Case report

Oral manifestations of Alagille syndrome

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SUMMARY

Alagille syndrome (AGS) is a multisystem disorder classically involving liver and heart failure, characteristic vertebral and facial features and ocular abnormalities. AGS is caused by heterozygous mutations in JAG1 or NOTCH2, with variable phenotype penetrance. We report two cases of AGS in children with tooth defects characterised by green discolouration and hypomineralisation. The role of hyperbilirubinaemia (HB) in this atypical colour, a classical feature of AGS, has been well described. However, it does not totally explain the dental phenotype. As JAG1 and NOTCH2 mutations can affect bone development and considering common physiological pathways between bone and tooth mineralisation, both mutations could participate in this unusual dental phenotype. The role of HB and genetics in the development of the dental phenotype of AGS is discussed in two prototypical cases. Future research should focus on the underlying genetic component of tooth abnormalities.

BACKGROUND

Alagille syndrome (AGS) is an autosomal dominant disorder involving defects of the Notch signalling pathway, most commonly a mutation in *JAG1*, a ligand of the Notch receptor. The estimated prevalence of AGS is from 1/100 000 to 1/70 000 births.[1]

The major diagnostic criteria of AGS are chronic cholestasis due to the paucity of intrahepatic bile ducts, pulmonary artery stenosis, 'butterfly-like' vertebrae (caused by lack of fusion of the anterior arch of the vertebra) and a posterior embryotoxon (thickening and central displacement of anterior border ring of Schwalbe in the eyes). Specific facial features include: prominent forehead, deep-set eyes, upslanted palpebral fissures, hypertelorism, flat nasal root and pointed chin.[2]

Besides these common clinical features, various affections (minor criteria) can be detected in the skeleton, blood vessels, kidneys, ears, gastrointestinal tract, lungs, central nervous system, skin or teeth.[1 3]

AGS represents 10%–15% of neonatal cholestasis cases and is associated with congenital hyperbilirubinaemia (HB),[4] secondary to biliary excretion dysfunction. Biliary stasis is due to the progressive destruction of interlobular bile ducts, resulting in increased plasma levels of bile acids, causing pruritus. Furthermore, lack of biliary excretion in the digestive lumen results in the malabsorption of fat-soluble elements, including vitamins A, D, E and K, subsequently responsible for other metabolism disorders (particularly, coagulation and phosphocalcic metabolism disorders). Various degrees and time courses of liver damage have been described in AGS, responsible for an impaired synthesis of 25-hydroxy vitamin D. In 15% of cases, hepatocellular insufficiency will ultimately lead to transplantation.[5]

During tooth development, HB has been suggested to cause green discolouration and structural abnormalities of affected teeth.[6–8] In addition, bilirubin accumulation in soft tissues leads to jaundice of skin and mucosa. Bilirubin also accumulates in mineralised tissues, although temporarily until complete remodelling. Conversely, as dental mineralised tissues do not undergo such remodelling, bilirubin deposits are not eliminated as bilirubinaemia decreases. Several case series have described the abnormal tooth phenotype in AGS but did not link green teeth and tooth defects to the disease's pathophysiology.[5 9–12] As dentin and bone share similar aspects and considering that dentin defects can reflect genetic bone mineralisation disorders,[13] investigating the bone system may thus help understand the teeth hypomineralisation observed in AGS patients. For instance, a study investigated bone fragility in children with AGS. These children had significant bone mineral deficits and more fractures than healthy controls. Vitamin D deficiency was not likely the sole explanation for the increased fracture risk, and the authors showed a direct genetic contribution to bone mineralisation.[14] Hence, tooth mineralisation also may be directly affected by the genetic mutations in AGS.

This article describes dental findings in two cases of AGS and discusses the role of HB and genetics in the development of the dental phenotype. We highlight the importance of further investigations focusing on Notch-mediated mineralised tissue alterations in AGS.

CASE PRESENTATION

Case 1

An 11-year-old Melanesian girl was referred to our paediatric dentistry department, to eliminate potential oral infectious foci before hepatic transplantation. AGS had been diagnosed at birth on neonatal cholestasis and was linked to a common heterozygous *JAG1* mutation. The girl presented all of the major criteria of AGS: cholestasis, bilateral embryotoxon and stenosis of the pulmonary artery branches. Her facial features comprised a domed forehead, moderate hypertelorism and flattened nose root (figure 1). Several minor criteria were also present: numerous cutaneous xanthomas, debilitating pruritus and stunted growth. The girl had muco-cutaneous jaundice and HB, with a total

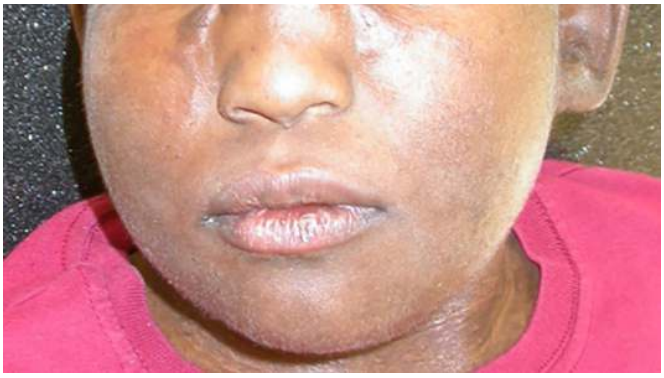

Figure 1 Flattened nose root and cutaneous xanthomas (case 1).

bilirubin level of 182 µmol/L (normally ≤20 µmol/L). Liver transplantation had been indicated early because of refractory pruritus, not resolved with usual oral treatments (rifampicin, ursodeoxycholic acid, hydroxyzine and sertraline).

Her height was less than the 3rd percentile and weight was in the 50th percentile. Cardiovascular examination revealed a 2/6 systolic murmur without other abnormalities. The abdomen was soft, with mild hepatosplenomegaly. Cutaneous examination revealed widespread xerosis.

Oral examination revealed a yellow-green discolouration of all permanent teeth (except for unerupted third molars and teeth #2, #15 and #31) (figure 2A). One carious first molar had been previously extracted (#3). The enamel of all erupted teeth had a smooth and regular surface. Oral hygiene was insufficient as evidenced by accumulation of plaque and debris on the cervical region of teeth. Severe gingival inflammation was associated. Prior deciduous teeth colouration had not been reported by attending physicians, although the mother did not notice any significant colouration changes between deciduous and permanent teeth. Oral soft tissues were healthy and normal. All first molars were restored with amalgams and active caries were observed on incisors and bicuspids. Areas of yellowish hypomineralisation were present on the labial cusps of the left lower second molar (#18), the right lower second bicuspid and the first molar (#29 and #30) (figure 2B). These hypomineralisations were not located in classical areas of tooth decay secondary to poor oral hygiene. Orthopantomography confirmed the presence of all teeth except #3 and wisdom teeth (figure 2C).

Figure 2 Intraoral photographs of case 1. Of note, all teeth are green with areas of hypomineralisation. (A) Frontal view: teeth show a yellow-green discolouration. Plaque and debris are noted (arrowheads). Gingival inflammation and bleeding are also present. (B) Mandibular view: areas of hypomineralisation are present on the labial cusps of #18, #29 and #30 (long black arrows). (C) Orthopantomogram: all permanent teeth are present except #3 and wisdom teeth.

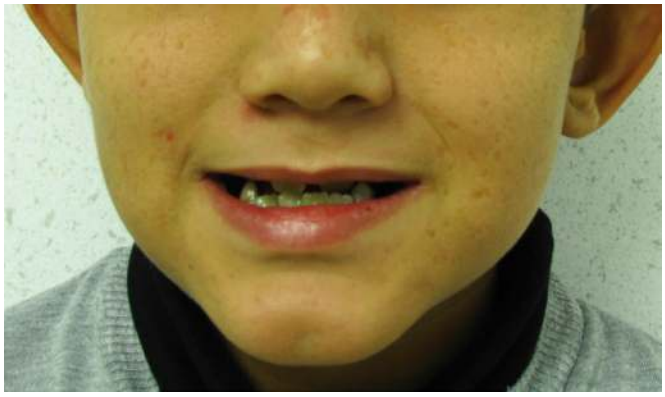

Figure 3 Jaundice and pointed chin in a 7-year-old Caucasian boy with Alagille syndrome (case 2).

Case 2

A 7-year-old Caucasian boy was initially referred to an orthodontist for management of an anterior cross-bite. AGS had been diagnosed at birth with neonatal cholestasis, associated with a *JAG1* mutation. The patient carried a c.3431 heterozygous insertion in the exon 24 of the *JAG1* gene which led to frameshift and a premature termination codon. He presented several major criteria of AGS: cholestasis, stenosis of the pulmonary artery branches, 'butterfly-like' vertebrae, associated to minor criteria: triangular face with slightly broad forehead and pointed chin (figure 3).

Additional findings included debilitating pruritus and horseshoe-shaped kidneys. The patient had presented jaundice and HB since birth. At age 7 total bilirubin level was 199 µmol/L (normally ≤20 µmol/L), with conjugated bilirubin level at 167 µmol/L (normally ≤5 µmol/L). Debilitating pruritus was controlled by symptomatic treatment: rifampicin, ursodeoxycholic acid, hydroxyzine and multivitamin supplementation.

His height was in the 50th percentile and his weight was less than the 25th percentile. Cardiovascular examination revealed a 4/6 systolic murmur with a patent arterial duct without haemodynamic repercussions as well as a supernumerary superior left vena cava. The abdomen was soft, with mild hepatosplenomegaly.

Dental examination showed a transitional dentition phase, with primary canine teeth present (#M and #R) (figure 4A,B). All primary molars had been extracted because of caries (figure 4C). Several oral abnormalities were observed. Primary and permanent teeth had a gray-green discolouration with white areas of hypomineralisation at the top of the cusps and vestibular surfaces. The enamel had a smooth and regular surface. Oral hygiene was insufficient and active carious lesions were observed on first molars and canine vestibular surfaces (International Caries Detection and Assessment System score 3) secondary to plaque accumulation. Occlusion was also disturbed (presence of an anterior crossbite between #9 and #24, #23, small maxillary arch and dento-maxillary disharmony). Oral soft tissues were normal, apart from severe gingival inflammation (figure 4A,B).

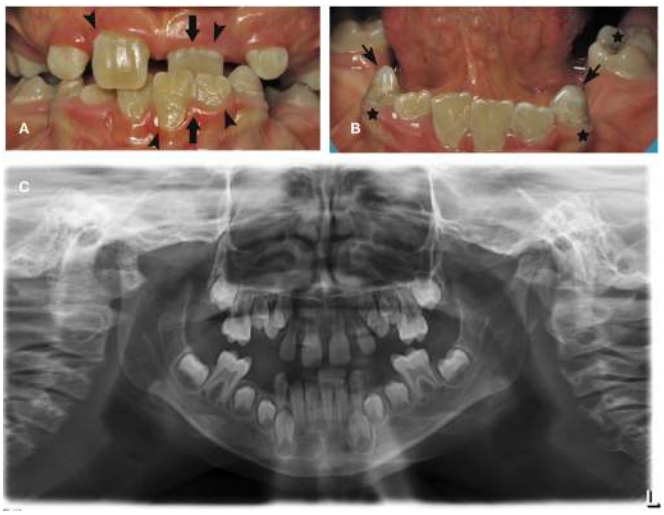

Figure 4 Intraoral photographs of case 2. (A) Frontal view: teeth are greyish green with multiple missing teeth secondary to extraction of carious teeth. Mild inflammation is present; plaque and debris are noted on a smooth and regular enamel surface (arrowheads). Crossbite of tooth #9 is observed (wide arrows). (B) Mandibular view: areas of hypomineralisation (arrows) and carious lesions located on #M, #R and #18 (star). (C) Orthopantomogram: all permanent teeth are erupted except wisdom teeth.

OUTCOME AND FOLLOW-UP

A 2 months postexamination, patient 1 received a liver transplant. She was able to return home a few months later. Unfortunately, chronic rejection occurred, requiring a new liver transplantation. No dental follow-up was possible during that period and will be resumed as soon as feasible.

After the treatment of his carious lesions, patient 2 had an orthodontic treatment. Oral cavity was checked every 6 months.

DISCUSSION

Two cases of green and/or hypomineralised teeth associated with AGS have been presented herein.

Most cases of 'green teeth' described in the literature are associated with newborn cholestasis.[15] In children with AGS, HB begins at birth. Only a liver transplantation, performed several years later in this indication, can restore normal serum bilirubin levels. Consequently, permanent teeth whose crowns mineralise from birth to the age of 9 may be affected. Conversely, in patients with biliary atresia (accounting for 50% of neonatal cholestasis cases[6]), only primary teeth and first erupted permanent teeth (incisors and first molars) are affected.[15] Indeed, the other permanent teeth are not affected as early surgery during childhood leads to rapid correction of bilirubinaemia.[5] Furthermore, permanent teeth that mineralise after this period are not concerned as well.

In a spectrophotometric analysis, Watanabe *et al* showed that the green discolouration results from bilirubin deposits in dentin and not in the enamel. The authors observed no abnormality in the dentin tubular structure, but bilirubin was incorporated in incremental parallel lines throughout the dentin.[16]

The colour ranges from light yellow to dark green and is difficult to accurately characterise.[17]

Only one study has tried to determine a minimal concentration of bilirubin and critical period leading to teeth discolouration, suggesting a mean duration of HB of 24.6 weeks with bilirubin concentrations >50 µmol/L.[17] However, the minimal concentration of bilirubin leading to teeth discolouration has still not been established.

Interestingly, some patients with high bilirubin levels for various amounts of time do not present with the green tooth discolouration.[18] Guimares *et al* suggested that HB alone does not predict the occurrence of discolouration on primary or permanent teeth.[17] Beyond the concentration of bilirubin, the level of unconjugated bilirubin could be a determinant in this discolouration.[19] Neves-Silva *et al* found a relation between childhood HB, green teeth and dentin morphology. Compared with teeth from healthy children, green teeth presented a decrease in dentine tubule density and in peritubular dentin thickness.[20]

An in vitro study carried out with stem cells from human exfoliated deciduous teeth showed that the teeth's structural morphology might be altered by HB. Indeed, a high bilirubin level induced cell death and impaired odontogenic stem cells via AKT, extracellular signal-regulated protein kinase (ERK1/2) and nuclear factor-kappa B (NF-κB) pathways. The authors suggested that such a process was reversible when bilirubin levels returned to normal.[21] Such mechanisms could explain the structural abnormalities (hypoplasia and hypomineralisation) observed in green teeth.

Most published cases of teeth discolouration and abnormalities related to HB were secondary to biliary atresia, AGS and sepsis.[19] Although the pathophysiology of green teeth is starting to be uncovered, a direct correlation cannot be made for the moment. In addition, structural abnormalities such as hypomineralisation are only partially related to HB.[22] Thus, one could look for a relationship between the systemic pathology and subsequent tooth phenotype. Other factors such as genetics could also be considered.

JAG1 mutations are found in most AGS cases.[22] More than 430 *JAG1* mutations have been described in the syndrome.[23] *JAG1* is located on chromosome 20p and encodes the Jagged 1 protein, a receptor of Notch2. A small number of patients (1%–2%) present mutations in *NOTCH2* instead.[24] Furthermore, some studies suggest that the location of pathogenic variants cannot predict the clinical manifestations of the disease. No genotype/phenotype correlation has yet been made.[25]

The Notch signalling pathway is involved in the development of many organs, particularly the skeleton and face, and also in tooth regeneration.[26] It plays a prominent role in controlling the differentiation and selection of cell fate.[25] In the absence of relevant data and in order to determine whether and how JAG1 and NOTCH2 mutations affect tooth development, we suggest investigating—by analogy—another mineralised tissue, bone.[13]

Skeletal anomalies, specific facial features and 'butterfly-like' vertebrae are classical clinical features of AGS. Vertebral abnormalities have been identified in 54% of AGS cases.[27] Additional skeletal abnormalities such as 'square shape to the proximal finger, ulna shortening, aseptic necrosis and temporal bone abnormalities' have also been reported.[28] Some studies reported a role of JAG1 and Notch signalling in bone development and homeostasis.[29] In a genome-wide association study of Chinese women, a single-nucleotide polymorphism identified in the *JAG1* gene was associated with a low risk for osteoporotic fractures. This polymorphism was also correlated with variations in bone mineral density.[30] Additional studies support *JAG1* and Notch signalling pathways' important roles in bone maintenance and remodelling. Animal studies suggest that the underlying genetic defect in AGS may lead to structural abnormalities of cortical and trabecular bones.[31–33]

Bales *et al* suggested that individuals with AGS might have an intrinsic bone formation defect, in addition to cholestasis-related

metabolic bone disease. AGS patients have increased risk of fracture and low bone density.[14]

Sokol and Stall showed greater growth deficiencies among children with AGS. However, as compared with other chronic liver diseases, this finding indicates a possible implication of *JAG1* mutations.[34] Furthermore, the frequency of clinical findings was compared in *NOTCH2*-related AGS and *JAG1*-related cohorts probands. AGS individuals with *NOTCH2* mutations showed decreased penetration of characteristic facial features and skeletal involvement as compared with *JAG1* probands.[35]

Learning points

- In Alagille syndrome, green teeth are essentially due to hyperbilirubinaemia, but a direct role of genetic disease or childhood illness on dentin structure cannot be excluded.
- Further studies are needed to characterise tooth defects for these individuals.
- In light of the role of NOTCH2 and JAG1 on bone physiology, one could investigate possible relevant consequences on tooth formation and mineralisation.

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