PITUITARY STALK INTERRUPTION SYNDROME: REPORT OF TWO CASES AND LITERATURE REVIEW

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Abstract

Pituitary stalk interruption syndrome (PSIS) consisting of the triad: ectopic posterior pituitary (EPP), thin or absent pituitary stalk and anterior pituitary hypoplasia is a rare pituitary malformation with variable degrees of pituitary insufficiency, from isolated growth hormone deficiency to TSH, gonadotropin and ACTH deficiency which may occur in time, with normo, hyper or hypoprolactinemia and central diabetes insipidus in up to 10% of cases. Also, extrapituitary malformations have been described in some cases. Genetic defects were identified only in 5% of cases. MRI findings are considered predictive for the endocrine phenotype.

We aim to describe two cases with PSIS without central diabetes insipidus, anosmia and extrapituitary malformations, except for minor head dysmorphic features. The first case was referred at the age of 4 years for short stature (-4SDS for height, bone age 2 years), diagnosed with severe GH deficiency and developed central hypothyroidism and hypoprolactinemia during five-years follow-up. The second case, a 26 year old male with birth asphyxia, cryptorchidism, poor growth in childhood and adolescence (-3 to -4 height SDS), absent puberty and normal adult height (-1.18 SDS; bone age 15.5 years and active growth plates) had GH, TSH, ACTH deficiency and low normal PRL levels.

Increasing medical awareness on PSIS clinical and endocrine heterogeneity may help a more early and accurate diagnosis. Corroboration of neuroimaging and endocrine data will improve our knowledge and understanding and will create premises for molecular diagnosis, genetic counseling and a better patients' management.

Key words: pituitary stalk interruption syndrome, hypopituitarism, magnetic resonance imaging.

INTRODUCTION

Pituitary stalk interruption syndrome (PSIS) was first described in 1987, soon after magnetic resonance imaging was used to explore pituitary disorders, in ten patients with congenital growth hormone (GH)

deficiency, which had an ectopic posterior pituitary and disrupted stalk. It was hypothesized that this anomaly was due to mechanical transection of the pituitary stalk during breech presentation or head trauma (1). Later, in 1992, a study performed on 101 GH deficient patients, of whom 59 had ectopic posterior pituitary (EPP), showed that the traumatic hypothesis can only explain 32% of the cases, and a new hypothesis emerged, that the cause is a genetic factor generating midline embryonic maldevelopment and breech presentation as well (2). Now it is considered that both genetic and environmental factors are responsible for PSIS.

PSIS belongs to the spectrum of holoprosencephaly related congenital defects, and it is characterized by the classic triad: thin or absent pituitary stalk, anterior pituitary hypoplasia and ectopic posterior pituitary, with a wide spectrum of clinical phenotypes and pituitary hormone deficiencies, of variable degree and timing of onset (3). Genetic defects were identified in less than 5% of cases, consisting of mutations, deletions or sequence variations in HESX1, OTX2, LHX4, LHX3, PROKR2, GPR161, CDON, TGIF, GLI2, FGFR1, ARNT2, CHD7 and chromosomal defects such as 18p deletion, X chromosome translocations affecting SOX3 (3, 4, 5) and 17q21.31 microdeletion (6). PSIS is more prevalent in males (1.7 – 2.3:1), and is rarely familial (5%) (7, 8). The real prevalence is not known, but PSIS is a common finding in patients with combined pituitary hormones deficiency (3, 8). In a MRI study of 85 GH deficient children, structural pituitary anomalies were found in 25.9% of cases, 14.4% with empty sella and 11.8% with PSIS – of whom, 41.66% had CPHD (9). PSIS was identified in 34.2% of cases in an Italian cohort of 144 patients with combined pituitary hormones deficiency (CPHD) (10); 15% of PSIS patients (with a more severely affected pituitary development and CPHD) are diagnosed in the neonatal period because of hypoglycemia, prolonged jaundice, micropenis and/

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or cryptorchidism in males. Most patients (70%) are referred during childhood for short stature due to GH deficiency; other pituitary hormones deficiencies may progressively appear. Some patients are diagnosed in adulthood.

Extrapituitary malformations are present in 30-50% of PSIS cases (7, 8). Considering the heterogeneity of PSIS presentation (11), it is crucial to establish the clinical, MRI and endocrine phenotype and to monitor closely every case. The aim of this paper is to present two different PSIS cases pointing out the diagnostic issues elicited by this rare disorder.

CASE REPORT

Case 1

The first case is a 9 years 9 months old girl, the second child of a healthy, unrelated couple (midparental height 165cm), full term born by vaginal delivery after an uneventful pregnancy, with birth weight 2600g and length 47cm. Psychomotor milestones were normal, but growth was poor, and she was referred at the age of four years for short stature- height 86cm (-4.57SD), height velocity

2cm in the previous 12 months, weight 12.7kg (Z score -1.94) and body mass index (BMI) 17.2kg/m² (Z score 1.24). Particular facial features were noticed: midface hypoplasia, large forehead, deep set eyes, telecanthus (canthal index 45), broad nasal bridge, bulbous nasal tip, long philtrum and narrow upper lip (Fig. 1). Skin was fair and thin, with visible vessels and the voice was high-pitched. Blood pressure and heart rate were normal. Ultrasound examination (heart, abdomen, pelvis, thyroid) was normal. Bone age was 1.5-2 years and skull X-ray revealed a 3/8mm “omega” shaped sella turcica. Routine laboratory investigations (CBC, urea, creatinine, liver enzymes, serum electrolytes, cholesterol, triglycerides) were normal, except mild anemia (haemoglobin 11.5g/dL), hypocalcemia (8.6mg/dL not corrected for albumin level), and a low fasting blood glucose (63mg/dL). Hormonologic assessment is shown in Table 1. Karyotype was normal, 46, XX. Head MRI was performed using a 1.5 T unit, with T1 and T2-weighted sagittal and coronal native scans and T1-weighted gadolinium-enhanced scans, showing anterior pituitary hypoplasia (2.5/6mm), interrupted pituitary stalk and ectopic posterior pituitary, located underneath the optic chiasm; iterative head MRI scan at the age of 9 years using a 3T unit identified an extremely thin pituitary stalk, and pituitary size measured on sagittal scan was 4/4.7mm (Fig. 2). No other brain anomalies were detected on both examinations. GH treatment was

Figure 1. Facial appearance of the first case- large forehead, deep set eyes, hypertelorism, broad nasal bridge, bulbous nasal tip, long philtrum, narrow upper lip.

Figure 2. Head MRI of the first case; sagittal T1 weighted section in the first case showing slight anterior pituitary hypoplasia, a thin pituitary stalk, and the bright spot of the posterior pituitary adjacent to the optic chiasm (arrow).

Table 1. Hormonal assessment of the first patient at the age of 4, 4.4 and 9 years (abnormal values in bold, * = under GH treatment, ** = under T4 treatment, ND = not done).

Hormone	Age 4 years < 25 ng/mL	Age 4.4 years 106 ng/mL*	Age 9 years	Normal range
IGF1				49-289 ng/mL (4-5 yr) 49-451 ng/mL (9-10 yr)
Peak GH after Arginine	0.56 ng/mL	-	280 ng/mL*	5-10 ng/mL
Peak GH after Insulin	0.46 ng/mL	-	-	5-10 ng/mL
Prolactin	ND	ND	94.54 -119.1 μ IU/mL	< 280 μ IU/mL
TSH	4.96 μIU/mL	0.683 μIU/mL	3.16 μ IU/mL**	0.6-4.84 IU/mL
FT4	16.17 pmol/L	11.23 pmol/L	12.76 pmol/L**	12.5-21.5 pmol/L
T4	170 pmol/L	ND	ND	77.1-178 pmol/L
T3	3.62 nmol/L	ND	ND	1.42-3.8 nmol/L
Anti TPO Ab	11.31 IU/mL	ND	ND	< 34 IU/mL
Anti TG Ab	12.8 IU/mL	ND	ND	< 115 IU/mL
ACTH	ND	ND	19.85-27.7 pg/mL	7.2-63.3 pg/mL
8am cortisol	ND	ND	289.4-386.5 nmol/L	171-536 nmol/L

started at the age of 4 years and 3 months. Five months later, central hypothyroidism occurred and thyroxine treatment was initiated. Fasting blood glucose remained low (55-75mg/dL) but without symptoms and signs of hypoglycemia and serum electrolytes remained in the normal range (evaluated at 6 months intervals). GH and thyroxine dosages were adjusted to maintain IGF1 and FT4 in the upper half of the normal range. At the

age of 9 years height was 120cm (-2.72SD), weight 23kg and natremia was low normal (132mEq/L, normal range 132-145mEq/L), requiring evaluation for adrenal failure, but both ACTH and 8 am cortisol were repeatedly normal. There were no signs of pubertal development.

Case 2

The second case –

Figure 3. Eunuchoid habitus, cryptorchidism, gynecomastia and absence of androgenic body hair due to hypogonadotropic hypogonadism in the second case. **Figure 4.** Second patient -large forehead, fine wrinkles, retrognathia, posteriorly rotated ears.

a 26 year-old male patient, on thyroid replacement therapy since childhood, was referred for specifying the etiology of a recent, mild, pericardic and pleural effusion with features suggestive for bacterial infection, because a pathogenic germ was not identified, and immune profile was normal. Blood pressure was low, so 5mg Prednisone/day was recommended when discharged from hospital. This patient was the second child of a nonconsanguineous couple (target height 177cm, SDS -0.15), full term born by vaginal delivery, with birth asphyxia. Birth weight (3400g) and length (51cm) were normal, and no other postnatal events were noticed. Psychomotor milestones were achieved normally. He was referred for short stature (93cm, height SDS -4) and bilateral cryptorchidism at the age of 5 years. Testes failed to descend after several courses of hCG (human chorionic gonadotropin) treatment, and his height remained at -3, -4SDS till this twenties. He was also inconstantly treated with oral testosterone in his late teenage years. At the age of 26 years height was 170cm (-1.18SD), with an eunuchoid body habitus, weight 56.8kg and BMI 20kg/m² (below the 5th percentile), but with central adiposity (waist circumference 84 cm) (Fig. 3), high pitched voice, thin, dry and pale skin, slightly pigmented and rugged scrotum, stretched penile length 6cm, bilateral cryptorchidism, gynecomastia, mild kyphosis and particular facial features- large forehead, fine wrinkles, retrognathia, canthal index 30, low-set, posteriorly rotated ears) (Fig. 4). Blood pressure was 110/80mmHg. Ophthalmologic evaluation was normal. Ultrasound examination revealed fatty liver, decreased thyroid volume (2mL), bilateral gynecomastia corresponding to B2 Tanner stage, small testes (0.65mL) within the inguinal canal. Bone age assessed by wrist X ray was 15.5 years and

Figure 5. Wrist X-ray of the 26 year-old patient showing a marked delayed bone age (15.5 years) and still active growth plates.

Figure 7. Marked virilization, penile growth and testicular complete descent in the second case, following percutaneous testosterone treatment.

growth plates (also on knee X ray) were still active (Fig. 5). Bone mineral density was decreased (lumbar spine DXA Z score -3.6). Contrast enhanced head MRI was performed using a 3T unit, showing ectopic posterior pituitary located adjacent to the optic chiasm, absent pituitary stalk, and severe anterior pituitary hypoplasia, the reduced pituitary parenchyma being visible only on contrast enhanced sequences (Fig. 6). No other brain anomalies were detected. Hormonologic assessment while on treatment (100µg thyroxine +

Figure 6. Contrast enhanced head MRI, T1 weighted sagittal section of the second case showing absence of the pituitary stalk, ectopic posterior pituitary, located near the optic chiasm, and reduced anterior pituitary parenchyma, visible on the bottom of the fluid filled sella turcica.

Table 2. Hormonal assessment of the second patient under treatment with T4 + T3 and Prednisone (abnormal values in bold).

Hormone	Normal range	Value
GH	< 5 ng/mL	< 0.05 ng/mL
IGF1	87-225 ng/mL	33.94 ng/mL
Prolactin	102-496 µIU/mL	113.3 µIU/mL
TSH	0.27-4.2 µIU/mL	< 0.005 µIU/mL
TSH after 200µg TRH iv.		< 0.005 µIU/mL
FT4	12-22 pmol/L	15.71 pmol/L
FT3	3.1-6.8 pmol/L	6.16 pmol/L
ACTH	7.2-63.3 pg/mL	4.3 pg/mL
8am cortisol	171-536 nmol/L	9.94 nmol/L
Cortisol after synacthen	> 551.8 nmol/L	343 nmol/L
DHEAS	160-449 µg/dL	17.4 µg/dL
FSH	1.5-12.4 mIU/mL	0.404 mIU/mL
LH	1.7-8.6 mIU/mL	< 0.1 mIU/mL
Testosterone	0.22-2.9 nmol/L	< 0.087 nmol/L
AFP	< 7 ng/mL	2.08 ng/mL
hCG	< 2.6 mIU/mL	0.1 mIU/mL

20µg triiodothyronine/day and 5mg Prednisone/day) is shown in Table 2. Laboratory panel was normal except for low normal natremia (136mEq/L), hypocalcemia (8.4mg/dL not corrected for albumin, Ca⁺⁺ 3.35mg/dL) and hypercholesterolemia (240mg/dL). The patient was switched on thyroxine replacement therapy and Prednisone dose was increased to 7.5mg/day (before Prednisone was indicated, fasting glycemia was constantly low, 70-75mg/dL, while serum electrolytes

were normal). 5 months after percutaneous testosterone treatment both testes completed descent and abundant terminal hair occurred (facial, axillary, pubic and body), penile length increased from 6 to 8 cm (Fig.7). Semen analysis showed azoospermia, and the patient recorded a weight gain of 10kg. Both patients had normal vision and a normal sense of smell. Written informed consent was obtained from first patient's parents and from the second patient to publish this paper.

DISCUSSION

These two cases reflect the heterogeneity of PSIS regarding age at diagnosis, clinical presentation, outcomes, and the specific management issues raised by this rare malformation. Magnetic resonance imaging represents the best choice in assessing structural changes of hypothalamo-pituitary morphology and associated extrapituitary malformations, as well. There is a correlation between the MRI phenotype and the endocrine phenotype; if the pituitary of a GH deficient patient is hypoplastic or normal, with no other anomalies, GHD is isolated and may be transient, if there are other pituitary anomalies, GHD is permanent and may associate other pituitary hormones deficiencies. Moreover, the presence of midline defects may outline certain genetic syndromes. The classic triad of pituitary stalk interruption syndrome is more frequently reported in patients with multiple pituitary deficits, and GHD is generally, permanent (12).

At birth both the anterior and posterior pituitary display a high intensity signal on T1-weighted images, which is retained by the posterior pituitary throughout adulthood, due to the presence of neurosecretory granules or neurophysins, while the anterior pituitary decreases in signal intensity by 6 weeks of age. At birth, the pituitary has a concave superior contour which flattens with age. During childhood, normal pituitary height ranges between 2 to 6mm, and up to 10mm at puberty (larger in females than in males). After intravenous contrast the pituitary enhances symmetrically, with the pituitary stalk best visualized on post contrast T1 weighted images (13).

In the first case, the thin pituitary stalk was visualized only on the second MRI examination using a 3T unit. Anterior pituitary hypoplasia must be distinguished from empty sella (infundibulum in its normal midline position, normal MRI signal and contrast enhancement of the flattened anterior pituitary, and normal position of the posterior pituitary) (13). The posterior brightspot is visible in 90% of normal

individuals and does not vary in size or signal intensity during childhood. Tridimensional MRI studies showed a weak correlation between pituitary height and pituitary volume, and also between measured and calculated pituitary volume, raising the question on the reliability of measuring pituitary height (12).

The pituitary arises in the first 4-5 weeks of life from two ectodermal primordia – Rathke's pouch which upgrowths from the roof of stomodeum and develops into pars distalis, pars intermedia and pars tuberalis, and a downgrowth of the diencephalic floor which forms the neurohypophysis (pars nervosa, infundibulum and median eminence). From the sixth week, Rathke's pouch separates from the oral epithelium by obliteration of the craniopharyngeal canal, and proliferates predominantly anteriorly, fully structuring the adenohypophysis by the tenth week (14).

The pituitary cells of the pouch, originating from the anterior and median portion of the primitive neural plate, induce generation of the Rathke's pouch from the oral-pharyngeal ectoderm (15).

Pituitary morphogenesis depends on signal molecules and transcription factors acting in synergy or opposing, creating gradients and programming cell responsiveness, in a coordinated spatiotemporal sequence. There are two main stages: early organogenesis and pituitary cells differentiation (16). Development of Rathke's pouch in mice is induced by the ventral diencephalon, which expresses Bmp4, FGF 8 and 10 which antagonize Shh and Bmp2 in the pharyngeal ectoderm (not in the portion that will become the pouch), that all would ultimately decline, to allow differentiation of pituitary cells. Transcription factors such as Ptx1 and 2, Lhx3 and 4, Isl1, Otx1, 2, Hesx1, Six1, 3, 4 and 6 are involved in cell proliferation, survival and differentiation. Pitx1 activates POMC, Pitx1 and 2 activate the promoters of genes coding for α subunit, β subunits of TSH and gonadotropins, GH and prolactin. Prop1 expression increases, as Hesx1 and Otx2 decline, and controls differentiation of pituitary cell by activating Pou1f1 and Notch2 (14). The Wnt signaling pathway affects Bmp and Fgf expression. Also, Sox2 and 3 activate Shh, and are blocked by the transcription factors Tbx2 and 3. Shh signals are transduced through transcription factors such as Gli2 which activates and Gli3, which represses transcriptional targets. It seems that Bmp and Fgf gradients regulate pituitary cells specification, Bmp2 favoring cells to become gonadotropes and Fgf to become somatotropes, but it is likely that signals intrinsic to Rathke's pouch may also play a role.

The Notch signaling pathway is very important for the anterior pituitary cell specification (17). Pou1f1 (Pit1), Ptx1 and 2 control tryptotrope, lactotrope and somatotrope differentiation, Tpit (Tbx19), Lif and NeuroD1 – corticotrope differentiation, Gata2 and Nr5a1 – gonadotrope differentiation (16).

Development of the portal system is crucial to pituitary function and it is controlled by VEGFA, FGFs (17) and the prokineticin pathway (18). Reduction of pituitary vascularisation affects pituitary cells. Moreover, spatial relationships between the portal system and pituitary cells impact secretory dynamics; cells closer to vessels are exposed to higher concentrations of releasing hormones and respond in a pulsatile, shorter and more frequent manner (17). Mice knockout for Hesx1, Lhx2, Nkx2.1, Rax and Tbx3, and double knockout Hex1/Hes5 fail to form a pituitary stalk (17).

Though animal data and animal models are very valuable for the discovery of genes implicated in pituitary development, there are differences between species that preclude extrapolation to human phenotypes; an obvious example is offered by PROP1 mutations which associate corticotroph deficiency in 40% of affected humans, but not in mice (4).

Mutations in genes resulting in abnormal human pituitary function may be categorized in three groups: 1) those affecting early development of the forebrain and pituitary, which result in syndromic hypopituitarism associated with extrapituitary defects, most often the eyes, optic nerves or midline forebrain structures; 2) mutations in genes that control early pituitary development which give rise to isolated pituitary hormones deficiencies; 3) mutations in genes involved in pituitary cell specification or coding for pituitary hormones which give rise to isolated deficiencies (19).

PSIS belongs to the spectrum of midline anomalies encompassing septo-optic dysplasia (septum agenesis or corpus callosum agenesis, optic nerve hypoplasia and pituitary deficiencies) and holoprosencephaly. Holoprosencephaly is caused by abnormal division of the prosencephalon between days 18 and 28 of gestation, giving rise to brain anomalies, facial anomalies (cyclopia, median or bilateral labial and/or palatal cleft, hypertelorism, single median incisor) and mental retardation (4).

Phenotypic variability of holoprosencephaly, to whom PSIS is more specifically related is explained by multifactorial synergy represented by other genes and environmental factors (3). This was recently

demonstrated in mice lacking Cdon (a hedgehog co-receptor acting with Ptch1), which develop holoprosencephaly only after in utero ethanol exposure (20). A CDON heterozygous mutation was reported in a patient with PSIS without holoprosencephaly, inherited from her mother, who had only strabismus (21). Beside environmental teratogenic influences, it is possible that genetic influences such as low cholesterol, maternal diabetes or coexisting molecular defects (the multiple hit hypothesis) to be implicated in PSIS pathogenesis; in holoprosencephaly heterozygous mutation carriers 37% have holoprosencephaly, 27% have microforms and 36% are normal.

A heterozygous TGIF mutation (TGIF is responsible for 1% of holoprosencephaly cases) was identified in a female patient with PSIS and no other brain defects (inherited from her asymptomatic father), a 18p deletion (encompassing TGIF gene) was described in another PSIS patient (22).

Several mutations in SHH pathway have been reported in patients with PSIS without holoprosencephaly. A GPR161 gain of function mutation (coding a G protein coupled receptor which represses the SHH pathway) with autosomal recessive transmission was reported in two siblings with partial alopecia, broad nasal root, hypotelorism, mental retardation, short fifth finger, ptosis of the left eye, one with diabetes insipidus and TSH deficiency, and the other with isolated GH deficiency (23). GLI2 mutations with autosomal dominant transmission, incomplete penetrance and variable expression are described in patients with PSIS without holoprosencephaly, with isolated GH deficiency or multiple pituitary deficits and, in some patients, with more severe mutations, postaxial polydactyly (24). SHH mutations usually have holoprosencephaly; a less severe mutation was described in a patient which had only anterior pituitary hypoplasia (22).

The family of LIM domain transcription factors is largely expressed during embryonic development, so patients with mutations of LHX4 and LHX3 manifest combined pituitary hormone deficiencies associated with other defects. Heterozygous LHX4 mutations were found in 2.4% of PSIS patients with corpus callosum hypoplasia and Chiari syndrome. A lethal case (respiratory distress) with homozygous LHX4 mutation and midface hypoplasia was also reported (5).

HESX1 mutations are responsible for 1% of cases with septooptic dysplasia. The phenotype is more severe in homozygous *versus* heterozygous cases. Ectopic or non visible posterior pituitary occurs in 50-

60% of cases, 80% have pituitary hypoplasia, 30% - optic nerve abnormalities and 25% corpus callosum agenesis or hypoplasia (4).

OTX2 is a transcriptional factor involved in brain and face development which activates HESX1, POU1F1 and GNRH1. OTX2 mutations have a variable phenotype, which associate hypothalamo-pituitary abnormalities with anophthalmia to nearly normal eye development (25).

Patients with eye defects (aniridia, foveal hypoplasia, anomalies of the anterior chamber) and pituitary deficiency should be screened for PAX6 heterozygous mutations. Five members of a family with a PAX6 mutation were shown to have only subtle corticotrope deficiency. One patient with GH deficiency, hypoplastic anterior pituitary, thin stalk, EPP, cleft palate, bilateral optic disc cupping was found to have a PAX6 enhancer deletion and another patient with GH deficiency, cryptorchidism, anterior pituitary hypoplasia, thin pituitary stalk, and no ocular anomalies inherited a heterozygous PAX6 mutation from his normal mother (26).

SOX2 mutations may also generate phenotypes belonging to the spectrum of septooptic dysplasia (microphthalmia, corpus callosum anomalies, gonadotropin, GH, TSH or ACTH deficiencies, pituitary hypoplasia in 80% of cases, EPP) and inconstant mental retardation (4, 19).

Altered dosage of SOX3 (a X-linked gene with HMG box highly homologue to SRY) deletions and duplications, polyalanine tract variations should be screened for in patients with hypopituitarism (from isolated GH deficiency to panhypopituitarism), with or without mental retardation, even in the context of a structurally normal pituitary. Most of patients have PSIS and some have clefts or brain anomalies (corpus callosum hypoplasia, persistent craniopharyngeal canal) or diverse phenotypes such as 46,XX testicular disorder of sex development, X-linked hypoparathyroidism, X-linked congenital hypertrichosis, minor facial dysmorphism, speech or hearing impairment (27, 28).

There are recent data supporting an etiologic overlap of congenital hypopituitarism and Kallmann syndrome; PROKR2 variants were reported in patients with variable phenotypes, from hypogonadotropic hypogonadism with septooptic dysplasia to panhypopituitarism without septooptic dysplasia and a normal sense of smell. Among the patients with PSIS caused by PROKR2 variants one patient also had central diabetes insipidus (18, 29). Similarity, FGFR1

and FGF8 heterozygous mutations (that account for 10% of Kallmann syndrome and 7% of normosmic hypogonadism) were also reported in 4% of patients with septooptic dysplasia. Patients with FGF8 mutations had a normal or hypoplastic pituitary, but in patients with FGFR1 mutations EPP was identified; given the fact that FGF signaling is largely implicated in embryonic development, ear, dental, limb anomalies and cleft palate were also encountered (4, 30, 31).

PSIS, TSH and ACTH deficiencies, GH dysregulation with poor development, postretinal visual impairment, microcephaly with frontotemporal hypoplasia, obesity, central diabetes insipidus, delayed brain myelination, renal malformations (hydronephrosis, vesicoureteral reflux) and dysmorphic facial features (prominent forehead, deep set eyes, well grooved philtrum and retrognathia) were found in five females and one male (with cryptorchidism) with a homozygous mutation in the ARNT2 gene (32).

Recently, epigenetic mechanisms' role in PSIS pathogenesis was reflected by the discovery of a variation in CHD7 gene (responsible for more than 65% of CHARGE syndrome cases – Coloboma of the eye, Heart malformations, choanal Atresia, growth Retardation, Genital and Ear anomalies) in a male patient with PSIS, thin corpus callosum, tetralogy of Fallot, deafness, micropenis and cryptorchidism, abnormal ears, squint, GH, TSH, and possible gonadotropin deficiency. CHD7 acts as a chromatin remodeler that binds to methylated histones, interacts with SOX2 and regulates target genes such as GLI2, FGFR1, BMP4 and OTX2 (33).

A mutation of a new candidate gene for congenital hypopituitarism – TCF7L1 (which codes for a regulator of the WNT/ β catenin pathway involved in Rathke's pouch progenitors induction and expansion) was found in a male patient with absent posterior pituitary, anterior pituitary and septum pellucidum hypoplasia, small optic nerves and chiasm and isolated GHD (34).

A complete description of the clinical, endocrine and imaging phenotype is useful in the attempt to identify the underlying genetic defect in PSIS patients, despite extreme phenotypic variability, which should always be kept in mind. In the two patients described, sense of smell and vision were normal, they had no diabetes insipidus, no associated malformations (except facial dysmorphic features in both, and ear anomaly in the second patient) and no other head MRI abnormalities beside PSIS. Nevertheless, absence of extrapituitary malformation

does not exclude the possibility of bearing a mutated gene responsible for a more severe phenotype. Both patients are currently under molecular investigations.

A study performed on 137 GH deficient patients, 73 with isolated GHD and 64 with multiple pituitary hormones deficiency showed that the canthal index (CI= inner canthal distance in mm x100/outer canthal distance in mm) is correlated with pituitary morphology and predicts EPP; in patients with MPHD and CI>39 93% of patients have EPP, *versus* 77% with CI< 39. In patients with isolated GHD and CI< 39 only 29% had EPP. This association can be explained by the fact that altered midline development affects both the pituitary and the facial structure (35). On the contrary, in our second patient CI was 30, but pituitary morphology was more severely affected than in the first patient whose CI was 45. Another study performed on patients of French-Canadian ancestry with combined pituitary hormone deficiencies showed that subjects and their parents had midface retrusion, shorter skull base width, underdevelopment of the mandible, and reduced inner canthal distance (36). Hypotelorism may also be a feature of the holoprosencephaly spectrum. While midface retrusion and small mandible were present in the male patient, the girl had also particular facial features resembling those described in 3 cases with pituitary aplasia (frontal bossing, broad nasal bridge, bulbous nasal tip, hypertelorism) (37).

Diabetes insipidus is uncommon (0-4%) in PSIS patients, as reflected by cohort studies (3,7,8,11), but may be encountered in cases with severe brain developmental defects, or with mutations of GPR161, ARNT2 or FGF8 and FGRF1 (23, 30, 32).

PSIS is usually diagnosed in childhood due to growth failure, but up to one third of cases may be diagnosed shortly after birth if breech presentation, hypoglycemia, cholestasis manifested as prolonged jaundice, and in boys – micropenis and cryptorchidism are not overlooked (3, 8, 11). In the second presented case, though he had asphyxia at birth, cryptorchidism and later – growth failure, diagnosis was established at the age of 26 years when skull MRI was performed. This patient achieved a normal height despite poor growth (-3 to -4 SDS for height up to the age of 20 years), due to associated hypogonadotropic hypogonadism, which prevented growth plates closure, and allowed prolongation of growth into adulthood. GHD in this patient was documented by the low IGF1 level at the age of 26 years. In 55 PSIS patients (all GH deficient, but not treated with GH), heights were in the normal range in 25% of cases (38), also PSIS patients diagnosed in

adulthood may have normal height (39). The first case was diagnosed at the age of four years, showing very low IGF1 and stimulated GH levels, so GH treatment was started.

An analysis of 5085 patients with short stature enrolled in GeNeSIS (Genetics and Neuroendocrinology of Short Stature International Study) showed that in patients with PSIS phenotype was more severe (including GH deficiency) and had a better response to GH than patients with isolated anomalies (1071 patients had pituitary abnormalities); moreover, this study revealed the progression to multiple pituitary hormone deficiency (deficiency of TSH - 72.9%, gonadotropin - 9.4%, AVP - 9.4%, ACTH – 2.1%), during 4 years follow-up in 10% of 708 patients initially diagnosed with isolated GH deficiency; CPHD was found in 35% of patients with structural abnormalities, especially in patients with septooptic dysplasia (40).

A recent study found that a GH response > 5ng/mL after insulin or clonidine stimulation is found in 20% of patients with EPP, so MRI imaging was proposed as a first line diagnostic approach of GH deficiency in these patients (41). Moreover, growth hormone deficiency may not be so evident in the first few years of life. Once diagnosis of PSIS is established based on MRI, patients should be carefully followed up for additional pituitary deficiencies (3), especially ACTH deficiency, which may be lifethreatening. Gonadotropin deficiency can be diagnosed in the first six months of life in males if micropenis and cryptorchidism are present, and in the first two years in PSIS females, otherwise investigations must be postponed after 12 years of age.

Surprisingly, our male patient, though unsuccessfully treated in childhood and adolescence with hCG, experienced testicular descent and pronounced virilization after testosterone treatment was initiated, at 26 years. Long lasting inguinal position of the testis is responsible for germ cell depletion and Sertoli cell dysfunction that may allow abnormal germ cell differentiation, increasing risk for carcinoma in situ and seminoma (42), so further follow-up is mandatory. In this case we assume the risk for germ cell tumors is not increased, because semen analysis, performed after testosterone was initiated, revealed azoospermia, resulting from both gonadotropin deficiency and cryptorchidism.

Both patients had TSH deficiency. The male patient had also gonadotropin and ACTH deficiency. These deficiencies may also appear in the other patient

with time, so she will be forward screened up on a six months basis for adrenal axis function and after 12 years, for hypogonadotropic hypogonadism, if pubertal signs do not occur. Hyponatremia may announce the occurrence of multiple pituitary hormones deficits; despite the normal aldosterone secretion, it is encountered in patients with hypopituitarism, especially in the elderly. Cortisol deficiency leads to increased AVP release and increased renal response to AVP, by upregulation of aquaporin-2 water channel in the collecting ducts. Hypothyroidism minimally contributes to the inappropriate antidiuresis; GH and gonadotropin deficiency may also be implied (43).

Prolactin level was low in the first patient and low normal in the second patient. In PSIS patients variable prolactin level was reported – hyperprolactinemia in 36.4% and none of the patients with hypoprolactinemia in a Chinese cohort (38) and 44% hyperprolactinemia, 12% hypoprolactinemia (8) in an European cohort, respectively, 16.9% hyperprolactinemia and 14.5% hypoprolactinemia in the GENHYPOPIT cohort (7). Increased prolactin levels suggest a hypothalamic origin of PSIS or stalk dysfunction.

In conclusion, PSIS is a developmental defect easily diagnosed by MRI, which often result in a gradual occurrence of multiple pituitary hormones deficiencies, requiring careful follow up of affected patients.

Diagnosis is often delayed if symptoms such as breech presentation, neonatal distress, hypoglycemia and prolonged jaundice are overlooked, especially in a male newborn with micropenis and cryptorchidism, or if MRI is not performed in a child with poor growth or in an adolescent with delayed puberty due to hypogonadotropic hypogonadism.

Thorough assessment of the clinical, malformative, endocrine and MRI phenotype in each case is mandatory for a proper management of the patients, and will help decipher the underlying pathogenic mechanism which may be multigenic and environmental.

Normal height can be reached out in untreated, GH and gonadotropin deficient PSIS patients due to prolonged growth into early adulthood.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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