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case report

Ectopic Posterior Pituitary, Polydactyly, Midfacial Hypoplasia and Multiple Pituitary Hormone Deficiency due to a Novel Heterozygous IVS11-2A>C(C.1957-2A>C) Mutation in GLI2 Gene

Demiral M et al. Congenital Panhypopituitarism due to a GLI2 Mutation

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What is already known on this topic?
Patients with *GLI2* mutation presented with MPHD accompany to ectopic posterior pituitary, polydactyly and midfacial hypoplasia. Heterozygous mutations of GLI2 gene cause a wide range of clinical phenotype ranging from asymptomatic cases to more severe clinical phenotypes including Culler-Jones Syndrome and holoprosencephaly (HPE) or HPE-like syndrome.

What this study adds?
We report a novel heterozygous IVS11-2A>C(c.1957-2A>C) mutation in *GLI2* gene which expanded the mutation database. Extremely distinct phenotypical expression and incomplete penetrance of the heterozygous GLI2 mutations may cause skipping MPHD in a generation, therefore, delay or missing the diagnosis of these life-threatening hormonal disorders. The response to GH replacement may be excellent and clinician should be encouraged for trial of GH therapy in cases with *GLI2* mutation who have GHD.

Abstract
We, report a novel heterozygous IVS11-2A>C(c.1957-2A>C) mutation in *GLI2* gene with an extremely distinct phenotypical expression in two siblings and their father from an unrelated family. The index case was a boy who developed cholestasis and hypoglycaemia at the neonatal period. He had postaxial polydactyly, mid-facial hypoplasia, high palatal arch, micropenis, and bilateral cryptorchidism. Laboratory examination revealed a diagnosis of multiple pituitary hormone deficiency. There were severe anterior pituitary hypoplasia, absent pituitary stalk and ectopic posterior pituitary on pituitary MR imaging which suggested pituitary stalk interruption syndrome (PSIS) with no other midline structural abnormality. In molecular genetic analysis, a novel heterozygous splicing IVS11-2A>C (c.1957-2A>C) mutation detected in *GLI2* gene. His father and a 6-year-old brother with the identical mutation also had unilateral postaxial polydactyly and mid-facial hypoplasia whilst no pituitary hormone deficiency. Present novel heterozygous mutation detected in the *GLI2* gene suggested an extremely variable clinical phenotype in individuals with identical mutation, even in those within the same family and incomplete penetrance of *GLI2* mutations.
Keywords: Growth hormone deficiency, polydactyly, GLI2 mutations, multiple pituitary hormone deficiency

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Introduction
Sonic hedgehog (SHH) signalling pathway regulates differentiation, proliferation, tissue polarity, stem cell population, and carcinogenesis at notochord and floor plate in the developing spinal cord (1,2). Sonic Hedgehog signalling pathway is mediated by three related zinc-finger transcription factors (GLI1, GLI2, and GLI3) which are members of the GLI-Kruppel family.
GLI2 is an activating zinc-finger transcription factor in the SHH signalling pathway which plays a crucial role in the development of diencephalon and distal extremities during embryogenesis. It is encoded by *GLI2* gene that is mapped to 2q14.2. *GLI2* is a large polymorphic gene. Therefore, it is very likely to detect variants of uncertain significance (VUS). Homozygous deletion of both *GLI1* and *GLI2* results in complete absence of the pituitary gland (3). Heterozygous mutations of *GLI2* gene cause a wide range of clinical phenotype ranging from asymptomatic cases to more severe clinical phenotypes including Culler-Jones Syndrome and holoprosencephaly (HPE) or HPE-like syndrome. Culler-Jones Syndrome refers to a clinical spectrum of MPHD, ectopic posterior pituitary, and postaxial polydactyly with or without midline defects and developmental delay (3). HPE refers to a more severe clinical spectrum with additional midline structural abnormality and forebrain cleavage defects. To date, about 25 different pathogenic *GLI2* mutations have been identified (4). Heterozygous *GLI2* mutations can be autosomal dominant inherited or *de novo* (51% maternal, 40% paternal, and 9% *de novo*) (5). We,

herein, report a novel heterozygous IVS11-2A>C(c.1957-2A>C) mutation in *GLI2* gene in two siblings and their father from an unrelated family suggesting an extremely distinct phenotypical expression and incomplete penetrance.

Patients and Methods

Index case

The proband was a male patient who was born after 40 weeks uneventful gestation via spontaneous vaginal delivery with a birth weight of 3700 gr. The parents were not consanguineous. Family history revealed that one of his brother, father and paternal grandfather had polydactyly and atypical facial appearance with no known hormonal disorders. He had postaxial polydactyly, mid-facial hypoplasia, high palatal arch, micropenis and bilateral cryptorchidism. At the age of 2 months, he developed cholestasis and hypoglycaemia episodes. Growth hormone, cortisol, and insulin levels were measured from critical blood samples revealed a diagnosis of congenital multiple pituitary hormone deficiency (Table 1). Hypoglycaemia and cholestasis resolved with replacement of hydrocortisone and Na-L-T4. He had severe anterior pituitary hypoplasia, absent pituitary stalk and ectopic posterior pituitary with no any other midline structural abnormality on pituitary MR imaging. A surgical orchiopexy was applied. Diagnosis of GH deficiency was confirmed at the age of 1 year, and GH replacement therapy was commenced at another paediatric endocrine centre. The patient was admitted to our hospital for the first time when he was 2.1 years old. He was on GH replacement therapy for 1 year and his weight was 9 kg (-3.3 SD) and height was 69 cm (-5.4 SD). During his follow up in our clinic response to the GH therapy was excellent (Figure 1). At his most recent follow-up visit when he was 10-years-old, his height was 133.5 cm (-0,46), weight was 28.7kg (-0.51 SD), body mass index was 16.1 kg/m2 (-0.4 SD). He had no signs of puberty. He had bilateral postaxial polydactyly, mid-facial hypoplasia, high palatal arch and moderate developmental delay. He was on Na-L-T4, GH (with a dose of 0.031mg/kg/day), hydrocortisone and antiepileptic therapy for focal epileptic seizures.

The patient's brother was a 6-year-old boy; his weight was 20.7 kg (-0.01 SD), height was 116.2 cm (0.01 SD). He had normal sized prepbertal testiscles with no history of undescended testis. He had left postaxial polydactyly and mid-facial hypoplasia with no pituitary hormone deficiency. The patient's father was 38-years-old; his height was 166 cm. He had also left postaxial polydactyly and mid-facial hypoplasia with no pituitary hormone deficiency (Table 1). Cranial MRI was not performed to the father and sibling as they have not pituitary dysfunction.

Molecular genetic analysis

Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All coding exons of the *GLI2* gene and their flanking splice site junctions were amplified using in-house designed PCR primers (available upon request). These were subsequently sequenced by the MiSeq next-generation sequencing (NGS) platform (Illumina, San Diego, CA, USA). The libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. Next-generation sequencing was carried on MiSeq (Illumina Inc.). Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.). The data were visualized with IGV 2.3 (Broad Institute) software. Sanger sequencing analysis was performed for confirmation of the variant detected at NGS analysis.

In silico prediction tools (MutationTaster and Human splicing finder) were used for evaluation of the novel unpublished variant. The variant was classified based on the 2015 American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines (ACMG-AMP)(6).

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Local Ethical Committee. Written informed consent was obtained from the participants and their legal guardians.

Results

We identified a novel heterozygous IVS11-2A>C (c.1957-2A>C) mutation in intron 11 of the *GLI2* gene in the proband (Figure 2). His father and 6-year-old brother who had postaxial polydactyly and facial dysmorphism with no hormonal deficiency, were also heterozygous for the identical mutation. The unaffected mother and sister had normal alleles. This variant was listed neither in the 1000 Genomes nor in the ExAC database (http://browser.1000genomes.org/index.html, http://exac.broadinstitute.org/, respectively). This mutation in *GLI2* disrupted the intron 11 acceptor splice-site and predicted resulting in aberrant splicing, thereby, synthesis of a truncated protein.

Discussion

We herein present a patient with congenital multiple pituitary hormone deficiency, midfacial hypoplasia, bilateral postaxial polydactyly, anterior pituitary hypoplasia and ectopic posterior pituitary due to a novel heterozygous splicing IVS11-2A> C (c.1957-2A> C) mutation in the *GLI2* gene. Clinical features were similar to Culler-Jones syndrome. Although his father and brother with the identical mutation had similar physical dysmorphisms such as postaxial polydactyly and mild facial hypoplasia, they had no hormonal deficiency (Table 2).

The heterozygous IVS11-2A>C(c.1957-2A>C) mutation detected in *GLI2* in the present family causes a splicing defect that results in aberrantly spliced transcripts, thereby the synthesis of a truncated protein. *GLI2* mutations leading to a truncated protein usually cause panhypopituitarism, polydactyly and midfacial hypoplasia which were also present in our index case while pituitary dysfunction was not detected in his father and brother who had the identical mutation suggesting incomplete penetrance and variable expressivity (3,5,7,8). Distinct clinical phenotype in subjects with identical heterozygous *GLI2* mutations have previously been reported and suggested as evidence for the incomplete penetrance and variable expressivity(3,9). The variable expression of the *GLI2* gene mutations has been attributed to the combination of genetic, environmental and epigenetic factors or contribution of the other genes involved in the "Sonic Hedgehog Pathway" such as *SHH*, *ZIC2*, *SIX3*, *PTCH1*, *GLI3* and *TGIF* genes (5,9,10,11).

The largest cohort with *GLI2* variant has been reported by Bear et al., where, a *GLI2* variant was detected in 112 of 400 patients with HPE spectrum, endocrine disorders or craniofacial anomaly(5). Of which, 43 were found to have a truncating mutation (frameshift, nonsense, large deletion) and 69 were reported to have a VUS (5). The clinical characteristics of cases with *GLI2* mutations reported so far are shown in Table 3 (Supplementary file).

The clinical spectrum of mutations in *GLI2* may vary from asymptomatic individuals to polydactyly, functional and structural abnormality in the pituitary gland, facial dysmorphism, Culler-Jones Syndrome, HPE-like syndrome, and frank HPE(4,8).

Besides, renal problems such as renal hypoplasia/dysplasia, urethral stricture, cardiac problems such as ASD/VSD have been reported in patients with *GLI2* mutations (4,8). HPE is the most common anterior brain anomaly characterized by incomplete separation of cerebral hemispheres and underdeveloped midbrain structures. However, the mutations in *GLI2* rarely associated with HPE phenotype (7,12). Indeed, in the study of Bear et al., only 3 of the 112 patients with *GLI2* mutations, had HPE (5,13). Also, neuroanatomical anomalies such as corpus callosum agenesis, abnormal cerebral periventricular venous system and abnormal gyri have been reported in patients with *GLI2* mutations (8,14,15,16,17). Inconsistent with the literature, our patient had severe anterior pituitary hypoplasia, multiple pituitary hormone deficiency, and ectopic posterior pituitary with no features of HPE or HPE like syndrome. Pituitary stalk interruption syndrome (PSIS) is a congenital anomaly of pituitary gland characterized with small or absent anterior pituitary lobe, interrupted or absent pituitary stalk, and ectopic posterior pituitary lobe(18). PSIS may be associated with isolated or syndromic features(18). Mutations in genes encoding transcription factors in signalling pathways; especially *GLI2* variants have been reported in PSIS as in our case (18,19). Pituitary dysfunction due to *GLI2* mutations may vary from idiopathic GHD to multiple pituitary hormone deficiency with/without ADH deficiency(3,5). Our index case had biochemical and hormonal features of complete anterior pituitary hormone deficiency including GH, TSH, ACTH, prolactin, FSH and LH (Table 1). The most common pituitary hormone deficiency is GHD (20). Although the response to the rhGH replacement has been reported poor in some cases with *GLI2* mutations, excellent response to rhGH replacement in our case and some other reports suggested that clinicians should be encouraged for trial of the rhGH therapy in cases with *GLI2* mutation who have GHD (Figure 1) (3,8,21). In addition, hypoglycaemia, cholestasis, recurrent seizures and intellectual disability have been reported in patients with *GLI2* mutations as a consequence of ACTH and GH deficiency (22). Hypoglycaemia episodes and cholestasis of our case had resolved after replacement of hydrocortisone and rhGH therapy. We also attributed the seizures and moderate developmental delay of our case to the neonatal hypoglycaemia episodes due to ACTH and GH deficiency. While micropenis of our case could be attributed to GH deficiency, having cryptorchidism and inappropriately low FSH, LH and testosterone levels at mini puberty suggested concomitant gonadotropin deficiency. Despite having an ectopic posterior pituitary on pituitary-imaging he had no diabetes insipidus at presentation neither developed during the follow-up.

In conclusion, extra-pituitary findings may provide clues for the diagnosis of particular gene mutations including *GLI2*, *HESX1*, *LHX4*, *SOX3*, and *OTX2* which involve in the development and differentiation of pituitary gland thereby relevant pituitary hormone deficiencies. In cases presented with MPHD accompany to ectopic posterior pituitary, polydactyly and midfacial hypoplasia, a diagnosis of *GLI2* mutation should be considered. Furthermore, extremely distinct phenotypical expression and incomplete penetrance of the heterozygous *GLI2* mutations may be associated with skipping MPHD in a generation; therefore, delay or missing the diagnosis of these life-threatening hormonal disorders. Thus, a genetic analysis of either asymptomatic or symptomatic relatives for the *GLI2* gene mutations and evaluation of carriers for panhypopituitarism is warranted.

Statement of Ethics

We state that the subject and his parents have given their written informed consent to publish their case, in accordance with the Declaration of Helsinki.

Disclosure Statement

The authors have no conflicts of interest to declare.

Authorship Contributions

Concept: Meliha Demiral, Hüseyin Demirbilek Mehmet Nuri Özbek Design: Meliha Demiral, Hüseyin Demirbilek, Mehmet Nuri Özbek Data Collection or Processing: Meliha Demiral, Hüseyin Demirbilek, Mehmet Nuri Özbek Analysis or Interpretation: Meliha Demiral, Edip Ünal, Ceren Damla Durmaz, Serdar Ceylaner Literature Search: Meliha Demiral, Edip Ünal Writing: Meliha Demiral, Hüseyin Demirbilek, Mehmet Nuri Özbek.

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Figure 1. Facial dysmorphism and polydactyly in the index case, brother and father (a-f). Good response to rhGH therapy in the index case (g).

Figure 2. Family pedigree and electropherogram of heterozygous IVS11-2A>C(c.1957-2A>C) mutation in *GLI2* gene (Full-black filled box indicates index case with Culler-Jones Syndrome phenotype, shaded boxes indicate father and brother who are also heterozygous for the identical mutation with incomplete phenotype, empty boxes indicate mother and sister with wild type.

Table 1. Biochemical and hormonal characteristics of index case

	Patient's values	Father's value	Brother's value	Lab normal
Na (mEq/l)	140	**138**	**137**	135-145
K (mEq/l)	4.5	**4.2**	**3.9**	3.5-5.5
Glu (mg/dl)	17	**97**	**85**	60-100
ALT (IU/L)	24	**38**	**44**	0-40
AST (IU/L)	34	**31**	**43**	0-40
GGT (IU/L)	501			10-61
Total bilirubin (mg/dl)	6.4	**1.1**	**0.8**	0-1.2
Direct bilirubin (mg/dl)	4.8	**0.3**	**0.2**	0-0.3
LDH (IU/L)	309	**181**	**192**	180-430
Calcium (mg/dl)	9.6	**9.2**	**9.5**	8.8-10.8
Phosphorus (mg/dl)	5.3	**4.1**	**3.9**	3.5-5.5
ALP (IU/L)	940	**110**	**147**	150-1076
Cortisol*(μg/dl)	1	**7.2**	**7.2**	5-25
GH* (ng/ml)	0.059	**NA**	**NA**	-
Insulin*	<2 mIU/	**NA**	**NA**	-
fT4(ng/dl)	0.4	**1**	**1.25**	0.8-1.9
TSH(IU/L)	0.84	**2.3**	**2.16**	0.4-8.6
Prolactin (ng/ml)	1.99	**7**	**14.5**	3-11
FSH** (mIU/ml)	0.05	**8**	**0.54**	0.7-11.4
LH** (mIU/ml)	0.1	**5.2**	**0.06**	0.8-7.6
Testosterone** (ng/dl)	<20	**450**	**NA**	12-21

* GH, cortisol and insulin were measured from critical blood sample collected during hypoglycaemia. Therefore, GH and ACTH deficiency considered due to inappropriate response **Gonadotropin (FSH, LH) and testosteron levels were considered low as were collected at minipuberty and found low.

Table 2. Clinical characteristics of index case were different from father and brother with identical *GLI2* mutation and similar to Culler-Jones Syndrome (N/A: Not Available)

Symptoms	Index case	Father	Brother	Culler-Jones syndrome
Mutation	IVS11-2A> C (c.1957-2A> C)	IVS11-2A> C (c.1957-2A> C)	IVS11-2A> C (c.1957-2A> C)	-
Inheritance pattern	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Facial dysmorphism	+	+	+	+/-
Polydactyly	Bilateral postaxial polydactyly	Unilateral postaxial polydactyly	Unilateral postaxial polydactyly	Unilateral/bilateral post-axial polydactyly
Cranial midline defect	-	-	-	-
Forebrain cleavage defect	-	-	-	-
Anterior pituitary hypoplasia	+	N/A	N/A	+/-
Posterior pituitary abnormality Pituitary stalk	Ectopic posterior pituitary Interrupted	N/A N/A	N/A N/A	Ectopic posterior pituitary +/-
GH deficiency	+	-	-	+/-
TSH deficiency	+	-	-	+/-
ACTH deficiency	+	-	-	+/-
Gonadotropin deficiency	+	-	-	+/-
Prolactin deficiency	+	-	-	+/-
ADH deficiency	-	-	-	+/-
Genitourinary system abnormality	Micropenis, cryptorchidism	-	-	+/-
Developemental delay	+	-	-	+/-