Intermittent Cholestasis as a Clinical Manifestation of Sodium Taurocholate Cotransporting Polypeptide (SLC10A1) Deficiency

Carolina Rivera-Nieto**, Veronica Angel² and Camilo Velandia²

1Chief of Medical Genetics. Pediatric Hospital. Cardioinfantil Foundation. Bogotá, Colombia
2Medical Genetics Intern, University of Rosario. Bogotá, Colombia
3Investigation Assistant, Molecular Biology, Cardioinfantil Foundation, Bogotá, Colombia

*Corresponding author: Carolina Rivera-Nieto, Chief of Medical Genetics. Pediatric Hospital. Cardioinfantil Foundation. Bogotá, Colombia. Email: carolinariveran@gmail.com

Abstract

The liver uptake of bile salts is accomplished in sodium-independent and sodium-dependent manners. The Na+-taurocholate cotransporting polypeptide SLC10A1 (NTCP) plays a key role in the enterohepatic circulation of bile salts. The identification of NTCP deficiency confirms that this transporter is the main import system for conjugated bile salts into the liver but also indicates that auxiliary transporters are able to sustain the enterohepatic cycle in its absence. NTCP deficiency has been described as new inborn error of metabolism with a relatively mild clinical phenotype. It is also a cause of hypercholanemia and cholestasis in children. Although NTCP was cloned as early as in 1994 and its function has been studied extensively, clinical description of NTCP deficiency remains rather limited thus far. The patient suffers from intermittent cholestasis and no signs of liver dysfunction despite transaminits. Pruritus was present only in cholestasis episodes. A homozygous point mutation was found in the SLC10A1 gene resulting in a truncated protein. We present the first adult latin american patient with a defect in NTCP.

Keywords: Sodium taurocholate cotransporting polypeptide deficiency, SLC10A1, NTCP, Bile acid, Cholestasis

Introduction

The production of bile depends on the adequate transport of solutes from the hepatocyte to the canalule through the apical membrane. The liver uptake of bile salts is accomplished in sodium-independent and sodium-dependent manners. Sodium-independent is mediated by the multiple organic anion transporting polypeptides (OATPs), while sodium dependent is mediated predominantly by the sodium taurocholate cotransporting polypeptide (NTCP) [1-3], which is encoded by the SLC10A1 gene [4,5]. NTCP is a transmembrane transporter located in the basolateral membrane of hepatocytes, and has an important role in the bile acid uptake, accounting for 80% of the hepatic uptake of bile salts by a sodium dependent pathway [6]. Deficiency of sodium taurocholate cotransporting polypeptide (NTCP) is an inborn error of bile acid metabolism caused by biallelic SLC10A1 variants, which impairs the NTCP function as the primary transporter of conjugated bile salts from the plasma into hepatocytes [7]. The first patient with NTCP deficiency was reported by Vaz et al. [8]. Since then, several papers about patients with NTCP deficiency have been published. To date, no latin american adult patients have been described with mutations in SLC10A1. We describe for the first time a latin american adult patient that suffers from intermittent cholestasis caused by a defect of NTCP. The patient was homozygous for a nonsense mutation in SLC10A1 gene that resulted in a premature stop codon at position 35 of the NTCP protein.

Materials and methods

Patient

A 28 year old Colombian female was referred to Medical Genetics service due to intermittent cholestasis, pruritus and jaundice. She complains about abdominal distention and pain, weight loss, insomnia and secondary amenorrhea. She is daughter of a non-consanguineous union with no family history of hereditary diseases. Physical examination revealed a body weight of 63.2 kg, a height of 163 cm (BMI 23.79 kg/m2). No jaundice was observed in the skin and sclera. No stridor, crackles or crepitus was heard in the two lungs, and the heart sound was normal without any murmurs. There was no abdominal distention, and the liver and spleen were non-palpable. No abnormal
movements or pathological reflexes could be found on nervous system examination. Elevation of transaminases and GGT, as other clinical biochemistry were normal (Table 1). A screening for metabolic liver diseases was performed, ruling out Wilson disease and hemochromatosis. Autoimmune diseases were also discarded. A disorder of bile acid metabolism was highly suspected.

**Next Generation Sequencing (NGS), Sanger sequencing and in silico analysis**

Total genomic DNA from the patient was extracted with the QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) from a blood sample. A Sure SelectXT (Aigilent Technologies, Santa Clara, CA, USA) target enrichment system kit was used for exome capture. Raw reads (length 150 bp) passing quality filters were multiplexed using Casava software (Illumina, San Diego, CA, USA). The BWAMEM algorithm was used for aligning high quality reads with the human reference genome (HG19 version GRCh37). Variant calling was performed using Samtools. 0.1.18. Variant filtering was performed directly on all exome data according to a clinical suspicion of hereditary cholestasis. Potentially pathogenic variants (missense, non-sense, splice site and frameshifts) having <0.05 minor allele frequencies (1000 genome project or Exome Variant Server-University of Washington-EVS) were considered for subsequent analysis. The homozygous variant c.104T>A (p.Leu35*) in SLC10A1 gene fulfilled the above criteria. It was confirmed by PCR and direct sequencing. Primer sequences and PCR conditions are available upon request. The pathogenicity of the variant was assessed using ANNOVAR (SIFT, PolyPhen2, Mutation Taster, Mutation Assessor, LRT, FATHMM, MetaSVM and CONDEL).

**Discussion**

Sodium taurocholate cotransporting polypeptide (NTCP) deficiency is caused by SLC10A1 mutations impairing the NTCP function to uptake plasma bile salts into the hepatocyte [9]. Sodium/bile acid cotransporters are integral membrane glycoproteins that participate in the enterohepatic circulation of bile acids. Two homologous transporters are involved in the reabsorption of bile acids, one absorbing from the intestinal lumen, the bile duct, and the kidney with an apical localization (SLC10A2), and the other being found in the basolateral membranes of hepatocytes (SLC10A1). The latter is known as sodium/taurocholate cotransporting polypeptide (NTCP)/solute carrier family 10 (sodium/bile acid cotransporter family), member 1 (OMIM *182396) [10].

Shiao et al. determined that the SLC10A1 gene contains 5 exons and spans about 23 kb. In contrast to the rat promoter, the human promoter has no consensus TATA or CAAT box sequences. A number of putative DNA-binding sites for the liver-enriched binding factors HNF3, HNF6, and CEBP were identified, as well as binding sites for numerous ubiquitous transcription factors [11]. In 1994, Na+/taurocholate cotransporting polypeptide cDNA was cloned from a rat liver to screen a human liver cDNA library. A 1,599-bp cDNA clone that encodes the human equivalent of NTCP was isolated. The deduced protein consists of 349 amino acids with a calculated molecular mass of 38 kD and exhibits 77% amino acid homology with the rat polypeptide. In vitro translation experiments indicated that the protein is glycosylated and has a molecular mass similar to that in the rat [12].

The patient described in our study suffers from intermittent cholestasis, hyperbilirubinemia, transaminosis and elevation of GGT. Intelligence was normal. Plasma concentrations of conjugated bile salts were elevated. The diagnosis was confirmed by means of a next generation sequencing targeting 67 genes associated with cholestasis. We documented the p.Leu35X variant in the SLC10A1 gene that had been reported only once in a clinical test. Although a report to ClinVar database was performed, that case was never published. Our bioinformatic analysis established that the variant c.104T>A (p.Leu35*) in SLC10A1 is a stop-gain and may lead to functional consequences due to protein truncation, degradation of the transcript by Nonsense-Mediated Decay (NMD). A sequence alignment of the protein was performed evaluating the level of conservation of individual amino acids. The amino acid leucine at position 35 of the NTCP is highly conserved, so is likely to have an important role either in structure or in function (Figure 1). We consider the variant c.104T>A (p.Leu35*) in SLC10A1 as pathogenic.

In addition, the heterozygous variants c.1436A>G in ABCG8 gene, c.2673C>T in JAG1 gene and c.3523-7A>G in NOTCH2 gene were identified. This variants were classified as variants of uncertain significance by the criteria provided by American College of Medical Genetics and Genomics (ACMG). The genes JAG1 and NOTCH2 have been related with Alagille syndrome but have never been reported in literature or ClinVar. The gene ABCG8 causes sitosterolemia, however this is an autosomal recessive

**Table 1:** Routine clinical biochemistry during cholestasis episodes.

<table>
<thead>
<tr>
<th>Clinical Biochemistry</th>
<th>02/22/12</th>
<th>11/04/17</th>
<th>01/29/18</th>
<th>07/18/18</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/I (10-49)</td>
<td>125</td>
<td>92</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>ALT U/I (0-34)</td>
<td>287</td>
<td>151</td>
<td>33</td>
<td>80</td>
</tr>
<tr>
<td>TBIL mg/dl (0.2-1.5)</td>
<td>1.4</td>
<td>0.86</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>DBIL mg/dl (0-0.5)</td>
<td>1.1</td>
<td>0.68</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>I Bil mg/dl (0-1.0)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>ALP U/I (40-150)</td>
<td>116</td>
<td>134</td>
<td>61</td>
<td>103</td>
</tr>
<tr>
<td>GGT U/I (9-36)</td>
<td>80</td>
<td>80</td>
<td>76</td>
<td>75</td>
</tr>
</tbody>
</table>

ALT alanine aminotransferase, AST aspartate aminotransferase, TB total bilirubin, DB direct bilirubin, IB indirect bilirubin, ALP alkaline phosphatase, GGT gamma-glutamyl transpeptidase.

**Figure 1:** Alignment of sequence representation demonstrating the nonsense mutation observed in our patient.
disease, and a single mutation is not enough for a diagnosis confirmation. Is important to highlight that the patient does not present the clinical symptoms of sitosterolemia.

The cholestasis could be caused by impaired NTCP-mediated bile salt uptake is compensated for by other basolateral bile salt uptake transporters, including members of the SLC01B family. In addition, there may be compensatory hepatic uptake of bile salts by way of the OSTα-OSTβ heterodimer, which can function as a bidirectional transport system [8]. In the patients with NTC, sodium independent pathways try to compensate this altered bile secretion. Our patient presents intermittent cholestasis, a feature not frequently described in NTCP deficiency. It is important to mention that the patient did not had neonatal jaundice or cholestasis in childhood. We did not identify a trigger for the symptoms. Modifier genes could explain the atypical phenotype and the late onset. Vaz et al. suggests that the bile salt synthesis could be reduced to compensate for the high serum bile salt levels [8]. Our patient has only one measure of bile salts, cholic acid (CA) was 200 µmol/ml, chenodeoxycholic acid (CDCA) 600 µmol/ml, and total bile salts 800 µmol/ml. To date, the assumption of Vaz et al. can’t be confirmed.

Moreover, our patient showed other clinical and laboratory findings. She suffers from polycystic ovary syndrome. Total cholesterol and LDL-C were elevated only during cholestasis episodes. The transaminases were elevated, specially ALT that was 5.8 ULN, ferritin levels were normal and the serum level of 25-OH-Vitamin D3 was decreased. Steatorrhea was no documented so fat malabsorption was ruled out. We also observed a preaxial polydactyly in left hand, feature that does not influence the phenotype. Although two variants of uncertain significance in the genes JAG1 and NOTCH2 were identified, we can’t confirm that the polydactyly is a manifestation of Alagille syndrome because the patient does not meet clinical criteria. Is important to mention that polydactyly has an incidence of 1:500 newborns and this could be a random association. The relationship between NTCP and polydactyly remains unknown. Analysis of the genotype-phenotype relation should be performed in individuals with NTCP deficiency.

In summary, the current report presents a Latin American adult patient who demonstrated intermittent cholestasis with otherwise unremarkable presentations. SLC10A1 gene analysis revealed the presence of a p.Leu35X homozygote. This variant produces a truncated protein that alters the functioning of NTCP. To the best of our knowledge, this is the first clinical description of a Latin American adult with NTCP deficiency associated to polydactyly and polycystic ovary.

References

5. Anwer MS, Stieger B. Sodium-dependent bile salt transporters of the SLC10A transporter family: more than solute transporters. Pflugers Arch. 2014;466:77-89.