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Mutation-in-Brief

Siblings of neonatal hyperbilirubinemia with *UGT1A1* double missense variants

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Highlights

- We present two siblings with hyperbilirubinemia between the levels associated with Crigler–Najjar syndrome type II and Gilbert syndrome, starting in the neonatal period.
- Siblings had *UGT1A1* p.[Gly71Arg;Arg403His];[Gly71Arg;Tyr486Asp] with p.Arg403His being previously uncharacterized.
- The *UGT1A1* p.R403H variant may be associated with neonatal hyperbilirubinemia at levels similar to those observed in Crigler-Najjar syndrome type II.

Key words: double, hyperbilirubinemia, missense variant, neonate, *UGT1A1*

Introduction

Crigler–Najjar syndrome type I (CNI), Crigler–Najjar syndrome type II (CNII), and Gilbert syndrome (GS) are caused by the biallelic loss-of-function variants of *UGT1A1,* and are characterized by unconjugated hyperbilirubinemia (1). Typically, serum bilirubin concentrations in patients with CNI, CNII, and GS correspond to severe (> 30 mg/dL), moderate (6–20 mg/dL), and mild (1–5 mg/dL) phenotypes, respectively. Patients with intermediate severity between CNII and GS have been reported to be of the intermediate type. The serum bilirubin levels depend on the residual activities of UGT1A1 associated with *UGT1A1* pathogenic variants. Most *UGT1A1* variants are single-nucleotide substitutions, although double-missense nucleotide substitutions have been identified in some patients (2). Herein, we report two cases of neonatal hyperbilirubinemia with an intermediate phenotype in siblings with compound heterozygous *UGT1A1* double-missense variants, including a rare variant that has not been previously reported as pathogenic.

Case Report

Case 1 was the first child of a family born to non-consanguineous Japanese parents with no history of jaundice or neonatal hyperbilirubinemia. The pregnancy

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was uncomplicated and the patient was delivered at 40 wk 5 d gestation, weighing 2.5 kg. At 4 d of age, she had jaundice, with a serum total bilirubin level of 17.9 mg/dL. Since jaundice was intractable, she required intermittent phototherapy four times by 26 d of life, eventually also requiring phenobarbital administration. At 39 d of age, phenobarbital was discontinued, and bilirubin levels improved (**Fig. 1**). She was fed breast milk and formula during infancy. At 5 yr, her serum total bilirubin concentration was 2.2 mg/dL, and she showed typical development.

Case 2 was the younger sister of Case 1. The pregnancy was uncomplicated, and the patient was delivered at 38 wk 3 d gestation weighing 2.3 kg. At 6 d of age, she was had jaundice, with a serum total bilirubin level of 16.7 mg/dL. The patient required three intermittent phototherapy sessions within 21 d of age. She was not treated with phenobarbital, and the bilirubin levels improved after 32 d of age (**Fig. 1**). She was fed breast milk and formula during infancy. At 1 yr of age, her serum total bilirubin concentration was 1.9 mg/dL, and she showed typical development.

Genetic analysis

After obtaining written informed consent from the patients' legal guardians, we performed a genetic analysis of *UGT1A1* in both the sisters and their parents. We found that the sisters had compound heterozygous double missense variants in *UGT1A1* (NM_000463.3), namely, one allele harboring c.211G>A (p.Gly71Arg) and c.1208G>A (p.Arg403His) and the other allele harboring c.211G>A (p.Gly71Arg) and c.1456T>G (p.Tyr486Asp). Each allele was transmitted to the parent (**Fig. 2A**). Both alleles showed the c.211G>A (p.Gly71Arg) variant with a high minor allele frequency (0.18 in the Japanese population (https://www.ncbi.nlm.nih.gov/snp/rs4148323#frequency_tab). The homozygous variant p.[Gly71Arg;Tyr486Asp];[Gly71Arg;Tyr486Asp] is known to cause CNII, while the homozygous variant p.[Gly71Arg];[Gly71Arg] causes GS (**Fig. 2B**) (2), and the compound heterozygous variant p.[Gly71Arg];[Gly71Arg;Tyr486Asp] causes the intermediate phenotype (**Fig. 2B**) (2). While p.[Gly71Arg;Arg403His] has not been previously reported in CNII or GS patients, c.1208G>A (p.Arg403His) is registered as rs140613392 and has been found to be present in one of the 77,442 alleles in the Japanese general population in ToMMo 38KJPN. Arg403 is evolutionarily conserved across species (data not shown). *In silico* analyses using PolyPhen-2, SIFT, and MutationTaster predicted that Arg403His is pathogenic (data not shown). We further conducted computational modeling of the Arg403His variant using the crystal structure data of wild-type human UGT1A1 (ModBase Model ID: 5b895a057652f90609f0f3d8c583d8dc) as a template in order to predict the disruption of hydrogen bonds between Asn400 and Arg403 (**Fig. 2C**).

Ethical statement

This study complied with all the relevant national regulations and institutional policies, was performed in accordance with the tenets of the Helsinki Declaration, and was approved by the ethical committee at Keio University School of Medicine (#20170130). Written consent was obtained from the patients' parents for the publication of this study.

Discussion

Herein, we have presented two siblings with unconjugated hyperbilirubinemia at levels similar to CNII in the neonatal period, reducing them to those of GS thereafter, in whom genetic testing revealed the variants p.[Gly71Arg;Arg403His];[Gly71Arg;Tyr486Asp]. Both siblings were classified as having an intermediate phenotype. A previous patient with the compound heterozygous variant p.[Gly71Arg];[Gly71Arg;Tyr486Asp], also classified as an intermediate phenotype, showed serum total bilirubin levels of approximately 5 mg/dL at 40 yr of age. However, this phenotype during the neonatal period has not been described before in

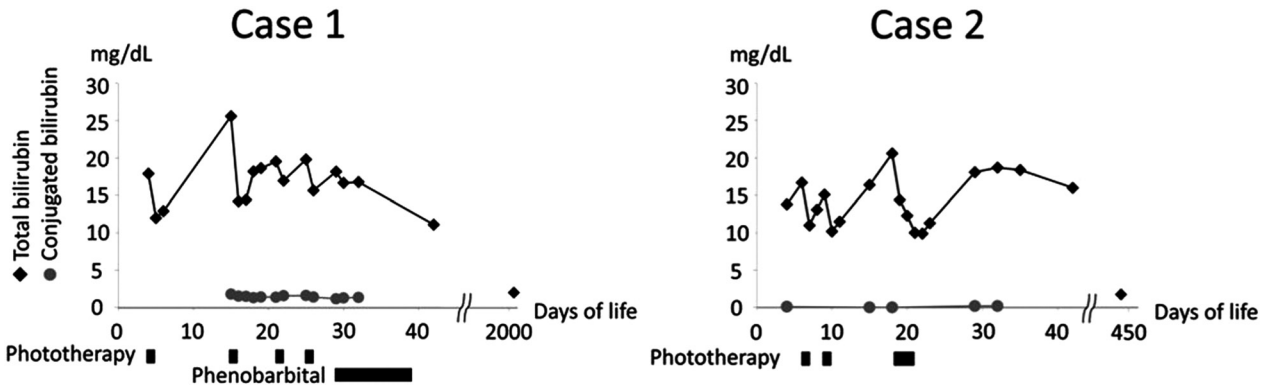

Fig. 1. Serum bilirubin concentrations and treatment strategies in the two siblings. Both patients presented with unconjugated hyperbilirubinemia during their newborn period. Case 1 required four sessions of phototherapy and phenobarbital therapy. Case 2 required three sessions of phototherapy. Hyperbilirubinemia in patients 1 and 2 improved after 1 mo of age.

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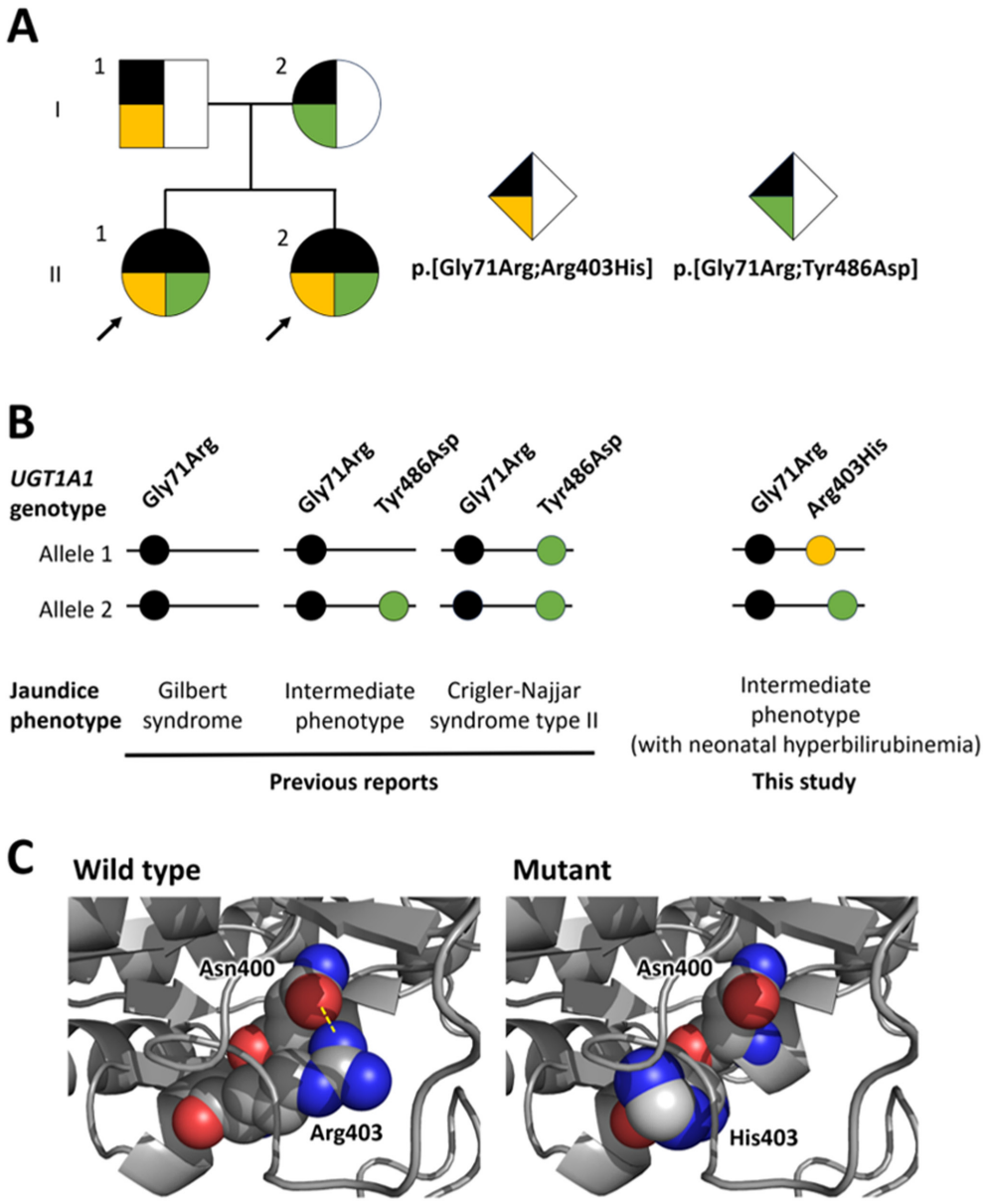

Fig. 2. Family tree and mutational effects on the molecular structure of UGT1A1 and *UGT1A1*-associated disorders. (A) Both of the variants p.[Gly71Arg;Arg403His] and p.[Gly71Arg;Tyr486Asp] in the siblings were each transmitted by one parents, resulting in compound heterozygosity for these variants in both probands. (B) Effects of the pathogenic variants on the jaundice phenotypes. (C) Computational prediction of R403H UGT1A1. Nucleotide substitution was predicted to cause the disruption of a hydrogen bond between Asn400 and Arg403 (dashed yellow line). Atom color codes: red, oxygen; blue, nitrogen; gray, other.

the literature (2). We speculate that the p.Arg403His variant plays an important role in the development of unconjugated hyperbilirubinemia in siblings at levels similar to CNII in the neonatal period. As shown in **Fig. 2C**, in wild-type human UGT1A1, Asn400 and Arg403 in the α-helix form hydrogen bonds. As such, Arg403His would result in a structural change of the α-helix due to the loss of the hydrogen bond, and the decrease in enzyme activity of UGT1A1. Patients with homozygous p.[Gly71Arg;Tyr486Asp];[Gly71Arg;Tyr486Asp] have been reported to show the CNII phenotype, and patients with p.[Gly71Arg;Tyr486Asp];[p.Gly71Arg] have been reported to show intermediate serum bilirubin concentrations between those of GS and CNII (**Fig. 2B**), suggesting that the decrease in enzyme activity due to p.Gly71Arg is modest (2). Gly71 and Arg403 are located in the domains involved in substrate specificity and interaction with uridine diphosphate glucuronic acid, respectively. We speculate that the double variants affecting the two domains simultaneously may have a more profound functional impact than a single variant alone, resulting in the development of neonatal unconjugated hyperbilirubinemia as high as that observed in CNII (3, 4).

In summary, we have identified sibling cases with an intermediate phenotype between CNII and GS,

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with compound heterozygosity for the double missense variants of *UGT1A1*, including a rare, previously uncharacterized variant, p.Arg403His. We considered p.[Gly71Arg;Arg403His] pathogenic and attributed it to the development of neonatal hyperbilirubinemia as high as that observed in CNII.

Conflict of interests: The authors have nothing to declare.

Acknowledgments

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