Hypertension and Biliary Ductopenia in a Patient with Duplication of Exon 6 of the JAG1 Gene

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Abstract: We describe a neonatal patient with biliary ductopenia featuring duplication of exon 6 of the JAG1 gene. Facial alterations were observed, consisting of a prominent forehead, sunken eyes, upward slanting palpebral fissures, hypertelorism, flat nasal root and prominent chin. From birth, these were accompanied by the development of haematuria and renal failure and by renal Doppler findings indicative of peripheral renal artery stenosis. JAG1 gene mutations on chromosome 20 have been associated with various anomalies, including biliary cholestasis, vertebral abnormalities, eye disorders, heart defects and facial dysmorphia. This syndrome, first described by Alagille, is an infrequent congenital disorder caused by a dominant autosomal inheritance with variable expressivity. Anatomopathological effects include the destruction and disappearance of hepatic bile ducts (ductopenia). The duplication of exon 6 of JAG1 has not previously been described as an alteration related to the Alagille syndrome with peripheral renal artery stenosis.

Keywords: hypertension, cholestasis, Alagille syndrome, chronic renal insufficiency, JAG1
**Introduction**

Alagille syndrome (AS) is a genetic disorder first described by Daniel Alagille in 1969. It is an autosomal dominant disorder with a broad spectrum of clinical presentations, which may vary even within the same family. Its prevalence is about 1/70,000–100,000. Criteria for diagnosis include histological findings after liver biopsy of bile duct paucity (ductopenia), in association with three of the five main clinical manifestations. These major diagnostic criteria include cholestasis, heart defects (usually involving the pulmonary artery and its branches), posterior embryotoxon, vertebral anomalies and characteristic facial features. Although these criteria are necessary to classify an individual with the syndrome, it has long been recognised that relatives of affected patients may present some of these characteristics and are likely to express milder forms (microforms) of the disease. It can manifest with prolonged jaundice due to conjugated hyperbilirubinaemia and/or cardiac signs and symptoms in newborns. Cardiac anomalies reported include pulmonary atresia or stenosis, atrial and/or ventricular septal defects, tetralogy of Fallot and patent ductus arteriosus. Cholestasis is manifested by conjugated hyperbilirubinaemia, hepatosplenomegaly, hypercholesterolaemia, hypertriglyceridaemia and coagulopathy. Pruritus and xanthomas may occur. Minor anomalies include butterfly vertebrae (approximately 50% of cases) and shortening of the radius, ulna and phalanges. The characteristic facial features, if present, are usually evident from childhood and include prominent forehead, sunken eyes, upward slanting palpebral fissures, hypertelorism and prominent chin. Ophthalmic anomalies include posterior embryotoxon (75% of cases), Axenfeld’s anomaly, retinitis pigmentosa and papillary and optic disc abnormalities. Patients show growth retardation, malabsorption of fat (rickets may occur) and, sometimes, failure to thrive. The kidneys may be small and dysplastic (common in type 2 syndrome), and hypothyroidism may be present. AS can be related to heart or lung disease or peripheral arterial stenosis. The reduced calibre of the peripheral renal arteries can cause hypertension and/or renal failure.

Type 1 AS (ALGS1, OMIM# 601920) is usually caused by mutations in the JAG1 gene (20p12), which encodes a ligand in the Notch signalling pathway. Type 2 AS (ALGS2, OMIM# 600275) is caused by mutations in the NOTCH2 gene (1p12). Transmission is autosomal dominant, but reduced penetrance is common (up to 50% of cases), as is somatic mosaicism (8%). Mutations in the JAG1 gene have been found in 88% of cases, while 7% have chromosomal deletions. Among the mutations found, 50%–70% are sporadic or de novo, while 30%–50% are hereditary.

In this paper, we report a case of duplication of exon 6 of JAG1 on chromosome 20, associated with bile duct paucity (ductopenia) and with peripheral renal artery stenosis.

**Patient and Methods**

In the case described, a premature newborn, referred from another hospital, presented the following characteristics: monochorionic diamniotic twin delivery at 34 weeks’ gestation; umbilical arterial pH of 7.09; birth by urgent caesarean delivery, due to alterations in the cardiotocographic trace; neonatal reanimation required, with tracheal intubation; Apgar score of 3 at one minute and of 5 at five minutes after birth. In the following hours of life, intense skin pallor and generalised hypotonia were observed, together with hypotension and haematuria (10350 cel/mcL). Facial abnormalities of hypertelorism, epicanthus and broad forehead were observed, but there was no evidence of any skeletal abnormalities. Anuria developed, and the following analytical results were obtained: creatinine 274 mcM/L, urea 22.3 mmol/L, sodium 124 mEq/L, potassium 6 mEq/L, chloride 91 mEq/L, calcium 1.85 mmol/L, total protein 33 g/L. In consequence, peritoneal dialysis was required from the third day of life and maintained for 24 days. The initial renal ultrasound showed the right kidney measured 50 mm and the left one, 45 mm. Doppler ultrasound showed an increase in arterial resistance and an almost total absence of diastolic flow, indicative of peripheral and bilateral renal artery stenosis (Fig. 1). The isotope renogram showed reduced cortical uptake. The coagulation study revealed prothrombin activity of 39%, activated partial thromboplastin time (APTT) 40 sec, INR 1.64, fibrinogen 114 mg/dL and D-dimer > 32 mg/L. A state of disseminated intravascular coagulation (DIC) required repeated infusions of packed cells, fresh plasma and platelets. Moreover, from the moment of admission, dopamine infusion was required in order to...
maintain blood pressure levels, and mechanical ventilation was performed for 28 days. Prior to the withdrawal of peritoneal dialysis, progressive jaundice was observed, developing to a cholestatic syndrome with peak direct bilirubin levels of 427.5 mcmol/L (N: 0–3.4 mcmol/L) and peak levels of serum ferritin of 6,600 mcg/L (N: 25–200 mcg/L), although these levels fell after treatment with chelating agents and vitamins. The newborn developed Enterobacter cloacae sepsis at 24 days of life, with raised levels of bilirubin, GGT and factor V. With the data then available, the existence of neonatal haemochromatosis was proposed as a diagnostic possibility, and a total blood exchange transfusion was performed, together with the infusion of intravenous immunoglobulin (1 g/kg). Hepatic Doppler ultrasound showed mild intrahepatic biliary dilatation. The liver scan showed biliary elimination delay and the liver biopsy revealed bile duct paucity, but the possible presence of hemosiderin deposits was discounted. A cardiological examination revealed a systolic murmur, and echocardiography highlighted the presence of a foramen ovale type atrial septal defect with left to right shunt, together with pulmonary artery stenosis. On admission, a tendency to metabolic acidosis (pH 7.16, pCO₂ 37, CO₂HNa 13.2) was observed, caused by the sustained decrease in serum bicarbonate levels (minimum 11.5 mmol/L), which required orally-administered supplementation. With respect to hydroelectrolytes, the levels of Na, K and Cl were normal, accompanied by hyperphosphataemia (3.68 mmol/L) and hypokalaemia (1.25 mmol/L), which improved after the oral administration of 1.25 hydroxycholecalciferol. Arterial hypertension (154/86 mmHg) required the administration of captopril (0.5 mg/Kg/8 h) and losartan (0.3 mg/Kg/day). Ophthalmologic examination did not reveal any presence of embryotoxon.

At discharge, the newborn showed icteric colouration, good vitality and slightly reduced muscle tone in the waist. The abdomen was soft and depressible with 3 cm hepatomegaly, with no splenomegaly. Breathing was spontaneous, and no supplemental oxygen was needed. Cardiorespiratory auscultation revealed persistence of the systolic murmur, but without repercussions, and the body weight was 3,200 g.

After discharge, periodic check-ups were carried out of phosphocalcic metabolism, renal function, acidosis and blood pressure, together with haematology and anaemia tests. In addition, liver enzyme values were tested, and GGT, ALT and AST were all found
to be normal. The infant is currently being treated with captopril, bicarbonate and losartan.

On ultrasound examination after two months, the right kidney measured 44 mm and the left one, 41 mm, with a highly echogenic cortex. The Doppler renal scan revealed arterial and venous flows, with a peripheral blood flow pattern of very high resistance and absence of diastolic flow. These findings are similar to those observed in the first week of life.

In subsequent examinations, a genetic study for Alagille syndrome was requested, which confirmed a heterozygous duplication in the exon 6 area of the JAG1 gene, located at 20p12.1-p11.23 with an autosomal dominant mode of inheritance. An identical study was requested of the newborn’s twin sister, which proved to be negative with respect to this duplication. The method used for this study was to examine DNA from the blood of the patient, following the protocol reported by Bertina et al., with some modifications. To study large gene deletions/duplications on chromosome 20p12.2, where the JAG1 gene is located, we used the MLPA (Multiplex Ligation-dependent Probe Amplification) technique; thus, ABI PRISM 310 visualisation enabled us to detect the existence of both heterozygous and homozygous mutations, although not that of isolated mutations.

**Discussion**

We present a case of biliary ductopenia and bilateral renal artery stenosis associated with duplication of exon 6 of the JAG1 gene on chromosome 20. The accepted clinical diagnosis for the “classic” form of AS includes the presentation of 3/5 clinical features such as anomalies of the liver, heart, vertebrae, eye or face together with bile duct paucity on liver biopsy. Diagnostic imaging (abdominal ultrasonography, cholangiography) helps to identify the biliary anatomy. A systematic diagnosis of ophthalmic, skeletal, vascular and endocrine (thyroid) abnormalities is required. The diagnosis can be confirmed by DNA sequencing. The differential diagnosis includes biliary atresia, congenital hepatic fibrosis, cystic fibrosis, neonatal jaundice, polycystic kidney disease, progressive familial intrahepatic cholestasis and tyrosinaemia. Treatment is non-specific and includes a diet high in carbohydrates and medium/long-chain triglycerides, with vitamin supplementation. It may be necessary to perform a liver transplant in patients with refractory disease, and cardiac or vascular procedures may be required for significant symptomatic lesions. The prognosis is usually favourable, but complications such as cirrhosis, variceal bleeding, refractory ascites and spontaneous bacterial peritonitis may occur. The disease usually stabilises by the age of 4–10 years. If cardiac insufficiency and/or lesions are present, the mortality risk is heightened.

Multisystem abnormalities of this syndrome are mainly due to mutations of the JAG1 gene in chromosome 20, but there are no published references on the role of the duplication of some exons of the gene during its production. We believe that acidosis may be responsible for the consumption coagulation observed at birth, although its presence associated with haematuria and very slight nephromegaly initially suggested the possibility of renal vein thrombosis. However, the persistence of the renal Doppler findings, beyond the acute period of the disease, seems to suggest peripheral renal artery stenosis as the cause of the high blood pressure and decreased glomerular filtration rates. Several authors have reported arterial stenosis in patients with AS, and it is known to cause renal failure and hypertension when located in renal arteries. However, the duplication of exon 6 of the JAG1 gene has not been reported previously.

Harendza et al described a new mutation of the JAG1 gene in a patient who developed hypertension and chronic renal failure.

The human Jagged1 (JAG1) gene on chromosome 20p12 has been identified as the gene for ALGS1. The JAG1 gene encodes a ligand in the Notch1 signalling pathway, the expression of which is related to the embryogenesis of the organs affected in this syndrome, including the cardiovascular system, the liver and blood vessels. Studies have shown that the phenotypes of patients with JAG1 mutations are indistinguishable from those of patients with deletions of chromosome 20p12 spanning the entire JAG1 gene. On the basis of these findings, it has been proposed that haploinsufficiency is the mechanism that generates the disease phenotype. In the Notch signalling pathway, of which JAG1 is a component, at least another seven members have been identified in humans. Moreover, mutations in one or more of these other components, or mutations in other (as yet unidentified) genes, may also influence the ALGS1 phenotype.
The expanded genetic study conducted on our patient shows a duplication of exon 6 of the JAG1 gene on chromosome 20. The result of this study is consistent with a heterozygous duplication in the exon 6 area of the JAG1 gene (Fig. 2). This duplication has not previously been described in relation to ALGS1. According to previous reports, renal malformations such as small hyperechoic kidneys, renal cysts, ureteropelvic obstructions, and even renal tubular acidosis, are relatively infrequent, but have been observed in 23%–74% of patients with ALGS1. In our patient, ALGS1 was associated with the chronic renal failure caused by bilateral arterial renal stenosis since birth. It is not known whether the genetic alteration observed in our patient is associated with renal alteration, although renal artery stenosis has been described in other patients with AS.

The prognosis for survival of these children depends on the associated renal and cardiac alterations, together with the degree of fibrosis present. It has been proposed that the disease may be addressed by liver transplantation, especially if there are signs of hepatic insufficiency or severe portal hypertension, conditions that have a major impact on the quality of life, and which may be caused by pruritus refractory to treatment, malnutrition, etc. These patients are also at risk of cardiovascular disease from prolonged hyperlipidaemia, as bypass surgery has not proved to be as effective as transplantation. Most untreated cases progress to cirrhosis and the histopathological finding is a sign of poor prognosis. Survival, when AS is associated with cardiac lesions, has been estimated at 40%, and when no such lesions are present (which is the case of our patient), at up to 80%. As the twin sister does not present this genetic disorder, presumably the parents will not present it either, and thus there would be de novo duplication.

With respect to the expected outcome for this patient, in view of the developments observed during the first 12 months of life, and taking into account the latest analytical tests made, the form of biliary ductopenia associated with the genetic pattern described is expected to evolve toward a more benign hepatic status, even if hypertension and renal failure persist.

Author Contributions
Analysed the data: JU, LM, AM-H. Wrote the first draft of the manuscript: JU, LM, AM-H. Contributed to the writing of the manuscript: JU, LM, AM-H. Agree with manuscript results and conclusions: JU, LM, AM-H. Jointly developed the structure and arguments for the paper: JU, LM, AM-H. Made critical revisions and approved final version: JU, LM, AM-H. All authors reviewed and approved of the final manuscript.

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