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To cite this version:
Raja Brauner, Joelle Bignon-Topalovic, Anu Bashamboo, Ken Mcelreavey. Pituitary stalk interruption syndrome is characterized by genetic heterogeneity. PLoS ONE, Public Library of Science, 2020, 15 (12), pp.e0242358. 10.1371/journal.pone.0242358. pasteur-03263613

HAL Id: pasteur-03263613
https://hal-pasteur.archives-ouvertes.fr/pasteur-03263613
Submitted on 17 Jun 2021

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Pituitary stalk interruption syndrome is characterized by genetic heterogeneity

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Abstract

Pituitary stalk interruption syndrome is a rare disorder characterized by an absent or ectopic posterior pituitary, interrupted pituitary stalk and anterior pituitary hypoplasia, as well as in some cases, a range of heterogeneous somatic anomalies. A genetic cause is identified in only around 5% of all cases. Here, we define the genetic variants associated with PSIS followed by the same pediatric endocrinologist. Exome sequencing was performed in 52 (33 boys and 19 girls), including 2 familial cases single center pediatric cases, among them associated 36 (69.2%) had associated symptoms or syndromes. We identified rare and novel variants in genes (37 families with 39 individuals) known to be involved in one or more of the following—midline development and/or pituitary development or function (BMP4, CDON, GLI2, GLI3, HESX1, KIAA0556, LHX9, NKX2-1, PROP1, PTCH1, SHH, TBX19, TGIF1), syndromic and non-syndromic forms of hypogonadotropic hypogonadism (CCDC141, CHD7, FANCA, FANCC, FANCD2, FANCE, FANCN1, IL17RD, KISS1R, NSMF, PMM2, SEMA3E, WDR11), syndromic forms of short stature (FGFR3, NBAS, PRMT7, RAFl, SLX4, SMARCA2, SOX11), cerebellum atrophy with optic anomalies (DNMT1, NBAS), axonal migration (ROBO1, SLIT2), and agenesis of the corpus callosum (ARID1B, CC2D2A, CEP120, CSPP1, DHCR7, INPP5E, VPS13B, ZNF423). Pituitary stalk interruption syndrome is characterized by a complex genetic heterogeneity, that reflects a complex phenotypic heterogeneity. Seizures, intellectual disability, micropenis or cryptorchidism, seen at presentation are usually considered as secondary to the pituitary deficiencies. However, this study shows that they are due to specific gene mutations. PSIS should therefore be considered as part of the phenotypic spectrum of other known genetic syndromes rather than as specific clinical entity.

Introduction

Pituitary stalk interruption syndrome (PSIS) is a rare disorder characterized by the combination of specific findings in magnetic resonance imaging (MRI) including an absent or ectopic posterior pituitary, absent or interrupted pituitary stalk and anterior pituitary hypoplasia [1]. This triad can be incomplete. The consequences of PSIS are a series of anterior pituitary
deficiencies including growth hormone (GH) deficiency that may be isolated or may be associated with other hormonal deficiencies including thyroid-stimulating hormone, adrenocorticotropic and/or luteinizing hormone/follicle stimulating hormone. Posterior pituitary deficiency leading to central diabetes insipidus is very rare in PSIS. The clinical presentation of PSIS is heterogeneous and can include hypoglycemia, seizures, jaundice, micropenis, cryptorchidism or later in age decreased growth rate [2]. PSIS can be associated with other congenital anomalies, mainly ophthalmic, which can be isolated or as a part of syndromes, as well as intellectual disability or epilepsy [2–6]. The latter are considered as secondary to hypoglycemic episode due to GH and adrenocorticotropic deficiencies but they are frequently (more frequent and more marked) than that due to the GH and adrenocorticotropic deficiencies.

Familial forms represent around 5% of the cases [7]. It is mainly in these forms that mutations have been identified in genes mainly associated with pituitary development including LHX4, OTX2, HEX1, SOX3, PROKR2, GPR161 [4,8–15]. Recently we identified mutations in the CDON and ROBO1 genes in patients with PSIS and ophthalmic anomalies [16,17].

Here, we used an exome sequencing approach to identify the underlying genetic causes of PSIS in 52 including 2 familial patients monitored for PSIS by the same pediatric endocrinologist. We identified pathogenic and potentially pathogenic mutations in a wide range of genes known to be involved in a wide range of biological processes.

Patients, family members, and controls

Written, informed consent was given by all the parents and by the patients aged more than 18 years for the clinical-biological evaluation (included in their hospital medical record) and for the molecular biology analyses with the use of protocols approved by local and national research ethics committees (Comité de Protection des Personnes Île de France III, n°3445). The pituitary evaluation and follow-up were conducted as previously described [2].

This retrospective single-center study was performed in 52 individuals (33 boys and 19 girls). This includes 2 families each with 2 affected children. PSIS patients monitored for hypothalamic-pituitary deficiency by a senior pediatric endocrinologist (R. Brauner) in a university hospital between 1978 and 2018 and for whom a DNA sample was available. The 7 patients in whom a mutation has been previously found (patients 7, 8, 10, 17 and 37, Table 1) and published (ROBO1, CDON, HEX1) were included in this study [11,16,17].

Methods

Hypoglycemia was defined as a blood glucose concentration below 3 mmol/L after 2 days of age. Decreased growth rate was defined as a height velocity during the previous year of more than one standard deviation score below the mean for chronological age or decrease in height standard deviation of more than 0.5 over 1 year in children older than 2 years. Micropenis was defined as a penis length of less than 30 mm.

The criterion for diagnosing GH deficiency was a GH peak response of less than 20 mU/L or 6.7 ng/mL after two pharmacological stimulation tests or during spontaneous hypoglycemia, excluding the response to GH-releasing hormone, with low insulin-like growth factor 1 concentration. As we used various GH assays over the study period, we expressed the GH peak concentration in mU/L using conversion factors (ng/mL to mU/L) that were specific of the international standard used to calibrate the GH assay.

Thyroid-stimulating hormone deficiency was diagnosed by plasma free thyroxin below 12 pmol/L. Adrenocorticotropic deficiency was diagnosed by basal plasma cortisol concentrations at 8 a.m. below 40 ng/mL (110 nmol/L) in neonates and below 80 ng/mL (220 nmol/L) in older children, with no increase during hypoglycemia and low/normal
Table 1. Phenotypes of 37 families of PSIS carrying potentially pathogenic genetic variants.

| Case | Ancestry         | Sex | Age at diagnosis (y) | Initial symptom (s) | Pituitary anomalies | Associated phenotypes                                                                 | Genitalia/Puberty | MRI features                                                                                     |
|------|------------------|-----|----------------------|---------------------|---------------------|---------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------------------------------------------|
| 1    | Afro-American M  | 4   |                      | Hypoglycemia, jaundice | T, C, PRL, HH       | Epilepsy, severe intellectual deficiency                                                 | Micropenis        | Left temporal dysplasia, no differentiation between white and black cerebral matter             |
| 2    | Indo-European M  | 1.3 |                      | Hypoglycemia         | T, C, partial HH    | Ptosis                                                                                | Cryptorchidism    | Chiari                                                                                         |
| 3    | European M       | 6.4 | Decreased GR         | None                | None                |                                                                                       |                   |                                                                                               |
| 4    | African M        | 8.3 | Decreased GR         | DI                  | T,DI, prepubertal   | Optic nerve atrophy                                                                   |                   | No sella turcica nor pituitary, splenium of corpus callosum hypoplasia, ethmoid meningocele, septum lucidum cyst |
| 5    | European M       | 3.1 | Decreased GR         | None                | T, prepubertal      | Fanconi syndrome with microphthalmia                                                   | Cryptorchidism    |                                                                                               |
| 6    | European F       | Neonate | Hypoglycemia, jaundice | T, C, HH | Cystic fibrosis                                                               |                     |                                                                                               |
| 7    | European M       | 1.0 | Decreased GR         | None                | None                | Left ptosis                                                                           |                   |                                                                                               |
| 8*   | European F/F     | 3.9 | Decreased GR         | T, partial HH       | Strabismus, transient cardiomyopathy                                                  | Secondary amenorrhea |                                                                                               |
| 9    | European F       | 4.2 | Hypoglycemia, seizures | T, C, HH | Optic nerve hypoplasia, epilepsy, intellectual deficiency |                     |                                                                                               |
| 10** | European M/F     | 2.6 | Decreased GR         | None                | Hypermetropia with divergent strabismus                                              |                     |                                                                                               |
| 11   | European M       | 2.7 | Hypoglycemia         | T, C, HH            | Cerebellar ataxia                                                               | Cerebellar hypoplasia |                                                                                               |
| 12   | European M       | Neonate | Hypoglycemia         | T, C, HH            | None                                                                              | Micropenis         |                                                                                               |
| 13   | European M       | 4.8 | Decreased GR         | None                | Cleft lip and palate with hypoplasia hemi premaxillary bone                       |                     |                                                                                               |
| 14   | European M       | 5.8 | Decreased GR         | T, prepubertal      | Intellectual deficiency                                                          | Cryptorchidism with very small testis |                                                                                               |
| 15   | European M       | 6.7 | Decreased GR         | None                | Bladder exstrophy, ano-rectal malformation                                      | Cryptorchidism     |                                                                                               |
| 16   | African M        | 0.4 | Jaundice             | None, prepubertal   | Fanconi syndrome, duodenal diaphragm, radial and thumb hypoplasia, microcornea, unic pelvic kidney, interventricular shunt | Cryptorchidism     | Delay in myelinisation,                                                                       |
| 17   | North African F  | Neonate | Hypoglycemia, jaundice, seizures | T, prepubertal | None                                | Mother: ptosis                     |                                           |
| 18   | North African F  | 3.5 | Decreased GR         | T, partial HH       | Cystic teratoma on right ovary                                                  | Secondary amenorrhea |                                                                                               |
| 19   | East African F   | Neonate | Hypoglycemia         | T                   | Strabismus                                                                       | Agenesis interventricular septum and corpus callosum |                                                                                               |
| 20   | European M       | Neonate | Hypoglycemia         | T, C, HH            | Strabismus, equinus foot deformity                                            | Micropenis, cryptorchidism | Abnormal signal in white matter of the brain                                                |
| 21   | European M       | 4.5 | Decreased GR         | None, prepubertal   | Deafness                                                                     | Cryptorchidism     |                                                                                               |
| 22   | European M       | Neonate | Hypoglycemia, jaundice, seizures | T, C, HH | Major dysphagia | Cryptorchidism |                                                                                               |
| 23   | European F       | 1.5 | Hypoglycemia, decreased GR | T, C, HH | Intellectual delay, major obesity                                |                     |                                                                                               |

(Continued)
Table 1. (Continued)

| Case | Ancestry | Sex | Age at diagnosis (y) | Initial symptom(s) | Pituitary anomalies | Associated phenotypes | Genitalia/Puberty | MRI features |
|------|----------|-----|---------------------|--------------------|-------------------|----------------------|-------------------|--------------|
| 24   | European | F   | 3.8                 | Intellectual delay, Hypoglycemia | T, C, HH          | Severe intellectual deficiency and obesity, no language, seizures, chooroaesthetic, thyroid dysfunction with basal and stimulated increased TSH | | Absence of sella turcica |
| 25   | North African | M   | 2.8                 | Decreased GR        | T, C, partial HH  | Diabetes mellitus, peripheral hypothyroidism, Father died suddenly when young | | Cryptorchidism, spermatogenic failure (inhibin B 0, micropenis and small testis) |
| 26   | West African | M   | Neonate             | Hypoglycemia, jaundice | T, C, HH          | Strabismus            | | Spermatogenic failure (inhibin B 0, micropenis, small testis) |
| 27   | North African | F   | 9                   | Decreased GR        | Partial HH        | None                 | Secondary amenorrhea | Post pituitary as a nodule in the stalk |
| 28   | North African | M   | 5.6                 | Decreased GR        | None              | None                 | Mother: kidney failure by nephroangiosclerosis |
| 29   | European | F   | 3                   | Decreased GR        | None              | Normal puberty but unexplained low inhibin B suggesting ovarian insufficiency |
| 30   | European | M   | 5.5                 | Decreased GR        | None              | None                 |
| 31   | European | M   | 3.5                 | Decreased GR        | None, early puberty | Learning difficulties, pharyngeal abnormality, hypertrophic piloric stenosis | Cryptorchidism |
| 32   | North African | M   | 3.4                 | Decreased GR        | None              | None                 | Temporal arachnoid cyst |
| 33   | European | M   | 2.6                 | Decreased GR        | None, prepubertal | Learning difficulties | Chiari, syringomelia |
| 34   | African | F   | 2                   | Decreased GR        | T, partial C, HH  | Unilateral papillary hypoplasia, strabismus |
| 35   | European | F   | 4.2                 | Decreased GR        | None              | None                 |
| 36   | Africa  | M   | 2.1                 | Decreased GR        | T, C, HH          | Single median incisor |
| 37   | European | M   | 3.5                 | Decreased GR        | None              | None                 | Micropenis |

Abbreviations: Deficiency of T, thyrotropin, C adrenocorticotropic, G gonadotropin hormones; DI diabetes insipidus, PRL prolactin; HH hypogonadotropic hypogonadism (all without anosmia); GR: Growth rate.

* Affected sibs,
** Affected Aunt.

https://doi.org/10.1371/journal.pone.0242358.t001

adrenocorticotropic concentration. Gonadotropin deficiency was diagnosed by the absence of pubertal development at 13 years in girls and 14 years in boys and no or partial gonadotropins response to a gonadotropin-releasing hormone stimulation test [18]. The plasma osmolalities were measured after water deprivation for 12 hours in 30 patients with concomitant urinary osmolality in 23 of them. All were normal (275 to 300 mosmol/kg in the plasma and between 700 and 1300 mosmol/kg in the urinary) except in one case who presented with diabetes insipidus.

The follow-up for each patient included measurements of plasma free thyroxin and cortisol concentrations at 8 a.m. every one or two years, if their concentrations had previously been normal to diagnose delayed deficiency.
Exome sequencing and array-CGH analysis

Exon enrichment was performed as described elsewhere using Agilent SureSelect Human All Exon V4 [19]. Paired-end sequencing was performed on the Illumina HiSeq2000 platform with an average sequencing coverage of x50. Read files were generated from the sequencing platform via the manufacturer’s proprietary software. Reads were mapped using the Burrows–Wheeler Aligner and local realignment of the mapped reads around potential insertion/deletion (indel) sites was carried out with the GATK version 1.6. SNP and indel variants were called using the GATK Unified Genotyper for each sample. SNP novelty was determined against dbSNP138. Datasets were filtered for novel or rare (MAF < 0.01) variants. Novel and rare variants were analyzed by a range of web-based bioinformatics tools using the Ensembl SNP Effect Predictor (http://www.ensembl.org/homo sapiens/userdata/uploadvariations). All variants were screened manually against the Human Gene Mutation Database Professional [Biobase] (http://www.biobase-international.com/product/hgmd). In silico analysis was performed to determine the potential pathogenicity of the variants using Polyphen (http://genetics.bwh.harvard.edu/pph), and SIFT (http://sift.jcvi.org/www/SIFT_chr_coords_submit.html) online tools that predict the effect of human mutations on protein function. We focused our analyses on non-synonymous coding, nonsense, and splice site variants, filtering out all known common variations contained in dbSNP (build 138) (www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes Project (http://www.1000genomes.org/) and in the gnomAD database (http://gnomad.broadinstitute.org/). An in-house database of 700 exomes from control individuals or individuals with unrelated pathologies were also screened for the potential pathogenic variants identified in the PSIS cohort. Variants were confirmed by visual examination using the IGV browser or by Sanger sequencing. Variants were classified according to ACMG guidelines [20]. In the vast majority of cases the parent’s DNA was unavailable for study, therefore trio analysis was not possible. Exome datasets were also compared to an in-house control dataset of >700 exomes. Karyotyping was performed using standard methods and chromosomes were observed after G and R banding. FISH analysis was carried out using FITC or rhodamin labeled probes localized in the chromosomal breakpoints regions. For the array-CGH, genome wide copy number analysis was performed using Illumina CytoSNP12 BeadChip arrays (Illumina, San Diego, California, USA). The samples were processed using the Infinium assay and results analyzed by Illumina Genome Studio software.

Results and discussion

The age at diagnosis of PSIS index cases ranged from birth to 10.8 years (Table 1). Among the 52 patients, there was consanguinity in two cases (cases 27 and 28) and the father deceased suddenly at 45 years in case 25. The initial symptom leading to the diagnosis of PSIS was hypoglycemia in 18 (34.6%) cases, seizures with concomitant hypoglycemia in 3 (5.7%), jaundice in 6 (11.5%), and/or decreased growth rate in 30 (57.7%). By MRI all patients had an ectopic posterior pituitary gland, except 6 patients where it was not seen and one with small posterior pituitary associated with interrupted stalk (case 1). Thus, the pituitary stalk was defined as interrupted (n = 19 with a nodule in case 27), not observed (n = 21), thin (n = 8), normal (n = 3) and large (n = 1). The sagittal median anterior pituitary height was <1 mm or not seen in 9 patients. The associated symptoms or syndromes (36 cases, 69.2%) are detailed in the Table 1. Ophthalmic malformations are present in 16 cases (30.8%).

The GH deficiency was isolated in 21 cases (40.4%), or associated with isolated thyrotropin deficiency in 4 cases (7.7%) or multiple deficiencies including gonadotropins deficiency in 23 (44.2% or 56.1% after excluding 11 in prepubertal age). Only one patient had diabetes insipidus (case 4). Two patients had early puberty. Three girls (cases 8, 18 and 27) had secondary...
amenorrhea, associated with thyrotropin deficiency, after normal pubertal development, despite a normal pubertal gonadotropins response to gonadotropin-releasing hormone test. These were considered as having partial gonadotropins deficiency [18].

The heterogeneity of the clinical presentation of patients with PSIS is explained by the wide variety of the genes carrying potentially pathogenic variants. In 39 individuals we identified genetic variants, which may contribute to the complex phenotypes seen in this series of patients (Table 2). Array-CGH analysis indicated normal ploidy and did not indicate changes in gene copy number associated with the phenotypes. However, exome sequencing identified rare and novel variants in genes known to be involved in one or more of the following—midline development and/or pituitary development or function (BMP4, CDON, GLI2, GLI3, HESX1, KIAA0556, LHX9, NKX2-1, PROP1, PTHCI, SHH, TBX19, TGF1), syndromic and non-syndromic forms of hypogonadotropic hypogonadism (HH; CCDC141, CHD7, FANCA, FANCC, FANCD2, FANCE, FANCG, IL17RD, KISS1R, NSMF, PMM2, SEMA3E, WDR11), syndromic forms of short stature (FGFR3, NBAS, PRMT7, RAF1, SLX4, SMARCA2, SOX11), cerebellar atrophy with optic anomalies (DNMT1), axonal migration (ROBO1, SLIT2), and agenesis of the corpus callosum (ARID1B, CC2D2A, CEP120, CSPP1, DHC7R, INPP5E, VPS13B, ZNF423). In the majority of cases, these variants were also absent from in-house controls or present at a very low frequency (Table 2).

Other variants, which contributed to the clinical phenotype of the patient, but not involved in PSIS include patient 6 with cystic fibrosis who is homozygous for the common deletion variant (del:p.507_508del), WT1 variants (p.P84S, p.A93G) associated with a familial history of renal anomalies in family 28 and an FSHR variant (p.R484H) in patient 29 associated with low inhibin B levels suggestive of ovarian insufficiency.

Several patients presented with complex syndromes, where PSIS has not been previously reported. In several of these cases the phenotype may be due to a combination of gene variants rather than single variant. Patient 1 presented with severe early epilepsy and intellectual deficiency, pituitary deficiencies as well as a micro penis. MRI showed PSIS leading to the clinical diagnosis of PSIS and Nicolaides-Baraitser syndrome. This child carried pathogenic and likely pathogenic variants in PROP1 and IL17RD, as well as a likely pathogenic variant in SMARCA2. Variants in latter are a known cause of Nicolaides-Baraitser syndrome [21] It is unclear if PSIS represents an extension of the phenotypic spectrum associated with the Nicolaides-Baraitser syndrome due to a SMARCA2 variant or if the PSIS is due to the PROP1 and/or IL17RD variants. Pituitary stalk anomalies have been reported in association with a homozygous, loss-of-function variant in PROP1, however the child described here is heterozygous for a LOF variant [22]. To resolve this, other patients with Nicolaides-Baraitser syndrome or hypogonadotropic hypogonadism will need to be screened for PSIS by MRI.

Patients 4 and 6 have biallelic, or homozygous variants in NBAS. Variants in NBAS are associated with autosomal recessive forms of either infantile liver failure syndrome 2 or short stature, optic nerve atrophy, and the Pelger-Huet anomaly [23,24]. Patient 4 presented with an undiagnosed syndromic form of short stature. Some aspects of the phenotype may be associated with the NBAS pathogenic variants including with diabetes insipidus at birth, left anophthalmia and optic nerve and chiasma agenesis. However, other features have not been reported in association with NBAS variants including the absence of the sella turcica and pituitary, hypoplasia of the splenium of the corpus callosum, ethmoidal meningocele and a septum lucidum cyst. The child also carried two rare variants in the KIAA0556 gene, where previously homozygous variants in KIAA0556 have been reported in association with micropenis, pituitary hypoplasia, pituitary stalk anomalies, cleft palate and cerebellar hypoplasia in Joubert syndrome 26 [25]. To add further complexity to the interpretation of the data, the child also carries a rare missense variant in ROBO1. ROBO1 variants are associated with PSIS and optic
| Case | Gene | Variant | ZY mutation | Predicted effect on protein | dbSNP | ACMG classification | MAF and population (GnomAD) | In-house controls (MAF) | MI | Associated phenotypes (OMIM) | PV | GV | PR |
|------|------|---------|-------------|-----------------------------|-------|---------------------|-----------------------------|---------------------------|----|---------------------------------|----|----|----|
| 1    | PROP1| NM_006261: c.63delG:p.L21fs | het frameshift | loss-of-function | rs780134345 | Pathogenic | 0.000096; African | Absent | AR | Pituitary hormone deficiency, combined, 2 (262600) | 44 |    |    |
|      | IL17RD| NM_017563: c.1256T>C:p.I419T | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.644 | rs145388838 | Likely pathogenic | 0.0001; NFE | Absent | AR/AD | Hypogonadotropic hypogonadism 18 with or without anosmia (606807) |    |    |    |
|      | SMARCA2| NM_00128939 6: c.787T>A:p.S263T | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.029 | Novel | Likely pathogenic | NA | Absent | AD | Nicolaides-Baraitser syndrome (601358) |    |    |    |
| 2    | GLI3 | NM_000168.5: c.4180C>T:p.R1394C | het missense | SIFT, deleterious; PolyPhen2, benign; REVEL 0.091 | rs577664817 | Uncertain significance | 0.00036; South Asian | Absent | AD | Greig cephalopolysyndactyly syndrome (175700); Pallister-Hall syndrome (146510); Postaxial polydactyly, types A1 and B (174200); Polydactyly, preaxial, type IV (174700); Hypothalamic hamartomas, somatic (241800) |    |    |    |
|      | IL17RD| NM_017563: c.794C>G:p.P265L | het missense | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.422 | rs148181557 | Uncertain significance | 0.000096; South Asian | Absent | AD | Hypogonadotropic hypogonadism 18 with or without anosmia (606807) |    |    |    |
| 3    | SHH | NM_000193.3: c.52G>T:p.V18L | het missense | SIFT, tolerated; PolyPhen2, unknown; REVEL 0.349 | rs148181557 | Uncertain significance | 0.000096; African | Absent | AD | Holoprosencephaly 3 (142945); Microphthalmia with coloboma 5 (611638); Schizencephaly (269160); Single median maxillary central incisor (147250) |    |    |    |
|      | CHD7 | NM_017780: c.6377A>T:p.D2126V | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.345 | rs1064794182 | Uncertain significance | NA | Absent | AD | CHARGE syndrome (214800); Hypogonadotropic hypogonadism 5 with or without anosmia (612370) |    |    |    |
| 4    | NBAS | NM_015909: c.1083+4C>T | hom essential splice site | Loss-of-function | rs112852390 | Uncertain significance | 0.014; African | 0.0032 | AR | Infantile liver failure syndrome 2 (614839); Short stature, optic nerve atrophy, and Pelger-Huet anomaly (614800) |    |    |    |
|      | KIAA0556| NM_015202: c.3346+8G>T | het essential splice site | Loss-of-function | rs374277288 | Uncertain significance | 0.0007; African | 0.001 | AR | Joubert syndrome 26 (616784) |    |    |    |
|      | KIAA0556| NM_015202: c.3180A>T:p.H727L | het missense | SIFT, deleterious; PolyPhen2, benign; REVEL 0.047 | rs139943989 | Uncertain significance | 0.0011; African | Absent | AR | Joubert syndrome 26 (616784) |    |    |    |
|      | ROBO1| NM_002941: c.1565G>A:p.R522Q | het missense | SIFT, tolerated; PolyPhen2, benign; REVEL 0.139 | rs138082446 | Likely pathogenic | 0.0061; African | Absent | AD | NA |    |    |    |

(Continued)
| Case | Gene | Variant | ZY mutation | Predicted effect on protein | dbSNP | ACMG classification | MAF and population (GnomAD) | In-house controls (MAF) | MI | Associated phenotypes (OMIM) | PV | GV | PR |
|------|------|---------|-------------|----------------------------|-------|---------------------|-----------------------------|--------------------------|----|----------------------------|----|----|----|
| 5    | CHD7 | NM_017780: c.6476C>A: p.S2159Y | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.202 | Novel | Uncertain significance | NA | Absent | AD | CHARGE syndrome (214800); Hypogonadotropic hypogonadism 5 with or without anosmia (612370) | 43 |
|      |      | NM_001286167: c.3971C>T: p. P1324L | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.714; rs182657062 | Pathogenic | 0.0001; NFE | Absent | AR | Fanconi anemia, complementation group A (227650) | 43 |
|      |      | NM_001286167: c.1193_1196del: p. V398fs | het frameshift | Loss-of-function | Novel | Pathogenic | NA | Absent | AR | Fanconi anemia, complementation group A (227650) | 43 |
|      | GLI3 | NM_000168: c.1346GG>A: p. R449Q | het missense | SIFT, tolerated; PolyPhen2, benign; REVEL 0.175 | rs745809543 | Uncertain significance | 0.000045; NFE | Absent | AD | Greig cephalopolysyndactyly syndrome (175700); Pallister-Hall syndrome (146510); Polydactyly, postaxial, types A1 and B (174200); Polydactyly, preaxial, type IV (174700); Hypothalamic hamartomas, somatic (241800) | 43 |
|      | SEMA3E | NM_012431.c. C1498T:p.R500W | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.488 | rs111300014 | Uncertain Significance | 0.0001; EAS | Absent | AD | CHARGE syndrome (214800) | 43 |
|      | SLX4 | NM_024444.c. C3143T:p.S1048F (FANCP) | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.174 | Novel | Uncertain Significance | NA | Absent | AR | Fanconi anemia, complementation group P (613951) | 43 |
| 6    | NBAS | NM_015909:c. G6311A:p.R2104Q | het missense | SIFT, tolerated; PolyPhen2, benign; REVEL 0.204 | rs773412024 | Uncertain Significance | 0.000097; African | Absent | AR | Infantile liver failure syndrome 2 (614643); Short stature, optic nerve atrophy, and Pelger-Huet anomaly (614800) | 43 |
|      |      | NM_015909:c. T1118C:p.L373P | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.211 | Novel | Uncertain Significance | NA | Absent | AR | Infantile liver failure syndrome 2 (614643); Short stature, optic nerve atrophy, and Pelger-Huet anomaly (614800) | 43 |
|      | CFTR | NM_000492: exon11: c.1520_1522del: p.507_508del | hom Deletion | NA | rs113993960 | Pathogenic | 0.0106; NFE | 0.0034 | AR | Cystic fibrosis (219700) | 43 |
| 7    | ROBO1 | NM_002941.c. G3450T:p.Y1150X | het nonsense | Loss-of-function | Novel | Pathogenic | NA | Absent | AD | NA | 17 | 17 |
| 8    | ROBO1 | NM_002941.c. G719C:p.C240S | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.542 | Novel | Pathogenic | 0.0001076; African | Absent | AD | NA | 17 | 17 |
| Case | Gene   | Variant                        | ZY  | mutation                      | Predicted effect on protein | dbsNP   | ACMG classification | MAF and population (GnomAD) | MI       | Associated phenotypes (OMIM) | PV | GV | PR |
|------|--------|--------------------------------|-----|-------------------------------|----------------------------|---------|---------------------|------------------------------|---------|--------------------------------|----|----|----|
| 9    | WDR11  | NM_018117.c:T109Gp.Y37D         | het | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.347 | rs776728184 | Likely pathogenic  | 0.00003; NFE | Absent | AD Hypogonadotropic hypogonadism 14 with or without anosmia (614858) | 13 |    |    |
|      |        |                                |     |                               |                            |         |                     |                              |         |                                |    |    |    |
|      | PMM2   | NM_000303:c.254_255delp.Q85fs   | het | frameshift                    | Loss-of-function           | Novel   | Uncertain Significance | NA | Absent | AR Congenital disorder of glycosylation, type 1a (212065) |    |    |    |
| 10** | ROBO1  | NM_002941:c.2928_2929delG,p.A977Qfs | het | Frameshift                    | Loss-of-function           | Novel   | Pathogenic          | NA | Absent | NA | 17 | 17 |
| 11   | DNMT1  | NM_001130823.3:c.A2858Gp.D953G  | het | Missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.780 | Novel   | Likely pathogenic   | NA | Absent | AD Cerebellar ataxia, deafness, and narcolepsy, autosomal dominant (604121); Neuroathy, hereditary sensory, type IE (614116) |    |    |    |
| 12 | NSMF   | NM_001130969.1:c.C53A:p.S18X    | het | Nonsense                      | Loss-of-function           | Novel   | Likely pathogenic   | NA | Absent | AD Hypogonadotropic hypogonadism 9 with or without anosmia (614838) |    |    |    |
| 13   | ARID1B | NM_020712.c:A5015T:p.N1672I    | het | Missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.610 | Novel   | Uncertain significance | NA | Absent | AD Coffin-Siris syndrome 1 (135900) | 47 |    |    |
|      |        |                                |     |                               |                            |         |                     |                              |         |                                |    |    |    |
|      | VPS13B | NM_017890.c:C4298Gp.S1433C     | het | Missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.532 | Novel   | Uncertain significance | NA | Absent | AR Cohen syndrome (216550) |    |    |    |
| 14   | LHX9   | NM_020204.c:T2C:p.M1T          | het | Missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.603 | rs201066309 | Uncertain significance | 0.00008637; AMR | Absent | NA |    |    |
|      |        |                                |     |                               |                            |         |                     |                              |         |                                |    |    |    |
|      | INPP5E | NM_018982.c:G907A:p.V303M      | het | Missense                      | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.699 | rs746212325 | Pathogenic          | 0.00003472; NFE | Absent | AR Joubert syndrome 1 (213300); Mental retardation, truncal obesity, retinal dystrophy, and microphrenis (610136) | 43 | 25 |    |
| 15   | BMP4   | NM_130851.c:C1001T:p.A334V     | het | missense                      | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.905 | rs550409227 | Uncertain significance | 0.00001499; NFE | Absent | AD Microphthalmia, syndromic 6 (607932); Orofacial cleft 11 (600625) |    |    |    |
|      |        |                                |     |                               |                            |         |                     |                              |         |                                |    |    |    |
|      | SLX4   | NM_032444.c:G248C:p.G83A      | het | missense                      | SIFT, tolerated; PolyPhen2, benign; REVEL 0.048 | rs771698977 | Uncertain significance | 0.00005994; NFE | Absent | AR Fanconi anemia, complementation group P (613951) |    |    |    |
| 16   | CDON   | NM_001243597.c: A1343G:p.H448R | het | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.119 | rs1209875838 | Uncertain significance | NA | Absent | AD Holoprosencephaly 11 (614226) | 16 |    |    |

(Continued)
| Case | Gene       | Variant               | ZY   | mutation                      | Predicted effect on protein | dbSNP         | ACMG classification | MAF and population (GnomAD) | In-house controls (MAF) | MI | Associated phenotypes (OMIM) | PV | GV | PR |
|------|------------|-----------------------|------|-------------------------------|-----------------------------|---------------|---------------------|----------------------------|-------------------------|----|--------------------------------|----|----|----|
| 17   | GLI2       | NM_005270:c.G2455A:p.V819M | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.348 | Novel         | Uncertain significance | NA                        | Absent                  | AD | Culler-Jones syndrome (615849); Holoprosencephaly 9 (610829) |    |    | 43 |
|      | PTC1       | NM_000264:c.G3929A:p.G1310D | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.692         | rs149486212   | Likely pathogenic    | 0.0002; NFE              | Absent                  | AD | Holoprosencephaly 7 (610828) |    |    | 13 |
|      | WDR11      | NM_018117:c.G3571A:p.G1191S | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.171         | rs111840592   | Uncertain significance | 0.0045; African           | Absent                  | AD | Culler-Jones syndrome (615849); Holoprosencephaly 9 (610829) |    |    | 43 |
| 18   | CDON       | NM_001243597:c.T2764C:p.E922X | het  | missense                      | Loss-of-function            | Novel         | Pathogenic          | NA                        | Absent                  | AD | Holoprosencephaly 11 (614226) |    |    | 16 |
| 19   | CHD7       | NM_017780:c.G7085A:p.S2362N | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.024         | rs148856258   | Uncertain significance | 0.00032; African          | Absent                  | AR | Fanconi anemia, complementation group G (614082) |    |    |    |
|      | GLI2       | NM_005270:c.G598A:p.A200T | het  | missense                      | SIFT, tolerated; PolyPhen2, benign; REVEL 0.171                      | rs111840592   | Uncertain significance | 0.0045; African           | Absent                  | AD | Culler-Jones syndrome (615849); Holoprosencephaly 9 (610829) |    |    | 43 |
| 20   | FANCG      | NM_032656:c.C788T:p.Q266X | het  | nonsense                      | Loss-of-function            | Novel         | Likely pathogenic   | NA                        | Absent                  | NA | Fanconi anemia, complementation group G (614082) |    |    |    |
|      | SLX4 (FANC) | NM_032444:3.c.G4445A:p.C1482Y | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.024         | rs148856258   | Uncertain significance | 0.000032; African         | Absent                  | AR | Fanconi anemia, complementation group P (613951) |    |    |    |
| 21   | FANCD2     | NM_003304:c.1277_1278+5del | het  | essential splice site         | Loss-of-function            | Novel         | Uncertain significance | NA                        | Absent                  | AR | Fanconi anemia, complementation group D2 (227646) |    |    |    |
|      | RAF1       | NM_002880:c.A1756T:p.S86S | het  | missense                      | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.372         | Novel         | Uncertain significance | NA                        | Absent                  | AD | Cardiomyopathy, dilated, 1NN (615916); LEOPARD syndrome 2 (611554); Noonan syndrome 5 (611553) |    |    |    |
| 22   | CCDC14I    | NM_173648:c.A2183G:p.N728S | het  | missense                      | SIFT, tolerated; PolyPhen2, possibly damaging; REVEL 0.044           | rs151185557   | Uncertain significance | 0.0001; NFE              | Absent                  | AR | NA                                  |    |    | 43 |
| 23   | TBX19      | NM_005149:c.603+6->GTGTTGT | homo | essential splice site         | Loss-of-function            | Novel         | Uncertain significance | NA                        | Absent                  | AR | Adrenocorticotropin hormone deficiency (201400) |    |    |    |
| 24   | PRMT7      | NM_019023:c.T1480C:p.W494R | het  | missense                      | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.598         | rs751670999   | Pathogenic           | 0.000086; AMR             | Absent                  | AR | Short stature, brachydactyly, intellectual developmental disability, and seizures (617157) |    |    | 36 |
| Case | Gene      | Variant                  | ZY mutation          | Predicted effect on protein | dbsNP     | ACMG classification | MAF and population (GnomAD) | In-house controls (MAF) | MI | Associated phenotypes (OMIM)                                      | PV | GV | PR |
|------|-----------|--------------------------|----------------------|----------------------------|-----------|---------------------|----------------------------|--------------------------|----|---------------------------------------------------------------|----|----|----|
| 25   | NKX2-1    | NM_001079668:c.67G>C     | p.G23R               | SIFT, tolerated; PolyPhen2, probably damaging; REVEL 0.332 | rs773410433 | Likely pathogenic  | 0.00003286; South Asian | Absent AD | Choreaathetosis, hypothyroidism, and neonatal respiratory distress (610978) | 41 |     |     |
| 25   | SOX11     | NM_0031108:c.295G>A      | p.D295E              | SIFT, deleterious; PolyPhen2, probably damaging; REVEL NA | Novel     | Uncertain significance | NA                        | Absent AD | Mental retardation, autosomal dominant 27 (615866)                   |     |     | 45 |
| 26   | TGIF1     | NM_170695:c.25T>CG       | p.S9P                | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.494 | rs148390122 | Uncertain significance | 0.0057; African            | Absent AD | Holoencephalgy 4 (142946)                                             |     |     |     |
| 26   | SOX11     | NM_001079668:c.67G>C     | p.G23R               | SIFT, tolerated; PolyPhen2, possibly damaging; REVEL 0.107 | rs199996939 | Uncertain significance | 0.00008639; AMR           | Absent AR | Fanconi anemia, complementation group C (227645)                     |     |     |     |
| 26   | FGFR3     | NM_001163213:c.875A>T    | p.E292V              | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.086 | rs75146768 | Uncertain significance | 0.00001499; NFE           | Absent AD | Achondroplasia (100800); CATCH syndrome (610474); Crouzon syndrome with acanthosis nigricans (612247); Hypochondroplasia (146000); LADD syndrome (149730); Muenke syndrome (602849); SADDAN (614642); Thanatophoric dysplasia, type I (187600); Thanatophoric dysplasia, type II (187601) |     |     |     |
| 27   | KIAA0556  | NM_015202:c.4836C>T      | p.E1612D             | SIFT, tolerated; PolyPhen2, benign; REVEL 0.078 | Novel     | Uncertain significance | NA                        | Absent AD | Joubert syndrome 26 (616784)                                         | 46 |     |     |
| 28   | CSPP1     | NM_024790:c.1972G>A      | p.R658G              | SIFT, deleterious; PolyPhen2, benign; REVEL 0.107 | rs199996939 | Uncertain significance | 0.001; AMR                | Absent AR | Joubert syndrome 21 (615636)                                         |     |     | 25 |
| 28   | CHD7      | NM_017780:c.1696G>A      | p.R566A              | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.088 | rs764518030 | Uncertain significance | 0.0001172; Latino          | Absent AD | CHARGE syndrome (214800); Hypogonadotropic hypogonadism 5 with or without anosmia (612370) |     |     |     |
| 28   | WT1       | NM_024426:c.278G>A       | p.R93G               | SIFT, deleterious; PolyPhen2, probably damaging; REVEL NA | Novel     | Pathogenic           | NA                        | Absent AD | Denys-Drash syndrome (194080); Fraser syndrome (136680); Meacham syndrome (608978); Mesothelioma, somatic (156240); Nephrotic syndrome, type 4 (256370); Wilms tumor, type 1 (194070) |     |     |     |
| Case | Gene       | Variant     | Mutation   | Prediction on | dbsNP   | ACMG Classification | MAF and Population (GnomAD) | In-house Controls (MAF) | MI | Associated Phenotypes (OMIM)                                      | PV | GV | PR |
|------|------------|-------------|------------|---------------|---------|---------------------|-----------------------------|---------------------------|----|---------------------------------------------------------------|----|----|----|
| WT1  | NM_024426.c. C250T:p.P84S | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.045 | Novel | Pathogenic | NA | Absent | AD | Denys-Drash syndrome (194080); Frasier syndrome (136680); Meacham syndrome (608978); Mesothelioma, somatic 156240; Nephrotic syndrome, type 4 (256370); Wilms tumor, type 1 (194070) |
| FANCE | NM_021922.c. G1379A:p.R460Q | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.056 | rs541746126 | Uncertain significance | 0.00009619 | African | bsent | AR | Fanconi anemia, complementation group E (600961) |
| 29   | DHC7       | NM_001163817: c.355delC:p.H119fs | het frameshift | Loss-of-function | rs747827699 | Pathogenic | 0.0000155 | NFE | Absent | AR | Smith-Lemli-Opitz syndrome (270400) |
| ZNF423 | NM_015069.c. T144C:p.S382P | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.145 | rs142835239 | Uncertain significance | 0.0014 | NFE | Absent | AR/AD | Joubert syndrome 19 (614844); Nephronophthisis 14 (614844) |
| FSHR | NM_000145.c. G1451A:p.R484H | het missense | SIFT, tolerated; PolyPhen2, benign; REVEL 0.250 | rs763241241 | Likely pathogenic | 0.0001 | EAS | Absent | AR/AD | Ovarian dysgenesis 1 (233300); Ovarian hyperstimulation syndrome (608115); Ovarian response to FSH stimulation (276400) |
| 30   | FGFR3      | NM_001163213.c. A2120G:p.K707R | het missense | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.622 | rs369813768 | Uncertain significance | 0.00001534 | NFE | Absent | AD | Achondroplasia (100880); CATCH syndrome (610474); Crouzon syndrome with acanthosis nigricans (612247); Hypochondroplasia (149730); Muenke syndrome (602849); SADDAN (616482); Thanatophoric dysplasia, type I (187600); Thanatophoric dysplasia, type II (187601) |
| 31   | CHD7       | NM_017780.c. A218Sgp.K729E | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.422 | rs41272437 | Uncertain significance | 0.0013 | AMR | Absent | AD | CHARGE syndrome (214800); Hypogonadotropic hypogonadism 5 with or without anosmia (612370) |
| FANCD2 | NM_001018115.c. G3290A:p.R1097Q | het missense | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.372 | rs755748094 | Uncertain significance | 0.00007493 | NFE | Absent | AR | Fanconi anemia, complementation group D2 (225746) |
| 31   | CEP120     | NM_153223.c. A2114Gp.Y705C | het missense | SIFT, tolerated; PolyPhen2, benign; REVEL 0.365 | rs373838092 | Uncertain significance | 0.00006083 | SAS | Absent | AR | Joubert syndrome 31 (617761); Short-rib thoracic dysplasia 13 with or without polydactyly (616300) |

(Continued)
| Case | Gene   | Variant               | ZY | mutation            | Predicted effect on protein | dbsNP         | ACMG classification | MAF and population (GnomAD) | In-house controls (MAF) | MI          | Associated phenotypes (OMIM) | PV | GV | PR |
|------|--------|-----------------------|----|---------------------|----------------------------|---------------|---------------------|-----------------------------|--------------------------|------------|--------------------------------|----|----|----|
| 32   | GLI2   | NM_005270:4.c:G2159A:p.R720H | het | missense            | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.446 | rs149091975   | Uncertain significance | 0.001366; African           | 0.002        | AD          | Culler-Jones syndrome (615849); Holoprosencephaly 9 (610829) | 43 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
|      | GLI2   | NM_005270:4.c:A538C:p.M180L     | het | missense            | SIFT, deleterious; PolyPhen2, benign; REVEL 0.249 | rs565813552   | Uncertain significance | 0.0003388; Latino           | Absent       | AD          | Culler-Jones syndrome (615849); Holoprosencephaly 9 (610829) | 43 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 33   | SLIT2  | NM_004787:3.c:T3095C:p.L1032S    | het | missense            | SIFT, tolerated; PolyPhen2, probably damaging; REVEL 0.742 | rs768269055   | Uncertain significance | 0.0001388; Other            | Absent       | NA          | NA                                            |    |    |    |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 33   | CC2D2A | NM_001080522:2.c:G2356A:p.E786K | het | missense            | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.266 | Novel        | Uncertain significance | NA                         | Absent       | AR          | COACH syndrome (216360); Joubert syndrome 9 (612285); Meckel syndrome (612284) | 43 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 33   | WDR11  | NM_018117:c.199-9T>C    | het | essential splice site | Loss-of-function         | rs65141290    | Uncertain significance | 0.000045; NFE              | Absent       | AD          | Hypogonadotropic hypogonadism 14 with or without anosmia (614858) | 13 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 34   | CCDC141| NM_173648:3.c:C1402T:p.R468W    | het | missense            | SIFT, deleterious; PolyPhen2, possibly damaging; REVEL 0.075 | Novel        | Uncertain significance | 0.0007; African             | 0.0007       | AD          | NA                                            |    |    |    |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
|      | KISS1R | NM_032551:5.c:G710C:p.R237P     | het | missense            | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.732 | Novel        | Likely pathogenic   | NA                         | Absent       | AR          | Hypogonadotropic hypogonadism 8 with or without anosmia (614837) | 43 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 35   | KIAA0556| NM_015202:2.c:G1232T:p.G411V    | het | missense            | SIFT, tolerated; PolyPhen2, benign; REVEL 0.080 | rs201073350   | Uncertain significance | 0.0002; NFE               | Absent       | AR          | Joubert syndrome 26 (616784)                  | 46 |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 36   | GATA5  | NM_080473:4.c:C566G:p.S19W     | het | missense            | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.732 | rs200383755   | Uncertain significance | 0.005443; NFE              | 0.0014       | AR/ AD      | Congenital heart defects, multiple types, 5 (617912) |    |    |    |
|      |        |                       |    |                     |                            |               |                     |                             |                          |            |                                  |    |    |    |
| 37   | HESX1  | NM_003865:2.c:G445A:p.E149K   | het | missense            | SIFT, deleterious; PolyPhen2, probably damaging; REVEL 0.937 | rs104893742   | Pathogenic             | 0.0005016; East Asian       | Absent       | AR/ AD      | Pituitary hormone deficiency, combined, 5 (182230) | 11 | 11 |    |

**Abbreviations.** ZY zygosity, MI reported mode of inheritance, PV variant previously associated with the PSIS (reference), GV gene variants previously associated with the PSIS (reference), PR, variant previously reported in association with a mendelian disorder (reference), NFE non-finns European, AMR mixed American, SAS South Asian, AR autosomal recessive, AD autosomal dominant, het heterozygous, hom homozygous, N/A not available.

https://doi.org/10.1371/journal.pone.0242358.t002
nerve anomalies [17,26,27]. It is possible that the complex phenotype may be due to contributions of each of these variants. However, the observation that patient 6 presented with neonatal hypoglycemia and jaundice due to acute liver failure and carried two rare NBAS variants implies that PSIS may be a part of the phenotypic spectrum associated with NBAS pathogenic variants [24]. The child also presented with cystic fibrosis due to a homozygous CFTR variant.

Patient 9 presented with a syndromic form of short stature consisting of hypoglycemia, seizures, hypothyroidism, hypogonadism and failure to thrive. MRI showed PSIS. Based on the clinical phenotype the child had an initial clinical diagnosis of GH and adrenocorticotropic deficiencies responsible for hypoglycemia and seizures and secondary intellectual deficiency. The girl carries a novel heterozygous frameshift variant in PMM2. However, congenital disorder of glycosylation, type 1a, which is caused by biallelic variants in PMM2 is an autosomal recessive disorder and PMM2 LOF variants are common in the general population ([28]; https://gnomad.broadinstitute.org/gene/ENSG00000140650?dataset=gnomad_r2_1). Thus, although aspects of the phenotype are consistent with congenital disorder of glycosylation the pathogenicity of the PMM2 p.Q85fs variant is uncertain. The girl also carries a predicted likely pathogenic rare missense variant in WDR11. WDR11 variants are associated with an autosomal dominant form of HH [29]. Recently a heterozygous p.L436V WDR11 variant was reported in a child with combined pituitary hormone deficiencies, a small anterior pituitary, ectopic posterior pituitary, and a thin, interrupted stalk [13]. This child also carried a loss-of-function PROKR2 (p.R85C) and the pituitary anomalies were considered to be due to both of these variants.

Patient 11 presented with a complex phenotype consisting of short stature, hypoglycemia and cerebellar ataxia with cerebellar hypoplasia. The patient was initially clinically diagnosed with two independent presentations: neurological features secondary to prematurity and perinatal anoxia and hypoglycemia and failure to thrive due hypothalamic pituitary deficiency. Exome sequencing revealed that the affected boy carries a novel missense variant, p.D953G, in the bromo-adjacent homology 2 (BAH2) domain of the methyltransferase DNMT1 [30]. The BAH2 domain is required for controlling the interaction of the methyltransferase with the DNA major groove [31]. Heterozygous DNMT1 pathogenic variants are associated with autosomal dominant neurodegenerative disorders affecting both the central and peripheral nervous systems. Interestingly, variants in exon 20 of DNMT1 leads to hereditary sensory and autonomic neuropathy type IE [32], and variants in exon 21 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. Both of these exons encode the replication focus targeting sequence (RFTS) domain [33]. Here, the boy presented with cerebellar ataxia with cerebellar hypoplasia, which was detected by MRI at 2.7 years. There is no evidence of either deafness or narcolepsy. The affected aspartic acid 953 residue is highly conserved in vertebrates. It is interesting to speculate that this phenotype, including PSIS, may be specifically due to pathogenic variants in this domain of the DNMT1 protein. Indeed, the DNMT1 protein has been shown to physically interact with HESX1, a protein which is essential for pituitary development, and it is co-expressed with Hesx1 during murine pituitary development [34]. Variants in HESX1 are associated with pituitary anomalies and we have previously described a variant in HESX1 associated with PSIS (case 37; 11).

Patient 21 presented with deafness diagnosed during the first year, cryptorchidism and then decreased growth rate. The clinical diagnosis was initially considered to be GH deficiency due to PSIS, which was considered to be independent of the deafness. However, the cryptorchidism was unexplained, as this was not due to gonadotropin deficiency. Analysis of the exome sequencing dataset revealed that he carried a novel RAF1 variant p.A586S located within the highly conserved region CR3. Pathogenic variants, including those located within the CR3 domain, are associated with a wide spectrum of phenotypes including Noonan
syndrome 5, LEOPARD syndrome and non-syndromic cardiomyopathy [35]. Pathogenic RAF1 variants tend cluster in two regional 2 hotspots (CR2 ser259 or CR3 ser612). Although pathogenic variants in CR3 are usually not associated with cardiomyopathy, the contribution of the RAF1 p.A586S variant to the phenotype patient 21 is unclear.

Patient 24 presented with severe intellectual deficiency, hypoglycemia, obesity, seizures, thyroid dysfunction and choreoathetosis. The PSIS was diagnosed on the MRI performed in the evaluation of the intellectual deficiency. Despite the complete GH deficiency, this girl had spontaneous normal statural growth with adult height above the mean. This unusual feature has been reported to the reported hypoglycaemia leading to overconsumption of glucides and obesity. This girl carries a known pathogenic variant in PRMT7 [36]. Biallelic variants in PRMT7 are associated with an autosomal recessive form of short stature, brachydactyly, intellectual developmental disability, and seizures [36] However, this variant is heterozygous and it is unlikely to be responsible for the phenotype. However, the girl also carries rare or novel missense variants in NXX2-1 and SOX11. SOX11 variants are associated with an autosomal dominant form of short stature with intellectual deficiency as part of the Coffin-Siris syndrome 9 [37,38]. However, all of the SOX11 pathogenic variants reported to date fall within the functional HM-box domain (amino acids 47–122). Variants outside the SOX11 HMG-box are associated with congenital anomalies of the kidney and urinary tract (CAKUT; [39]). Hence, we consider that the SOX11 variant is unlikely to be responsible for the phenotype. Pathogenic variants in NXX2-1 are associated with choreoathetosis, hypothyroidism, and neonatal respiratory distress syndrome [40]. The association of pituitary anomalies with NXX2-1 variants has been reported rarely in the literature. However, both point mutations involving NXX2-1 as well as a deletion of the entire gene [41,42] have been reported with pituitary and/or pituitary stalk anomalies. An affected father and his daughter were reported with respectively low LH levels, leading to hypogonadism, or low GH levels, causing short stature. Both patients had motor developmental delay and chorea. Thyroid-stimulating hormone levels were normal in the affected daughter. Both cases carried nonsense variant in NXX2-1. Based on the similarity between this family and patient 24 we suggest that there is now evidence to support the inclusion of pituitary anomalies with NXX2-1.

Genetic variation in several of the genes reported here have been recently suggested to contribute to PSIS including ARID1B, BMP4, CC2D2A, CCDC141, CDON, CHD7, DHCRT7, GLI2, GLI3, INPP5E, KIAA0556, PROP1, PROKR2, SHH, TGIF1 and WDR11 [13,16,17,43–48]. However, we identified new candidate genes for PSIS including seven families, who carried variants in genes known to be involved in Fanconi anemia (patients, 5, 15, 20, 21, 25, 28 and 30), although only one case (patient 5) presented with Fanconi syndrome and microphtalmia. These findings may not be surprising considering that a proportion of Fanconi anemia patients present with hormone deficiencies (GH deficiency, hypogonadism) and short stature [3,49]. Other novel findings include a rare SLIT2 missense variant with two rare GLI2 variants (patient 32). SLITs are a conserved family of secreted proteins that were originally discovered in the nervous system where they signal through ROBO receptors to mediate axonal guidance and branching [50,51]. SLIT2 is the ligand for ROBO1 that we and others have previously shown to be involved in PSIS suggesting a contribution to the development of the phenotype.

The most common genetic finding in this group was rare/novel variants associated with anomalies pituitary development and/or HH with variants observed in 14 of the 29 families (excluding the patients in prepubertal age). Of these 29 cases, 14 carried either heterozygous mutations in more than one gene or potentially biallelic mutations in the same gene. This is consistent with previous findings of autosomal dominant causes of pituitary anomalies and di- or oligogenic causes of HH [52]. In the entire cohort 17 of the patients were diagnosed with hypogonadotropic hypogonadism. Of these cases 6 did not harbor variants in genes known to
cause HH. 11 of the 23 carried rare or novel variants in genes known to cause HH. Surprisingly, a further 6 patients with potentially pathogenic variants in HH genes did not present with HH.

These variants may explain the majority of the symptoms/syndromes presented by the patients included in this series and in other reported series. It is important to point out that it not possible to exclude that only one variant in patients carrying several variants is responsible for the full phenotype and that the remainder may have a minimal contribution to the phenotype. However, a proportion of patients are not explained by genetic variants in these genes. These cases may be due to mutations in other genes involved in pituitary development or function, which are currently unrecognized or may be due to variants in non-coding sequences including copy number variants that would not have been detected in this study. In some cases, described above, the clinical presentation is not fully explained by our current knowledge of biological function of the genes. However, our data expand the phenotypic spectrum associated with many of the genes described in this study and also suggest that PSIS may be associated with heterozygous carriers of autosomal recessive disorders. Similar findings were reported a large cohort of individuals with unexplained short stature [53]. Hauer et al., observed a heterozygous variant in FGFR3 in an individual, who at the initial clinical presentation had no obvious skeletal anomalies that are associated with pathogenic FGFR3 variants. Similarly, they found heterozygous variants in genes that were previously reported to cause autosomal recessive skeletal dysplasias [53]. In this study, we also observed heterozygous variants in genes reported to cause autosomal recessive disorders, which were associated with PSIS and atypical clinical presentations (e.g. case 24, PRMT7; case 29, DHCR7). As suggested by the authors, current descriptions of genotype-phenotype relationships are incomplete and the phenotypic spectrum needs to be expanded [53].

The initial presentation, leading to the diagnosis of PSIS, like seizures, intellectual disability, micropenis or cryptorchidism, are usually considered as secondary to the pituitary deficiencies. This study shows that they are likely due to pathogenic variants responsible for epilepsy, cerebral or cerebella development, thyroid development or hypogonadotropic hypogonadism. In conclusion, the phenotypic heterogeneity seen in association with PSIS is reflected in a complex genetic heterogeneity. In many circumstances PSIS may be considered as part of the phenotypic spectrum of other known genetic syndromes rather than as a specific clinical entity.

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