*JAGGED*1 expression in human embryos: correlation with the Alagille syndrome phenotype

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Abstract

Alagille syndrome (AGS, MIM 118450) is an autosomal dominant disorder with a variable phenotype characterised by hepatic, eye, cardiac, and skeletal malformations and a characteristic facial appearance. Mutations within the gene *JAGGED*1 (*JAG*1), which encodes a ligand for NOTCH receptor(s), has been shown to cause Alagille syndrome. Interactions of NOTCH receptors and their ligands influence cell fate decisions in several developmental pathways. We report the tissue expression of *JAG*1 in human embryos.

We have performed tissue in situ hybridisation on human embryos aged 32-52 days using ^{35}S labelled riboprobes for *JAG*1. *JAG*1 is expressed in the distal cardiac outflow tract and pulmonary artery, major arteries, portal vein, optic vesicle, otocyst, branchial arches, metanephros, pancreas, mesocardium, around the major bronchial branches, and in the neural tube. We conclude that *JAG*1 is expressed in the structures affected in Alagille syndrome, such as the pulmonary artery, anterior chamber of the eye, and face.

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Keywords: Alagille syndrome; arteriohepatic dysplasia; *JAGGED*1; NOTCH signalling

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Alagille syndrome (AGS, MIM 118450) is an autosomal dominant disorder associated with abnormalities of the liver, heart, eye, skeleton, and a characteristic facial appearance described first in 1969.[1] It is one of the most common paediatric causes of chronic liver disease and has an estimated incidence in a mixed white population of 1 in 100 000 live births with no gender differences when ascertained on the basis of neonatal liver disease.[2 3] The diagnosis is based on the finding of paucity of the interlobular bile ducts associated with three to five major features: chronic cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities, and a characteristic facial phenotype (table 1). However, the phenotype is very variable and within the same family one patient may present with life threatening congenital heart disease, whereas others may have only mild cholestasis. As yet, no specific phenotype has been associated with a particular genotype.[4] The clinical features of AGS are shown in table 1.

Table 1 Clinical features of Alagille syndrome and the frequency of occurrence[5–8]

Clinical feature	*% of affected patients*	*Comments*
Intrahepatic bile duct paucity	91	This increases in frequency with age
Chronic cholestasis	94	
Cardiac murmur or defect	92	Peripheral pulmonary stenosis is the most common defect but a structural cardiac defect is present in 24%
Characteristic facies	91	Prominent forehead, deep set eyes, pointed chin, and a saddle or straight nose with a bulbous tip
Eye defects	80	Anterior chamber defects, eg posterior embryotoxon, Axenfeld's anomoly, or Rieger anomoly, but retinal pigmentary changes and optic disc drusen have also been reported
Skeletal abnormalities	51	Butterfly vertebrae
Renal abnormalities	40	Renal tubular acidosis
Growth retardation	87	
Pancreatic insufficiency	41	Exocrine
Chronic otitis media	35	
Intracranial bleeding	14	
Small bowel stenosis/atresia	7	

Interstitial deletions within chromosome 20p were initially identified in a small number of Alagille cases and subsequently mutations in *JAG*1 were identified, showing that this was the disease gene.[9–11] *JAG*1 stretches over 36 kb of genomic DNA on chromosome 20p12 and comprises 26 exons varying in size from 28 to 2284 bp to produce a 5.9 kb mRNA transcript.[9] The *JAG*1 gene encodes a protein belonging to the family of NOTCH ligands. These contain conserved sequences: a DSL domain (named after Delta, Serrate, and Lag-2 ligands for *C elegans* NOTCH), a varying number of EGF-like repeats (16 in human *JAG*1), a cysteine rich NOTCH region, and a transmembrane domain. In a recent study by Crosnier *et al*,[12] mutational analysis was performed on samples from 109 unrelated patients; 63% had intragenic mutations including 14 nonsense mutations, 31 frameshifts, 11 splice site mutations, and 13 missense mutations. Mutations were de novo in 40 of 57 probands. All the mutations mapped to the extracellular domain of the protein and the majority are predicted to give rise to truncated proteins.

Transgenic mice homozygous for the *Jag*1 mutation die from haemorrhage during early embryogenesis, exhibiting defects in remodelling of the embryonic and yolk sac vasculature. Mice heterozygous for the *Jag*1 null allele exhibit an eye defect similar to that seen in AGS patients, but do not have a similar hepatic or cardiac phenotype.[13] Therefore, there is a discrepancy between the mouse and the human phenotype.

NOTCH and its ligands are well conserved between species and are involved in the determination of cell fate.[14] However, the differences in phenotype between *JAG*1 mutations in human and mouse suggest that their roles may not be identical or there is a greater degree of redundancy in mice. Northern blot analysis of human adult RNA indicates that *JAG*1 is widely expressed and most abundant in ovary, prostate, pancreas, placenta, and heart. Lower levels are seen in colon, small intestine, spleen,

and skeletal muscle and the lowest levels in testis, thymus, leucocytes, kidney, liver, and lung.[9] Human *JAG*1 studies have shown that *JAG*1 is expressed in the developing ductal plate of the fetal liver at 14 weeks, but earlier embryonic material was not examined.[15] A recent study by Loomes *et al*[16] has shown that the expression of *JAG*1 in the developing mouse heart correlates with the picture of cardiovascular disease seen in Alagille syndrome. This study also included examination of a 56 day old embryonic human heart. Unfortunately, this is at the end of the embryonic period and cardiogenesis will almost have been completed. We have investigated *JAG*1 in earlier human embryonic development in order to determine the gene expression pattern and correlate it with the defects seen in Alagille syndrome.

Methods

EMBRYO COLLECTION

Collection and use of human embryonic tissue was carried out following ethical approval from the Newcastle Health Authority. Embryos were collected following medically (RU486) or surgically induced termination of pregnancy, staged immediately according to the Carnegie classification system by stereomicroscopy, fixed overnight in 4% paraformaldehyde in phosphate buffered saline, and embedded in paraffin wax.[17–19]

IN SITU HYBRIDISATION

A 550 bp fragment of exon 26 of the human *JAG*1 gene was used as a template to make sense and antisense RNA probes (IMAGE clone 430227) and also a 500 bp fragment of a similar region of mouse *Jag*1 (IMAGE clone 1227823). RNA probes were labelled with ^{35}S using RNA in vitro transcription.[19] Paraffin embedded mouse embryos between 9 and 13 ed and human embryos between 32 and 52 days post ovulation (dpo) were sectioned at 5 μm intervals and tissue in situ hybridisations were performed as previously described.[19]

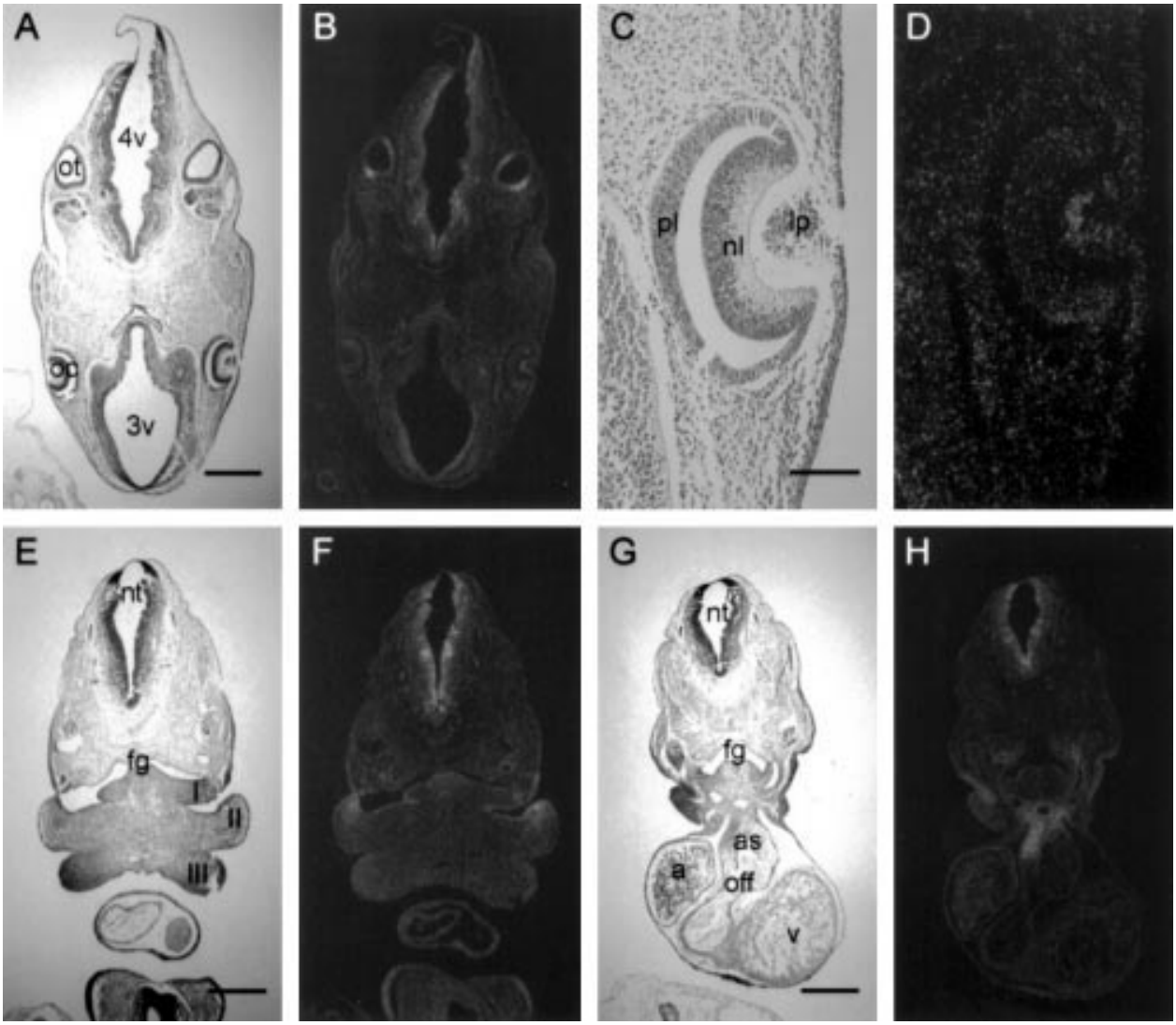

Figure 1 JAG1 expression in transverse sections of a Cs14 (32 dpo) human embryo. (A, C, E, G) Haematoxylin and eosin stain. Dark field images of tissue in situ hybridisation showing JAG1 expression in optic cup, otic vesicle, and neural epithelium (B), lens placode (D), branchial arches and neural tube (F), and distal cardiac outflow tract, aortic sac, atrial walls, branchial arches, and neural tube (H). Scale bar 500 μm (A, E, G), 125 μm (C). oc=optic cup, ot=otic vesicle, 4v=4th ventricle, 3v=3rd ventricle, lp=lens placode, pl=future pigment layer of retina, nl=neural layer of optic cup, nt=neural tube, fg=foregut, I, II, III=1st, 2nd, and 3rd branchial arches, as=aortic sac, a=atria, v= ventricle, off=cardiac outflow tract.

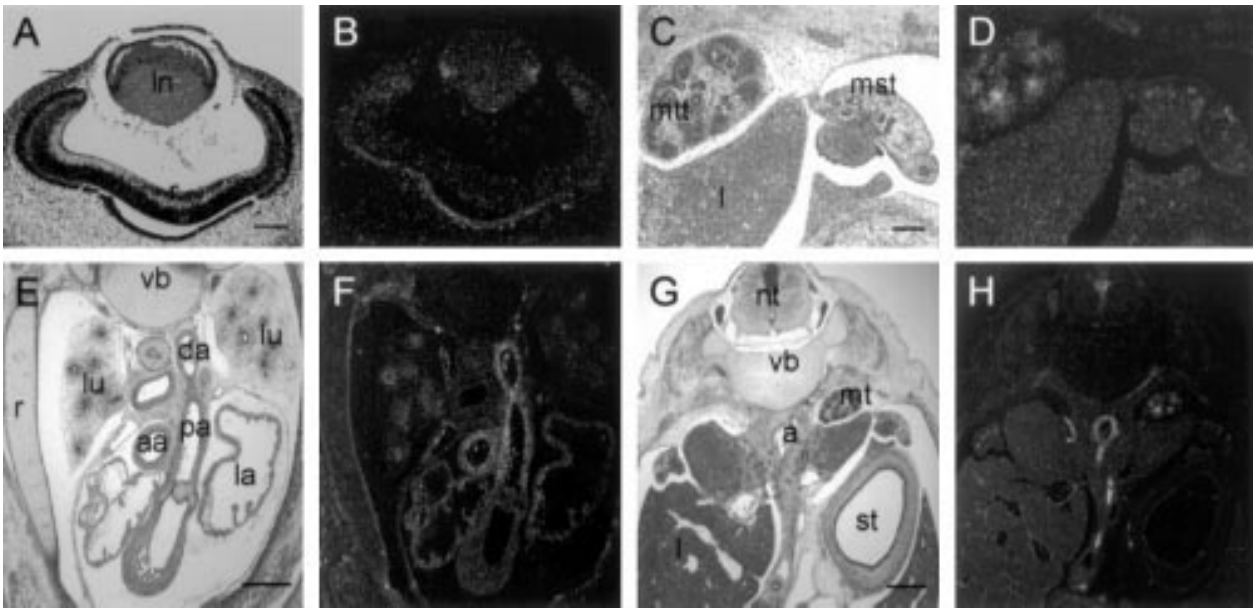

Figure 2 JAG1 expression in transverse sections of a Cs21 (52 dpo) human embryo. (A, C, E, G) Haematoxylin and eosin stain. Dark field images of tissue in situ hybridisation showing JAG1 expression in the circumferential part of the lens and anterior part of the visual layer of the retina (B), in metanephric and mesonephric tubules (D), in pulmonary artery, aortic outflow tract, and atria (F) and in aorta, metanephros, and mesonephros (H). Scale bar 125 μm (A, C), 500 μm (E,G). ln=lens, r=retina, mst=mesonephric tubules, mtt=metanephric tubules, l=liver, vb=vertebral body, aa=ascending aorta, da=descending aorta, pa=pulmonary artery, la=left atrium, lu=lung, r=rib, nt=neural tube, a=aorta, mt=metanephros, and st=stomach.

Results

At 32 dpo expression is seen in the lens vesicle and the tip of the optic cup (fig 1B, D). By 52 dpo expression was confined to the circumference of the developing lens and the anterior part of the visual layer of the retina (fig 2B).

JAG1 is expressed strongly in the distal cardiac outflow tract, aortic sac, and dorsal aortae at 32 dpo (fig 1H). A lower level of expression was seen in the atria, particularly the right, but no convincing expression has been seen in the cardiac ventricles (fig 1H). By 52 dpo expression is detected in the pulmonary outflow tract, pulmonary artery, and aorta (fig 2F), but at lower levels than 32 dpo.

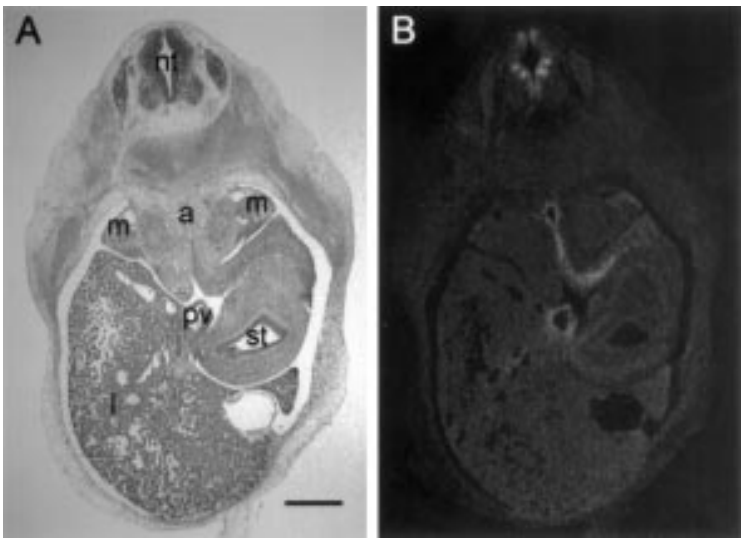

Figure 3 JAG1 expression in transverse sections of a Cs17 (41 dpo) human embryo. (A) Haematoxyllin and eosin stain. (B) Dark field image of tissue in situ hybridisation showing JAG1 expression in portal vein, aorta, splenic artery, and neural tube. Scale bar 500 μm. nt=neural tube, m=mesonephros, a=aorta, pv=portal vein, st=stomach, l=liver.

*JAG*1 is expressed in the portal vein and hepatic artery by 41 dpo (fig 3B). It is not expressed in the hepatic parenchyma during the age range studied.

*JAG*1 is expressed in the mesonephric tubule at 32 dpo and later in the metanephric tubules (52 dpo) (figs 3B and 2D, H).

Embryos aged 32 dpo showed strong *JAG*1 expression in the left and right sinus horns, branchial arches (fig 1F), rhombencephalon, otocyst (fig 1B), and neural tube (fig 1F and fig 3B). There was also expression around the bronchi. A striking feature was the strong expression of *JAG*1 in all the major arteries, such as the aorta and iliac and vertebral arteries. By 52 dpo *JAG*1 was expressed strongly in the ependymal layer of the spinal cord and the omentum of the midgut. At 13 weeks there was also expression in the pancreas (results not shown).

*Jag*1 expression was also examined in tissue sections of mouse embryos (embryonic day 9-13) and the distribution of expression was similar to that seen in human embryos.

Discussion

We have shown that *JAG*1 expression during human embryonic development predominately localises to the organs or tissues that are affected in Alagille syndrome. The expression in the outflow tract, particularly the developing pulmonary trunk, correlates with common cardiac manifestations; 67% of patients with AGS have either right outflow tract obstruction (for example, pulmonary valve stenosis) or peripheral pulmonary artery stenosis.[5] It is interesting that although *JAG*1 is also expressed in the developing aorta, the majority of cardiac defects affect the right side of the heart.

The peripheral margin of the optic cup differentiates into the ciliary body and iris.[20]

*JAG*1 is expressed in the presumptive ciliary body region before it is morphologically distinguishable from the adjacent neural retina and iris. Therefore, it is not surprising that ophthalmological findings in patients with AGS predominately affect the anterior chamber. Posterior embryotoxon, although not entirely pathognomonic of AGS, occurs in 55% of patients. It is characterised by a prominent Schwalbe's line (a line of material in the anterior chamber at the junction of the cornea and the uveal trabecular network). Although usually asymptomatic, such anterior chamber malformations can result in glaucoma. By 52 dpo *JAG*1 is expressed in the circumference of the lens and in the anterior part of the visual layer of the retina. This may explain the findings reported by Emerick *et al*[5] of retinal pigmentary changes and optic disc drusen.

*JAG*1 is expressed in the portal vein and hepatic artery by 41 dpo. The ductal plate forms around the branches of the portal vein at about 56 dpo and is subsequently remodelled to produce mature bile duct architecture. During development, *JAG*1 is expressed in the ductal plate and postnatally in the biliary epithelium.[15] The classical histopathological lesion in AGS is bile duct paucity but this lesion is progressive and is often not evident in the newborn period.[21] The initial formation of the bile ducts appears to be macroscopically normal and then there is a gradual loss of these bile ducts. Initial correct formation of the ductal plate may well be in keeping with the phenotype of AGS. However, a defect in the development of the ductal plate may lead to later regression of the bile ducts. This is in contrast to the structural lesions in the heart and eye, which are primary malformations. It is possible that the bile duct loss is not the result of abnormal bile duct formation, but rather a later insult that affects the integrity of the bile ducts. This may be because of haploinsufficiency of *JAG*1 in the bile duct epithelium or a vascular defect.

One of the striking findings of the expression pattern of *JAG*1 was the strong expression in all the major arteries. This is interesting as diffuse vascular abnormalities are a minor feature of Alagille syndrome. Renal artery stenosis,[22] middle aortic syndrome,[23] and intracranial bleeding[8] have all been associated with Alagille syndrome. Additionally, patients sometimes have ileal stenosis or atresia suggesting possible prenatal ischaemic injury. Thus, it is possible that defects in the NOTCH signalling cascade result in vascular anomalies leading to these manifestations in Alagille syndrome.

Mutations in the gene for *NOTCH*3 receptor cause adult onset CADSIL which is associated with intracranial bleeding.[24] Patients with CADSIL have small, deep cerebral infarcts, leucoencephalopathy, and a non-atherosclerotic, nonamyloid angiopathy involving the media of small cerebral arteries. Histopathological analysis shows major lesions of vascular smooth muscle cells that eventually disappear. Thus, NOTCH signalling pathways have roles in adult vertebrate tissues and in mature cells. If these pathways are perturbed then serious disease may result. The mechanism of the vasculopathy in AGS may be similar to that seen in CADSIL.

Patients with AGS have clefting of their vertebrae known as butterfly vertebrae. *Notch*1 and 2 are expressed in the presomatic mesoderm and *Notch*1 mutant mice undergo disorganised somitogenesis.[25] Thus, the vertebral anomalies may be the result of disrupted NOTCH signalling. However, as *JAG*1 is not expressed in the developing vertebrae but is expressed in the vertebral arteries it is possible that the clefting is the result of vascular compromise during development.

*JAG*1 is expressed in both the meso- and metanephros. Renal anomalies have been reported in 23-74% of patients in series that have examined renal function.[6] In the study of Emerick *et al*,[5] the commonest abnormality was renal tubular acidosis but a number of structural abnormalities were also noted. These included small, hyperechogenic kidney, uretopelvic obstruction, renal cysts, and infantile onset renal insufficiency, which may be considered either structural or functional. Therefore, it is possible that more than one mechanism of disease may play a part in the renal abnormalities.

*JAG*1 is also expressed at sites that do not appear to be affected in AGS, such as in the neural tube. Structural defects of the nervous system are not commonly seen in AGS. It is possible that there is functional redundancy in the NOTCH signalling system and other ligands may substitute for *JAG*1. For instance, in mice, *Jag*2 is also expressed in the spinal cord.[26]

In summary, the embryonic expression pattern of *JAG*1 shows correlation with many of the tissues affected in Alagille syndrome and gives clues to the pathogenetic mechanisms operating in this disease.

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