A novel heterozygous *GLI2* mutation in a patient with congenital urethral stricture and renal hypoplasia/dysplasia leading to end-stage renal failure

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Abstract

Glioblastoma 2 (*GLI2*) is a mediator of Sonic hedgehog signaling pathway that plays an important role in development of the central nervous system and limbs. Heterozygous *GLI2* mutations have been associated with postaxial polydactyly, various pituitary dysfunction, and holoprosencephaly-like phenotype. Herein, we report a Japanese boy who presented with isolated growth hormone deficiency with ectopic posterior pituitary, postaxial polydactyly, atrioventricular septal defect, intellectual disability and dysmorphic facial features including mid-facial hypoplasia. The patient was also complicated with congenital urethral stricture with megacystis, hydronephrosis, and renal hypoplasia/dysplasia, which led to end-stage renal failure by the age of 8 years. Trio-whole-exome sequencing showed a novel de novo heterozygous frameshift mutation in *GLI2* (c.3369delG, p.Met1123Ilefs*7) in the patient. This is the first report of possible association between *GLI2* mutation and the phenotype of congenital anomalies of the kidney and urinary tract, and subsequent end-stage renal failure. Further studies on the urogenital phenotype in patients with *GLI2* mutations may clarify a role of *GLI2* in embryonic development of the urinary tract.

Keywords *GLI2* · CAKUT · Urethral stricture · Renal failure · Polydactyly · GH deficiency

Introduction

During embryogenesis, Sonic hedgehog (Shh) signaling pathway plays an important role in the central nervous system and limb development. This signaling pathway is

mediated by three related zinc finger transcription factors, Gli1, Gli2 and Gli3. Of these, Gli1 and Gli2 act as activators for target genes, while Gli3 acts as a suppressor [1].

Mutations in *GLI2* were firstly identified by analyzing a large cohort of patients with holoprosencephaly (HPE) and/or with HPE-like phenotypes, because *SHH* and other genes involved in the Shh pathway have already been identified as causative genes for HPE [2]. To date, over 25 pathological *GLI2* mutations have been identified in patients with postaxial polydactyly, various pituitary dysfunction, and dysmorphic facial features including mid-facial hypoplasia; however, the phenotype of HPE has been infrequently found in the patients with *GLI2* mutations. Since the phenotypes of these patients are highly variable, the clinical spectrum and genotype–phenotype correlations remain to be determined [3].

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common form of malformations at birth and approximately 50% of pediatric end-stage renal disease has been attributed to this condition [4]. Recently,

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many genes involved in the development of syndromic and non-syndromic CAKUT have been identified thus far; however, pathogenic mechanisms of the majority of CAKUT cases remain to be determined [5].

We herein report a Japanese boy with a novel heterozygous frameshift mutation in the *GLI2* gene. The patient presents not only isolated growth hormone deficiency and post-axial polydactyly but also congenital urethral stricture and renal hypoplasia/dysplasia leading to end-stage renal failure.

Case report

A Japanese male patient was born to non-consanguineous parents at 38 weeks of gestation. During the gestation period, bilateral hydronephrosis and mega bladder had been detected by fetal echography and Magnetic Resonance Imaging (MRI). At birth, his length was 48 cm [-0.32 standard deviations (SD)], and weight 3.1 kg ($+0.57$ SD). He had postaxial polydactyly of bilateral fingers and toes (Fig. 1a) and distinctive facial features consisting of midface hypoplasia and a bulbous nose. He also had congenital atrioventricular septal defect, which was surgically repaired at 2 years of age. While urethral stricture with megacystis, severe hydronephrosis of the left kidney, and cystic dysplasia of the right kidney were still detected after birth, the right kidney and ureter were

undetectable by voiding cystourethrogram (VCUG) (Fig. 1b). He had no malformations of the external genitalia such as hypospadias, micropenis, and undescended testes. A clean intermittent catheterization technique for dysuria was needed to maintain an appropriate urination. Although he underwent several endoscopic transurethral incision procedures for urethral stricture and recurrent episodes of pyelonephritis, his renal function had gradually deteriorated. A peritoneum dialysis therapy was started at 9 year of age, when his estimated glomerulus filtration rate came to be less than 15 mL/min/1.73 m². A living donor kidney transplantation was performed at 11 years of age.

At 15 years of age, he had severe short stature [123.7 cm (-7.0 SD)] and remained prepubertal (bilateral testicular size: 2 mL). Endocrine studies indicated severe growth hormone (GH) deficiency [peak serum GH values: 2.49 ng/mL at Arginine stimulation test (cut off value: <3 ng/mL), and 4.79 ng/mL at GH releasing peptide-2 stimulation test (cut off value: <9 ng/mL)], and his bone age was assessed as 8 years and 2 months. The results of provocation tests for other anterior pituitary hormones and testosterone were within normal ranges. Thus, he was diagnosed as isolated GH deficiency and possible delayed puberty. A brain MRI examination revealed an ectopic posterior lobe and anterior pituitary hypoplasia (Fig. 1c). He had severe intellectual disability and autism spectrum disorder.

Fig. 1 Clinical and genetic findings in the patient. **a** Postaxial polydactyly. **b** Voiding cystourethrogram at 4 years of age showed megacystis and high-grade left vesicoureteral reflux. **c** Magnetic resonance imaging of brain showed ectopic posterior pituitary and anterior pituitary hypoplasia. **d** Electrochromatogram of the *GLI2* gene in the patient. Wild-type and mutant sequences are shown. **e** The

structure of *GLI2* and the position of the mutation (black arrow). The black and white boxes on the genomic DNA (gDNA) denote the coding regions on exons 1–14 and the untranslated regions, respectively. The black arrow and the dashed line indicate positions of the mutation and the produced stop codon, respectively. DBD DNA-binding domain, TA transactivation domain

Whole-exome sequencing

DNA was obtained from peripheral blood samples from the patient and the phenotypically normal parents. Whole-exome sequencing using a SureSelect Human All Exon V5 (Agilent Technologies, CA, USA) ran on a HiSeq 2500 platform (Illumina, San Diego, CA, USA). The mutation was confirmed via Sanger sequencing using a BigDye terminator and 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA, USA). Based on the pedigree, autosomal dominant, recessive, and X-linked recessive modes of inheritance were assumed for the analysis. The Online Mendelian Inheritance of Man (<http://www.omim.org>) database was used to determine any known disease associations. In the strategy, a de novo heterozygous frameshift mutation in *GLI2* (c.3369delG, NM_005270) was proposed as the sole candidate in association with the previously reported phenotypes (Fig. 1d). This *GLI2* mutation is predicted to cause a frameshift at codon 1123 and the resultant termination at codon 1129 (p.Met1123Ilefs*7, NP_005261) (Fig. 1e). Other variants or mutations in the known causative genes associated with development of CAKUT were not identified in the patient.

Discussion

We identified a novel heterozygous frameshift mutation in the *GLI2* gene in a Japanese boy with isolated growth hormone deficiency, postaxial polydactyly, intellectual disability, autism, and congenital urethral stricture with renal hypoplasia/dysplasia leading to end-stage renal failure. Since the mutation is located at the last exon in the *GLI2* gene, the transcript from the *GLI2* termination mutation was predicted to escape nonsense-mediated mRNA decay, producing a variant GLI2 protein lacking a part of the C-terminal transactivation domain [6] (Fig. 1e). In fact, several truncating mutations in the last exon of *GLI2* have been associated with patients with polydactyly and/or pituitary dysfunction in previous studies [3, 7].

The patient with the *GLI2* mutation in the present study exhibited congenital urethral stricture, resulting in upper urinary tract obstruction, left renal hypoplasia, right renal dysplasia, and ultimately, end-stage renal failure. Although such phenotypes of urinary tract have not been reported in patients with *GLI2* mutations, the phenotypes are likely associated with the *GLI2* mutation for several reasons. First, Shh is expressed in the urethral plate epithelium, and its signal is mediated through Gli2 in the mesenchyme [8]. Second, a drastic genital defect, which may be due to an open urethral groove-like structure and preputial

fusion defects, was identified in male *Gli2*-mutant mice [8]. These findings indicate that Shh signaling, including GLI2, is indispensable for the establishment of male external genitalia and urinary tract characteristics in mice [8]. In this regard, a patient with a heterozygous *GLI2* mutation has been reported to have perineal hypospadias, indicating that *GLI2* mutations can also cause male urinary tract anomalies in humans [7]. However, the penetrance of the phenotype should be incomplete, because only some of those with *GLI2* mutations exhibit such urinary tract anomalies. Further studies are needed to clarify the relationship between *GLI2* mutations and CAKUT.

Post-renal obstruction during the fetal period, such as congenital urethral stricture in the present patient, likely causes renal hypoplasia and dysplasia, leading to end-stage renal failure, based on several findings in previous reports. First, a fetal sheep model with obstruction of bladder outflow demonstrated renal morphologic changes as early as 2 days after surgery and progressed over 20–30 days to cystic renal dysplasia [9]. Second, posterior urethral valve (PUV), in which the bladder outflow is obstructed, is one of the most common causes of end-stage kidney disease in childhood. Animal models of PUV have demonstrated varieties of renal dysplasia and reductions in the nephron number [10, 11]. Finally, both mechanical and humoral effects have been seen in rat models of obstructed kidney. These effects are followed by impaired growth and maturation with a decrease in the renal mass, a reduced number of nephrons, and immature renal vasculature, glomeruli, tubules, and interstitium. Mechanical stretching of the distal tubular epithelial cells stimulates tubular cell apoptosis, partially via an increased expression of TGFβ1 and reduced expression of EGF. During obstruction, the renin-angiotensin system is activated, resulting in apoptosis via angiotensin 2 receptors. There is also a reduction in the renal blood flow and GFR that is observed secondary to the humoral effects and peritubular capillary compression [12].

In conclusion, we herein reported for the first time a patient with a *GLI2* mutation who was suffering from congenital urethral stricture and end-stage renal failure. Further studies are needed to elucidate the association between the phenotypes and *GLI2* mutations.

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Compliance with ethical standards

Conflict of interest Yuko Hamasaki belongs to a department endowed with sponsorship by Asahi Kasei Pharma Corporation, Novartis Phar-

ma K.K., Chugai Pharmaceutical Co., and Astellas Pharma. The other authors have declared that no Conflict of interest exists.

Human and animal rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional Review Board at Nagasaki University Graduate School of Biomedical Sciences at which the study was conducted (IRB approval number 151027331) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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