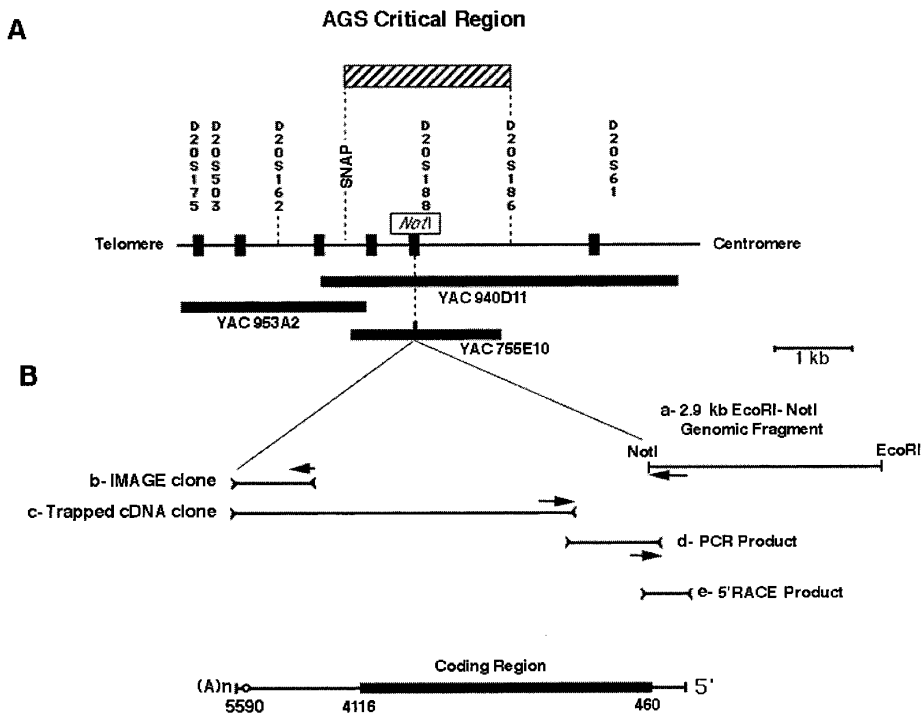


FIG. 1. Cloning of the human *Jagged1* cDNA from the Alagille syndrome (AGS) critical region. (A) The location of the 1.1- to 1.4-Mb AGS critical interval flanked by *SNAP* and *D20S186* on a 3.7-Mb physical map for the chromosome 20p12 region. The map indicates the locations of six CpG islands (filled boxes) based on the YAC contig described by Pollet *et al.* (13). Three overlapping YAC clones, YAC 940D11 (2.1 Mb), YAC 953A2 (1.3 Mb), and YAC 755E10 (1 Mb), encompassing the AGS critical region and the only *NotI* restriction site associated with a CpG island, are shown. (B) Schematic representation of full-length cDNA cloning of *JAG1* starting from the CpG island associated *NotI*-*EcoRI* genomic fragment. The orientation of the 5.9-kb cDNA suggests the direction of transcription (centromeric to telomeric). The coding region from nt 460 to 4116 is indicated by a thick bar, and the location of the first poly(A) signal at nt 5590 is shown as a circle. The full-length cDNA was assembled from the 2.9-kb genomic DNA fragment (a), the 1-kb cDNA clone identified from the IMAGE database (b), the 4.3-kb cDNA clone isolated by the Genetrappor system from the human liver Superscript cDNA library (c), the PCR products amplified from heart poly(A) RNA (d), and from the 5'RACE reaction product (e). The locations of the primers used for Genetrappor cDNA screening, for amplification of the 1 kb cDNA, and for the 5'RACE reaction are indicated by small arrows.

library. This clone hybridized to a single 5.9-kb transcript in nearly all the tissues (Fig. 2), albeit at variable levels of expression, with highest expression occurring in ovary, prostate, pancreas, placenta, and heart. The level of expression was somewhat lower in the colon, small intestine, spleen, and skeletal muscle, much lower in testis and thymus, and barely visible in leukocyte, kidney, liver, and lung. Northern blots contained poly(A) RNA (2 μ g) isolated from adult tissues. The low

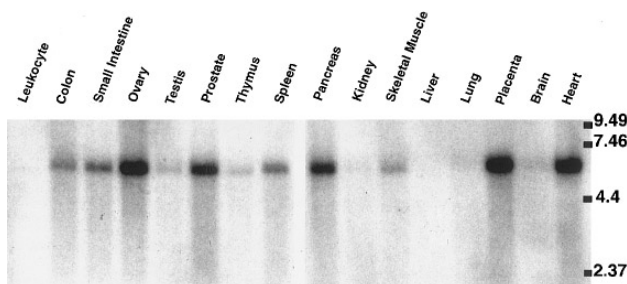


FIG. 2. Northern blot analysis of *JAG1* expression. Two multiple tissue Northern blots (human poly(A) blots, MTN and MTN II; Clontech) were probed with the 4.3-kb cDNA clone (Fig. 1B, c). The source of poly(A) RNA in each blot is indicated at the top. Each lane contained 2 μ g of poly(A) RNA isolated from corresponding adult tissues.

level of expression in adult liver may not appear to support the possible involvement of *JAG1* in AGS, a syndrome with prominent liver abnormalities. However, AGS is primarily a developmental disorder, and the levels of *JAG1* expression in adult tissue may not necessarily reflect its role in liver development.

An additional nearly 1-kb cDNA (Fig. 1B, d) was amplified starting from a cDNA template generated from human heart poly(A) RNA (Clontech) using flanking primers: one located near the 5' end of the 4.3-kb trapped cDNA clone and the other near the *NotI* end of the 2.9-kb genomic clone (Fig. 1B, c and a). To amplify this GC-rich region, hot start PCR was carried out under the conditions described by Valdes *et al.* (18). Finally, a 5'RACE reaction (GIBCO BRL) was performed using an antisense primer generated from the 5' end of the 1-kb PCR cDNA on human heart poly(A) RNA (Clontech). A 500-bp RACE product (Fig. 1B, e) provided the 5' end of the transcript.

The complete cDNA sequence (GenBank Accession No. AF003837) of human *Jagged1* (5942 bp) shown in Fig. 3 has 88.8% homology to the rat *Jagged1* cDNA sequence. The coding region (ORF) consists of 3657 nt and shows 97.6% identity with that of the rat *Jagged1*.

only one transcript is observed on the Northern blot, the differential polyadenylation could be related to the regulation of *JAG1* in specific tissues.

The human *Jagged1* (*JAG1*) is the only gene identified so far within the AGS critical region defined by the *SNAP* gene and the marker *D20S186*. Recently, the same human transcript (U77720) was identified by differential display, and its role in hematopoiesis was reported (19). Additionally, two other complete cDNA sequence entries have also been deposited in GenBank (U73936 and U61276). The sequence reported here is a full-length cDNA including 459 nucleotides in the 5'-untranslated region. The role of the *Notch1* gene product, and its ligands in cell fate determination (12, 17) as well as the clinical features of the Alagille syndrome patients, makes *JAG1* a strong candidate gene for Alagille syndrome. Further investigation is needed to determine whether this gene is altered in Alagille syndrome patients.

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