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ORIGINAL ARTICLE

Truncating and zinc-finger variants in *GLI2* are associated with hypopituitarism

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Abstract

Variants in transcription factor *GLI2* have been associated with hypopituitarism and structural brain abnormalities, occasionally including holoprosencephaly (HPE). Substantial phenotypic variability and nonpenetrance have been described, posing difficulties in the counseling of affected families. We present three individuals with novel likely pathogenic *GLI2* variants, two with truncating and one with a de novo missense variant p.(Ser548Leu), and review the literature for comprehensive phenotypic descriptions of individuals with confirmed pathogenic (a) intragenic *GLI2* variants and (b) chromosome 2q14.2 deletions encompassing only *GLI2*. We show that most of the 31 missense variants previously reported as pathogenic are likely benign or, at most, low-risk variants. Four Zn-finger variants: p.(Arg479Gly), p.(Arg516Pro), p.(Gly518Lys), and p.(Tyr575His) were classified as likely pathogenic, and three other variants as possibly pathogenic: p.(Pro253Ser), p.(Ala593Val), and p.(Pro1243Leu). We analyze the phenotypic descriptions of 60 individuals with pathogenic *GLI2* variants and evidence a morbidity spectrum that includes hypopituitarism (58%), HPE (6%) or other brain structure abnormalities (15%), orofacial clefting (17%) and dysmorphic facial features (35%). We establish that truncating and Zn-finger variants in *GLI2* are associated with a high risk of hypopituitarism, and that a solitary median maxillary central incisor is part of the *GLI2*-related phenotypic variability. The most prevalent phenotypic feature is post-axial polydactyly (65%) which is also the mildest phenotypic expression of the condition, reported in many parents of individuals with systemic findings. Our approach clarifies clinical risks and the important messages to discuss in counseling for a pathogenic *GLI2* variant.

KEYWORDS
combined pituitary hormone deficiency, Culler–Jones syndrome, *GLI2*, holoprosencephaly, solitary median maxillary central incisor

1 | INTRODUCTION

Culler and Jones (1984) described a cohort of individuals displaying a syndrome of post-axial polydactyly (PAP), hypopituitarism, and dysmorphic facial features with autosomal dominant pattern of inheritance. Heterozygous pathogenic genetic variants were later characterized in the transcriptional activator gene GLI-Kruppel family member 2 (*GLI2*, OMIM *165230) (Roessler et al., 2005). *GLI2* encodes a downstream transcription activator within the Sonic Hedgehog (*SHH*, OMIM *600275) signaling cascade. *GLI2* belongs to the GLI family, which is a C2H2-type zinc-finger protein subclass as indicated by the presence of five tandem zinc fingers connected by histidine-cysteine links, that constitute the Kruppel-like Gli motif (Figure 1). Variants in the SHH pathway are known to cause holoprosencephaly (HPE), as well as a spectrum of craniofacial malformations considered to constitute HPE microforms (Heussler et al., 2002).

The phenotypes of pathogenic *GLI2* variants vary from isolated PAP or short stature, to the triad of Culler–Jones Syndrome (CJS), through to more severe phenotypes including structural brain abnormalities and facial malformations (Bertolacini et al., 2012). In particular, the relationship between *GLI2* variants and hypopituitarism has been well established in a number of case reports and cohort studies (Arnhold et al., 2015; Babu et al., 2019; Bear et al., 2014; Cohen, 2012; Demiral et al., 2020; Elizabeth et al., 2020; Elward et al., 2020; Flemming et al., 2013; França et al., 2010, 2013; Gregory et al., 2015; Juanes et al., 2016; Kremer Hovinga et al., 2018; Martín-Rivada et al., 2019; Roessler et al., 2003; Solomon et al., 2012; Vishnopolska et al., 2021). *GLI2* variants have also been associated with HPE in the literature. However, Bear et al. (2014) suggest that *GLI2* pathogenic variants cause a phenotype that is distinct from HPE. In addition, substantial phenotypic variability and nonpenetrance have been described, posing difficulties in counseling affected families.

We present three individuals with variable CJS caused by novel *GLI2* variants. Furthermore, we review the literature for comprehensive phenotypic descriptions of individuals with pathogenic *GLI2* variants to better define the clinical risks for affected families.

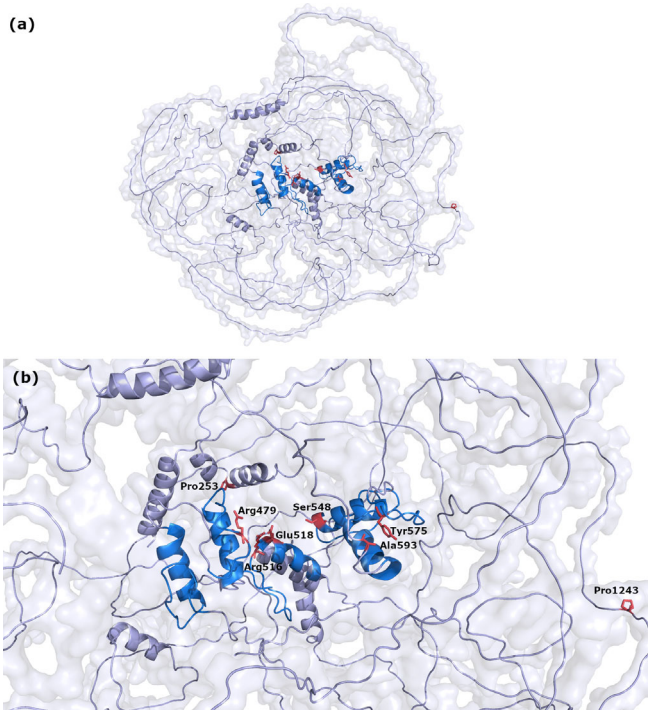

FIGURE 1 (a) GLI2 as seen in the AlphaFold artificial intelligence-generated structure (https://alphafold.ebi.ac.uk/). A pdb file containing the structural coordinates was downloaded and visualized using PyMOL molecular graphics system v.2.2. The five Zn-fingers are colored blue, with amino acids containing variants found in patients visualized as red sticks. (b) Close-up view of the amino acid residues containing variants. Note that all the likely pathogenic (LP) variants are centrally located and predict changes of the annotated amino acids Arg479, Arg516, Glu518, Ser548, and Tyr575 (see Table 2). The VUS+ variants in Pro253 and Ala593 are also in the same category, but not as central as the LP variants. The VUS+ variant in Pro1243 is located to a region with low confidence of the structural prediction and more likely to be in the periphery of the protein

2 | CLINICAL REPORT

2.1 | Clinical descriptions

2.1.1 | Individual 1

This boy was the second child of nonconsanguineous parents. His older brother was born with transposition of the great arteries, which was surgically corrected when he was 10 days old. A younger brother was healthy. Oligohydramnios was identified at 32 weeks of gestation, and delivery was induced due to intrauterine growth retardation. He was born at 37 weeks of gestation with a birth weight (BW) of 2.48 kg (the 9th percentile). Shortly after birth, he was diagnosed with unilateral, membranous, posterior choanal atresia, and was treated with two dilatations.

Psychomotor development was normal: he achieved independent sitting at 6 months, crawling at 10 months, walking at 1 year and first words at 9/10 months. At 2 years and 6 months, he had short stature, with a height of 77 cm, weight of 8.9 kg, and head circumference of 47.2 cm; all below the 0.4th centile. He had rudimentary PAP of both hands, left cupped ear deformity, fusion of the helical rim of the right ear, midface hypoplasia, and a solitary median maxillary central incisor (SMMCI).

He was referred to endocrinology due to poor weight gain and short stature in infancy. Biochemical studies initially suggested isolated growth hormone (GH) deficiency. Although later testing was not conclusive, GH supplementation was initiated due to the clinical presentation. Brain Magnetic Resonance Imaging (MRI) showed anterior pituitary hypoplasia with ectopic posterior pituitary gland on the underside of the optic chiasm, an 8-mm Arnold-Chiari type 1 malformation with no cervical syrinx or hydrocephalus, and an abnormal left frontal cortex vein draining into the ventricular venous system.

2.1.2 | Individual 2

This boy was the first child born to nonconsanguineous, healthy parents. A cleft lip and palate were diagnosed prenatally, at 20 weeks. He was born at 41 + 6 weeks of gestation weighing 3.67 kg (50th centile). Vaginal delivery was assisted by forceps due to prolonged nonadvancing labor and shoulder dystocia. Postnatally, he was transferred to the Neonatal Intensive Care Unit due to oxygen desaturations, severe hypoglycemia, and hyponatremia. He had prolonged jaundice with no hepatic cause found. He had feeding difficulties due to severe gastroesophageal reflux and was commenced on nasogastric tube feeding with supplementary oral feeding. He was investigated for a systolic heart murmur, with a normal echocardiogram. At 5 weeks of age, he presented with further apneic episodes and hypoglycemia. Endocrinology investigations evidenced combined hypopituitarism (CPHD), and MRI demonstrated a hypoplastic anterior pituitary gland and an ectopic posterior pituitary gland. He was started on replacement hydrocortisone, levothyroxine, testosterone, and GH. Despite appropriate levels of hormone replacement, he later developed hypertension of uncertain cause requiring control with amlodipine and atenolol.

His psychomotor development was normal. His head circumference, length and weight were all below the 0.4th percentile at 6 months of age. He had a bilateral cleft lip with partial clefting of the hard palate, a slightly depressed nasal bridge, slight hypotelorism, a micropenis, right cryptorchidism, and a left varicocele.

2.1.3 | Individual 3

Individual 3 is the daughter of nonconsanguineous, healthy parents and has two healthy, maternal siblings. Her mother had asymptomatic dilated cardiomyopathy (OMIM # 613172). She was delivered by caesarean section due to breech position at 40 weeks with birth growth parameters as follows: weight 4625 g (2.08 SDS), length 51 cm (0.28 SDS) and OFC 38.5 cm (2.51 SDS) (all references according to Norwegian growth charts), and Apgar score 9,7,7. She was transferred to the neonatal unit due to compromised respiration, and was found to have a blood glucose of 0.9 mmol/L. At 14 hours of age, she had early-onset jaundice with a bilirubin of 114 μmol/L, and was stable but lethargic and hypotonic with feeding difficulties. She was treated with IV glucose, UV light, and tube feeding. At 11 days of age, TSH and T4 were borderline, high and low, respectively, not adjusted for weight. She had a slightly enlarged heart and a small PFO, normal EEG and cerebral ultrasound, unstable hips by Ortolani's test, and ongoing mild hypoglycemic episodes. She was treated with vitamin B12 due to elevated homocysteine, fat-soluble vitamin supplements, and a Frejka-type hip pillow. Metabolic and TORCH screens were negative. She was discharged at 3 weeks of age, with persistent jaundice, lethargy, and feeding difficulties. Upon follow up at 4 weeks of age, she had persistently borderline, low T4 and ongoing, unconjugated hyperbilirubinaemia, with normal liver ultrasound. Bilirubin was 360 μmol/L on day 4 and normalized at 4 months of age. Direct bilirubin peaked at 3 months and normalized within a month. At 5 weeks of age, the genetic test result was completed. The following morning, she was re-admitted to the hospital with cortisol deficiency, treated with immediate supplementation followed by levothyroxine after 24 h. During this period, the parents describe that the child rapidly became more alert. One week later, GH supplementation was initiated based on several, undetectable IGF-1 measurements, combined with abnormal pituitary imaging. MRI showed a tiny dysplastic anterior pituitary stalk in the sella turcica, with an ectopic posterior pituitary gland in the third ventricle. There was also partial agenesis of posterior parts of corpus callosum, a narrow aqueduct, rotated hippocampi and slightly dysmorphic mesencephalon. At 4 months of age she was a well-nourished and happy child, with mild hypotelorism, alternating esotropia, a slightly small midface, and a visible neck fold. She presented with mild gross motor delay attributed to 4 months of hip splinting. The previous hyperbilirubinaemia was considered secondary to her hypothyroidism. At 5 months, her weight was −0.20 SDS, length −0.46 SDS, and OFC 0.86 SDS. Endocrine analysis established undetectable FSH and LH levels and a normal prolactin level. At 9 months, her weight was −0.67 SDS, length −0.11 SDS, and OFC 0.42 SDS; a cleft in one of the deciduous maxillary incisors was visible.

2.2 | Laboratory investigations

Individual 1 had an initial microarray (OGT CytoSure Constitutional v3 Array; analysis software CytoSure Interpret v4.8) which demonstrated a chromosome 3q21.2 duplication inherited from his asymptomatic father. This was of uncertain significance and, although possibly contributing to the familial speech articulation problems, was not thought to explain the rest of the features of the proband. Targeted D-HPLC/sequencing for variants known to cause SMMCI demonstrated no deleterious variant in *SHH* (NM_00193.2), *ZIC2* (NM_007129.2), *SIX3* (NM_0015413.2), and *TGIF* (NM_003244.2). Exome capture and sequencing performed as part of the Deciphering Developmental Disorders Study (Firth et al., 2011) evidenced heterozygosity for the *GLI2* c.1643C>T p.(Ser548Leu) variant (DECIPHER individual number DDD-SMH: 277648:15-04-21). This variant was confirmed by Sanger sequencing, and it predicts a change of a highly conserved serine in Zn-finger number 4. Carrier testing in parents evidenced that the variant occurred de novo.

Individual 2 was investigated with a microarray (OGT CytoSure Constitutional v3 Array; analysis software CytoSure Interpret v4.8) with normal results. A targeted clinical exome (Agilent SureSelectXT Human Focused Clinical Exome and Illumina NGS) for variants in genes associated with bilateral cleft lip and panhypopituitarism identified a heterozygous frameshift variant in *GLI2*: c.922delT p.(Ser308Glnfs*9), predicted to disrupt the translation of *GLI2*. Carrier testing in parents evidenced that the variant was inherited from his asymptomatic father.

Individual 3 was investigated with CytoScanHD copy number array (Thermo Fisher Scientific) and methylation-sensitive MLPA for

TABLE 1 Summary of genetic variants and clinical characteristics of study patients

	Individual 1	Individual 2	Individual 3
Sex	Male	Male	Female
Age at examination	2 years 8 months	6 months	3 weeks
Variant location	g.120982840C > T	g.120970469del	g.120989488C>T
Allele	c. 1643C>T	c.922del	c.3574C>T
Amino acid change	p.(Ser548Leu)	p.(Ser308Glnfs*9)	p.(Gln1192*)
Inheretance	De novo	Paternal	De novo
Clinical features	Intrauterine growth retardation and oligohydramnios, unilateral posterior choanal atresia, left cupped ear deformity, fusion of the helical rim of the right ear, single median maxillary central incisor, rudimentary postaxial polydactyly	Bilateral cleft lip with partial clefting of palate, slightly depressed nasal bridge, mild hypotelorism, micropenis, right cryptorchidism, left hydrocele, hypertension (under investigation)	Neonatal hypoglycaemia and hyperbilirubinaemia, mild hypotelorism, somewhat small midface, visible neck fold. Hip dysplasia and mild motor delay ascribed to 4 months in a hip splint
Endocrine features	Isolated GH deficiency	Deficiencies in TSH, ACTH, IGF-1, FSH and LH	Deficiencies in cortisol, IGF-1, Free T4, FSH and LH
Brain MRI	8 mm Arnold-Chiari type 1 malformation with no cervical syrinx or hydrocephalus. Anterior pituitary hypoplasia with ectopic posterior pituitary on the underside of optic chiasm. Abnormal left frontal cortex vein draining into the ventricular venous system.	Hypoplastic (nearly invisible) anterior pituitary with ectopic posterior pituitary	Tiny dysplastic anterior pituitary gland stalk in the sella turcica, ectopic posterior pituitary gland in the third ventricle. Partial agenesis of posterior parts of corpus callosum, narrow aqueduct, rotated hippocampi and slightly dysmorphic mesencephalon.
Other information	Speech articulation disorder, also present in father		Slightly enlarged heart and small PFO

BWS (MRC Holland ME-030 v C3), both with normal results. A large NGS-based panel (about 2500 genes) on Illumina NextSeq500 with trio filtering showed a heterozygous de novo pathogenic nonsense variant in *GLI2* (NM_001371271.1) c.3574C>T p.(Gln1192*). The variant was predicted to introduce a premature termination codon in the last exon, escaping NMD but leading to a truncated protein with loss of the last 395 amino acids.

The genotype and phenotype of the three individuals are summarized in Table 1 while the detail of the biochemical investigations performed can be found in Table S1.

2.3 | Genotype–phenotype evaluation

We reviewed the literature for comprehensive phenotypic descriptions of individuals with confirmed pathogenic (a) intragenic *GLI2* variants and (b) chromosome 2q14.2 deletions encompassing only *GLI2*. We re-evaluated the pathogenicity of previously published *GLI2* missense variants (Table 2) (Arnhold et al., 2015; Babu et al., 2019; Bear et al., 2014; Bertolacini et al., 2012; Brauner et al., 2020; Flemming et al., 2013; França et al., 2013; Gregory et al., 2015; Juanes et al., 2016; Meng et al., 2019; Rahimov et al., 2006; Vaaralahti et al., 2012; Vishnopolska et al., 2021). This re-evaluation was primarily based on population variant frequency in the gnomAD database (Karczewski et al., 2020). Variants with minor allele frequencies (MAFs) >20 per 10,000 (>0,2%) were considered benign, variants with MAFs in the range 2–20 per 10,000 were considered likely benign, and variants with MAFs of less than 2 per 10,000 were considered to be a variant-of-unknown-significance (VUS) or a VUS+, that is, a VUS that is more likely to be pathogenic. The latter distinction was based on in silico variant prediction tools (SIFT and Mutation Taster) and variants' position and structural constraints in the predicted GLI2 protein structure, determined by AlphaFold (Tunyasuvunakool et al., 2021). Since CPHD is rare in newborns and the etiology is heterogeneous, these MAF boundaries were considered appropriate for a presumed monogenic disorder, even when taking reduced penetrance into account. It goes beyond the scope of this work to consider population frequent *GLI2* variants in a multifactorial setting or as modifiers of the pituitary phenotype. For comparison, all variants were also classified according to the ACMG system, using a Bayesian framework (Tavtigian et al., 2018) (Table 2). This classification was based on available information from the case descriptions and data on amino acid conservation and protein functional domains. Of note, only one variant was considered likely pathogenic by ACMG classification: the de novo p.(Ser548Leu) variant found in this study. In contrast, by our MAF/structure/incidence-based classification, all the variants in the

TABLE 2 Evaluation of reported (likely) pathogenic missense variants in *GLI2* (NM_005270.4)

Missense variant	MAF per 10,000 in gnomAD	Number of alleles in gnomAD	SIFT and mutation taster	Gene-specific classification, see footnote[a]	ACMG[b]	References
p.(Met180Leu) *inherited*	8	22	Tolerated Polymorphism	Likely Benign	Likely Benign[A]	Brauner et al. (2020)
p.(Ala200Thr) *inherited*	22	62	DeleteriousDisease causing	Benign	VUS[C]	Brauner et al. (2020)
p.(Arg226His) *inherited*	3	7	Tolerated Disease causing	Likely Benign	VUS[B]	Bear et al. (2014)
p.(Arg226Leu) *N/A*	0	0	Tolerated Disease causing	VUS	VUS[C]	Juanes et al. (2016)
p.(Arg231Gln) *inherited*	0	1	Deleterious Disease causing	VUS	VUS[E]	Juanes et al. (2016)
p.(Pro253Ser) *N/A*	0	1	Deleterious Disease causing	VUS+	VUS[E]	França et al. (2013)
p.(Ala268Val) *inherited*	82	232	Deleterious Disease causing	Benign	Likely Benign[H]	Bear et al. (2014)
p.(Arg374His) *inherited*	5	13	Deleterious Disease causing	Likely Benign	Likely Benign[H]	Bear et al. (2014)
p.(Pro386Leu) *inherited*	2	5	Deleterious Disease causing	VUS	VUS[D]	Babu et al. (2019)
p.(Val432Met) *N/A*	154	436	Deleterious Disease causing	Benign	Likely Benign[H]	Bear et al. (2014)
p.(Arg479Gly)[c] *inherited*	1	2	Deleterious Disease causing	Likely Pathogenic	VUS[F]	Bear et al. (2014)
p.(Arg516Pro)[c] *inherited*	0	0	Deleterious Disease causing	Likely Pathogenic	VUS[F]	Flemming et al. (2013)
p.(Glu518Lys)[c] *N/A*	0	0	Deleterious Disease causing	Likely Pathogenic	VUS[F]	Gregory et al. (2015)
p.(Ser548Leu)[c] de novo	0	1	Deleterious Disease causing	Likely Pathogenic	Likely Pathogenic[K]	This study
p.(Tyr575His)[c] *inherited*	0	0	Deleterious Disease causing	Likely Pathogenic	VUS[F]	Babu et al. (2019)
p.(Ala593Val) N/A	0	0	Deleterious Disease causing	VUS+	VUS[E]	Babu et al. (2019)
p.(Glu629Lys) *N/A*	1	2	Deleterious Disease causing	VUS	VUS[E]	Bertolacini et al. (2012)
p.(Arg720His) *inherited*	43	121	Tolerated Disease causing	Benign	Likely Benign[A]	Arnhold et al. (2015)
p.(Arg754Gln) *inherited*	13	37	Tolerated Disease causing	Likely Benign	Likely Benign[I]	Bear et al. (2014)
p.(Leu761Phe) *inherited*	0	0	Deleterious Disease causing	VUS	VUS[E]	Bear et al. (2014) Vishnopolska et al. (2021)
p.(Val819Met) *N/A*	0	1	Tolerated Polymorphism	Likely Benign	VUS[C]	Brauner et al. (2020)
p.(Gly837Lys) *N/A*	8	17	Tolerated Disease causing	Likely Benign	VUS[B]	Vaaralahti et al. (2012)
p.(Ala895Val) *inherited*	0	0	Tolerated Disease causing	VUS	VUS[C]	Meng et al. (2019)
p.(Ser1213Tyr) *N/A*	0	0	Deleterious Polymorphism	VUS	VUS[C]	Bear et al. (2014)
p.(Met1241Ile) *inherited*	0	0	Tolerated Polymorphism	VUS	Likely Benign[J]	Bear et al. (2014)

(Continues)

TABLE 2 (Continued)

Missense variant	MAF per 10,000 in gnomAD	Number of alleles in gnomAD	SIFT and mutation taster	Gene-specific classification, see footnote[a]	ACMG[b]	References
p.(Pro1243Leu) *N/A*	0	0	Deleterious Polymorphism	VUS+	VUS[C]	Rahimov et al. (2006)
p.(Leu1445Phe) *N/A*	920	2598	Deleterious Disease causing	Benign	Likely Benign[H]	Bear et al. (2014)
p.(Pro1485Ala) *inherited*	2	6	Tolerated Polymorphism	Likely Benign	Likely Benign[A]	Bear et al. (2014)
p.(Asp1520Asn) *inherited*	918	2594	Deleterious Disease causing	Benign	Likely Benign[G]	Bear et al. (2014)
p.(Arg1543His) *inherited*	53	151	Tolerated Disease causing	Benign	VUS[B]	Bear et al. (2014)
p.(Pro1554Leu) *N/A*	4	11	Deleterious Disease causing	Likely Benign	VUS[D]	Bear et al. (2014)
p.(Ser1555Pro) *inherited*	105	298	Tolerated Disease causing	Benign	Likely Benign[I]	Bear et al. (2014)

Note: Missense variants judged benign by Bear et al. (2014) are not listed.

Abbreviations: N/A, not assessed; inherited, variant known to be inherited in at least one case; VUS, Variant of Unknown Significance; VUS+, variant considered possibly pathogenic but not likely pathogenic (i.e., not >90% likelihood).

[a]Variant classification is based on MAF (= Minor Allele Frequency) in gnomAD: >20 benign, 2–20 likely benign, 0–1 VUS or VUS+, and if the predicted amino acid exchange represents a major change to a conserved residue in a known functional domain or not, also taking the AlphaFold-predicted protein structure into account.

[b]ACMG criteria used: A: BP1, BP4; B: BP1; C: BP1, PM2; D: BP1, PP3; E: BP1, PP3, PM2; F: BP1, PP3, PM2. PM1; G: BP1, BS1, PS2; H: BP1, BS1, PP3; I: BP1, BS1; J: BP1, BP4, PM2; K: BP1, PS2, PM1, PM2, PP3.

[c]All variants in bold affect highly conserved residues in one of the five Zn-fingers in the Zn-finger domain.

five DNA-binding Zn-fingers remained likely pathogenic: p.(Arg479Gly) in Zn-finger number 2, and p.(Arg516Pro) and p.(Gly518-Lys), both in Zn-finger number 3, and p.(Tyr575His) in Zn-finger number 5 (Figure 1). Three variants were considered possibly pathogenic (VUS+): p.(Pro253Ser), p.(Ala593Val) and p.(Pro1243Leu). Variants p.(Pro253Ser) and p.(Ala593Val) exchange amino acids close to the Zn-finger domain in the AlphaFold-predicted 3D structure of GLI2, while p.(Pro1243Leu) did not reside in a part of the protein with a confident structure prediction (Figure 1). Of note, both p.(Pro253Ser) and p.(Ala593Val) were found in individuals with hypopituitarism. It should also be noted that the number of VUS increased from 11 to 20 (from 31% to 62%) when going from our classification to ACMG classification (Table 2).

In contrast to missense variants, the predicted consequence of truncating variants is usually clear, that is, complete loss-of-function (LoF). Despite such variants being rare in the population database gnomAD (39 LoF variants recorded in 250,000 alleles, pLI = 0.97), most of the LoF variants ascertained due to a *GLI2*-related phenotype were inherited: Of the 19 truncating variants where inheritance was known, only 5 (26%) were de novo (Table S2). Of the five likely pathogenic missense variants in Table 2, only 1 (Individual 1, this report) was de novo.

We analyzed the phenotypic descriptions of 60 individuals with pathogenic *GLI2* variants, including the three reported here, for common findings (Figure 2) (Babu et al., 2019; Bertolacini et al., 2012; Culler & Jones, 1984; Demiral et al., 2020; Elizabeth et al., 2020; Elward et al., 2020; Flemming et al., 2013; França et al., 2010; Kremer Hovinga et al., 2018; Martín-Rivada et al., 2019; Rahimov et al., 2006; Roessler et al., 2003; Shirakawa et al., 2018; Solomon et al., 2012; Vishnopolska et al., 2021). Hypopituitarism affected 58% (33/57) of the individuals. Among them, two thirds (75%) presented CPHD while the rest presented isolated GH deficiency. Two (6%) individuals had HPE: one with the p.(Pro1243Leu) (lobar HPE) and one with the p.(His289Profs*6) (semilobar HPE) *GLI2* variants. Five out of 33 (15%) individuals with brain imaging findings reported had other brain structure abnormalities affecting the corpus callosum (Patient 2, Rahimov et al., 2006 and present Individual 3); the ventricular system (Patient 2 from Rahimov et al., 2006 and Individual 1, this report); one individual had diminished brain size (III.8 from França et al., 2010). Ten individuals had orofacial clefting (17%) and one third (35%) were reported with dysmorphic facial features. The most prevalent phenotypic feature is PAP (37/57 individuals, 65%). Of the 13 parents with a pathogenic *GLI2* variant and reported phenotypes, nine had PAP, two presented minor dysmorphic craniofacial features (hypotelorism or midface hypoplasia) and one had abnormalities on pituitary imaging. The detail of the phenotypic features can be found in Table S2.

3 | DISCUSSION

Understanding the prevalence of pituitary dysfunction within the *GLI2*-related phenotype is important for individual and family

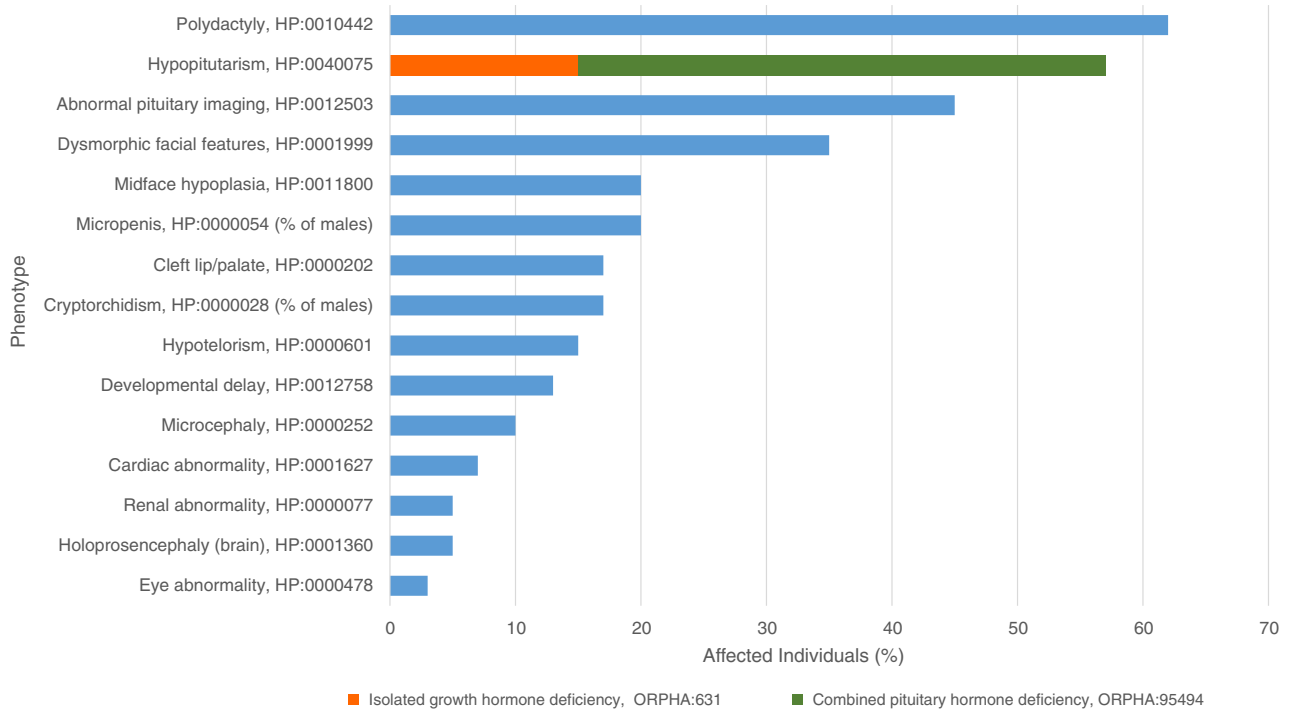

FIGURE 2 Clinical presentation of individuals with confirmed, pathogenic, intragenic *GLI2* variants

management and to prevent neurological damage. Early detection of hypopituitarism and vigilance for evidence of complications such as hypoglycemia or secondary adrenal insufficiency may prevent poor neurodevelopmental outcomes. Older literature may, however, be misleading due to false positive associations with presumed pathogenic missense variants. *GLI2* is a gene with frequent normal missense variation. The *z*-score in the gnomAD database is 0.82, which indicates high tolerance for missense variation. In contrast, the tolerance for LoF variation is low, indicated by the pLI score which was 0.97 (Karczewski et al., 2020). This fits with Cohen (2012), who observed that hypopituitarism tended to be more strongly associated with truncating *GLI2* variants than with missense ones. França et al. (2013) reported 15 novel missense *GLI2* variants predicted to be deleterious in individuals with pituitary dysfunction, but all these missense variants are likely benign with one exception: p.(Pro253Ser) (Table 2). Most of the missense variants reported by Bear et al. (2014) in individuals referred to an endocrine clinic, were also found to be likely benign or at most low-risk upon our re-evaluation (Table 2). Bear et al. (2014) reported that 24% of those with *GLI2* truncating variants had pituitary abnormalities. Our analysis shows that hypopituitarism is more than twice as common in this group of individuals: 58% had this phenotypic feature.

Bear et al. (2014) also reported that HPE is an uncommon manifestation of pathogenic *GLI2* variants. Our analysis confirms a low risk for HPE in affected individuals (approximately 5%). Individual 1 reported here had an SMMCI, a finding described just once previously, in an individual with a reportedly pathogenic variant in *GLI2* (Roessler et al., 2003) (Table S2). A further case of SMMCI was documented in one of the individuals originally described by Culler and Jones (1984); however, this individual was not genotyped to our knowledge. Isolated SMMCI is considered a microform of HPE, defined as the presence of characteristic craniofacial features of HPE without appreciable forebrain abnormalities on neuroimaging. Cohen (2012) provides a molecular explanation for the lack of a strong association between *GLI2* variants and HPE. He suggests that cleavage of the ventral forebrain and eye field still occurs despite *GLI2* dysfunction owing to the overlapping and compensatory actions of *GLI3* in these regions. Importantly, we evidence the possibility of *GLI2*-related, non-HPE, structural brain abnormalities in 15% of affected individuals (França et al., 2010; Rahimov et al., 2006). While alobar HPE is an uncommon finding related to pathogenic *GLI2* variants, clinicians should discuss the low but possible risk of severe pathology with affected families, including pituitary dysfunction, HPE and developmental delay.

On the other hand, the most prevalent *GLI2*-related endophenotype is PAP that is also the mildest phenotypic expression of the condition, reported in many parents of individuals who instead presented systemic findings. This presents a particularly challenging genetic counseling scenario as most of the minimally affected parents discover their *GLI2* genotype following, e.g., the diagnosis of a more significantly affected first child. Moreover, the prevalence of PAP may be underestimated as it may be rudimentary and hence missed on examination, or it is not recorded by the affected individuals as it is frequently managed surgically at a very young age. It would be helpful to understand the prevalence of *GLI2* pathogenic variants in populations of individuals with isolated PAP.

Finally, our study has shown that the *GLI2* missense variants affecting the Zn-fingers are the ones more likely to be pathogenic,

while other reported missense variants are likely/certainly benign or more rarely a VUS (unknown significance) or VUS+ (possible significance), see in Table 2. The same picture emerges when we examine missense variants in other GLI-family members. Regarding *GLI1*, variants segregating with dominant nonsyndromic A/B-type PAP were recently reported, and all the missense variants (6 in all) were in the Zn-fingers of *GLI1* (Palencia-Campos et al., 2020; Yousaf et al., 2020). Variable digital and other abnormalities in a large family segregated with a missense *GLI3* variant Cys609Tyr in Zn-finger number 5 (Crapster et al., 2017), and the same Zn-finger was affected in a four-generation family with Greig cephalopolysyndactyly syndrome segregating with a *GLI3* p.(Arg625Trp) variant (Debeer et al., 2003). Missense variants outside the Zn-finger domain of *GLI3* can also be pathogenic, e.g., p.(Ala934Pro) (Elson et al., 2002), and like *GLI2*, most reported pathogenic *GLI3* LoF variants are also of the truncating type (Démurger et al., 2015).

Importantly, although we do not formulate conclusive genotype–phenotype correlations, we believe that our variant overview is of clinical importance as *GLI2* is regularly included in diagnostic sequencing panels, and likely benign missense variants will then be a more frequent finding than true LoF variants (gnomAD z-score 0,82). As most *GLI2* missense variants have not been functionally studied, and as such studies are cumbersome and not readily available in most diagnostic genetic laboratories, we show that protein structure prediction (by AlphaFold) can be helpful when evaluating *GLI2* variants. Moreover, functional data are not necessary to make conclusions about likely genotype–phenotype correlations (i.e., according to ACMG, the threshold for likely pathogenic is >90%).

4 | CONCLUSION

Although this is a limited cohort and our results may also be subject to ascertainment and publication bias, there has been no systematic approach so far in delineating the *GLI2*-related morbidity in the context of counseling affected individuals and families. Our approach clarifies clinical risks related to LoF and missense *GLI2* variants, and the important messages to discuss in counseling for a pathogenic *GLI2* variant.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Sofia Douzgou, Siren Berland, Jostein A. Førsvoll, Indraneel Banerjee, and Phil Murray examined, investigated and cared for the individuals and the families; provided clinical information; obtained the families' informed consent. Eirik Bratland, Siren Berland, David Gokhale, and Gunnar Houge performed variant interpretation; Megan L. Corder performed the literature review and phenotypic analysis and wrote the manuscript. Gunnar Houge re-evaluated previously reported *GLI2* variants. Sofia Douzgou conceived and designed the approach and submitted the manuscript. All co-authors reviewed and approved the submitted version of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

ETHICS STATEMENT

Because all findings were a consequence of routine clinical evaluation and diagnostics, and further research did not require further individual investigations, ethical review board evaluation is not required according to Norwegian rules (Houge, 2015). Written and oral consent from all individuals' parents was obtained.

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