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TWO NOVEL HETEROZYGOUS MISSENSE VARIATIONS WITHIN THE *GLI2* GENE IN TWO UNRELATED ARGENTINE PATIENTS

MATÍAS JUANES*, ISABEL DI PALMA*, MARTA CIACCIO, ROXANA MARINO, PABLO C. RAMÍREZ, NATALIA PÉREZ GARRID, MERCEDES MACEIRAS, JUAN M. LAZZATI, MARCO A. RIVAROLA, ALICIA BELGOROSKY

Servicio de Endocrinología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina

Abstract Several heterozygous *GLI2* gene mutations have been reported in patients with isolated GH deficiency (IGHD) or multiple pituitary hormone deficiency (MPHD) with or without other malformations. The primary aim of this study was to analyze the presence of *GLI2* gene alterations in a cohort of patients with IGHD or MPHD and ectopic/absent posterior pituitary. The coding sequence and flanking intronic regions of *GLI2* gene were amplified and directly sequenced from gDNA of 18 affected subjects and relatives. *In silico* tools were applied to identify the functional impact of newly found variants (Polyphen2, SIFT, Mutation Taster). We identified two novel heterozygous missense variations in two unrelated patients, p.Arg231Gln and p.Arg226Leu, located in the repressor domain of the protein. Both variations affect highly conserved amino acids of the Gli2 protein and were not found in the available databases. *In silico* tools suggest that these variations might be disease causing. Our study suggests that the *GLI2* gene may be one of the candidate genes to analyze when an association of pituitary hormone deficiency and developmental defects in posterior pituitary gland. The highly variable phenotype found suggests the presence of additional unknown factors that could contribute to the phenotype observed in these patients.

Key words: GLI2 gene, IGHD, MPHD, ectopic posterior pituitary

Resumen ***Dos nuevas variantes heterocigotas en el gen* GLI2 *en dos pacientes argentinos no relacionados.*** Mutaciones heterocigotas en el gen *GLI2* fueron previamente comunicadas como causa de déficit aislado de hormona de crecimiento (IGHD) o déficit múltiple de hormonas hipofisarias (MPHD), con o sin otras malformaciones. El objetivo del estudio fue analizar la presencia de alteraciones en el gen *GLI2* en un grupo de pacientes con IGHD o MPHD acompañado de neurohipófisis ectópica o ausente. La secuencia codificante y las regiones intrónicas flanqueantes del gen *GLI2* fueron amplificadas y secuenciadas de manera directa a partir de ADN genómico extraído de sangre periférica proveniente de 18 sujetos afectados y sus familiares. Se utilizaron herramientas informáticas para predecir el impacto funcional de las nuevas variantes encontradas (Polyphen2, SIFT, Mutation Taster). Identificamos dos nuevas variantes heterocigotas con pérdida de sentido en dos pacientes no relacionados, p.Arg231Gln y p.Arg226Leu, localizadas en el dominio represor de la proteína. Estas variantes afectan aminoácidos altamente conservados en la secuencia proteica de GLI2 y no se encuentran informadas en las bases de datos disponibles. Las herramientas informáticas utilizadas sugieren que estas variantes pueden ser la causa del desarrollo de la enfermedad. Nuestro resultados indican que el gen *GLI2* es uno de los genes candidatos a estudiar cuando existe una asociación entre déficit de hormonas hipofisarias y alteraciones en el desarrollo de la neurohipófisis. Se sugiere la existencia de otros factores adicionales que podrían contribuir a la variabilidad del fenotipo observado

Palabras clave: gen GLI2, IGHD, MPHD, neurohipófisis ectópica

Mutations in the *GLI2* gene have been described associated with a diverse range of phenotypes, including pituitary anomalies as well as classic holoprosencephaly (HPE), a neuroanatomic anomaly resulting from incomplete cleavage of the developing forebrain. However, *GLI2* mutations rarely extend to HPE, but more commonly includes, pituitary abnormalities and/or polydactyly[1-3]. Several heterozygous *GLI2* mutations have been reported in patients with isolated growth hormone deficiency (IGHD) or multiple pituitary hormone deficiency (MPHD) with or without other malformations, most often ectopic posterior pituitary and postaxial polydactyly[4-6]. Initially, nonsense and frameshift mutations within the *GLI2* gene were cited as the cause of HPE- or HPE-like features[3, 7]. Nevertheless, Bear *et al*[8] reported, in a large cohort, that Individuals with truncating and non-truncating variants in the *GLI2*

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*Matías Juanes and Isabel Di Palma contributed equally to the study

Postal Address: Dra. Alicia Belgorosky, Servicio de Endocrinología, Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Combate de los Pozos 1881, 1245 Buenos Aires, Argentina
e-mail: abelgo@netizen.com.ar

gene typically present pituitary anomalies, polydactyly, and subtle facial features rather than HPE.

The Sonic Hedgehog signaling (SHH) pathway plays an important role in pituitary development and growth, acting early in ventral forebrain development. It mediates its effects through three zinc finger proteins (Gli1, Gli2, and Gli3), leading to the activation or repression of target genes[9]. Gli1 and Gli2 have activating effects, in contrast to Gli3, which has a repressive effect on SHH pathway activity[10]. In GLI2-deficient animal models, it has been shown that Gli2 plays a specific role in pituitary development in early gestation, with variable loss of normal pituitary development. Conversely, mice with inactivated Gli1 or Gli3 had no pituitary abnormalities[11].

In this study, we report the clinical, hormonal, and pituitary features of two Argentinean families with IGHD and MPHD respectively, with alterations in posterior pituitary who carry two novel heterozygous missense variations within the *GLI2* gene in the repressor domain of the protein.

Material and Methods

As shown in Table 1, we analyzed *GLI2* gene variants in 18 non-related prepubertal patients (median age, 4.05 years; range, 0.03-12.72 years), 16 with congenital MPHD and 2 with IGHD followed-up at our clinic from 2000 to 2015. Patients were selected according to the following criteria: diagnosis of IGHD or MPHD associated with ectopic or absent posterior pituitary on MRI. Growth hormone deficiency was defined as follows: maximal serum GH response below 6.10 ng/ml (IRP IS80/505) or 4.70 ng/ml (IRP IS 98/5742) to two pharmacological tests. Microcephaly was defined as a head circumference at or more than two standard deviations below the mean for age and gender.

This study was approved by the Ethics Committee of the Garrahan Pediatric Hospital. Written informed consent for the study was obtained from parents.

Height, weight, and head circumference were measured with standard equipment. Anthropometric standard deviation score (SDS) was calculated on the basis of an Argentinean reference population[12]. Bone age was determined using the Greulich and Pyle method[13]. Routine biochemical parameters (complete blood count, serum protein, serum electrolytes, liver and kidney function, and screening for celiac disease) were measured by standard techniques.

Serum GH was measured using the Immulite assay and the GH standard IRP IS80/505 (cut off 6.10 ng/ml) or IRP IS98/574 (cut off: 4.70 ng/ml)[14]. Serum IGF-1 and IGFPB3 were determined by automated chemiluminescent assay systems (Immulite®, Diagnostic Products Corp, Los Angeles, CA, USA) which use monoclonal murine anti-IGF-1 and anti-IGFBP3, respectively, and values were converted to SDSs based on normative data from our laboratory[15].

The MRIs were done with 1.5 T MRI equipments, at different centers.

Serum prolactin, TSH, T3, T4, and fT4 levels were determined using Architect i2000 (Abbott Diagnostic, Illinois, Estados Unidos). Thyroid autoantibodies (antithyroglobulin antibodies - ATG and/or thyroid peroxidase antibodies - TPO) were determined with antibodies chemiluminescence Immulite Siemens. Serum levels of ACTH, cortisol, estradiol and testosterone were determined with chemiluminescence Immulite 2000 (Siemens Healthcare Diagnostics LTD., UK) and serum levels of LH and FSH with MEIA Abbott Axsym.

Genomic DNA was isolated from mononuclear cells of the affected subjects and relatives according to standard procedures. The coding sequence (exon 1-13) and flanking intronic

Table 1.– *Clinical characteristics and hormonal studies of the study population*

Patient	Sex	Age (years)	Height (SDS)	Weight (SDS)	BMI SDS (kg/m^2)	GH peak (ng/ml)	Pituitary hormone deficiencies	Posterior pituitary MRI	Anterior pituitary MRI	APS/TPS	Clinical data	Variants
P1	F	2.00	(-)3.50	(-)2.14	0.06	2.57	GH	EP	APH	APS	microcephaly, high forehead, low nasal bridge, hypoplastic nostrils, hypotelorism, mild facial asymmetry, left eye strabismus, mild neurodevelopmental delay	p.Arg231Gln(ht)
P2	M	0.64	(-)1.99	(-)0.70	0.36	0.75	GH, TSH, ACTH	APP	APH	APS	microcephaly, micropenis, undescended testes, neurodevelopmental delay, hypoglycemia	p.Arg226Gln(ht); p.Met1444Ile(hm) p.Leu1445Phe(hm)
P3	F	2.16	(-)4.34	(-)1.38	–	0.89	GH, TSH, ACTH	EP	APH	APS	high forehead, low nasal bridge	N
P4	F	2.64	(-)3.29	(-)0.19	0.26	0.10	GH, TSH, ACTH	EP	APH	APS	mild neurodevelopmental delay	N
P5	M	0.03	NA	0.84	NA	3.49	GH, TSH, ACTH, LH/FSH	EP	APH	APS	micropenis, hypoglycemia	N
P6	F	0.56	(-)2.00	(-)0.52	0.93	1.09	GH,TSH, ACTH	EP	APH	APS	hypoglycemia	N
P7	M	1.24	(-)6.10	(-)4.53	(-)2.02	0.38	GH,TSH,LH/FSH*	APP	APH	APS	micropenis, high forehead, low nasal bridge	N
P8	F	1.40	(-)4.44	(-)5.30	(-)1.16	0.15	GH,TSH	EP	APH		scoliosis, syringomyelia	N
P9	F	6.00	(-)3.90	(-)1.17	2.00	0.55	GH,TSH, ACTH	EP	APH	APS	micropenis, high forehead, low nasal bridge, reedy voice	N
P10	F	0.16	(-)4.66	(-)3.42	(-)3.17	0.44	GH,TSH, ACTH	APP	AAP	TPS	Hypoglycemia,	N
P11	M	12.72	(-)3.92	(-)3.43	(-)1.87	0.33	GH,TSH, ACTH, LH/FSH	EP	APH	–	micropenis	N
P12	M	10.20	(-)2.74	(-)2.26	(-)0.32	1.40	GH,TSH	EP	APH	APS		N
P13	M	12.16	(-)3.12	(-)2.68	(-)0.97	2.42	GH,TSH	EP	APH	–	low nasal bridge	N
P14	M	6.50	(-)4.27	(-)2.27	0.86	0.35	GH	EP	AAP	–		N
P15	F	5.40	(-)1.53	(-)1.98	(-)1.19	4.18	TSH,GH	EP	APH	–		N
P16	F	7,4	(-)6.54	(-)4.72	(-)1.98	0.70	GH,TSH	EP	APH	TPS	A. Chiari,high forehead, low nasal bridge, convergent strabismus,	N
P17	M	0.16	(-)0.45	(-)1.13	(-)1.73	6.00	GH,TSH; ACTH	EP	APH	APS	hypoglycemia, jaundice,micropenis, neurodevelopment al delay	N
P18	M	7.00	(-)2.72	(-)1.59	(-)0.73	3.19	GH,TSH	EP	APH	–		N

*PRL, prolactin; ACTHp, partial ACTH deficiency; EP, ectopic posterior pituitary; APP, absent posterior pituitary; APH, anterior pituitary hypoplasia; AAP, absent anterior pituitary; APS, absent pituitary stalk; TPS, Truncated pituitary stalk; N, normal; NA, not available; BA, bone age; NB new born. *At 15 years prepubertal with no detectable LH and FSH*

regions of *GLI2* gene were PCR amplified from genomic DNA using specific primers[2]. Each purified product (QIAquick PCR purification kit, Qiagen, Germany) was directly sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, California, Estados Unidos) and 3130 Genetic Analyzer capillary DNA sequencer (Applied Biosystems, California, Estados Unidos). The nucleotide sequence obtained was compared with those from GenBank accession number: NG_009030.1.

In silico tools were applied to identify the potential functional impact of newly found variants. *In silico* was assessed using online tools, i.e. sequence homology-based tool, SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/) version 2_0_6, the structure-based tool, PolyPhen2 (Polymorphism Phenotyping; *http://genetics.bwh.harvard.edu/pph2*) and mutation Taster (*http://www.mutationtaster.org/*).

Results

Out of 18 patients studied, we identified two novels heterozygous and two previously described homozygous missense *GLI2* gene variations by DNA sequencing in two unrelated patients. In P1, we found the heterozygous p.Arg231Gln variation in exon 5. In P2, we found the p.Arg226Leu variation in heterozygous state in exon 5 and the p.Met1444Ile and p.Leu1445Phe homozygous variants in exon 13, as described in Fig. 1. Sixteen of the 18 pituitary deficient patients had non-syndromic pituitary hormone deficiency. MPHD was found in all but two patients. P1 and P2 had microcephaly as was defined above, but in both patients the head circumference to height ratio was within the normal reference range for age and sex[16] (Table 2).

P1, an affected girl, was the second child of healthy, non-consanguineous parents. She was born at term after a normal pregnancy and delivery; her birth weight was 3.18 kg. On physical examination a right cleft lip and palate with a natal tooth were observed. The right cleft lip and palate was corrected at 7 months of age. At 2 years of age she was admitted to our unit because of short stature. Clinical phenotype and auxological parameters are shown in Table 2. Severe growth retardation (Height -3.50 SDS), delayed bone age (1.24 years), microcephaly (SDS -2.50), high forehead, low nasal bridge, hypoplastic nostrils, hypotelorism, mild facial asymmetry, left eye strabismus, mild neurodevelopmental delay at the language area, normal 46,XX female karyotype, and normal routine biochemical parameters were observed. MRI showed hypoplastic anterior pituitary, ectopic posterior lobe and absent pituitary stalk. Hormonal studies (Table 2) revealed very low serum GH response to pharmacological test as well as low serum basal IGF-1 and IGFBP-3 concentrations. Serum cortisol, prolactin, and thyroid hormone concentrations were within normal reference age value, confirming IGHD.

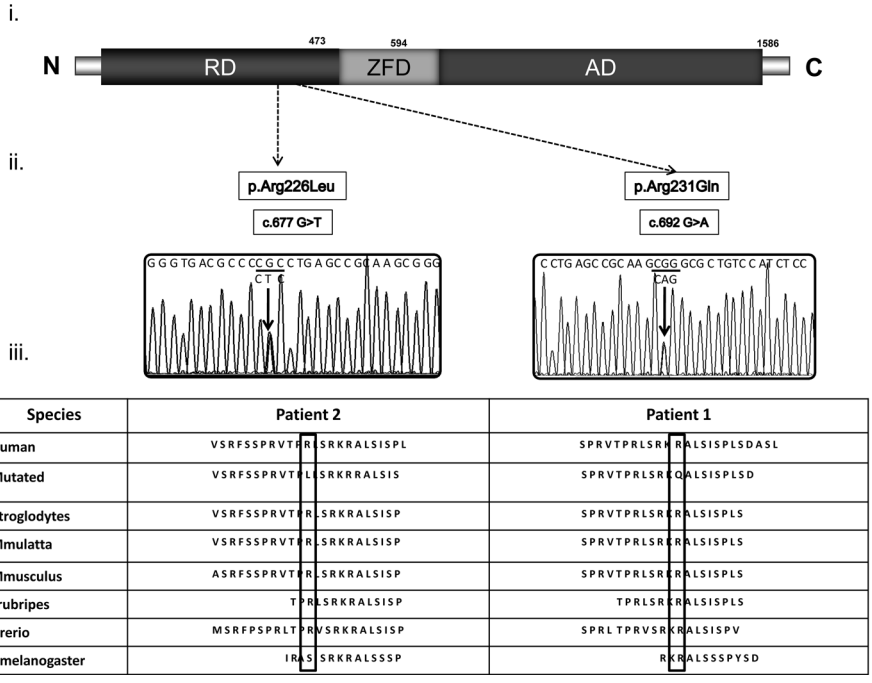

Fig. 1.– Panel (i) Schematic representation of the GLI2 zinc-finger protein Protein: RD, repressor domain, ZFD, zinc finger domain, AD, activator domain. (ii) Chromatograms of the novel GLI2 gene variations present in two unrelated patients. Electropherograms showing replacement of Arginine to Leucine, at position 226 (c.677 G > T) in P2 and Arginine to Glutamine at position 231 (c.692 G > A) in P1, both in exon five of GLI2 gene. (iii) Conservation of the affected amino acids in different species. The pictures revealed that the two amino acid substitutions affect a highly conserved amino acid of the GLI2 protein.

TABLE 2.– *Clinical characteristics and hormonal studies of two patients with GLI2 variations*

Patient	P1	P2
Height of the father	0.76	ND
Height of the mother	-1.75	ND
Target height	-0.48	ND
Head circumference (HC, SDS)	-2.50	-2.00
HC/Height	0.60	0.65
Glycemia mg/dl (RV 60-110)	70	38
IGF-1 (SDS)	-4.20	-4.00
IGFBP3 (SDS)	-2.09	ND
TSH µUI/ml (RV: 0.84-4.31)	3.98	3.84
T4 µg/d (RV: 5.3-14.3)	8.00	4.60
fT4 ng/dl (RV: 0.86-1.9)	1.14	0.36
T3 ng/ml (RV: 1.3-2.3)	1.39	1.88
Cortisol µg/dl RV: > 18 under hypoglycemia	NA*	3.40
Basal cortisol 5-25 µg/dl	10.80	2.30

ND: note done, ht: heterozygous, hm: homozygous, RV: reference value.
** Hypoglycemia was not detected*

Recombinant human growth hormone (rhGH) replacement therapy was started at 4.80 years of age (24 µg/kg/day). After 6 months of rhGH treatment a 0.70 SDS ∆height was observed.

P2, now a 14.80 year-old boy, was the fifth child of healthy, non-consanguineous parents. He was born at term after a normal pregnancy and delivery; his birth weight was 3.48 kg. During the neonatal period he presented severe hypoglycemia, seizures and respiratory distress requiring mechanical ventilation.

At 8 months of age he was first seen at our department. The following relevant clinical features were found: height 64.80 cm (-1.99 SDS), weight 7.530 kg (-0.70 SDS), head circumference 42.50 cm (-2 SDS), micropenis (1.50 cm length phallus), bilateral undescended testes (right and left 0.5 / 1 cc, respectively) and neurodevelopmental delay. MRI revealed small sella turcica with a hypoplastic anterior pituitary; the posterior pituitary lobe and stalk were absent. Hormonal studies are shown in Table 2. Low serum thyroid hormone concentrations and inadequate serum TSH concentrations were found confirming the diagnosis of central hypothyroidism. Central adrenal insufficiency was confirmed by an inadequate serum cortisol response to hypoglycemia. Levothyroxine and hydrocortisone replacement therapy was started. Normal serum prolactin concentrations were found, while lack of serum GH response to pharmacological test was observed. At 0.64 years of age, recombinant human GH treatment was started (dose: 35 µg/kg/day). At last evaluation, height and bone age were -1.33 SDS and 12 years, respectively. Signs of sexual development were not present yet, and testicular volume was still prepubertal (right 0.5 and left 1 cc). Serum cortisol and thyroid hormones concentrations with lack of response of serum GH confirm the diagnosis of MPHD.

The nucleotide sequences of genomic DNA in P1 revealed a heterozygous variation in exon 5, substituting G for A at cDNA nucleotide position 692, changing Arginine to Glutamine at codon 231 (p.Arg231Gln) in the repressor domain of the Gli2 protein. The mother was found to be heterozygous for the same *GLI2* gene variation; her height was 150 cm (-1.93SDS). In P2, a heterozygous variation in exon 5, substituting G for T at cDNA nucleotide position 677 was found, changing Arginine to Leucine at codon 226 (p.Arg226Leu) in the repressor domain of the molecule. Furthermore, in this patient we found two previously described homozygous variations in the activation domain of the Gli2 protein, p.Met1444Ile and p.Leu1445Phe (Figure1)[8]. P2´s parents were not available for studies.

In order to determine if the novel variants were present in the general population, 60 control subjects (120 alleles) were screened by DNA sequencing and no allele carrying these variations was detected. Furthermore, these variations were not found in the databases of NCBI, Ensembl genome browser and ExAc browser beta, except for p.Arg231Gln variation that was found in Exac browser beta database with a very low frequency of 0.000008289 (1/120642). These data suggest that these variations are not common polymorphisms.

In order to analyze the evolutionary conservation of the amino-acids affected by the novel variations, the sequence alignment of Gli2 proteins from different species was examined. This approach revealed that the amino acid substitutions affect highly conserved amino acids of the Gli2 protein (Fig. 1), suggesting that these novel variants might be deleterious for protein activity.

Other tools such as, 1) PolyPhen2 which predicts that the variations found were probably damaging with a score of 1.000 (score 0 to 1.000) and 2) Mutation Taster which predicts that the alterations might be disease causing, suggest that variations did affect protein function. Additionally, 3) SIFT tool showed that both variations affect protein function, having the highly deleterious tolerance index score of 0.00.

Discussion

In this report we describe the clinical and molecular findings of two novel variations in the *GLI2* gene in two patients with IGHD and MPHD. Both of them have abnormalities in the posterior lobe of the pituitary gland, developmental delay and microcephaly on admission.

The etiology of congenital MPHD is unknown in the majority of the series studied so far. *PROP1* mutations are the most commonly known genetic cause of MPHD but

with a variable incidence according to the series[17]. In the last years several studies reported variants in *GLI2* gene as a frequent cause of MPHD, particularly in patients with an ectopic posterior lobe[1-8]. Franca *et al*[2] reported that nonsense and non-synonymous *GLI2* alterations were present in 27/207 (13%) patients with MPHD; among the 125 patients with an ectopic posterior lobe, *GLI2* variants were found in 18 patients (14%). The frequency in our small group of patients (2/18) was 11%, in agreement with the previous report.

Bear *et al*[8] have recently published the largest review of *GLI2* variants so far. They found that all loss-of-function mutations reported were heterozygous and the pattern of inheritance was dominant with incomplete penetrance. They considered variants to have high evidence for pathogenicity if they resulted in truncation of the predicted protein, were not found in the public data base, and were predicted to be probably damaging using software prediction. They compared the phenotypes of individuals with mutations predicted to lead to loss-of-function (such as nonsense or frameshift mutations, or large deletions) to those with missense variants of unknown significance (non-truncating variants), showing a more frequent association with pituitary abnormalities and polydactyly in the former. The two novel variations present in our patients were also found to be heterozygous; indeed, they are non-truncating missense variants, while P1´s mother was also found to be heterozygous for the same variation. Neither of our two patients had polydactyly, consistent with the report of an incidence of only 3% of non-truncating mutations in *GLI2*[8]; and both of them had pituitary abnormalities, similarly to 58% of the patients with non-truncating mutations.

Only 1/43 individuals with truncating mutations in *GLI2*, reviewed by Bear *et al*[8], presented with HPE, revealing that frank HPE might not be a common part of the spectrum of abnormalities found in patients with *GLI2* variants as previously suspected. The patients with dysmorphic features, only 30%, have a specific well-defined combination of facial features (midface hypoplasia, cleft lip/palate, and hypotelorism), associated with loss of function mutations. In our cohort, facial abnormalities were observed in one patient (P1) who carried a *GLI2* variant, but also in patients in whom no *GLI2* variants were detected, suggesting that facial abnormalities might not be a useful tool to suspect *GLI2* gene variations.

Bertolacini *et al*[5] described one patient with the p.Ala268Val mutation and clinical findings similar to P1. This mutation is in the same protein domain as the novel variations present in P1 and P2, confirming that the repressor domain of the Gli2 protein is important for the normal function of the SHH pathway and the development of the pituitary gland. Bear *et al*[8] reported an individual who had a missense variant (c.677 G>A; p.Arg226His) in the same position as the one found in P2 in the presence of semilobar HPE; however, this individual was also found to have a deleterious mutation in *ZIC2* gene, which is clearly established as a cause of HPE[18]. It is possible that the p.Arg226His mutation might cause a deleterious effect on the function of the GLI2 protein which would be hidden by the presence of the *ZIC2* gene mutation. Therefore, it could be proposed that in the absence of a ZIC2 gene mutation, the p.Arg226His variant could lead to a milder phenotype, as observed in our patient. In P2, we also found the missense variations p.Met1444Ile and p.Leu1445Phe, previously described by Franca *et al*[2]. Similar to this study, these *GLI2* variants were found in five families with MPHD and alterations on posterior and anterior pituitary MRI. They were also found in the databases of the NCBI, the Ensembl genome browser, and the Exac browser beta[2, 8]. Based on these reports, we hypothesized that the variations p.Met1444Ile and p.Leu1445Phe are benign single nucleotide polymorphisms while p.Arg226Gln has a deleterious effect on Gli2 protein function and might be the cause of the phenotype observed in our patient. Similar to the reports in which variations in *GLI2* gene are investigated, in this study functional assays were not performed[2, 4-6, 8]. However, the clinical, biochemical, molecular findings and *in silico* predictions strongly suggest that the variations may cause the phenotype observed in our patients. Most patients with idiopathic MPHD have an ectopic posterior pituitary on MRI but the genetic etiology remains unclear[3, 5]. Romero *et al* screened candidate genes (*HESX1, LHX4, OTX2, LHX3, SOX3*) in patients with hypopituitarism and pituitary stalk interruption syndrome and concluded that mutations in the transcription factors are extremely rare[17]. In this line, it could be proposed that the association of MPHD or IGHD with an ectopic or absent posterior pituitary lobe might be a clinical marker of a deleterious variant in the *GLI2* gene, causing this clinical phenotype.

Even though it has been proposed that variants in the repressor domain of *GLI2* gene might be deleterious to the SHH pathway[5], the molecular mechanism remains unknown. In addition, Bear *et al*[8] identified mutations in the three domains of the GLI2 protein but found no correlation between the location of these variations and patient phenotype.

In summary, this study reports two novel heterozygous missense variations in the *GLI2* gene that affect the repressor domain of the protein in two affected non-related patients with different clinical phenotypes, supporting the concept that mutations in the *GLI2* gene have a highly variable phenotype ranging from IGHD to MPHD. Finally, the presence of an ectopic or absent posterior pituitary lobe might improve the odds of finding deleterious variants in the *GLI2* gene.

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LA TAPA

Grand Prismatic Spring and Midway Geyser Basin (Yellowstone, Wyoming, EE.UU.). Fotografía de James St. John, de Newark, Ohio, EE. UU.; 5 June 2013, 18:04 h. Fuente: Flickr, Wikipedia. *Creative Commons*. En: *https://en.wikipedia.org/wiki/Grand_Prismatic_Spring#/media/File:Grand_Prismatic_Spring_and_Midway_Geyser_Basin_from_above.jpg*; consultado el 19/4/2016.

La *Grand Prismatic Spring* del Parque Nacional Yellowstone, detrás el geiser. El vapor de color azul se eleva del centro caliente y casi estéril de la fuente termal, lo rodean grandes mantos de algas, bacterias y *archaea*. El color se debe a las moléculas de clorofila o de carotenoides producidas por los microorganismos. En el verano el contenido de clorofila de los organismos es bajo, y los colores son naranja, rojo o amarillo por los carotenoides; durante el invierno los mantos son usualmente verde oscuro, porque la luz solar es poca y los microorganismos producen más clorofila, y predominan las moléculas de clorofila sobre las de carotenoides. (Geilling N. The Science behind Yellowstone's Rainbow Hot Spring. *Smithsonian,* May 7, 2014. En: *http://www.smithsonianmag.com/travel/science-behind-yellowstones-rainbow-hot-spring-1809*; consultado el 16/4/2016).

Este lago, o lagos similares, son los escenarios más probables que, en el ambiente primitivo de la Tierra, nutrieron al Origen de la Vida (Szostak JW. On the Origin of Life. *Medicina (B Aires)* 2016; 76: 199-203).