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Clinical findings in patients with *GLI2* mutations – phenotypic variability

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Abstract

Mutations in the human *GLI2* gene were first reported in association with defective anterior pituitary formation, pan-hypopituitarism, and forebrain anomalies represented by typical holoprosencephaly (HPE) and holoprosencephaly-like (HPE-L) phenotypes and postaxial polydactyly. Subsequent, anophthalmia plus orbital anomalies, heminasal aplasia, branchial arch anomalies and polydactyly have also been incorporated into the general phenotype. Here we described six Brazilian patients with phenotypic manifestations that range from isolated cleft lip/palate with polydactyly, branchial arch anomalies to semi lobar holoprosencephaly. Novel sequence variants were found in the *GLI2* gene in patients with marked involvement of the temporomandibular joint (TMJ), a new clinical finding observed with mutations of this gene. Clinical, molecular and genetic aspects are discussed.

Keywords

branchial arch anomalies; cleft lip and palate; *GLI2*; HPE; HPE-L; polydactyly; *SHH* signaling pathway; temporomandibular joint

INTRODUCTION

Holoprosencephaly (HPE) is a common developmental defect affecting both the forebrain and the face. It is characterized by the incomplete separation of the cerebral hemispheres into distinct right and left halves. Clinical expression is extremely variable ranging from minor facial signs to complex craniofacial anomalies such as cyclopia (1), and both genetic and environmental causes have been attributed as responsible for the wide phenotypic variability (2).

Pathological mutations in holoprosencephaly have been identified in several genes that include: *SHH*, *GLI2*, *PTCH1*, *TGIF1*, *ZIC2*, *SIX3*, however, other candidate genes such as *GAS1*, *EYA4*, *DISP1*, *TDGF1*, *FOXA2*, *LRP2*, *LSS*, *HHIP*, *SMO*, *BMP4*, *CDON*, *CDC42*, *ACVR2A*, *OTX2*, and *WIF1* have been proposed (3,4,5,6,7,8).

Three *Gli* genes have been implicated in vertebrate Shh signal modulation. In animal models, of the three *Gli* genes, *Gli2* has been shown to act as the central transcriptional

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Conflict of interest

None of the authors have conflict of interest with regard to the manuscript submitted.

activator. More recently, it has been shown that the amino-terminal transcriptional repressor domain of *GLI2* plays a central role in the pathogenic dominant-negative activity resulting from mutations (9). In humans, *GLI2* mutations have been reported in patients with HPE and/or HPE-like phenotypes with pituitary anomalies and post axial polydactyly (9,10) however, other craniofacial anomalies involving the first branchial arch and/or the orbits have also been reported (11). Here we described the clinical findings of 6 new patients with *GLI2* mutations, possibly pathogenic, who presented additional clinical manifestations related to first branchial arch, temporomandibular joint, and preaxial ray involvement.

MATERIALS AND METHODS

We studied 110 individuals with diverse craniofacial anomalies mainly involving central nervous system, midline, face, and eye anomalies, which were ascertained by the Hospital of Rehabilitation of Craniofacial Anomalies, Bauru/SP, Brazil. This study was approved by the institutional review board of the Hospital and written informed consent was obtained from parents and from each person included in the study as controls. All patients had a normal karyotype. These individuals were previously screened for mutations in the *SHH*, *TGIF*, and *SIX3* genes. Control samples were obtained from 96 Brazilian people with no history of familial structural anomalies of the central nervous system.

Genomic DNA was extracted from whole blood by standard methods. Sequence was generated for the coding region and 3'UTR region of the candidate gene *GLI2*. The primers used to amplify the coding region (exons 6–13) were based on Roessler et al., 2003 (10). The 3'UTR region was divided in 5 regions and the primers were designed using the primer 3 Web site (<http://frodo.wi.mit.edu>). The templates for sequencing were generated by Polymerase Chain Reaction (PCR) in an Applied Biosystems Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA). The 10µl reactions contained 1.5 mM Mg₂₊, 200mM dNTPs, 0.3 mM each primer, and 0.25 units of Bioexact or Biolase reaction buffer (Bioline USA, Inc., Randolph, MA) and 20 ng to 40 ng of DNA. The PCR conditions were variable according to the primers used. Sequencing then was performed with the DNA sequencing kit, Big Dye™ Terminator Cycle Sequencing (Applied Biosystems, Foster City, CA). The 10µl sequencing chemistry reactions contained Big Dye Terminator Mix, Big Dye Terminator Buffer reaction, 0.075 mM of the corresponding primer, 1.25 ng per 100 bp of the PCR product, and 5% of DMSO, and were run using at least 40 cycles. Sequencing reactions were resolved on an ABI Prism 3700 analyzer (Applied Biosystems, Foster City, CA) and analyzed by Consed (<http://www.genome.washington.edu/UWGC/analysistools/consed.cfm>) and manually inspected to confirm possible mutations found.

RESULTS

We performed mutational analysis on the coding region of the *GLI2* gene and 3'UTR region in 110 individuals with diverse craniofacial anomalies mainly involving face, midline and eye anomalies. We identified fourteen variants in the coding region of *GLI2* gene in fourteen unrelated individuals: one deletion, one insertion, nine nonsynonymous variants, and three synonymous variants. Among the nonsynonymous variants, four were predicted as damaging, and five were predicted as benign by the PolyPhen (<http://genetics.bwh.harvard.edu/pph/>).

None of the individuals presented mutations in *SHH*, *TGIF*, and *SIX3* genes previously screened. These sequence variants were not identified in the control population and only two of them have been reported as variant in online sequence databases. Results are summarized in table 1.

Among these 14 individuals, we described the clinical findings of 6 patients who presented a possible pathogenic *GLI2* mutation, and also additional clinical manifestations related to first branchial arch, TMJ, and preaxial ray involvement.

Patient 1 presented high forehead, flat facial profile, low nasal bridge, broad nasal ridge, hypoplastic nasal septum, abnormal and short philtrum, short columella, right cleft lip with preserved palatal structures (Fig. 1A). Reevaluation at age 4y showed normal neuropsychological development. Cranial computerized tomography (CT scan) was normal. The variant c.803 C>T (p.Ala268Val) in the 3'UTR region was observed. Parents were not available for study.

Patient 2 at age 3 months showed flat face, maxillary hypoplasia, arched eyebrows, down slanted palpebral fissures, epicanthus inversus, large ears, low nasal bridge, flat nose, bilateral cleft lip/palate, hypoplastic columella and philtrum, and right hand preaxial polydactyly (Fig. 1B). Reevaluation at age 28 months showed normal development for age. CT scan was normal. She presented a sequence variant c.4663T>C (p. Ser1555Pro). This alteration was inherited from her mother who had isolated hypotelorism with no other noted anomalies.

Patient 3 (Fig. 1C), first examined at age 28 years old, presented normal neuropsychological development, long and flat profile, hypotelorism, broad nasal tip, wide nasal cavity, hypoplastic septum, thin columella, long philtrum, surgically corrected midline cleft, agenesis of the pre-maxilla, and bilateral surgical scars from postaxial polydactyly correction. Brain MRI was normal. She presented the variant c.1530_1531insC leading to a stop codon. This sequence variant was inherited from her mother who presented isolated hypotelorism only.

Patient 4 at age 3 months showed microcephaly, large cleft lip/palate involving partially the premaxilla, bilateral postaxial polydactyly (Fig. 1D). Reevaluation at age 8 years old showed severe neurodevelopmental delay. Brain MRI showed semi-lobar holoprosencephaly. A deletion of two bases (c.864_866delCC) leading to stop codon was identified in the proband and her mother who also presented postaxial polydactyly.

Patient 5. First evaluation at age 5 years old showed high forehead, facial asymmetry with hypoplastic right side, right epibulbar dermoid, thin and asymmetric nose, bilaterally abnormally modeled ears with pre auricular skin tags, Tessier cleft number 7 at right, and short neck (Fig. 2A–C). The pre auricular tags and epibulbar dermoid have been surgically corrected. Neuropsychological development was normal. Cranial computerized tomography was normal. Panoramic X-rays of the mandible showed abnormally developed right condyle and coronoid process, short right ramus with abnormal opening of the angle of the mandible, and short mandible body at right. The sequence variant c.1886G>A (p.Glu629Lys) was observed. He has 2 normal half sibs, and a cousin through maternal side who presented pre-axial polydactyly. Parents and relatives were not available for study.

Patient 6 (Fig. 2D–F) was first evaluated at age 5 months presenting a high forehead, facial asymmetry with hypoplastic left side, left sided anophthalmia, abnormally modeled ears, bilateral pre auricular skin tags greater at left, Tessier cleft number 7 at left. Re-evaluation at age 3 1/2 years-old pre auricular tags surgically corrected. She had normal neuropsychological development. Computerized tomography of mastoid bone and brain were normal. The alteration c.4558G>A (p.Asp1520Asn) was observed as a *de novo* variant with both parents unaffected.

DISCUSSION

Shh signaling pathway plays an important role in craniofacial development including the first branchial arch (12). *Gli2* is one of three vertebrate transcription factors implicated as obligatory mediators of *Shh* signal transduction (10). *Gli2* is necessary for normal endochondral bone development and has been also implicated in limb development (13). From the facial bones, the temporomandibular joint is a special and complex endochondral structure essential for jaw movement and a specific mammalian attribute (14). Deficient *Gli2* mice display aberrant TMJ development with abnormal disk morphogenesis, condyle initiation, and disk-condyle separation (14,15). In addition, *Gli2* mutations are associated with defects in developing ventrolateral neurons in CNS and with postaxial extra digits (16,17).

In humans, *GLI2* mutations have been reported in HPE and/or HPE-like phenotypes with pituitary anomalies and post axial polydactyly (9,10). The primary craniofacial phenotype in most of the reported cases was predominately related to the so called HPE-like phenotype.

Abnormalities involving eye, orbits, nose, first branchial arch, and a variable degree of gural development were previously described in patients with *GLI2* mutations (11). The facial phenotype involving the first branchial arch most likely is related with the extended action of the *SHH* signaling pathway to rhombomere differentiation (18,19) and to temporomandibular joint and upper jaw formation (14,20).

In the present study, novel or previously reported alterations were identified in the coding region of the *GLI2*. The reported Asp1520Asn variant was found in a patient with anophthalmia and first branchial arch anomalies (patient 6). In a previous study this variant was found in a patient with split hand-split foot malformation who presented with two translocation breakpoints separated by 2.5 Mb on chromosome 2q14.1–q14.2 (21). The unreported alteration p.Glu629Lis found in patient 5 who also presented with first branchial arch anomalies was not found in a control population and was predicted as damaging by PolyPhen.

The variants p.Ser1555Pro and p.Ala268Val were found in patients presenting with primary features of cleft lip and preaxial polydactyly (patients 1 and 2, respectively). Although only a CT scan and not an MRI were available for analysis, the evolution of the facial gestalt has been shown as an important parameter toward diagnostic definition, since in a first instance, some patients with *GLI2* mutations could be misdiagnosed as having isolated cleft lip and palate. Isolated cleft lip/palate has been also observed in patients with mutations in other known HPE causative genes such as *PTCH1* (22). In contradistinction, the absence of classic HPE facial gestalt with typical imaging of holoprosencephaly has been reported in patients with *ZIC2* mutations (23). To date, all patients described with *GLI2* mutations who present with polydactyly had the involvement of the post axial ray suggesting a preferential ray involvement, however, preaxial polydactyly observed in patient 3, suggesting that both rays can be involved.

In conclusion, mutations in the *GLI2* gene, analogous to variants reported in the *PTCH1* gene may be considered as a rare cause of isolated cleft lip. Attention should be given to patients with first branchial arch anomalies that could have *GLI2* mutations and misdiagnosed as OAV spectrum. Follow-up with special attention to phenotypic subtleties should be valuable for a more precise diagnosis and adequate genetic counseling. The diversity of phenotypes in *GLI2* mutations reported here expands the phenotypic spectrum associated with this gene.

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Fig. 1.
A–D. Facial aspects of patients 1–4. Note the wide phenotypical variability ranging from cleft lip/palate with minor facial anomalies (patients 1 and 2), HPE-like phenotype (patient 3), and HPE facies (patient 4).

Fig. 2.

A–F. Facial aspects of patients 5 and 6. Note the branchial arch involvement manifested by asymmetric face, unilateral mandibular hypoplasia, and pre auricular skin tags.

Table 1

Sequencing results of the *GLI2* gene

Case	Exon	Nucleotide change	Predicted Amino Acid change	PolyPhen analysis	Status
1	5	c.803C>T	p.Ala268Val	Damaging	Unreported
2	13	c.4663T>C	p.Ser1555Pro	Damaging	Unreported
3	10	c.1530_1531insC	p.Ser511fsX536	-	Unreported
4	6	c.864_865delCC	p.P288fsX301	-	Unreported
5	11	c.1886G>A	p.Glu629Lys	Damaging	Unreported
6	13	c.4558G>A	p.Asp1520Asn	Damaging	Reported (21)*
7	12	c.1999G>A	p.E667K	Benign	Unreported
8	13	c.3018C>T	p.G1066G	-	Unreported
9	13	c.4081G>T	p.Val1361Leu	Benig	Unreported
10	13	c.3682G>A	p.Thr1294Thr	-	Unreported
11	13	c.3590G>A	p.Gly1197Asn	Benig	Unreported
12	12	c.2088A>G	p.Ala696Ala	-	Unreported
13	13	c.3626G>T	p.Ser1209Ile	Benig	Unreported
14	13	c.4054A>G	p.Met1352Val	Benig	Reported (21)*

* reference