SCREENING OF PROP-1, LHX2 AND POU1F1 MUTATIONS IN PATIENTS WITH ECTOPIC POSTERIOR PITUITARY GLAND

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Abstract

Objective. Ectopic posterior pituitary gland (EPP) is usually characterized by an abnormal pituitary stalk and hypoplasia of the anterior hypophysis. The genetic mechanisms involved in the development of EPP remain uncertain. The aim of this study is to determine whether mutations in the three genes, PROP-1, LHX2, and POU1F1, are associated with the risk for and the characteristics of EPP.

Methods. In the Endocrinology Outpatient Clinic of “Dr. Behcet Uz” Children’s Hospital, 27 patients with EPP were submitted to sequencing analyses of the PROP-1, LHX2, and POU1F1 genes.

Results. Growth hormone, thyrotropin, corticotropin, gonadotropin, and vasopressin deficiency were observed in 22 (81.5%), 23 (85.2%), 17 (63%), 14 (51.9%), and two (7.4%) patients. Thirteen patients (48.1%) presented with hyperprolactinemia. Fourteen patients (51%) had a history of birth dystocia, and 12 cases (42.1%) had a history of breech presentation. Central nervous system abnormalities included five cases with corpus callosum agenesis, one case with schizencephaly, and one case with Chiari type 1 malformation. We identified a homozygous p.S109* mutation in exon 2 in one male patient with EPP and two different PROP1 gene polymorphisms (A142T or c.109+3 G>A polymorphism) in thirteen patients.

Conclusions. Our results suggest that PROP1 gene abnormalities might explain the genetic mechanisms involved in the development of EPP.

Key words: PROP 1 mutation, ectopic posterior pituitary gland, ectopic neurohypophysis, multiple pituitary hormone deficiency.

INTRODUCTION

An ectopic posterior pituitary gland (EPP) is known as an abnormal pituitary stalk and a small

anterior pituitary gland in magnetic resonance imaging (MRI) (1). Abrahams *et al.* demonstrated that patients with abnormalities in the hypothalamo–hypophyseal axis, who had growth hormone deficiency, could be divided into two groups: 1) those with an ectopic neurohypophysis (43%), including a neurohypophysis near the median eminence, absence of the infundibulum, and absence of the normal posterior pituitary bright spot; and 2) those with a small anterior pituitary gland (43%), which was either an isolated finding or associated with an ectopic neurohypophysis (2). The disease of EPP may range from isolated GH deficiency (IGHD) (48%) to multiple pituitary hormone deficiency (MPHD) (52%) (1). Although the etiology is unclear, traumatic birth, breech delivery, and genetic factors have been considered possible factors. Some genetic factors, including LHX4, HESX1, GLI2, GH-1, GH releasing hormone receptor, POU1F1, PROP-1, and SOX3 mutation, were found important in the development of the pituitary gland (3). These mutations can lead to progressive hormone deficiencies that affect the somatotroph, lactotroph, thyrotroph, gonadotroph, and corticotroph axes (3). Different mutations in these genes have been reported in patients with EPP (4–6). Patients with HESX1 mutation exhibit relatively variable manifestations of septo-optic dysplasia, with or without optic nerve hypoplasia and the occurrence of EPP on MRI (5). Patients with EPP had heterozygous OTX2 mutations (6). Patients with over or underdosage of SOX3 presented with IGHD or MPHD, with or without mental retardation or learning difficulties and EPP on MRI (7). No mutations in LHX4 and HESX1, or in three new polymorphisms in LHX4, were identified in patients with EPP (8). No loss of function mutations

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in the LHX3 and PROP-1 genes were detected in patients with EPP (9). POU1F1 gene mutations were not found in patients with multiple pituitary hormone deficiency associated with EPP (10, 11). To the best of our knowledge, no study has been conducted to assess the relationship between LHX2 expression and ectopic posterior pituitary gland in patients with MPHD. In addition, there have been rare studies regarding the relationship between PROP-1 and POU1F1 expression and ectopic posterior pituitary gland. The purpose of this study is to determine whether three mutations, PROP-1, LHX2, and POU1F1, are related to the risk for and characteristics of ectopic posterior pituitary gland.

PATIENTS AND METHODS

This study was conducted between January 2012–October 2014 at “Dr. Behçet Uz” Children’s Hospital, Division of Pediatric Endocrinology, Izmir. Twenty-seven patients (15 males and 12 females; aged 2–22 years) with EPP, were evaluated consecutively according to MRI of the pituitary gland. No control group was used. The inclusion criteria for the children with EPP were as follows: they had regular follow-ups from the time of diagnosis and a disease duration > 24 months. The clinical characteristics of the cohort were collected from the medical files of the patients, including gestational age, birth order, major incident during pregnancy, history of traumatic delivery, admission to neonatal intensive care unit, and hormonal profile. The wall-mounted “Harpender” stadiometer, which is similar to that designed by Tanner and Whitehouse, was used to measure height. The published standards of the Greulich–Pyle method were used to assess bone age from radiographs of the left hand. The parental target height was readily ascertained by calculating the mean parental height and adding or subtracting 6.5 cm for male or female children, respectively.

Written informed consent was acquired from the legal guardians of the children. The study was conducted according to the Declaration of Helsinki and the study protocol had been approved by Ethics Committee for “Dr. Behçet Uz” Children Disease and Surgery Training and Research.

Hormonal evaluation

Hormonal assays were carried out using commercial radioimmunoassay (RIA) kits. The results of anterior pituitary function tests were recorded, according to basal hormone levels (GH levels following

insulin tolerance and L-dopa stimulation tests, basal free T4 and TSH by TRH stimulation, cortisol levels by low dose synacthen test [Blood samples for cortisol at +20, +30 and +40 min were taken after IV 1 µg Synacthen (ACTH 1–24)], LH and FSH levels by gonadotrophin releasing hormone stimulation, prolactin). Growth hormone level was taken as less than 10 µg/L for growth hormone deficiency (12). The level was subsequently further classified into moderate (3–5 µg/L) or severe (< 3 µg/L) (12). When the serum T4 level was <12 pmol/L (0.93 ng/dL), if the serum thyroid stimulating hormone (TSH) concentration was inappropriately low (TSH <15 µIU/mL), it was defined as TSH deficiency (13). When the basal plasma cortisol level measured at 08:00 a.m. was <15 µg/dL, a low-dose adrenocorticotrophic hormone (ACTH) stimulation test was performed, and a peak cortisol < 19.6 µg/dL was used to define adrenal insufficiency. Serum prolactin (PRL) levels were also measured (normal range: 5–25 ng/mL). Hypogonadism was defined by the absence of physical signs of pubertal development at advanced bone age (12 years in girls and 13.5 years in boys) (14) and confirmed by biochemical data (prepubertal baseline serum gonadotropin concentrations). GnRH tests were performed when euthyroid status was accomplished by T4 supplementation. In the case of ACTH deficiency, hydrocortisone treatment was supplemented in a physiological dosage (12 mg/m² per day).

MRI evaluation of the pituitary

1.5 Tesla unit using T1 and T2-weighted sagittal and coronal scans were obtained, showing gadolinium contrast in all patients at the time of diagnosis. Maximal height of the pituitary gland was assessed perpendicular to the sella turcica and considered hypoplastic when it was less than -2 SD, compared with the normal controls (15). The anterior lobe of the pituitary gland was measured to determine the following: site, maximum height, maximum antero-posterior (AP) diameter, and SA [calculated using the formula: $\Pi \times (\text{maximum diameter}/2) \times (\text{maximum height}/2)$]. The absence of the normal posterior lobe hyperintense signal in the sella turcica and the presence of a hyperintense nodule in an ectopic location were classified as EPP. The authors identified a normal pituitary stalk when they found a normal diameter from the level of the optic chiasm to its insertion into the pituitary gland. The stalk was considered thin when it had a continuous but extremely thin appearance and its proximal and distal diameter size were below normal (16). Additional and variable combinations of brain abnormalities,

including hypoplasia of anterior commissure and/or the mesencephalon and/or pons hypoplasia, optic nerves and midline structures, such as septum pellucidum and corpus callosum and/or absence, hypoplastic optic chiasm and/or absence of interthalamic mass were examined. All measurements were recorded on at least three occasions, and all images were evaluated with the same method used by the same experienced neuroradiologist. A second experienced neuroradiologist assessed 10 scans in order to assess inter-observer error. The inter-observer error included measurement of the pituitary gland, calculation of anterior lobe of the pituitary gland and interpretation of scan for evaluating posterior lobe of pituitary gland, ectopic posterior pituitary gland and brain abnormalities.

Genomic analysis

Peripheral blood samples, which were collected in EDTA tubes and stored fresh (-20 °C), were obtained with informed consent from all 27 patients. Genomic DNA was extracted from peripheral leukocytes using the QIAamp DNA mini kit (Qiagen, 51304, Dusseldorf, Germany), according to the manufacturer's protocol. Genomic fragments encompassing the coding regions and adjacent intronic regions of the PROP1 (three fragments), POU1F1 (six fragments), and LHX2 (five fragments) genes were amplified by PCR using the specific primers described previously (17,18) (Table 1). The amplicons from individual exons were purified and analyzed by cycle sequencing with ABI BigDye Terminator Cycle Sequencing Kit v3.1 (ABI Applied Biosystems, Foster City, CA) on an automatic DNA sequencer (ABI 3130 Genetic Analyzer, Applied

Biosystems). The sequencing results in patients and the consensus sequences in the NCBI Human Genome Database [NCBI Reference Sequence: NG_015889.1 (PROP1), NG_008225.2 (POU1F1)] were imported into the ABI SeqScape program and aligned to identify variations. Sequence variations in LHX2 were numbered with the adenine of the ATG initiation codon as the first nucleotide (NCBI Reference Sequence: NM_004789.3).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS Version 18, SPSS Inc., Chicago, IL, USA) for Windows program was used to perform the analysis. In addition to graphical methods, the Kolmogorov–Smirnov test was used to check the distribution of the numerical data. Normally distributed numeric variables were expressed as mean±standard deviation, whereas medians and interquartile ranges (IQRs) were used for variables with non-normal distributions. Categorical variables are presented as numbers and percentages. Chi Square and Fisher's Exact tests were used for the comparison of the categorical data. A p value < 0.05 was considered significant.

RESULTS

Patient demographics

The study population consisted of 27 patients with EPP. The auxological data and basal characteristics of the patients are shown in Table 2. The median age at first referral was 8.5 (4.5). In the study group, 55%

Table 1. Primers used in PCR amplification of fragments containing exons of PROP1, POU1F1 and LHX2 genes

Gene	Fragment	Forward	Reverse
PROP1	Exon 1	TGTAAAACGACGGCCAGTCAG GTGTGGAGAGGAGCT	CAGGAAACAGCTATGACCATGCT TTCAGCCTCACAC
	Exon 2	AGGCACATGTGGTCCAGCACC TGTAAAACGACGGCCAGTTCAC	GAT AGC ACC AAA GAA ATC TGC CAGGAAACAGCTATGACCTCAGA
	Exon 3	CCCTACAGCCATGC CC GAATCGGCCCTTTGAGACAG	CTGGTGTGACAAAGC CCCGGTCATATGTAACTGT
	Exon 1	GAATCGGCCCTTTGAGACAG GATCCAAACTCCTAAATGTTTG	GTGTCCCCAAATTCAATAACA CAGGAAACAGCTATGACCAGATT
	Exon 2	TGTAAAACGACGGCCAGTGAG AATGACAAATGGACT	TGCAAACCAA GTT TCCTACTTATTGAAGCCATTA
	Exon 3	GATACACAGATTTGTGTGAC TGTAACGACGGCCAGTGTC	CAGGAAACAGCTATGACCACCAG TTTTGTCCTC TAGTAAC
POU1F1	Exon 4	TGTAAAACGACGGCCAGTGTC TACTTTGTGTTTGTA	CAGGAAACAGCTATGACCATGAA ACGGGAGAAAAAGGCT A
	Exon 5	TGTAAAACGACGGCCAGTATT CAACCTACCTTAATT	GGAGCCACCGCCTTGCATT GCCAAACTGTAAGACTGTGCCTG
	Exon 6	CAACCTACCTTAATT TGAGGCGGGGGCAAGCCCT	GCCAAACTGTAAGACTGTGCCTG CCGTCGAGGCCGCACACTTT
LHX2	Exon 1	TGAGGCGGGGGCAAGCCCT ATGTCCTGGCAGCCCCCTCC	GCCAAACTGTAAGACTGTGCCTG CCGTCGAGGCCGCACACTTT
	Exon 2	ATGTCCTGGCAGCCCCCTCC CCGTGTGTTCCACAGCCCC	CCGTCGAGGCCGCACACTTT GTCCTTCCAAGGCCACGGC
	Exon 3	CCGTGTGTTCCACAGCCCC TGGGTGGGGCGAGTGTGGAT	GTCCTTCCAAGGCCACGGC ATGTGGTTAGTTAGTTGCTC
	Exon 4	TGGGTGGGGCGAGTGTGGAT CTCACCAGCCCTTCCCTGTC	ATGTGGTTAGTTAGTTGCTC

Table 2. Baseline characteristics of patients with Ectopic posterior pituitary gland

	(n=27)
Median Age at Diagnosis. y (IQR) *	8.5(4.5)
Male/Female	15/12
Number of patients who had Caesarean Delivery (%)†	15(%55)
Number of patients who had Breech Presentation (%)†	12(%44)
Number of patients with difficult Birth (%)†	14(%51)
Median Weight. kg (IQR)*	18 (8)
Median Weight SDS (IQR) *	-2.4(2.3)
Height (cm)**	110 ± 18
Height SDS*	-3.2 (1.4)
BMI(kg/m ²)**	16.3 ± 3.3
BMI SDS*	-0.34 (1.6)
Height Age (year)*	5.1 (3)
Bone Age (year)*	5 (4)
Height Velocity (cm/year)*	3(1.4)
Height Velocity SDS**	-2.7 (1.7)
Target Height (cm)**	163 ± 7.6
Target Height SDS*	-1.1 (0.6)
IGF-1 (ng /mL)*	25(12)
IGF-1 SDS*	-2.2 (0.7)
IGFBP-3 (ng/mL)*	1300 (930)
IGFBP-3 SDS*	-2.6 (1.5)

* Data are presented as median(interquartile range); ** Data are presented as mean ± standard deviation; † Data are presented as number (percent age); BMI: Body mass index, IGF: Insulin-like growth factor-1, IGFBP-3: Insulin-like growth factor-binding protein-3.

Figure 1. Columns represent number of cases with deficiencies for each hormone HPRL=hyperprolactinemia. DI= Diabetes insipidus. **Figure 2.** Brain magnetic resonance imaging findings in patients with ectopic posterior pituitary gland.

of the patients were male, but no gender prevalence was found in the demographic and MRI data. Fourteen cases (51%) had a history of birth dystocia, and 12 cases (42.1%) had a history of breech presentation.

Pituitary hormone status

Growth hormone, thyrotropin, corticotropin, gonadotropin, and vasopressin deficiency were observed in 22 (81.5%), 23 (85.2%), 17 (63%), 14 (51.9%), and two (7.4%) patients, respectively (Fig. 1). Seven patients with MPHD had two hormone deficiencies, five patients had three hormone deficiencies, nine patients had four hormone deficiencies and two patients had five hormone deficiencies. Thirteen patients (48.1%) presented with hyperprolactinemia (Fig. 1). In patients with growth hormone deficiency, the median (interquartile range) IGF-1 and IGFBP-3 values were -2.2 (0.7) and -2.6 (1.5), respectively. Table 3 shows the serum hormone levels of all patients.

Anatomical brain abnormalities

In this cohort of patients with EPP, abnormalities in the central nervous system included five cases with corpus callosum agenesis, one case with schizencephaly, and one case with Chiari type 1 malformation (Fig. 2). There was no intraobserver variability between experienced neuroradiologist and second neuroradiologist for evaluating images.

Table 3. Baseline hormone levels and clinical presentations of patients with Ectopic posterior pituitary gland

	(n=27)
Peak GH (ng/mL)*	0.3 (0.6)
fT4 (ng/dL)**	0.8 ±0.2
TSH(μIU/mL)*	0.7 (2.7)
ACTH (pg/mL)*	14.6(25)
Peak cortisol (μg/dL) (ITT)*	6.2 (8)
Prolactin (ng/mL)*	24 (22)
GH Deficiency n(%)	22 (%81.5)
ACTH Deficiency n(%)	17 (%63)
FSH-LH Deficiency n(%)	14 (%51.9)
TSH Deficiency n(%)	23 (%85.2)
Diabetes Insipidus n(%)	2 (%7.4)
Multiple Hormone Deficiency n(%)	22(%81.5)
Hyperprolactinemia n(%)	13(%48.1)

* Data are presented as median(interquartile range); ** Data are presented as mean ± standard deviation; GH: Growth Hormone, fT4: Free Thyroxine, TSH: Thyroid-stimulating hormone; ACTH: Adrenocorticotrophic hormone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone.

Table 4. Detected gene abnormalities of PROP-1 gene in 27 patients with Ectopic posterior pituitary gland

	Frequency n (%)
No Mutation	14 (51.9)
A142 Homozygous	2 (7.4)
A142 Heterozygous	4 (14.8)
A142 Heterozygous + C109 Heterozygous	2 (7.4)
C109 Homozygous	2 (7.4)
C109 Homozygous +A142 Heterozygous	2 (7.4)
S109X Homozygous	1 (3.7)
Total	27 (100)

PROP-1: PROP paired-like homeobox 1.

Mutations in PROP-1, LHX2 and POU1F1

No mutation was found in the first exon of the PROP-1 gene in any patient. In one male patient only, a homozygous p.S109* mutation was found in the second exon. He was born at a birth weight of 2.650 kilograms to first-degree consanguineous parents (first cousins) after an uneventful term pregnancy. This patient had GH, ACTH, TSH, FSH, LH, vasopressin deficiency, and a mild hyperprolactinemia (46.2 ng/mL [5–25]). Brain magnetic resonance imaging revealed corpus callosum agenesis.

In the third exon of the PROP-1 gene, a heterozygous A142T [c.424G>A (rs1800197)] polymorphism was found in four patients, and a homozygous A142T polymorphism was found in two patients. Homozygous c.109+3 G>A [c.109+3G>A (rs4072924)] polymorphism was detected in two patients. Heterozygous A142T + heterozygous c.109+3 G>A polymorphism was found in two patients, and homozygous c.109+3 G>A + heterozygous A142T polymorphism was found in two patients. Table 4 shows the detected mutations in the PROP-1 gene.

Figure 3. Columns represents number of cases with deficiencies for each hormone HPRL=hyperprolactinemia. DI= Diabetes insipidus.

Growth hormone, thyrotropin, corticotropin, gonadotropin, and vasopressin deficiency were observed in 11 (84.6%), 12 (92.3%), 10 (76.9%), 8 (61.5%), and one (7.7%) in EPP patients with PROP-1 gene abnormalities, respectively (Fig. 3).

No mutations were found in the LHX2 and POU1F1 genes in this cohort.

DISCUSSION

We performed mutational analysis of the PROP-1, LHX2, and POU1F1 genes in a cohort of 27 patients with EPP. In thirteen patients with EPP, we identified two different PROP1 gene polymorphisms (A142T or c.109+3 G>A polymorphism) and found a homozygous p.S109* mutation in the second exon. However, we found no mutations in the LHX2 and POU1F1 genes.

Vieira *et al.* studied 40 patients with idiopathic hypopituitarism and found that those with EPP showed no mutations in the HESX1, LHX4, and PROP1 genes (19). However, Vallette-Kasic *et al.* performed PROP1 gene

abnormality in 23 patients with multiple pituitary hormone deficiencies and found that A142T gene polymorphism was responsible for 23% of multiple pituitary hormone deficiencies; however, data about EPP were not included (20). In addition, Kandemir *et al.* reported that 14 patients with MPHD had heterozygous A142T polymorphism, and a homozygous A142T polymorphism was found in three patients with MPHD (21). Diri *et al.* analysed 44 patients with Sheehan's syndrome and 43 healthy women in terms of the mean expression values of pituitary genes. Diri *et al.* found higher expression values of the HESX1, TLE1, TLE3, and MSX2 genes in patients with Sheehan's syndrome, when compared with healthy women (22). Our study showed that the frequency of A142T polymorphism was about 10 in 27, and thus about 2-fold higher than the frequency of this polymorphism in the general population (about 20 in 100). In contrast, our study yielded a frequency of 6 in 27 for the c.109+3 G>A polymorphism, and the frequency of this polymorphism is higher in the general population (about 49 in 100).

Deladoëy *et al.* studied 73 patients with combined pituitary hormone deficiency and demonstrated that 13 patients had homozygous p.S109* mutation in the second exon (23). Kandemir *et al.* found the homozygous p.S109* mutation in two brothers, who had growth hormone and thyroid-stimulating hormone deficiencies and normal prolactin levels; however, data about the magnetic resonance imaging were not included (21). In the present study, our patient with homozygous p.S109* mutation had growth, thyroid-stimulating, adrenocorticotrophic, follicle-stimulating, and luteinizing hormone deficiencies and a high prolactin level.

Although MPHD was found in 23 (85%) of the patients in the present study, previous studies have reported lower rates for MPHD of 48% (24,25). Thyrotropin, corticotropin, and gonadotropin deficiency were observed in 23 (85.2%), 17 (63%), and 14 (51.9%) of our patients with EPP. Previous studies reported TSH, ACTH and gonadotropin deficiency in patients with EPP as follows: 44 to 68%, 18 to 43%, and 0 to 81% (1,7,26-28). Although 13 patients (48.1%) in our study presented with hyperprolactinemia, Jagtap *et al.* found hyperprolactinemia in 12.9% of patients with EPP (24). However, Chen *et al.* studied 25 patients with EPP and found that only one patient had hyperprolactinemia (1).

Because abnormal delivery and perinatal and postnatal complications were reported more frequently in patients with invisible infundibulum and EPP, birth injury is thought to cause these morphological abnormalities (29,30). Most (42.1%) of our 27 patients with EPP had breech delivery. This finding correlates

with that of Jagtap *et al.* (24), who reported that the ectopic posterior pituitary gland could be associated with breech deliveries. Some researchers speculated that hypoxemia or anoxia due to breech delivery might cause injury to the pituitary gland and stalk (31). Other investigators suggested that because fetal endocrine system is an important trigger for the induction during birth, genetic disorders involving hypothalamic-pituitary abnormalities could cause the malposition of the fetus, resulting in breech delivery. (32). However, the results of our study indicated that EPP with abnormalities in the PROP-1 gene was not associated with breech delivery.

Midline defects, such as absence of corpus callosum, central cleft lip and palate, absence of septum pellucidum, and optic nerve atrophy, are associated with pituitary hormonal deficiencies (33). In a retrospective study, Mehta *et al.* analyzed 170 patients with hypopituitarism and found that MPHD with EPP was associated with abnormal corpus callosum and stalk abnormalities (34). The present study found no septo-optic dysplasia. While we found central nervous system abnormalities, including corpus callosum agenesis, schizencephaly, and Chiari 1 malformation in 26% of our cohort with EPP, Chen *et al.* reported midline abnormalities (Arnold Chiari type I malformation, agenesis of corpus callosum, and cranio-pharyngeal canal) in 75% of their 25 patients with EPP (1).

Although our study group was relatively small for detecting PROP-1, LHX2, and POU1F1 gene variants in patients with EPP, we observed for the first time that genotype-phenotype associations exist between PROP-1 gene variants and patients with EPP. Examining the PROP-1 gene variants in a large cohort group would be interesting. Another limitation of our study was the absence of an evaluation of other transcription factors that have a role in the development and function of the pituitary gland.

In conclusion, we found PROP-1 gene abnormalities in 37% of patients with EPP. The results of this study further indicated that rare gene variants in patients with EPP could provide novel insights into the development of the pituitary gland. Further studies of PROP-1 gene variants and clinical EPP cases are needed to explore genetic disorders involving hypothalamic-pituitary abnormalities.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial disclosure

The authors declared that this study has received no financial relationship with the organization.

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