

Case report

A novel missense mutation in human Receptor Roundabout-1 (ROBO1) gene associated with pituitary stalk interruption syndrome

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What is already known on this topic?

Pituitary stalk interruption syndrome (PSIS) is a rare, congenital anomaly of the pituitary gland characterized by pituitary gland insufficiency, thin or discontinuous pituitary stalk, anterior pituitary hypoplasia, and ectopic positioning of the posterior pituitary gland. The underlying genetic etiology for the vast majority of cases remains to be determined.

What this study adds?

DNA sequence analysis revealed a novel missense mutation (c.1690C>T, p.Pro564Ser) in human Receptor Roundabout-1 (ROBO1) gene associated with pituitary stalk interruption syndrome.

Abstract

Pituitary stalk interruption syndrome (PSIS) is characterized by the association of an absent or thin pituitary stalk, an absent or hypoplastic anterior pituitary lobe and an ectopic posterior pituitary lobe. The causes of this anatomical defect include both genetic and environmental factors. Molecular defects in genes have been identified in a small number of patients with PSIS. A 4-year-old boy presented with hypoglycemia and hyponatremia as associated with growth hormone (GH), thyroid stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH) deficiencies. The patient had strabismus on his left eye. MRI images showed pituitary hypoplasia, EPP and absent pituitary stalk. A novel ROBO1 missense mutation (c.1690C>T, p.Pro564Ser) that may contribute to the disorder was found in this patient and his mother.

Key words: Receptor Roundabout-1 gene; pituitary stalk interruption syndrome; combined pituitary hormone deficiency; missense mutation

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Conflict of interest: None declared.

25-Feb-2019

16-Aug-2019

Introduction

Pituitary stalk interruption syndrome (PSIS) is a rare disorder due to the blocked transportation for hormones from hypothalamus to pituitary. The estimated incidence of this disorder is 0.5/100,000 births (1, 2). The patients with PSIS were characterized by a combination of specific pituitary hormone deficiencies on magnetic resonance imaging (MRI): interrupted pituitary stalk, absent or ectopic posterior pituitary, and anterior pituitary hypoplasia (3, 4). The diagnosis of this disease mostly relies on MRI imaging along with classical clinical and laboratory findings.

Despite the intensive research on PSIS, the etiology of PSIS still remains unknown in 95% of cases, and genetic causes are suspected. In human, mutations in *LHX4*, *OTX2*, *HESX1*, *SOX3*, *PROKR2*, *GPR161* and *CDON* have been postulated to be involved in PSIS. Recently, mutations in *ROBO1* gene have been reported in five patients with PSIS (5), confirming its genetic roles in PSIS.

Here, we report a case of PSIS with multiple anterior pituitary deficiencies and classical triad of MRI findings in which whole exome sequencing (WES) analysis identified a novel heterozygous mutation in *ROBO1* gene. This mutation is also carried by her mother, who also had abnormal pituitary function and short stature, thereby indicating a genetic mechanism as the basis for this condition.

Case Report

The patient was a 4-year-old boy who was admitted to our hospital with complaints of episode of generalized tonic-clonic seizures. The episode was not associated with fever or any sign of infection. He was born after an uneventful pregnancy and delivered with normal birth parameters (weight 3860 g, length 50cm). Psychomotor development was normal. The height of patient was 97 cm (<3rd percentile, -2.10 SDS) and weight was 16 kg (10-25rd percentile). Findings on physical examination were unremarkable apart from left strabismus (Figure 1). The penis was 2 cm (stretched) and the testicular volume was 1ml bilaterally. The patient was the first child of this non-consanguineous Chinese parents. The father was healthy and of normal stature (170.5 cm, 25rd percentile), while the mother had a short stature (146cm, <3rd percentile). No further details of the mother's medical history were available. His maternal grandmother also presented with short stature and strabismus. The patient was found to have extremely hypoglycemia with a blood glucose concentration of 0.92mmol/l (normal, 3.3–5.5) and hyponatremia (Na 117mmol/l, normal 135-155). The measurements of hormones during hypoglycemia are as follows: serum insulin 0.9 mU/L, serum cortisol 0.55ug/dl (normal, 6.2–19.4), adrenocorticotropic hormone (ACTH) 9.8pg/mL, urine ketone bodies (KET): negative, plasma lactate 1.8mmol/L (normal, <2), serum ammonia 52.2mol/L (normal, <80). The level of ck, ck-mb, organic acids, amino acids, acylcarnitines and free carnitine in plasma were normal. Blood gas analysis: PH 7.44, PCO₂ 29.6 mmHg, HCO₃ 22.4 mmol/L, BE -0.6 mmol/L. No abnormalities were detected on complete blood count. HbA1C 5.6% (normal, 4-6). GH deficiency attributed to this patient after insulin tolerance test and L-dopa test, with a peak GH of 0.3 and 0.05 ng/mL, respectively. IGF-1 was 25 ng/ml (normal, 66–427), and prolactin were in normal range. In addition to GH deficiency, he was diagnosed with central hypothyroidism (free T4, 8.6 pmol/l [normal, 10.8–20], TSH 0.489 ulU/ml [normal, 0.8–5]). Luteinizing hormone (LH) 0.131 U/L, follicle-stimulate hormone (FSH) 0.71U/L. The karyotype was 46 XY. Echocardiogram, Electroencephalogram (EEG) and video-EEG showed no abnormalities. Brain MRI revealed a small anterior pituitary gland, invisible stalk, ectopic posterior lobe (Figure 2).

From above, a diagnosis of PSIS was made. The patient presented with combined pituitary hormone deficiency (GHD, central hypothyroidism and central adrenocortical insufficiency). He was then treated with saline and hydrocortisone and responded well to the treatment with stabilized blood sugar and natremia levels. After that, Levo T4 and GH replacement therapy were started.

Genetic Analysis

The family DNA was sequenced to discover the causal gene using whole-exome sequencing. DNA was isolated from peripheral blood using DNA Isolation Kit (Biotek, AU1802). 1ug genomic DNA were fragmented into 200-300bp length by Covaris Acoustic System. The DNA fragments were then processed by end-repairing, A-tailing, adaptor ligation and a 4-cycle pre-capture PCR amplification, after which all exons and the 50bp bases in their adjacent introns were captured by SeqCap EZ Med Exome Enrichment Kit (Roche, USA). The DNA library were performed post-capture amplification and purification, and then sequenced on Illumina HiSeq X Ten platform (Illumina, USA) manually. The raw data produced were then filtered and aligned with the human genome reference (hg19) using the BWA Aligner (<http://bio-bwa.sourceforge.net/>) and variants were called by using NextGene V2.3.4 software (Soft genetics, USA). The data had a 151.24× mean read depth and about 97.95% of the targetbases were covered at 20× average read depth.

The filtered variants were then annotated by using NextGene V2.3.4 and lab's own scripts to get related information, including the conservation of nucleotide bases and amino acid, prediction of the biological functions, frequency in normal populations (1000 Genomes, ExAC, dbSNP database and local specific databases), and the data from HGMD, Clinvar and OMIM. Their potential effect of the variants were predicted by SIFT, Polyphen-2 (6-8). All variants of pathogenicity were interpreted according to ACMG (9) standards and categorized. *ROBO1* gene has three transcripts in National Center for Biotechnology Information (NCBI), of which NM_002941.3 was used as the reference sequence. Potentially pathogenic variant were verified using Sanger sequencing.

WES data filtering identified a heterozygous c.1690C>T, p.Pro564Ser variant (RefSeq: NM_002941.3; Chr3:78717393) in the *ROBO1* gene (Figure 3). Segregation studies revealed that the mother is also the carrier of the same mutation. This rare sequence variant was further predicted to be “probably damaging” with a score of 0.999 by polyphen-2, “damaging” with score 0.01 by SIFT. Multiple amino acid sequence alignments showed that p.Pro 564 is highly conserved in human, ptroglodytes, mmusculus, drerio, xtropicalis and ggallus (Table 1).

The mother, who is short stature, also carried the same *ROBO1* variant was then performed endocrine evaluation. ACTH was measured as 38.8 pg/mL, cortisol as 8.1 ug/dl, IGF1 as 188 ng/mL(115-307 ng/mL), FT4 as 9.4 pmol/l, TSH as 2.16 uIU/ml, LH as 2.97 IU/L, FSH as 5.34 IU/L and E2 as 67 pg/ml. Her pituitary MRI showed a thin pituitary stalk, hypoplasia of the adenohypophysis (Figure 4).

Discussion

The patient reported here had left strabismus and combined pituitary hormone deficiencies (GH, ACTH and TSH deficiencies). His mother, who also carried the variant, had abnormal pituitary function, short stature but normal eye structure. While the patient's grandmother presented with short stature and strabismus. Though DNA was not available from the grandmother who had passed away, this may reflect phenotypic variability in this family. The phenotypic variability found in the patient and his mother could be due to the impact of other genes in pituitary development or gene-environment interactions (10), which is similar to the missense variants involving the *HESX1* and *LHX4* genes (11), of which the heterozygous variants are characterized by highly variable phenotypes amongst family members.

To date, several etiological factors have been proposed for PSIS, and a polygenic etiology has been highly suggested. *HESX1*, *LHX4*, *OTX2*, *SOX3*, and *PROKR2* have been reported to be associated with PSIS (12-14). In 2017, 5 unexplained PSIS cases including 2 familial cases identified one nonsense, one missense and one frameshift mutation in heterozygous state by WES (5), which first identified the novel heterozygous frameshift, nonsense and missense variants (p.Ala977Glnfs*40, two affected sibs; p.Tyr1114Ter, sporadic case and p.Cys240Ser affected child and paternal aunt) in *ROBO1* gene (15) (Table 2). In these 5 cases, three of them showed isolated GH deficiency and the rest two presented with combined GH and TSH deficiencies. Sumito Dateki et al identified a novel homozygous splice site mutation in *ROBO1* (c.1342+1G>A) in a 5 years boy. The patient had combined pituitary hormone deficiency, psychomotor developmental delay, severe intellectual disability, sensorineural hearing loss, strabismus, and characteristic facial features (15). Their findings suggest *ROBO1* gene as one of the potential causative genes of PSIS and the heterogenesis of disease that caused by *ROBO1* mutations. In our report, by using next generation sequencing (NGS) technology, we identified a maternal missense mutation (c.1690C>T, p.Pro564Ser) in the *ROBO1* gene in a case diagnosed with PSIS and CPHD. This variant was predicted to be possibly pathogenic by Polyphen-2, SIFT and PROVEAN. Multiple amino acid sequence alignments showed that p.Pro 564 is highly conserved across various species. All these findings suggested that this variant could play an important role in disease causation. Patients who harbor *ROBO1* mutations shared some phenotypes with the present patients. 4 cases presented with strabismus and 1 case presented with ptosis. These data suggest that mutations in *ROBO1* contribute to ocular anomalies. Cardiomyopathy was seen in one patient, and one patient had psychomotor developmental delay. More cases are needed to elucidate the relationship between genotype and phenotype. The receptor Roundabout-1 (*ROBO1*) and its ligand Slit are

known to influence axon guidance and central nervous system (CNS) patterning in both vertebrate and nonvertebrate systems (16). Missing expression of *ROBO1* could lead to ectopic differentiation of forebrain neurons. The chemo repulsive ligand Slit and its receptors of the Robo family are expressed in the developing and adult brain (17) and are crucially involved in the formation of midline commissures. Slit2 and Slit1/2 double knockout animals display defects in corticothalamic and thalamocortical targeting, callosal and hippocampal commissure projections (18) and defects in the formation of the optic chiasm. S.F.Calloni *et al.* described a 9-year-old boy with severe intellectual disability, absence of the transverse pontine fiber, thinning of the anterior commissure and corpus callosum, and compound heterozygous variants in the *ROBO1* gene (19). These findings strongly suggest that human *ROBO1* variants could result in neurodevelopmental disorders. Our patient was subsequently found to induce a wide range of symptoms, including classic combined pituitary hormone deficiency (CPHD) and left strabismus. Thus *ROBO1* gene may be one of the potential causative genes for PSIS and CPHD. BJORKE B *et al* (20) showed that Slit signaling is necessary to inhibit the initiation of oculomotor . Oculomotor axons (midline crossing) are led by an axon-like process that forms from the cell body as a secondary axon. Perhaps this repolarization is subject to Robo regulation. Overall, the introduction of NGS technology in the diagnostic workflow will lead to the identification of novel genetic determinants in pediatric patients with pituitary defects (21-22). Additional examples of *ROBO1* variants and clinical PSIS cases are needed to explore the function of *ROBO1* and its functions during human embryogenesis and organogenesis. ROBO were shown to be responsible for ocular as well as pituitary abnormalities.

Ethics

Informed Consent:It was taken.

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: Ziqin Liu , Design: Xiaobo Chen, Data Collection and/or Processing: Ziqin Liu, Analysis and/ or

Interpretation:Ziqin Liu, Literature Research: Xiaobo Chen, Writing: Ziqin Liu. Financial Disclosure: The authors declared that this study has received no financial support.

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Fig.1 The facial image of the patient.

Image of the patient shows left strabismus.

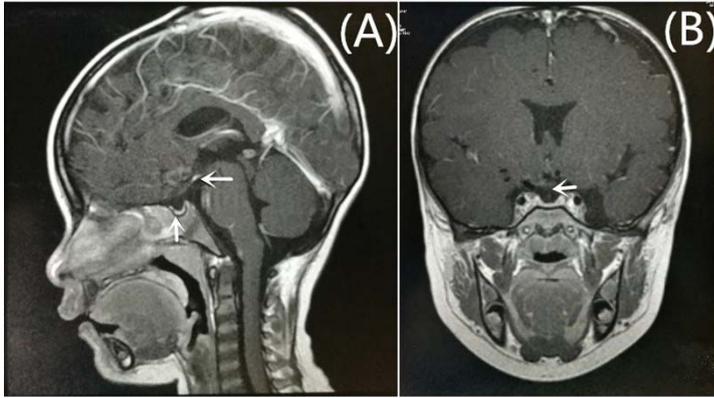


Fig.2 The sagittal and coronal pituitary on MRI confirming PSIS.

(A) Sagittal view. The small anterior pituitary (vertical arrow) and the posterior lobe was localized at the hypothalamic region (horizontal arrow). (B) Coronal view. The pituitary stalk is absent.

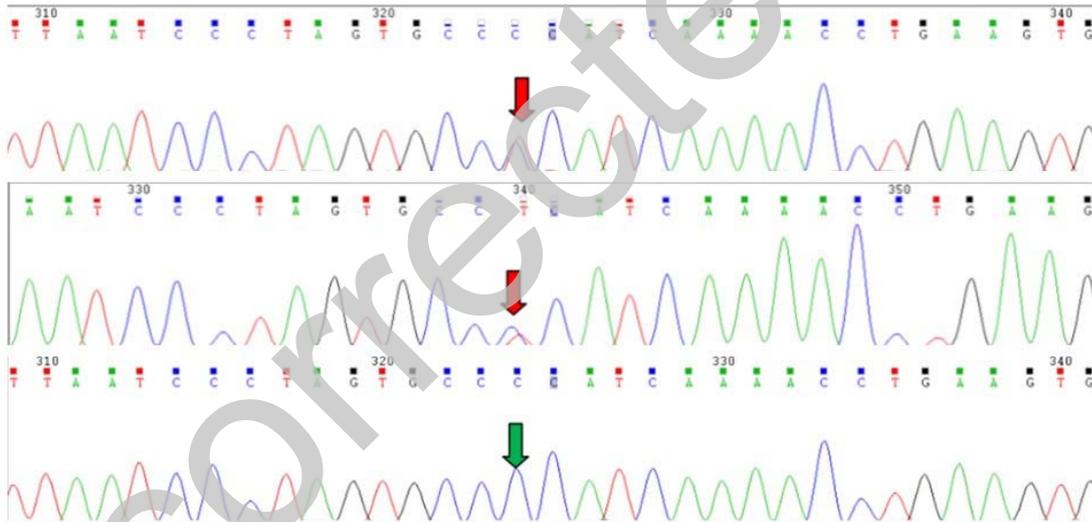


Fig.3 Sanger Sequencing Results of the family.

The heterozygous c.1690C>T, Pro564Ser ROBO1 mutation is found in the patient (top) and his mother (middle). The father (bottom) has no mutation. The red arrows show the mutation.

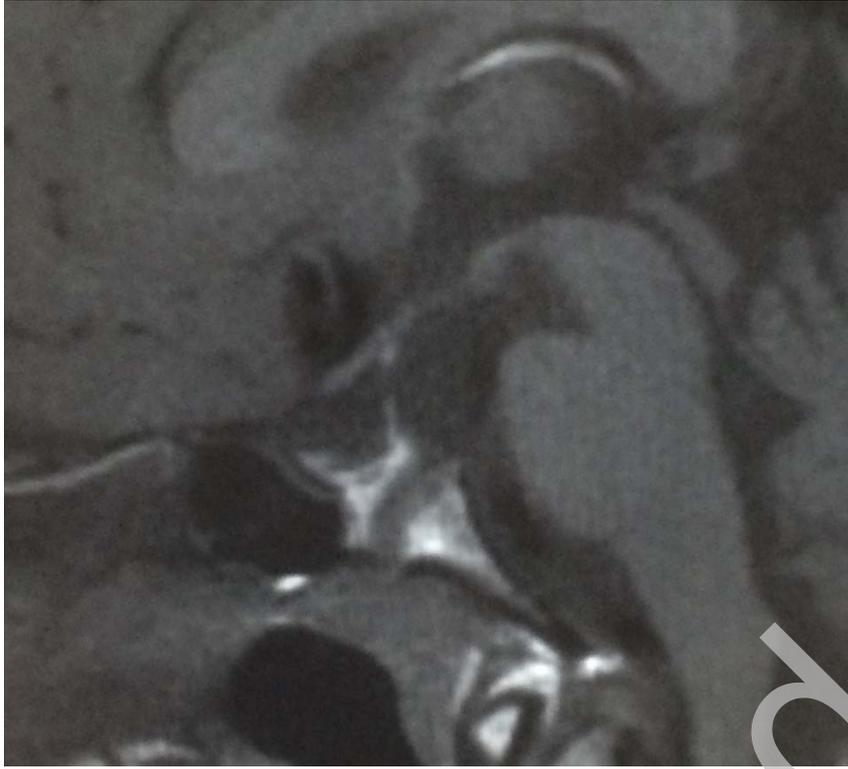


Figure 4. MRI image from the patient's mother. Her pituitary MRI showed a thin pituitary stalk, hypoplasia of the adenohypophysis.

Table 1 Alignment of amino acid sequences encoded by the *ROBO1* gene from different species.

Species	Aa alignment
Human	RPTDPNLIPSA P SKPEVTDVSRNT
Ptrogodytes	RPTDPNLIPSA P SKPEVTDVSRN
Mmusculus	RPTDPNLIPSA P SKPEVTDVSKN
Drerio	RPTDPNLIPSA P SKPDVTDVSRT
xtropicalis	RPTDPNLIPSA P SKPE
Ggallus	RPTDPNLIPSA P SKPEVTDVSRN

Note: The P residue is highlighted in red for each sequence.

Table 2. Clinical and genetic features of patients with ROBO1 mutations in pituitary stalk interruption syndrome

Case#	M/F	Mutation	Eyes	Pituitary function	Clinical finding
1(5)	female	c.2928_2929delG	strabismus	Isolated GH deficiency	NA
2(5)	male	c.2928_2929delG	strabismus	Isolated GH deficiency	NA
3(5)	male	c.3450G>T	ptosis	Isolated GH deficiency	NA
4(5)	female	c.719G>C	strabismus	combined GH and TSH deficiencies	NA
5(5)	female	c.719G>C	-	combined GH and TSH deficiencies	Cardiomyopathy
6(15)	male	c.1342+1G>A	strabismus	Combined GH, TSH, PRL, ACTH, LH/FSH deficiencies	psychomotor developmental delay, severe intellectual disability, sensorineural hearing loss, characteristic facial features
Present case	male	c.1690C>T	strabismus	Combined GH, TSH, ACTH deficiencies	Micropenis