Role of *GLI2* in hypopituitarism phenotype

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Abstract

GLI2 is a zinc-finger transcription factor involved in the Sonic Hedgehog pathway. Gli2 mutant mice have hypoplastic anterior and absent posterior pituitary glands. We reviewed the literature for patients with hypopituitarism and alterations in *GLI2*. Twenty-five patients (16 families) had heterozygous truncating mutations, and the phenotype frequently included GH deficiency, a small anterior pituitary lobe and an ectopic/undescended posterior pituitary lobe on magnetic resonance imaging and postaxial polydactyly. The inheritance pattern was autosomal dominant with incomplete penetrance and variable expressivity. The mutation was frequently inherited from an asymptomatic parent. Eleven patients had heterozygous non-synonymous *GLI2* variants that were classified as variants of unknown significance, because they were either absent from or had a frequency lower than 0.001 in the databases. In these patients, the posterior pituitary was also ectopic, but none had polydactyly. A third group of variants found in patients with hypopituitarism were considered benign because their frequency was $\geq$0.001 in the databases. *GLI2* is a large and polymorphic gene, and sequencing may identify variants whose interpretation may be difficult. Incomplete penetrance implies in the participation of other genetic and/or environmental factors. An interaction between *Gli2* mutations and prenatal ethanol exposure has been demonstrated in mice dysmorphology. In conclusion, a relatively high frequency of *GLI2* mutations and variants were identified in patients with congenital GH deficiency without other brain defects, and most of these patients presented with combined pituitary hormone deficiency and an ectopic posterior pituitary lobe. Future studies may clarify the relative role and frequency of *GLI2* alterations in the aetiology of hypopituitarism.

Key Words
- GLI2
- hypopituitarism
- growth
- mutations

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Introduction

This paper reviews the evidence for *GLI2* mutations as a cause of hypopituitarism.

Hypopituitarism may manifest as a deficiency of a single pituitary hormone, such as isolated growth hormone deficiency (IGHD) or combined pituitary hormone deficiency (CPHD), and it may be congenital or acquired (Kelberman *et al.* 2009). Congenital hypopituitarism may have a genetic, environmental or

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combined aetiology. Knowledge about genetic causes of congenital hypopituitarism has been advanced by the study of pituitary embryogenesis in animal models (natural and transgenic) and by candidate gene analysis in patients with pituitary hormone deficiencies. Although animal models have been very useful, one has to bear in mind that there may be differences when the results are extrapolated to the human (Kelberman *et al*. 2009).

GLI2 is a gene of the GLI-Kruppel family, which received its name because it was initially amplified in brain gliomas, and it is also known as *GLI2* oncogene (OMIM*165230). *GLI2* is located on the long arm of chromosome 2 at position q14 (genomic coordinates GRCh37: 2:121,493,440-121,750,228). The coding region is 6.8 kb in length and spans 13 exons which encode for the GLI2 protein with 1586 amino acids. GLI2 is a transcription factor that contains a zinc-finger region responsible for binding to DNA (aa437–594), an amino-terminal (-NH2) region with repressor activity and a carboxy-terminal (-COOH) domain responsible for transcriptional activation (Roessler *et al*. 2003, 2005). Gli2 is a member of the Gli family of proteins (including Gli1 and Gli3) which are involved in the Sonic Hedgehog (Shh) signalling pathway.

SHH (Shh, OMIM*600725) induces tissue-specific cellular proliferation during embryogenesis (Villavicencio *et al*. 2000). After cleavage and the addition of a cholesterol molecule, Shh binds to the membrane receptor Patched releasing Smoothened, which in turn activates the Gli family of transcription factors. Gli1 and Gli2 usually act as activators, and Gli3 usually acts as a repressor; however, divergence of Gli protein function among tissues and species has been observed (Ruiz i Altaba *et al*. 2002).

Role of the Shh/Gli2 pathway in pituitary embryogenesis

Most of our knowledge about pituitary embryogenesis derives from murine models. In mice, the development of the pituitary results from the timely complex interaction of transcription factors and signalling molecules acting as activators or repressors, which play a crucial role in cell proliferation, cell patterning and terminal differentiation (Zhu *et al*. 2007). The posterior pituitary lobe derives from the neural ectoderm of the ventral diencephalon, which is part of the anterior neural placode, whereas the anterior lobe (and intermediate lobe in mice) derives from the oral ectoderm adjacent to the diencephalon (extensively reviewed by Kelberman *et al*. (2009)). In early stages of pituitary organogenesis, the close interaction between the invagination of the oral ectoderm to form the rudimentary Rathke's pouch and the evagination of the neuroectoderm of the ventral diencephalon to form the posterior pituitary lobe is critical for pituitary gland formation. Extrinsic signals from the ventral diencephalon and surrounding structures (WNT, BMP, FGF, Notch and Hedgehog pathways) play an important role in the early stage of pituitary embryogenesis (Zhu *et al*. 2007, Kelberman *et al*. 2009). The oral ectoderm thickens and invaginates upwards to form the Rathke's pouch, and the diencephalon evaginates downwards (Treier *et al*. 2001). Rathke's pouch separates and detaches from the oral ectoderm to form the pituitary gland.

During early vertebrate embryogenesis, Sonic Hedgehog (Shh, OMIM*600725) is expressed in midline tissues, such as the notochord and floor plate of the neural tube, and it is critical for distal elements of the developing limbs. *Shh* is expressed in the ventral diencephalon and in the adjacent oral ectoderm but not in the primordial Rathke's pouch (Treier *et al*. 2001). In contrast, the Shh receptor Patched is expressed in Rathke's pouch. Shh signalling is mediated by three related zinc-finger transcription factors, Gli1, Gli2, and Gli3, which are expressed in the ventral diencephalon and in the developing Rathke's pouch and lead to activation of target genes (Hui *et al*. 1994). *Gli2* is expressed in the ventral diencephalon, where it induces *Bmp4* and *Fgf8* expression, and it is also expressed in the oral ectoderm, where it induces pituitary progenitors (Wang *et al*. 2010). In mice, inactivation of *Gli2* caused severe ventral patterning defects in the hindbrain, diminished pituitary progenitor cells, caused a normally patterned but hypoplastic anterior pituitary as a result of diminished cellular proliferation and reduced expression of *Bmp4* and *Fgf8* with an absent posterior lobe (Lebel *et al*. 2007, Wang *et al*. 2010). The numbers of somatotropes, lactotropes and corticotropes were also diminished (Wang *et al*. 2010). In contrast, mice with inactivated *Gli1* or *Gli3* had no pituitary abnormalities (Wang *et al*. 2010).

By *in situ* hybridisation and immunostaining, Gregory *et al*. (2015) showed widespread expression of *GLI2* within the neural epithelium, including the hypothalamus, head mesenchyme and developing Rathke's pouch, in human embryos at Carnegie stages 13 and 15. Widespread *Gli2* expression has previously been described in mouse embryos at comparable developmental stages (11–12 days post coitum) (Hui *et al*. 1994), which demonstrates that *GLI2* is expressed during human development at the critical stages when Gli2 activity is required in the mouse (Gregory *et al*. 2015).

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A mutant zebrafish with loss-of-function *gli2* (*yoo-too*, *yot*) was shown to have anterior pituitary hypoplasia and abnormal patterning of the ventral CNS (Karlstrom *et al.* 2003).

Human mutations

SHH mutations are a genetic cause of holoprosencephaly (HPE), which is characterised by a failure of midline division of the forebrain. Clinical manifestations are variable, ranging from closely spaced eyes (hypotelorism) to the failure of the eye field and forebrain to separate, which is associated with cyclopia (Roessler *et al.* 2003). GLI2 is a mediator of SHH action and is, therefore, a candidate gene for HPE. Roessler *et al.* (2003, 2005) isolated the human *GLI2* gene and searched for mutations in 390 patients who met clinical criteria for HPE. Very few patients had *GLI2* mutations that, in addition to HPE, were noted to have polydactyly, midfacial and/or pituitary abnormalities. The authors observed an autosomal dominant inheritance with incomplete penetrance and inferred that pituitary and facial structures were more sensitive to a reduction in GLI2 activity than the ventral forebrain was. Subsequently, Rahimov *et al.* (2006) screened *GLI2* in patients with isolated midfacial defects, and França *et al.* (2010) screened patients with isolated pituitary hormone deficiency without HPE. In this review, we concentrate on the *GLI2* mutations in patients that have a pituitary phenotype with or without additional abnormalities.

A comprehensive review of *GLI2* mutations also in subjects without hypopituitarism that included patients with HPE and/or midfacial defects or their normal relatives has been published by Bear *et al.* (2014).

Several different heterozygous *GLI2* variants have been reported in patients with hypopituitarism, and some of these variants have been reported in databases of normal individuals. It is not clear if all of them have clinical significance. *GLI2* mutations with a functional effect may be found in normal individuals because the penetrance is incomplete and its percentage is presently unknown. Incomplete penetrance also indicates that the mutation alone is probably not enough to cause the phenotype but instead must be coupled to other genetic and/or environmental factors.

In this review, we take a conservative approach and classify variants in three groups: i) highly likely to cause the phenotype: complete gene deletions, nonsense or frameshift mutations that result in protein truncation, mutations in the universal splicing sites (−2, −1, +1 or +2) and mutations within the zinc-finger region with impaired functional test (Table 1); ii) variants of unknown significance: non-synonymous variants with normal or absence of functional tests but absent or with an allele frequency lower than 0.001 in Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC browser, http://exac.broadinstitute.org; updated 18/02/2015) and/or local control individuals (Table 2); and iii) probably benign: variants with a frequency ≥0.001 (equivalent to 0.1%) in the EVS or ExAC browser.

Table 1 displays 25 patients from 16 families where the *GLI2* mutation has led to a severe alteration in the GLI2 protein, including: one family with a complete gene deletion, six with termination codons, six with frameshift mutations that resulted in stop codons, one with a mutation in the universal splicing site and two with mutations in the zinc-finger region and functional tests that show impaired transactivation.

Roessler *et al.* (2005) performed luciferase-based reporter assays in cultured mouse C3H cells to test GLI2 transcriptional activity. The human GLI2 carboxy-terminal domain had transcriptional activity, whereas the amino-terminal domain had transcriptional repressor activity, which was similar to reported findings in mice Gli2 (Sasaki *et al.* 1999, Roessler *et al.* 2005). Constructs that represent pathogenic human GLI2 mutants with intact amino-terminal and zinc-finger domains and a truncated carboxy-terminal domain showed no intrinsic Gli2 transcriptional activity. Cotransfection of these mutants, together with WT *GLI2*, revealed strong dominant negative activity as compared to WT alone. Intact zinc-finger as well as amino-terminal domains were important for the preservation of dominant negative activity (Roessler *et al.* 2005). The GLI2 p.Arg516Pro mutation reported by Flemming *et al.* (2013) in patient 4a is located in the third zinc finger of the DNA-binding domain. Electromobility shift assay demonstrated a lack of binding to a consensus GLI-binding site, and luciferase reporter assay revealed a complete loss of transactivation in mouse embryonic fibroblast (NIH-3T3) as well as murine corticotrophinoma (At-T 20) cell systems. Further experiments showed no dominant negative behaviour of this mutation, which confirmed previous studies that required integrity of the DNA-binding domain for this effect (Roessler *et al.* 2005, Flemming *et al.* 2013). The authors suggested a dose effect or even autosomal random monoallelic expression. The two-amino-acid-apart GLI2 p.Glu518Lys mutation reported by Gregory *et al.* (2015) had normal binding on the electromobility shift assay but

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Table 1 Patients with pituitary phenotype with heterozygous *GLI2* mutations likely to be pathogenic

Family/ patient	Mutation	Hormonal deficiencies	Brain/ pituitary MRI	Additional findings	Inheritance	Comments	References
1a	2q14.2(121,412,559–121,530,829)!1 mat	GH	Chiari I, ectopic PP	Mild midface hypoplasia, R postaxial PD	Inherited from mother		Solomon *et al.* (2012)
1b	2q14.2(121,412,559–121,530,829)!1 mat	GH	Unknown	Bilateral postaxial PD	Sister of 1a		Bear *et al.* (2014)
2a	c.1138G>T, p.E380X	GH, TSH, ACTH, ADH	Small AP, absent PP		Normal mother with mutation		França *et al.* (2010)
3a	c.1323G>A, p.Trp441X	GH	Small AP	Bilateral CL/P, SCI, MC, postaxial hexadactyly	*De novo* (gonadal mosaicism)	Deceased sister had alobar HPE, no genetic testing	Roessler *et al.* (2003)
4a	c.1547G>C, p.Arg516Pro	GH, TSH, LH/FSH	Small AP, ectopic PP	Unilateral PD	Normal father with mutation	Absent transcriptional activity, located in zinc-finger domain	Flemming *et al.* (2013)
5a	c.1552G>A, p.E518K	GH, TSH?	Small AP, normal stalk absent PP		Relatives not tested	Normal binding, reduced transactivation, located in zinc-finger domain	Gregory *et al.* (2015)
6a	c.1683G>A= IVS11+1G>A	GH		CL, MC, single nares, midface hypoplasia, DD	Apparently normal father with mutation	Splice-site	Roessler *et al.* (2003)
7a	c.1908dupC, p.Val637Argfs*42	GH	Widening L temporal horn	Bilateral postaxial PD in hands and feet, MC, upper slanting palpebral fissures, high palate, atrioventricular septum defect			Bear *et al.* (2014)
8a	c.2081_2084del, p.Leu694fsX722 also c.1760C>T, p.Pro608Leu	GH	Small AP, ectopic PP	CL/P, unilateral cryptorchidism	Normal father with both mutations	Truncating, absent transcriptional domain, missense variant after the stop codon	França *et al.* (2010)
9a	c.2362_2368del, p.Leu788fsX794 also c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH, TSH, ACTH, LH/FSH	Small AP, ectopic PP, small brain size, asymmetrical cerebral hemispheres	Bilateral postaxial PD	Mother with mutation and PD	Brain anomalies probably secondary to repeated hypoglycaemias, missense variants after the stop codon	França *et al.* (2010)
9b	c.2362_2368del, p.Leu788fsX794 also c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH	Small AP, ectopic PP	L postaxial PD	Uncle of 9a	Missense variants after the stop codon	França *et al.* (2010)
9c	c.2362_2368del, p.Leu788fsX794 also c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH	Small AP, thin stalk, ectopic PP	Bilateral postaxial PD	Uncle of 9a and brother of 9b	Father of four children, low testosterone after head trauma, missense variants after the stop codon	França *et al.* (2010)
9d	c.2362_2368del, p.Leu788fsX794 also c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH, TSH, LH/FSH	Small AP, thin stalk, ectopic PP, anomalous venous development	Bilateral postaxial PD of hands and feet	Daughter of 9c	Missense variants after the stop codon	França *et al.* (2010)

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Table 1 *Continued*

Family/ patient	Mutation	Hormonal deficiencies	Brain/ pituitary MRI	Additional findings	Inheritance	Comments	References
9e	c.2362_2368del, p.Leu788fsX794 also c.4315G>A, p.Ala1439Thr c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH	Small AP, ectopic PP, normal stalk	Maxillary SCI	Distant relative of 9a–d		Bear *et al*. (2014) and Paulo *et al*. (2015)
9f	c.2362_2368del, p.Leu788fsX794 also c.4332G>A, p.M1444I and c.4333C>T, p.L1445F	GH	Small AP, ectopic PP	Cleft palate, maxillary SCI	Father of 9e, distant relative of 9a–d		Bear *et al*. (2014) and Paulo *et al*. (2015)
10a	c.2773C>T, p.Gln925X		Aplasia of pituitary	Postaxial PD		Two additional family members with mutation and PD	Bear *et al*. (2014)
10b	c.2773C>T, p.Gln925X	Panhypopituitarism	Empty sella	Postaxial PD	Paternal uncle of 10a	Two additional family members with mutation and PD	Bear *et al*. (2014)
11a	c.3258del1, p.Tyr1086fs*42	Panhypopituitarism	ONH, absent pituitary	CL/P, postaxial PD	Father with mutation and PD	Other family members with mutation CL/P and PD	Roessler *et al*. (2003)
11b	c.3258del1, p.Tyr1086fs*42	Panhypopituitarism	NA	Normal hands	Brother of 11a, father with mutation and PD	Deceased dizygotic twin had CL/P, PD, no molecular testing	Roessler *et al*. (2003)
12a	c.3294_3295delAC, p.Arg1098Serfs*43	Panhypopituitarism	Aplastic AP	Midface hypoplasia, sandle gap toes, micropenis, cryptorchidism, bilateral postaxial PD, bifid epiglottis			Bear *et al*. (2014)
13a	c.3351C>A; 3555delC; p.Pro1184Gln; p.Tyr1186Thrfs*34	Panhypopituitarism	Pituitary hypoplasia, Chiari I malformation	Maxillary SCI	Two mutations inherited from apparently normal mother		Bear *et al*. (2014)
14a	c.3382C>T, p.Gln1128*	Panhypopituitarism	Small AP, ectopic PP	DD, epicathus, broad nose, wide mouth, brachydactyly, unilateral postaxial PD, dislocated hips		Also with 324 kb deletion at 6p26, which deletes PARK2	Bear *et al*. (2014)
15a	c.3502C>T, p.Gln1168X	Panhypopituitarism	Small AP, ectopic PP, pontocerebellar hypoplasia, temporobasal dysgyria	DD, microcephaly, brachycephalia, low frontal hairline, thin upper lip, fleshy nasal tip, unilateral postaxial PD	*De novo*		Bear *et al*. (2014)
16a	c.3768C>T, p.Gln1256X	Panhypopituitarism	Empty sella	Bilateral postaxial PD, MC, bilateral cryptorchidism	Father and brother with mutation and PD		Roessler *et al*. (2005)
16b	c.3768C>T, p.Gln1256X	Panhypopituitarism	Pituitary aplasia	Bilateral postaxial PD, patent ductus arteriosus, microphallus, small testes	Nephew of 16a		Roessler *et al*. (2005)

AP, anterior pituitary; CL, cleft lip; CL/P, cleft lip and palate; DD, developmental delay; MC, microcephaly; PD, polydactyly; PP, posterior pituitary; SCI, single central incisor.

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Table 2 Patients with pituitary phenotype with heterozygous non-synonymous *GLI2* variants of unknown significance

Family/ patient	Mutation	Hormonal deficiencies	Brain/ pituitary MRI	Additional findings	dbSNP number MAF in ExAC	Comments	References
18a	c.547G>A, p.Val183Met	GH	Small AP, stalk abnormality, ectopic PP		0.000008298	Normal father and brother with variant	Bear *et al.* (2014) and Paulo *et al.* (2015)
19a	c.757C>T, p.Pro253Ser	GH, TSH, ACTHp	Small AP, ectopic PP		0.000008262	Breech delivery	França *et al.* (2013)
20a	c.1418G>A, p.Arg473His	GH, TSH, ACTHp, LH/FSH	Small AP, ectopic PP		rs150170739 0.00008255		França *et al.* (2013)
21a	c.2159G>A, p.R720H	LH, FSH	Small AP, normal PP	Cleft palate, cervical vertebral fusion, sensori-neural hearing loss, Klippel–Feil syndrome?	rs149091975 0.000465	Normal transactivation, asymptomatic father with variant	Gregory *et al.* (2015)
22a	c.2281C>T, p.Leu761Phe	GH, TSH, ACTH	ONH, absent septum pellucidum, CC hypoplasia, schizencephaly, normal pituitary		Not reported		Bear *et al.* (2014) and Paulo *et al.* (2015)
23a	c.2339C>T, p.Ala780Val	GH, TSH, ACTH	NA		rs374016746 0.00005253		França *et al.* (2013)
24a	c.2488T>C, p.Phe830Leu	GH, TSH, ACTHp, LH/FSH	Small AP, ectopic PP		0.0002581	Forceps delivery	França *et al.* (2013)
25a	c.2509G>A, p.Glu837Lys	LH/FSH	Absent olfactory bulbs, hypoplastic olfactory sulci, no signs of HPE	Absent sense of smell, Kallman syndrome	rs193090538 0.00003277	Also had a variant in *SIX3* (c.428G>A, p.Gly143Asp)	Vaaralahti *et al.* (2012)
26a	c.2798G>A, p.Arg933His	GH, TSH, ACTHp	Small AP, ectopic PP		Not reported	Breech preterm delivery, mother with mutation had short stature and normal hormones	França *et al.* (2013)
27a	c.2840G>A, p.Gly947Asp	GH, TSH, ACTH, ADH	Small AP, absent PP, abnormal cerebral periventricular venous system	Perineal hypospa-dias, cryptorchid-ism, epicanthus, palpebral ptosis, micrognathia	Not reported	Breech preterm delivery, mother with same variant had short stature and normal hormones	França *et al.* (2013)
28a	c.3723G>A, p.Met1241Ile;	GH, ACTHp	Small AP, ectopic PP		rs138191075 0.0001456	Born by Caesarean section, father with variant had short stature and normal hormones	França *et al.* (2013)
28a	c.4453C>G, p.Pro 1485Ala	GH, ACTHp	Small AP, ectopic PP		rs145958673 0.00001651	Born by Caesarean section, father with variant had short stature and normal hormones	França *et al.* (2013)

ACTHp, partial ACTH; CC, corpus callosum; HPE, holoprosencephaly; ONH, optic nerve hypoplasia; MAF, minor allele frequency; ExAC browser, Exome Aggregation Consortium.

significantly reduced transactivation in a reporter assay that also uses NIH 3T3 cells.

Seventeen (68%) of the 25 patients in Table 1 had polydactyly, whereas none of the patients in Table 2 had polydactyly, which suggests that only major GLI2 abnormalities result in polydactyly. Similar findings have been observed by Bear *et al*. (2014): 16/26 (62%) of severe mutations had polydactyly in addition to pituitary abnormalities, whereas only 1/58 (2%) with milder variants had both hand and pituitary defects. The presence of polydactyly in a patient with hypopituitarism or in one of his relatives may be an additional indication that the *GLI2* gene should be studied (França *et al*. 2010). Interestingly, mice with targeted disruption of Gli2 have not been shown to have digit abnormalities unless Gli1 is also deleted, which emphasizes the difference between Gli protein functions in vertebral species (Roessler *et al*. 2005).

Molecular testing was performed in several parents of the probands with hypopituitarism in Table 1: of the 11 parents who carried the same mutation, two had hypopituitarism (one with polydactyly), three had only polydactyly and six were apparently completely normal. This indicates that even with the most severe mutations, the penetrance is incomplete. Therefore, in addition to the *GLI2* mutation, another genetic and/or environmental factor is probably responsible for the pituitary phenotype.

An interaction between specific genetic and environmental risk factors has been demonstrated in mice by Kietzman *et al*. (2014). Heterozygous Shh and Gli2 mice exposed to prenatal ethanol had, respectively, a 3.2- and a 6.6-fold increase in their dysmorphology score as compared to WT littermates that were exposed to the same ethanol protocol. The authors also observed a close correlation between the severity of facial dysmorphology and midline forebrain abnormalities in affected animals (Kietzman *et al*. 2014).

The phenotypic expressivity of *GLI2* mutations is variable. The largest family with *GLI2* mutations reported to date had the p.Leu788fsX794 mutation (França *et al*. 2010, Paulo *et al*. 2015). In the family reported by França *et al*. (2010), the ten subjects who tested positive for the mutation also had polydactyly, whereas in the two distant relatives with the same mutation studied by Paulo *et al*. (2015) in a different city, polydactyly was not observed. Among the six patients with hypopituitarism, four had IGHD and two had CPHD, independent of age. The two patients reported by Paulo *et al* (2015). had a median solitary maxillary incisor, one with cleft palate, but this was not observed in the patients studied by França *et al* (2010). Therefore, the phenotypic variability in this family with the same mutation ranged from polydactyly only to IGHD or CPHD, with the presence or absence of cleft palate and of a median solitary maxillary incisor. None of the patients had brain abnormalities characteristic of HPE. Interestingly, all of the patients with hypopituitarism in this family had a small anterior lobe and an ectopic posterior lobe on pituitary magnetic resonance imaging (MRI).

After an extensive review of more than 400 patients, Bear *et al*. (2014) concluded that frank HPE resulting from pathogenic *GLI2* mutations is rare. The absence of forebrain abnormalities in patients with *GLI2* mutations, as opposed to those with *SHH* mutations, might be related to the maintained activity of *GLI3* in the former. Shh-dependent ventral patterning in the neural tube is preserved in *Gli1/Gli2* double homozygous mutant mice embryos, which suggests that *Gli3* partly compensates for the function of *Gli2* (Sasaki *et al*. 1999).

An ectopic or undescended posterior pituitary lobe is a frequent finding in patients with hypopituitarism resulting from *GLI2* mutations. An ectopic or undescended posterior pituitary lobe is also a frequent finding in patients with GH deficiency, especially CPHD. Its presence on MRI has been increasingly used for the diagnosis of GH deficiency in light of the limitations of GH stimulation tests. Among most patients with an ectopic or undescended posterior pituitary lobe, rare mutations in factors involved in early pituitary embryogenesis have been reported, including: *HESX1* (Dattani *et al*. 1998, Alatzoglou & Dattani 2009), *LHX4* (Machinis *et al*. 2001, Alatzoglou & Dattani 2009), *SOX3* (Woods *et al*. 2005, Solomon *et al*. 2007) and *OTX2* (Diaczok *et al*. 2008, Dateki *et al*. 2010). In addition, mutations in these early acting factors may present as part of a syndrome in patients that manifest abnormalities in extra-pituitary structures that share a common embryological origin with the pituitary gland, such as the eye and forebrain. However, mutations in these factors have been identified in only a minority of patients that have hypopituitarism with an ectopic posterior lobe, and *GLI2* mutations might be a significant genetic cause of additional cases (França *et al*. 2013).

Mutations in *PROP1* are the most common genetic cause of CPHD in many geographical areas, but the inheritance is autosomal recessive with complete penetrance, and in all patients, the posterior pituitary lobe has been shown to have a normal position (Wu *et al*. 1998, Kelberman *et al*. 2009). Interestingly, the posterior pituitary lobe has been consistently in a normal position in patients with mutations in transcription factors that act late, and expression is restricted to the Rathke's pouch, such as PROP1 and POU1F1 (PIT1) (Alatzoglou & Dattani 2009).

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Two patients (2a and 27a, Tables 1 and 2) also had diabetes insipidus. ADH deficiency might be explained because *GLI2* is also expressed in the ventral diencephalon, and it is important for hypothalamus, infundibulum and posterior pituitary lobe formation (Wang *et al*. 2010). All of the mice with *Gli2* mutations had no posterior pituitary, which suggests that *Gli2* is a requirement for ventral diencephalon formation (Wang *et al*. 2010). Interestingly, patient 2a with a nonsense mutation (p.E380X) had diabetes insipidus at the age of 18 months and reached a urinary osmolality of 613 mOsm/kg H_2O during follow-up at the age of 30 months (França *et al*. 2010).

In one patient, diabetes insipidus and semilobar HPE were initially attributed to a GLI2 p.Arg226His variant (Roessler *et al*. 2003). However, the patient was later found to also have a truncating mutation in *ZIC2*, and there was stronger evidence that this was the cause of HPE and ADH deficiency in that individual (Solomon *et al*. 2009).

Diabetes insipidus is common in typical HPE, whereas in patients with *GLI2* mutations, there is frequent anterior pituitary hormone deficiency (Bear *et al*. 2014).

It is noteworthy that patient 27a with the GLI2 p.Gly947Asp variant had, in addition to hypopituitarism, incomplete masculinisation of the external genitalia. Miyagawa *et al*. (2011) have shown that Shh is expressed in the urethral plate epithelium of mice during embryogenesis and that its signal is mediated through Gli2 in the mesenchyme. *Gli2* mutants had hypoplasia of the genital tubercle and a cleft at the proximal ventral midline without androgen deficiency. This murine phenotype resembles that patient's hypospadias. However, the boys with other *GLI2* sequence variants or nonsense mutations had normal external genitalia, which indicates that additional, as-yet-unidentified factors modulate the phenotype.

The greater prevalence of GH deficiency among hypopituitary patients that harbour *GLI2* mutations might be explained by an ascertainment bias, because more cohorts of patients with GH deficiency have been studied. Furthermore, *Gli2* knockout mice have a reduced number of somatotrophs, and these represent the largest population of pituitary hormone-secreting cells (Wang *et al*. 2010).

Vaaralahti *et al*. (2012) screened HPE genes in 19 patients with Kallman syndrome who were negative for known candidate gene mutations. One patient (25a, Table 2) had the GLI2 p.Glu837Lys variant (Table 2) in addition to a SIX3 p.Gly143Asp. He had gonadotrophin deficiency but no evidence of other characteristics of patients with *GLI2* mutations, such as GH hormone deficiency, ectopic posterior pituitary lobe or polydactyly. Although the pathogenicity of these variants was not proven, the authors suggested an overlap between HPE and Kallman syndrome genes.

Larger deletions of chromosome 2q, including *GLI2* and several additional genes, have been described. Kevelam *et al*. (2012) reported a heterozygous submicroscopic 1.3 kb deletion of 2q14.2 in a patient with a cleft lip and palate and panhypopituitarism. Gustavsson *et al*. (2006) described a balanced translocation and a

Table 3 Heterozygous GLI2 variants identified in patients with hypopituitarism and found with a frequency of more than 0.001 in updated databases

		Minor allele frequency		
Mutation	**dbSNP number**	EVS	ExAC browser	**References**
c.607G>A, p.Ala203Thr	rs147044066	0.0013090	0.0007559	França *et al*. (2013)
c.803C>T, p.Ala268Val	rs146992756	0.0022347	0.0007070	França *et al*. (2013)
c.1294G>A, p.Val432Met	rs142296407	0.0042467	0.001509	França *et al*. (2013), Bear *et al*. (2014) and Paulo *et al*. (2015)
c.3349G>T, p.Val1117Leu	rs147580961	0.0022347	0.0005250	França *et al*. (2013)
c.3590G>A, p.Gly1197Asp	rs114823319	0.0049115	0.001446	França *et al*. (2013)
c.3943C>T, p.Pro1315Ser	rs114376238	0.0192021	0.01649	França *et al*. (2013)
c.4054A>G, p.Met1352Val	rs149140724	0.0108065	0.009916	Flemming *et al*. (2013), França *et al*. (2013) and Paulo *et al*. (2015)
c.4332G>A, p.Met1444Ile	rs146467786	0.000769	0.008259	Flemming *et al*. (2013), França *et al*. (2013) and Paulo *et al*. (2015)
c.4333C>T, p.Leu1445Phe	rs146207623	0.000769	0.008258	Flemming *et al*. (2013), França *et al*. (2013) and Paulo *et al*. (2015)
c.4558G>A, p.Asp1520Asn	rs114814747	0.0110385	0.01005	Flemming *et al*. (2013), França *et al*. (2013) and Paulo *et al*. (2015)
c.4628G>A, P.Arg1543His	rs138987487	0.0004615	0.0005028	Flemming *et al*. (2013)

EVS, Exome Variant Server; ExAC browser, Exome Aggregation Consortium.

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submicroscopic deletion of 2q14.2–2q22.1 that included 42 known genes in addition to *GLI2* with a complex phenotype that included, among others, polydactyly, GH deficiency, hypospadias and undescended testes.

Table 3 shows heterozygous non-synonymous *GLI2* variants that were reported in isolation or in combination in patients with hypopituitarism and were probably benign, because their frequency in updated databases of normal individuals was $\geq$0.001. However, the 0.001 threshold is arbitrary, because the true prevalence of hypopituitarism resulting from *GLI2* mutations is unknown, as is the penetrance of *GLI2* mutations, which may also vary according to each specific mutation. Therefore, it is premature to conclude that these variants have no effect on the phenotype. Functional studies are useful, but they also have limitations because of their artificial nature. It is possible that after a few years of whole exome and genome sequencing of large cohorts of patients with hypopituitarism, the relative contribution of variants in different genes will be better understood. There is also a selection bias of the types of patients that are screened with the candidate gene approach. Because the *GLI2* gene will be included in the analysis in different conditions that are submitted to whole exome/genome sequencing in future studies, the range of phenotypes associated with each *GLI2* mutation will likely be further expanded.

The exact frequency of GLI2 alterations in patients with hypopituitarism is still difficult to determine because of the ascertainment bias in and the phenotypic heterogeneity of studied patient cohorts as well as different *GLI2* screening methods and a lack of proof of pathogenicity for many *GLI2* variants. Among 284 patients with hypopituitarism from three cohorts in whom the complete coding region of *GLI2* was sequenced, 15 patients (5%) had either loss-of-function or non-synonymous *GLI2* variants found in updated databases with a frequency lower than 0.001 (França *et al*. 2013, Gregory *et al*. 2015, Paulo *et al*. 2015).

The aetiology of congenital GH deficiency in most cases is still unknown. The presence of familial cases suggests a genetic origin instead of a traumatic/ischaemic origin secondary to perinatal insults (Phillips & Cogan 1994, Triulzi *et al*. 1994). However, in most cases of GH deficiency, the family history is apparently not positive for similar cases. A disease model of partial penetrance, as with *GLI2* mutations, indicates that the possibility of hypopituitarism and/or polydactyly should be investigated even in distant relatives. One of the index patients had two uncles with IGHD who were unaware of their condition (França *et al*. 2010).

GLI2 is a large and polymorphic gene, and sequencing may identify variants of unknown significance whose interpretation may be difficult. *GLI2* variants might interact with environmental and other genetic factors to modulate the phenotype. Functional analyses of *GLI2* variants are important to add evidence to their pathogenicity.

In conclusion, a relatively high frequency of *GLI2* mutations and variants was identified in patients with congenital GH deficiency without other brain defects, and most of these patients presented with CPHD and an ectopic posterior pituitary lobe. Future studies may clarify the relative role and frequency of *GLI2* alterations in the aetiology of hypopituitarism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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