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Case Report / Olgu Sunumu

The first Turkish family with Rotor syndrome diagnosed at the molecular level

Moleküler düzeyde tanısı konulmuş olan ilk Türk Rotor sendromlu aile

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The known about this topic

Although Rotor syndrome is a disease that has been known for about 70 years, its genetic background was elucidated only after the 2010s.

Contribution of the study

The individuals presented in our study are the first Turkish patients with Rotor syndrome whose diagnoses were demonstrated using molecular methods.

Abstract

Rotor syndrome is defined as a self-limiting hyperbilirubinemia characterized by jaundice that does not need treatment, cause any morbidity or affect life expectancy. As far as the literature is evaluated, the number of patients with Rotor syndrome diagnosed at the molecular level is less than 20 until today. In this case presentation, we aimed to present two siblings with Rotor syndrome who were diagnosed at the molecular level. To the nest of our knowledge, these patients are the first Turkish patients with Rotor syndrome diagnosed at the molecular level.

Keywords: Hyperbilirubinemia, rotor syndrome, *SLCO1B1*, *SLCO1B3*

Öz

Rotor sendromu, tedaviye gereksinim göstermeyen, kendi kendini sınırlayabilen, sarılık ile seyreden, herhangi bir morbiditeye neden olmayan, beklenen yaşam süresini etkilemeyen, hiperbilirubinemi olarak tanımlanmaktadır. Dizinde değerlendirilebildiği kadarı ile bugüne kadar moleküler temeli gösterilmiş Rotor sendromu hasta sayısı 20'den azdır. Bu olgu takdiminde moleküler temeli gösterilmiş Rotor sendromu olan iki kardeşi sunmayı amaçladık. Dizinde değerlendirebildiğimiz kadarı ile bu bireyler Rotor sendromu tanıları moleküler yöntem ile gösterilen ilk Türk hastalardır.

Anahtar sözcükler: Hiperbilirubinemi, rotor sendromu, *SLCO1B1*, *SLCO1B3*

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Introduction

Rotor syndrome (RS; OMIM#237450) is defined as a rare (<1/1 000 000), nonhemolytic, benign hyperbilirubinemia, it is generally diagnosed in the neonatal period and has autosomal digenic recessive inheritance. In individuals who have this disease, aminotransferase levels and liver histology are observed to be normal. Due to the effects on hepatobiliary transport/storage, an increase in both direct and indirect bilirubin levels is observed in this disease. Serum total bilirubin levels are generally found as 2–5 mg/dL (0.3–1 mg/dL), and sometimes this value may show an increase. The increase in direct bilirubin levels is more prominent compared with the increase in indirect bilirubin. In addition, urinary excretion of coproporphyrin, 65% of which is composed of coproporphyrin 1, showing a 2–5–fold increase also supports the diagnosis of RS and helps to differentiate this syn-

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drome from the other nonhemolytic hyperbilirubinemia syndromes (1, 2).

Although RS is a disease that has been known for about 70 years, its genetic background was elucidated only after the 2010s. In a study conducted by van de Steeg et al. (3) in 2012, it was shown that *SLCO1B1* and *SLCO1B3*, which were found considerably close to each other on the 12th chromosome, were responsible for the disease in a total of 11 individuals in eight different families. In this article, we aimed to present the first Turkish patients with RS who were diagnosed genetically in company with their clinical and laboratory characteristics.

Case

In this article, we aimed to evaluate two children who were being followed up with a prediagnosis of RS. Their 27-year-old mother and 30-year-old father had consanguinity (Fig. 1a), in company with their clinical, genetic and laboratory characteristics. The first child of the family, previous to our patients, died at the age of one year as a result of an accident and another sibling died of sepsis at the age of one year. The first child of the family had no symptoms related to RS, while the second child had jaundice, which was observed in the first week after birth. The parents were evaluated to be clinically normal and they had no chronic diseases. This study was designed in accordance with 2013 Helsinki Declaration and the parents gave written consent for thre use of all analyses belonging to the patients with the condition that their identities be kept private.

Case 1

The 2-year-old male child was consulted by our department with a prediagnosis of RS. He was born as the third child of the family by cesarean section at the 38th gestational week with a birth weight of 2050 g (3rd percentile). He had jaundice, which started on the postnatal 5th day. Dysmorphic examination performed after excluding other diseases that cause hemolysis revealed no findings except for a mild icteric appearance. His height and weight were found at the 50–75th percentile. Laboratory findings were as follows: aspartate aminotransferase (AST): 32 U/L (5–34 U/L), alanine aminotransferase ALT: 18 U/L (0–55 U/L), gamma-glutamyl transpeptidase (GGT): 15 U/L (12–64 U/L), alkaline phosphatase (ALP): 124 U/L (40–150 U/L), total bilirubin: 2.53 mg/dL (0.2–1 mg/dL), and direct bilirubin: 1.77 mg/dL (0–0.5 mg/dL). Whole abdominal ultrasonography revealed no pathologic findings. Chromosomal microarray analysis was performed on a DNA sample obtained from peripheral blood using an Affymetrix CytoScan Optima (315k) device (Thermo Fisher, MA, USA). All data were analyzed using the ChAS 3.1 program (Thermo Fisher, MA, USA). A homozygous deletion of 340 kilobase

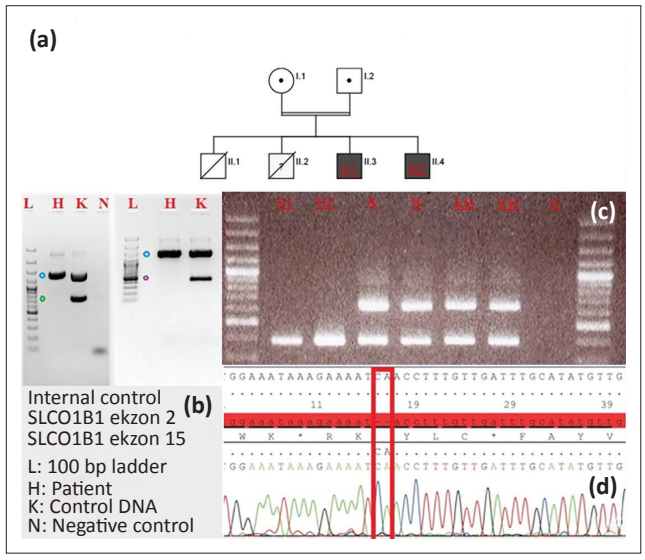

Figure 1. (a) Family pedigree. (b) Gel appearance of whole gene deletion belonging to *SLCO1B1*. (c) Gel appearance of duplex PCR. (d) Sequence electropherogram of deletion breakpoints ([hg19] g.(21,017,795–21,402,024)x0)

(kb) was observed in the short arm of the 12th chromosome on microarray analysis: arr[hg19] 12p12.2p12.1 (21,017,795–21,357,948)x0. This region involved the first 10 exons of *SLCO1B1* and the final eight exons of *SLCO1B3*.

Case 2

The six-month-old younger brother was born by cesarean section with a birth weight of 2900 g (10–25th percentile) and followed up under intensive care conditions for a period because of jaundice observed in the postnatal period. Dysmorphic examination performed after excluding other diseases that cause hemolysis revealed no findings except for a mild icteric appearance. Laboratory findings were as follows: AST: 27 U/L (5–34 U/L), ALT: 17 U/L (0–55 U/L), GGT: 39 U/L (12–64 U/L), ALP: 154 U/L (40–150 U/L), total bilirubin: 2.7 mg/dL (0.2–1 mg/dL), and direct bilirubin: 1.92 mg/dL (0–0.5 mg/dL). Whole abdominal ultrasonography revealed no pathologic findings. Chromosomal microarray analysis was performed on a DNA sample obtained from peripheral blood using an Affymetrix CytoScan Optima (315k) device (Thermo Fisher, MA, USA). All data were analyzed using the ChAS 3.1 program (Thermo Fisher, MA, USA). A homozygous deletion of 384 kilobase (kb) was observed in the short arm of the 12th chromosome on microarray analysis:arr[hg19] 12p12.2p12.1 (21,017,795–21,402,024)x0. This region of deletion involved the whole of SLCO1B1 and the final eight exons of *SLCO1B3*. Both parents had heterozygous deletion for this region. Polymerase chain reaction (PCR)-based genotyping showed full deletion of *SLCO1B1*, also in case 1 (Fig. 1b). Because the region of

deletion identified was similar to the region of deletion described by van de Steeg et al. (3) we aimed to amplify all exons encoding protein belonging to *SLCO1B1* and *SLCO1B3* using the same PCR conditions and primers. Our objective was to identify the final exons that did not amplify and the first exons that amplified in SLCO1B1, and the final exons that amplified and the first exons that did not amplify in *SLCO1B3*. As a result of our study, we found that only the first two exons belonging to *SLCO1B3* amplified. Subsequently, we confirmed our finding with gel electrophoresis pattern in our two patients, their parents, and in two individuals who were previously proven to be heterozygous (Fig. 1c). In the next step, we aimed to identify the part that was exposed to deletion using sequence analysis (Fig. 1d). According to the results of both analyses, we concluded that the mutation in our patients was completely the same as in six Saudi Arabian individuals in the relevant publication (3) and an individual from Middle Europe and thus, we confirmed the diagnosis of RS at the molecular level with multiple methods.

Discussion

A blood total bilirubin level above 1 mg/dL is defined as hyperbilirubinemia. Differential diagnosis of the causes of hyperbilirubinemia (RS, Dubin-Johnson syndrome, Gilbert syndrome, Crigler-Najar syndrome, hemolytic anemias, transient jaundice of the newborn and cholestatic diseases) is vital in terms of changing treatment and follow-up approaches. RS, which is among these differential diagnoses, is defined as a self-limiting hyperbilirubinemia that does not need treatment, cause significant symptoms or any morbidity or influence life-expectancy, and is characterized by life-long jaundice not accompanied by pruritus.

Rotor syndrome is inherited in an autosomal recessive manner due to the involvement of *SLCO1B1* and *SLCO1B3* in association. Four pathologic variants related to RS have been identified in these genes so far. These variants include a small deletion as observed in our patient, two non-sense mutations, and one splice-site involvement. Various involvements observed have not shown any difference in terms of clinical reflection; patients have carried classic RS findings. These genes are required for the production of organic anion transport polypeptide 1B1 and 1B3 (OATP1B1 and OATP1B3). In a healthy liver, the majority of bilirubin is conjugated by hepatocytes and released back into the blood. Subsequently, it is reabsorbed by OATP1B1 and OATP1B3 proteins in hepatocytes. Because OATP1B1 and OATP1B3 proteins are abnormal in RS, bilirubin reuptake and excretion by the liver is less efficient, and this causes accumulation of bilirubin in the blood and urine; this is reflected in the phenotype as jaundice and dark colored urine (4–7).

Although histopathologic findings of the liver are helpful in the differential diagnosis of benign syndromic hyperbilirubinemias, there is no need to perform such an invasive procedure. In cases where the amount of excretion of coproporphyrin subtypes cannot be examined (we could not perform this analysis because of technical causes) or is not suggestive, it is valuable to make the diagnosis by genetic analysis and to give clear genetic counselling to the family. As far as the literature shows, the number of patients with RS whose genetic basis could be demonstrated is less than 20, till today. In this case presentation, we aimed to present two siblings with RS whose genetic bases were demonstrated. As far as we know, these individuals are the first Turkish patients whose diagnoses of RS were shown using molecular methods. Notably, the mutation we found in our patients was the same as in the seven patients who were previously described, and the geographic locations of these patients were close. In this context, we think that this study, which is the first study in the area of RS, could be instructive in the evaluation of patients and patient groups followed up with a diagnosis of RS in our country, and might open the way for studies including large patient series.

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