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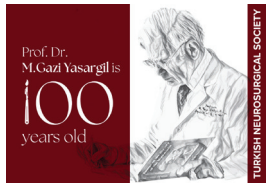

Genetic Characterization of Turkish Patients with Pituitary Neuroendocrine Tumors

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ABSTRACT

AIM: To examine the genetic results of patients diagnosed with pituitary neuroendocrine tumors (PitNETs) with respect to clinical, radiological, and pathological findings.

MATERIAL and METHODS: A total of 53 patients (30 men and 23 women) diagnosed with PitNETs were included in the study. The clinical findings, family history, imaging, and pathology results were recorded. The DNA was isolated from the peripheral blood. A customized panel test with the highest number of genes (28 genes associated with PitNET) found in the literature was used. Sequencing was conducted using the next-generation sequencing method, and the variants were analyzed according to current guidelines.

RESULTS: A total of 22 variants were identified in 20 patients, two of which were determined to be pathogenic. Pathogenic variants were detected in *AIP* (c.468+1G>A) and *MEN1* (c.1102_1104del) genes, which showed the most common pathogenic variant. Variants of unknown clinical significance were most frequently detected in the *MSH6, RET*, and *CDH23* genes.

CONCLUSION: Although the number of studies that conducted multigene testing in patients with PitNETs is limited, all studies, including ours, have shown that the patient's age at diagnosis and family history are the most important determinants of germline variant detection.

KEYWORDS: PitNETs, *AIP, MEN1*, NGS, Panel

ABBREVIATIONS: PitNET: Pituitary neuroendocrine tumor, **MRI:** Magnetic resonance imaging, **PRL:** Prolactin, **GH:** Growth hormone, **IGF-1:** Insulin-like growth factor-1, **ACTH:** Adrenocorticotropic hormone, **FSH:** Follicle-stimulating hormone, **LH:** Luteinizing hormone, **E2:** Estradiol, **TSH:** Thyroid stimulating hormone, **fT3:** Free triiodothyronine, **fT4:** Free thyroxine, **GnomAD:** The Genome Aggregation Database, **1000G:** 1000 genomes, **ESP5400:** Exome Sequencing Project, **HSF:** Human Splicing Finder, **ACMG:** American College of Medical Genetics and Genomics, **VUS:** Variant of uncertain significance, **MEN1:** Multiple endocrine neoplasia type 1, **HNPCCS:** Hereditary nonpolyposis colorectal cancer syndrome, **CNVs:** Copy number variations, **MLPA:** Multiplex ligation-dependent probe amplification, **A-CGH:** Array-comparative genomic hybridization, **IGV:** Integrative Genomics Viewer, **B:** Benign, **LB:** Likely benign

INTRODUCTION

Pituitary neuroendocrine tumor (PitNET) is an almost entirely benign tumor, typically arising from the clonal proliferation of cells as a result of pathogenic variants or chromosomal abnormalities (3). These tumors exhibit a wide range of clinical manifestations, depending on their size, hormonal activity, and specific location within the pituitary gland (17). PitNETs are the third most common intracranial

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tumor, following meningioma and glioma (2). However, tumors of the central nervous system seen in children, adolescents, and young adults (aged 18–39 years) commonly originate in the pituitary region (10). While they used to be classified based on the hormone they secrete, the new classification by the World Health Organization is based on the tumor cell lineage, cell type, and related characteristics (4).

Familial predisposition is detected in 5% of PitNET patients, while the majority of cases are sporadic (31). Some of the genes whose germline pathogenic variants have been associated with PitNETs are *AIP, MEN1, PRKAR1A, GPR101, SHDA, SDHB, SDHC, SDHD, CABLES1*. *AIP* is the gene with the most frequently detected pathogenic variants, especially in familial PitNET cases, with the rate of detecting pathogenic variants of 15% (22). The burden of somatic variants in PitNETs is very low compared to other tumors (21). Somatic variants are most commonly detected in the *GNAS* and *USP8* genes, and they are most commonly detected in somatotroph (15) and corticotroph tumors (23), respectively.

Understanding the genetic basis of PitNETs and their correlation with clinical, radiological, and pathological findings is extremely important for optimizing patient care and treatment strategies. This study thus aimed to examine the genetic results of patients diagnosed with PitNETs in the light of clinical, radiological, and pathological findings.

MATERIAL and METHODS

The study approval was obtained from the Ethics Committee of Marmara University Faculty of Medicine (Decision No: 09.2021.1291/Date: 05.11.2021).

We recorded participant's age, gender, smoking, alcohol consumption, previously known disease, regular drug use, surgery history, physical examination findings, age at diagnosis, pituitary magnetic resonance imaging (MRI), prolactin (PRL), growth hormone (GH), insulin-like growth factor-1 (IGF-1), adrenocorticotropic hormone (ACTH), cortisol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone, thyroid stimulating hormone (TSH), free triiodothyronine (fT3) and free thyroxine (fT4) values, and pathology results.

Following DNA isolation from peripheral blood leukocytes of the patients, pituitary adenoma-related genes were sequenced via Illumina Nextseq 550 platform (San Diego, CA, USA) using the TrueSight One Expanded kit (Illumina, San Diego, California, USA). We analyzed *AIP, MEN1, CDKN1B, PRKAR1A, SDHA, SDHB, SDHC, SDHD, SDHAF2, DICER1, MLH1, PMS2, MSH2, MSH6, VHL, NF1, CDH23, CABLES1, RET, MAX, TMEM127, GNAS, TSC1, TSC2, CDKN1A, CDKN2A, CDKN2B*, and *PRKACB*. The Sophia DDM-version 4 platform was used for the analysis of variants (Sophia Genetics SA, Switzerland). The following filtering methods were applied to evaluate the pathogenicity of the obtained variants: 1. variants with a fraction between 15% and 100% were analyzed; 2. variants with minor allele frequency <0.1% in databases such as The Genome Aggregation Database (gnomAD) (16), 1000 genomes (1000G) (1) and Exome Sequencing Project (ESP5400) (27) were selected; 3. variants reported as "Benign (B)" and/or "Likely benign (LB)" in the ClinVar database were excluded; 4. synonym variants that are not predicted to affect splicing according to the "Mutation Taster" (26) and "Human Splicing Finder (HSF)" (11) in silico programs were excluded; 5. All missense, nonsense, frameshift, in-frame indel variants, and variants within 20 bp of the exon-intron junction region were selected; 6. the pathogenicity of the remaining variants following filtration was evaluated using the American College of Medical Genetics and Genomics (ACMG) criteria (24), and Varsome (13) and Franklin (Genoox Ltd, Tel Aviv, Israel) programs were used for this task: variants evaluated as "Benign" and/or "Likely benign" were eliminated; and 7. the remaining variants were evaluated through patients' clinical and segregation studies, and variants that could be clinically relevant were reported. Confirmation using the Sanger sequencing was performed for exons that were not read sufficiently. After the PCR was conducted under appropriate conditions using primers specific to the exons, sequencing was performed using the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, USA). Data analysis was performed using the Chromas (Technelysium Pty Ltd, Australia).

RESULTS

Patients' Demographic Characteristics

A total of 53 patients were enrolled in this study. Of those, 30 (57%) were male and 23 (43%) were female (Figure 1). The participants' ages ranged from 13 to 72, with a mean of 42.8 ± 13.8 years (mean ± SD). The age at diagnosis ranged from 8 to 67 years, with a mean of 36.07 ± 14.2 years (mean ± SD). There were four patients diagnosed under the age of 18.

One patient had a positive family history of PitNET. Other patients did not have a family history of PitNET, any endocrine neoplasia, or any cancer diagnosis.

PitNET Characteristics

All 48 patients whose pituitary MRI results were available had macroadenomas (>10mm in diameter). According to Hardy classification, the distribution of PitNETs was macroadenoma (>10mm in diameter) in 22 patients (45.8%),

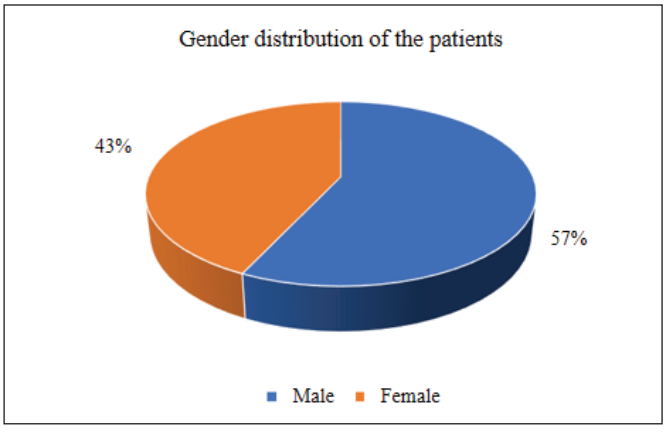

Figure 1: Gender distribution of patients.

large macroadenoma (>20mm in diameter) in 25 patients (52%), and giant macroadenoma (>40mm in diameter) in one patient (2%). In addition, 22 patients (45.8%) were classified as grade II (within the sella turcica but with bulging margin), 16 (33.3%) were classified as III (localized sella destruction), and 10 (20.8%) were classified as grade IV (diffuse sella destruction) (Figure 2).

Pathology results for 51 patients were accessible. The hormonal immunohistochemistry results of PitNETs showed that the most common subtypes consisted of isolated GH (n=17, 33.3%), plurihormonal (n=8, 15.6%), and isolated ACTH (n=8, 15.6%) staining, respectively. Isolated TSH staining was observed in only one patient's tissue (1.9%). There was also one case (1.9%) with no staining. When the immunohistochemical staining status of PitNETs according to age was examined, it was seen that isolated GH staining was most common in pediatric patients. Isolated GH and plurihormonal staining were the most common among the patients aged 18–64. Isolated ACTH staining in PitNETs was most frequently observed in patients aged 65–80. Sixteen patients had silent adenomas. The most common subtype was silent gonadotropinoma, consistent with the literature (12). While silent corticotropinomas are the second most common type in the literature (12), PIT-1 lineage and silent somatotrophinoma were seen more frequently in this study. Six patients had atypical PitNETs (ki-67> 3%, increased mitosis, and positive staining with p53). Immunohistochemical stainings in atypical PitNETs were GH/PRL (n=2, 33.3%), plurihormonal (n=1, 16.6%), GH (n=1, 16.6%), ACTH (n=1, 16.6%), and LH (n=1, 16.6%).

Demographic characteristics, clinical findings, MRI, and pathology results are summarized in Table I.

Results of Genetic Studies

The pathogenic and likely pathogenic variant was detected in two patients, and a variant of uncertain significance (VUS) was detected in 18 patients. No clinically relevant variants were detected in the remaining 33 patients. In the single case of familial PitNET in our study, a pathogenic variant was detected. No pathogenic variants or VUS were detected in the four pediatric patients. Of the six patients with atypical PitNET in our cohort, only one case had pathogenic variant in the *MEN1* gene.

Patient #3

A 52-year-old male patient with acromegaly was referred to our clinic. At the age of 32, the patient consulted a doctor when he had noticed that his hands, feet, and jaw continued growing. The patient underwent surgery after the MRI showed an 18x16 mm PitNET in the right half of the pituitary. The pathology results were compatible with somatotropinoma. There was no consanguinity between parents. The mother was 76 years old, and the father was 74. The patient had two sons, aged 27 and 10. His younger son had been diagnosed with cerebral palsy. His two sisters and mother also had somatotropinoma diagnosed at the ages of 30, 18, and 67, respectively. The patient's pedigree is shown in Figure 3A.

As a result of genetic analysis, heterozygous c.468+1G>A pathogenic variant was detected in the *AIP* (NM_003977) gene (Figure 3B). The variant was not reported in gnomAD, 1000G, and ESP5400. It was reported as likely pathogenic in the ClinVar. Varsome predicted the variant as pathogenic (PVS1, PM2, PP5), and Franklin as likely pathogenic (PVS1, PM2, PP5). Segregation analysis showed that the patient's mother and two sisters diagnosed with PitNET carried the same variant in a heterozygous state. Additionally, the patient's son, who had cerebral palsy, was also heterozygous for this variant (Figure 3C-F). However, no PitNET was detected in his younger son. The patient's elder son, healthy brothers, and sisters did not carry this variant.

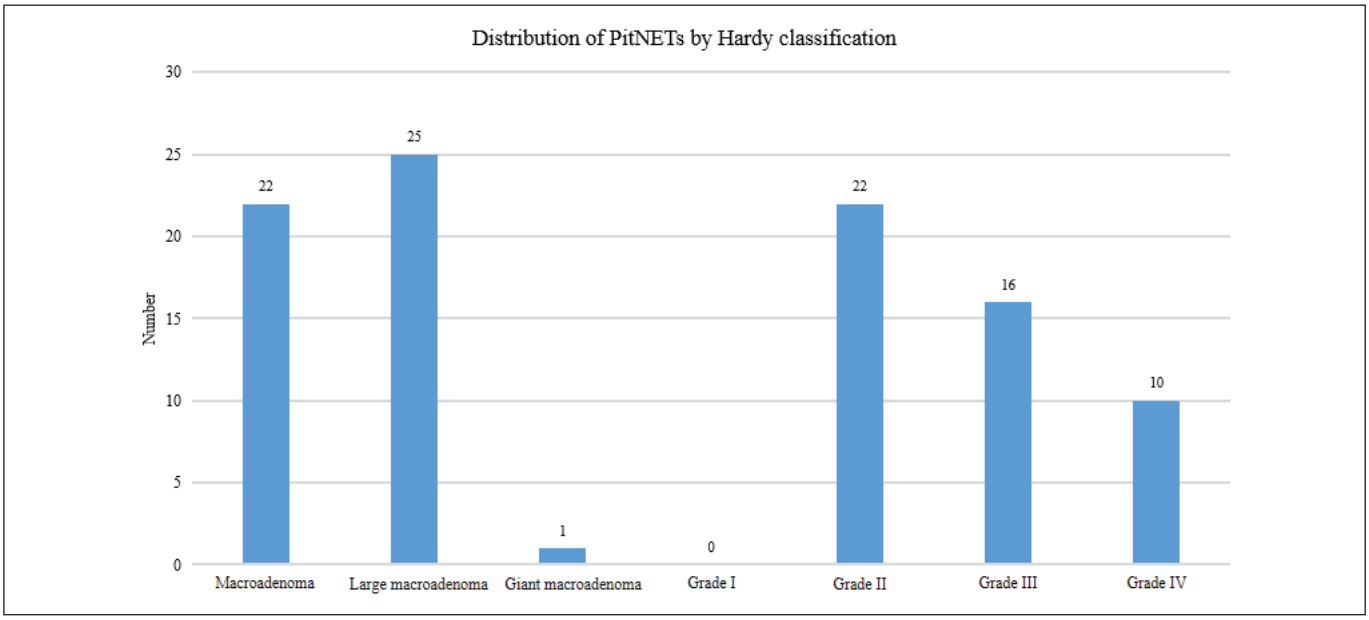

Figure 2: Distribution of PitNETs by Hardy classification.

Table I: Demographic characteristics, clinical findings, MRI, and pathology results of the patients.

Patient no.	Age (years)	Gender	Age at Diagnosis (years)	Clinical features	PitNET size (mm)	Invasion	Hormonal immunohistochemistry results						P53 (%)	Ki67 (%)	Transcriptional factors			
							BH	PRL	FSH	LH	ACTH	TSH			PIT-1	T-PIT	G-2	ERα
1	17	Male	11	Headache	N/A	N/A	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
2	44	Male	42	Acromegaly	18x14	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
3	54	Male	32	Acromegaly	18x16	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
4	43	Female	41	Headache	23x24	Left cavernous sinus	-	-	+	+	-	-	-	<1	-	-	+	N/A
5	50	Female	49	Acromegaly	20x15	-	+	-	-	-	-	-	1-2	1-2	+	-	-	N/A
6	57	Female	55	Headache	16x15x14	-	-	-	-	+	-	-	15	3	-	-	+	N/A
7	63	Male	60	Cushing	10x5	-	-	-	-	-	+	-	3-4	<1	-	+	+	N/A
8	49	Female	47	Acromegaly	16x10	-	+	-	-	-	-	+	10-15	2	+	-	+	N/A
9	29	Male	27	High PRL	25x19	-	-	+	-	-	-	-	4-5	2-3	+	-	-	N/A
10	30	Male	29	Headache	17x16x16	-	+	+	-	-	-	-	10	2	+	-	-	N/A
11	66	Female	65	Cushing	25x14	Right cavernous sinus	-	-	-	-	+	-	5-10	1-2	+	+	-	N/A
12	36	Female	35	Headache	20x20	-	-	-	+	-	-	-	-	2	-	-	-	N/A
13	41	Female	37	Cushing	10x8	-	-	-	-	-	+	-	-	2	-	+	-	-
14	38	Female	36	Acromegaly	10x10	-	+	-	-	-	-	-	1-2	2-3	+	-	-	N/A
15	36	Male	35	Acromegaly	15x13	-	+	Rare	-	-	Rare	-	15-20	2-3	Rare	+	-	N/A
16	52	Male	48	Headache	21x19x16	-	-	-	+	+	-	-	3-5	1	-	+	N/A	-
17	26	Male	22	Headache	23x20	Right ICA, sphenoid sinus	+	+	-	-	-	-	10	5	+	-	N/A	-
18	32	Female	31	Acromegaly	25x22x19	Pressure on the optic chiasm	+	-	-	-	-	-	15-20	5-10	+	-	-	N/A
19	17	Male	8	Headache	22x28x20	-	-	-	Rare	-	-	+	70	8	N/A	N/A	N/A	N/A
20	42	Female	36	Cushing	17x12	-	-	-	-	-	+	-	-	2	N/A	N/A	N/A	N/A
21	49	Male	47	Headache	26.5x25	Pressure on the optic chiasm	-	-	-	+	-	-	3	3	-	-	+	N/A
22	53	Female	48	Headache	17x12x13	-	-	-	-	-	+	-	5-10	2	+	-	-	Rare
23	72	Male	67	Headache	25x20x17	Right cavernous sinus	-	-	+	+	-	-	2-3	1	Rare	-	N/A	-
24	25	Female	20	Acromegaly	12x11x14	-	+	-	-	-	-	-	5	2	+	-	N/A	-
25	56	Male	51	Headache	28x30x31	-	-	-	-	-	-	-	-	1	-	-	-	N/A
26	48	Male	47	Headache	26x26x25	-	-	-	+	+	-	-	-	-	-	-	-	-

Table I: Cont.

Patient no.	Age (years)	Gender	Age at Diagnosis (years)	Clinical features	PitNET size (mm)	Invasion	Hormonal immunohistochemistry results						P53 (%)	Ki67 (%)	Transcriptional factors			
							BH	PRL	FSH	LH	ACTH	TSH			PIT-1	T-PIT	G-2	ERα
27	20	Female	15	Headache	17x17	-	-	+	-	-	-	-	5-10	2	+	-	N/A	Rare
28	44	Female	41	Cushing	20x8	-	-	-	-	-	+	-	-	1	+	+	N/A	Rare
29	31	Male	29	Cushing	30x25x33	-	-	+	-	-	Rare	-	4-5	2-3	+	-	-	N/A
30	65	Male	50	High PRL	19x16	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
31	51	Male	43	Acromegaly	N/A	N/A	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
32	49	Female	38	Acromegaly	25x25	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
33	44	Female	33	Acromegaly	N/A	N/A	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
34	33	Male	22	Acromegaly	30x30	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
35	36	Male	33	Acromegaly	30x38	-	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
36	49	Male	46	Acromegaly	12x10	-	+	+	-	-	-	-	8-10	3	+	-	-	N/A
37	65	Female	62	Cushing	15x10	-	-	-	-	-	+	-	2-3	1	-	+	N/A	-
38	36	Male	35	Acromegaly	18x12	-	+	+	-	-	-	Rare	1-2	1-2	+	-	-	N/A
39	26	Female	23	Cushing	13x9	-	-	-	-	-	+	-	15	15	-	+	-	N/A
40	38	Male	23	Acromegaly	N/A	N/A	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
41	25	Female	24	Headache	26x16x21	-	+	Rare	-	+	-	-	4-5	1-2	+	N/A	N/A	N/A
42	43	Female	38	Acromegaly	20x15	-	+	+	-	-	-	+	3	3	+	N/A	N/A	+
43	28	Female	18	Headache	10x15	-	+	+	-	-	-	+	-	1	N/A	N/A	N/A	N/A
44	49	Male	47	Acromegaly	17x13	Right cavernous sinus	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
45	46	Male	45	High PRL	20x20x20	Right cavernous sinus, supracellar cistern, pressure on the optic chiasm	-	+	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
46	36	Male	35	Headache	48x39x45	Pressure on the optic chiasm	+	+	-	-	-	-	20-30	2-3	+	-	-	N/A
47	42	Male	32	Acromegaly	At its widest point 36	Pressure on the optic chiasm	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
48	51	Male	51	High PRL	At its widest point 20	-	-	+	-	-	-	-	<1	<1	+	-	-	N/A
49	44	Male	22	Headache	N/A	N/A	+	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
50	16	Male	16	High PRL	33x18	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
51	13	Female	10	Headache	10x10	-	+	-	-	-	+	-	1-2	1-2	-	+	N/A	N/A
52	19	Male	18	Headache	26x22x2	-	+	-	-	-	-	-	-	3	N/A	N/A	N/A	N/A
53	46	Female	37	High PRL	15x13	-	-	+	-	-	-	-	N/A	1	N/A	N/A	N/A	N/A

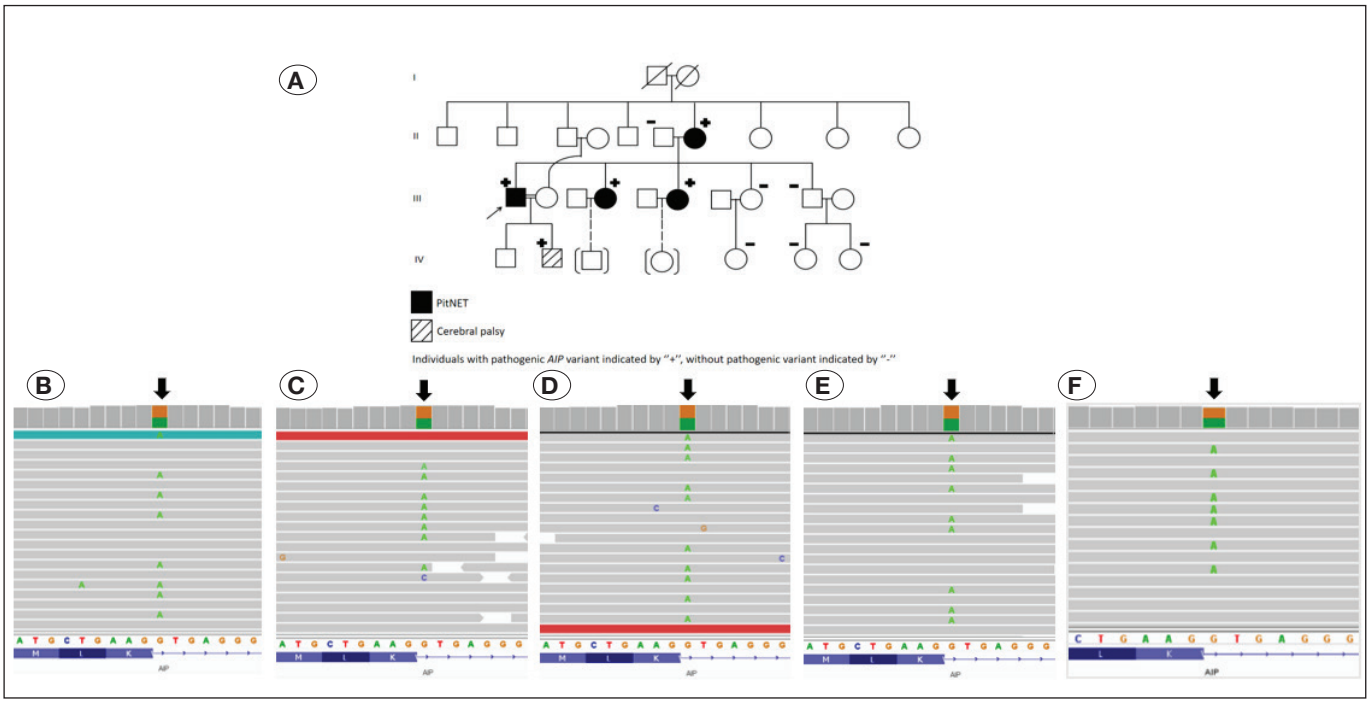

Figure 3: A) Pedigree of the Patient 3, **B)** Integrative Genomics Viewer (IGV) visualization of heterozygous c.468+1G>A variant in the *AIP* gene (NM_003977) in the Patient 3, **C, D, E, F)** IGV visualization of heterozygous c.468+1G>A variant in the *AIP* gene in individual II-5, III-2, III-3, and IV-2, respectively.

Patient #18

A 33-year-old female patient referred to our department had menstrual irregularity for the past 1.5 years and vision problems that developed in the last 9 months. The patient was primary infertile. Her parents were healthy and from the same village. The patient had three elder, healthy sisters. The family history was unremarkable (Figure 4A). In the pituitary MRI, a 30 × 20 mm macroadenoma in the pituitary gland, infiltrating the cavernous sinus on the right lateral, and surrounding the right internal carotid artery at 300 degrees was detected. The pathology showed a dense granular somatotropinoma. Based on immunohistochemistry, it was evaluated as an atypical PitNET because ki-67 and p53 were 5%–10% and 15%–20%, respectively. GATA-2 and T-PIT were negative, and PIT-1 was positive.

Since the patient's parathormone level was 108 pg/mL (Normal value: 15–65 pg/mL) and calcium was 11.8 mg/dL (Normal value: 7.6-10.4 mg/dL), 8 × 7 mm parathyroid adenoma was detected in the thyroid scintigraphy.

In the genetic analysis, a heterozygous c.1102_1104del (p.Glu368del) in-frame pathogenic variant was detected in the 8th exon of the *MEN1* gene (NM_000244) (Figure 4B). The detected variant was not reported in gnomAD, 1000G, and ESP5400. This variant was reported as pathogenic in the ClinVar. Varsome predicted this variant as pathogenic (PM1, PM2, PM4, PP5), and Franklin predicted it as likely pathogenic (PM2, PM4, PP5). Segregation analysis showed that this variant had arisen *de novo* (Figure 4C, D).

In total, 20 VUS were detected in 18 patients. Of those, 7 were female and 11 were male. The most common VUS-detected genes were *MSH6* (n=3, 15%), *CDH23* (n=3, 15%), and *RET* (n=3, 15%). Two different VUS were detected in *MLH1* and *GNAS* genes. One of the patients (patient 14) carried two different VUS in the *RET* gene. In another patient (patient 19), VUS was detected in both the *CDH23* and the *MSH6* gene. No significant correlation was found between the detection rates of VUS and immunohistochemical staining.

The pathogenic, likely pathogenic, and VUS variants detected in the patients and the clinical characteristics of the patients are summarized in Table II.

DISCUSSION

A pathogenic/likely pathogenic variant was detected in two (3.7%) and VUS in 18 patients (33.9%). In the literature, studies reporting genetic panel testing had a 4.5% (20) pathogenic/likely pathogenic variant detection rate in their unselected groups, similar to our findings. In the studies conducted with the pediatric group (28) and patients with acromegaly (25), these rates were 9.1% and 46%, respectively. This result shows that the age at diagnosis and adenoma pathology significantly affect pathogenic/likely pathogenic variant detection rates. Although almost all study participants in this study were diagnosed with macroadenoma, it was found that it did not directly affect the variant detection rate.

In this study, a pathogenic variant was found in the *AIP* gene in one case with familial isolated PitNET (100%). Although

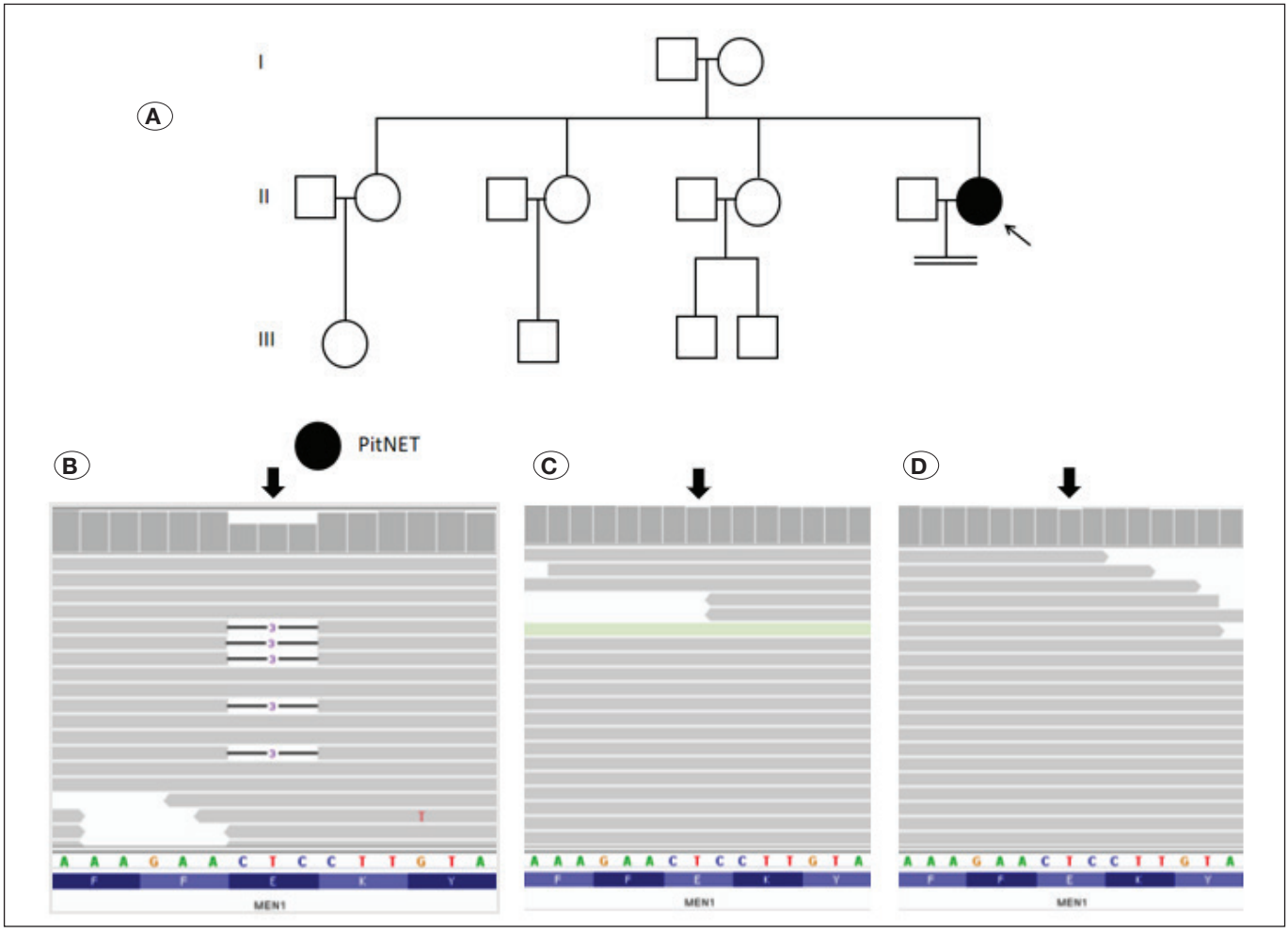

Figure 4: A) Pedigree of the Patient 18, **B)** IGV visualization of heterozygous c.1102_1104del (p.Glu368del) variant in the *MEN1* gene (NM_000244) in the Patient 18, **C, D)** Segregation analysis revealed that this variant had arisen *de novo.*

this rate is quite high compared to that in the literature, it was not considered reliable due to the small number of patients. Nevertheless, this result highlights the importance of the *AIP* gene in the etiology in familial cases. There is an important study that reveals a risk score for which patients with the AIP gene should be analyzed, and this score can be easily applied by clinicians (6). According to this risk score, family history, age of onset, presence of GH excess, and PitNET size can predict the probability of the *AIP* gene pathogenic variant detection with sufficient sensitivity and specificity. Furthermore, according to this risk category system, it is recommended to analyze the *AIP* gene in patients with a risk >20%, while it is recommended that genetic analysis can be performed for people with a risk between 5% and 19%, but the risk should be individualized. The *AIP* gene screening was not recommended in patients with <5% risk. When the patient with the *AIP* gene variant in this study was assessed according to this risk score, the presence of a family history, excessive growth hormone, the presence of a macroadenoma, and diagnosis at the age of 32 led us to the risk of 18%, which is an intermediate risk. This result also points to the need for improving the risk score by including more patients.

Pathogenic variant was detected in the *MEN1* gene in a patient with PitNET and parathyroid adenoma. Although the detection of pathogenic variants in our single case (100%) with multiple endocrine neoplasia type 1 (MEN1) clinical findings is quite high compared to the rate of 24% reported in the literature (29), this is attributable to the small sample. This patient is also a good example of why all patients with PitNET should be investigated for other endocrine abnormalities. No pathogenic/likely pathogenic variant was found in the *MEN1* gene in any of the isolated PitNET cases. In a recent study conducted with isolated PitNET cases, a variant in the *MEN1* gene was detected in 3.4% of the patients (9). However, this rate may be higher due to the inclusion of only patients younger than 30 who were diagnosed with macroadenoma. Although patients with macroadenoma were included in this study, the mean age at diagnosis was 36.07.

The c.149A>G (p.His50Arg) variant detected in the patient 28 was investigated in the in-house database that included 181 patients. These patients had different clinics and were previously studied with the same kit. Only one patient in in-house database had the variant. The case was 9 years old,

Table II: The Pathogenic, Likely Pathogenic, and VUS Variants Were Detected in the Patients

Patient no.	Gender	Age at Diagnosis (years)	Clinical features	Gene (NM number)	Variant/Zygosity	Clinvar	Varsome	Franklin
1	Male	11	Headache	-	-	-	-	-
2	Male	42	Acromegaly	-	-	-	-	-
3	Male	32	Acromegaly	*AIP* (NM_003977)	c.468+1G>A heterozygous	LP	P	LP
4	Female	41	Headache	*MSH6* (NM_000179.3)	c.1484G>A (p.Arg495Gln) heterozygous	VUS	VUS	VUS/LP
5	Female	49	Acromegaly	-	-	-	-	-
6	Female	55	Headache	-	-	-	-	-
7	Male	60	Cushing	*MSH6* (NM_000179.3)	c.1069G>A (p.Asp357Asn) heterozygous	VUS	VUS	VUS
8	Female	47	Acromegaly	-	-	-	-	-
9	Male	27	High PRL	-	-	-	-	-
10	Male	29	Headache	-	-	-	-	-
11	Female	65	Cushing	*MLH1* (NM_000249.4)	c.1612T>C (p.Trp538Arg) heterozygous	-	LP	VUS
12	Female	35	Headache	-	-	-	-	-
13	Female	37	Cushing	-	-	-	-	-
14	Female	36	Acromegaly	*RET* (NM_020975.6)	c.3094G>A (p.Gly1032Ser) Heterozygous	VUS	VUS	VUS/LP
				RET (NM_020975.6)	c.1946C>T (p.Ser649Leu) heterozygous	VUS	VUS	VUS
15	Male	35	Acromegaly	*CDH23* (NM_022124.6)	c.205G>A (p.Val69Met) heterozygous	VUS	VUS	VUS
16	Male	48	Headache	*TSC2* (NM_000548.5)	c.745G>A (p.Val249Ile) heterozygous	VUS	VUS	VUS
17	Male	22	Headache	-	-	-	-	-
18	Female	31	Acromegaly	*MEN1* (NM_000244)	c.1102_1104del (p.Glu368del) heterozygous	P	P	LP
19	Male	8	Headache	*MSH6* (NM_000179.3)	c.2074A>C (p.Lys692Gln) heterozygous	VUS	VUS	VUS
				CDH23 (NM_022124.6)	c.5692G>A (p.Ala1898Thr) heterozygous	VUS	VUS	VUS
20	Female	36	Cushing	*MSH2* (NM_000251.3)	c.557A>G (p.Asn186Ser) heterozygous	LB/VUS	VUS	VUS
21	Male	47	Headache	-	-	-	-	-
22	Female	48	Headache	-	-	-	-	-
23	Male	67	Headache	*TSC1* (NM_000368.5)	c.2247G>T (p.Met749Ile) heterozygous	-	VUS	VUS
24	Female	20	Acromegaly	*VHL* (NM_000551.4)	c.86G>T (p.Gly29Val) heterozygous	-	VUS	VUS

Table II: Cont.

Patient no.	Gender	Age at Diagnosis (years)	Clinical features	Gene (NM number)	Variant/Zygosity	Clinvar	Varsome	Franklin
25	Male	51	Headache	*MLH1* (NM_000249.4)	c.1360G>C (p.Gly454Arg) heterozygous	B/LB/ VUS	VUS	VUS
26	Male	47	Headache	*CDH23* (NM_022124.6)	c.3301A>G (p.Ile1101Val) heterozygous	VUS	VUS	VUS
27	Female	15	Headache	-	-	-	-	-
28	Female	41	Cushing	*SDHD* (NM_003002.4)	c.149A>G (p.His50Arg) heterozygous	B/LB/ VUS	B	B
29	Male	29	Cushing	-	-	-	-	-
30	Male	50	High PRL	-	-	-	-	-
31	Male	43	Acromegaly	-	-	-	-	-
32	Female	38	Acromegaly	-	-	-	-	-
33	Female	33	Acromegaly	-	-	-	-	-
34	Male	22	Acromegaly	-	-	-	-	-
35	Male	33	Acromegaly	-	-	-	-	-
36	Male	46	Acromegaly	-	-	-	-	-
37	Female	62	Cushing	*GNAS* (NM_016592.5)	c.133C>T (p.Pro45Ser) heterozygous	-	VUS	VUS
38	Male	35	Acromegaly	-	-	-	-	-
39	Female	23	Cushing	-	-	-	-	-
40	Male	23	Acromegaly	-	-	-	-	-
41	Female	24	Headache	-	-	-	-	-
42	Female	38	Acromegaly	-	-	-	-	-
43	Female	18	Headache	-	-	-	-	-
44	Male	47	Acromegaly	-	-	-	-	-
45	Male	45	High PRL	*PMS2* (NM_000535.7)	c.953A>G (p.Tyr318Cys) heterozygous	B/LB/ VUS	LP	VUS
46	Male	35	Headache	-	-	-	-	-
47	Male	32	Acromegaly	-	-	-	-	-
48	Male	51	High PRL	*GNAS* (NM_016592.5)	c.577G>A (p.Glu193Lys) heterozygous	-	VUS	VUS
49	Male	22	Headache	-	-	-	-	-
50	Male	16	High PRL	-	-	-	-	-
51	Female	10	Headache	-	-	-	-	-
52	Male	18	Headache	*RET* (NM_020975.6)	c.2372A>T (p.Tyr791Phe) heterozygous	B/LB/ VUS/LP	B	B
53	Female	37	High PRL	*RET* (NM_020975.6)	c.2330A>G (p.Asn777Ser) heterozygous	VUS	VUS	VUS/LP

and the test indication was different. Consequently, the frequency of this variant in the in-house database was calculated as 0.55%. The frequency of the same variant in community databases was 0.64% and 0.66% in the gnomAD and 1000G, respectively. In Kytölä et al. (14), the variant (H50R) we detected in our patient was found to be heterozygous in two patients with Merkel cell carcinoma and mid-intestinal carcinoid and evaluated as pathogenic. Cascón et al. reported in their letter to the editor that they detected this variant in 6 out of 200 healthy subjects in their cohort and that this variant is a rare polymorphism rather than a pathogenic variant (7). We agree with Cascón and colleagues (7); however, the 9-year-old patient will be followed to determine whether PitNET develops.

In a recent study (8), 211 patients diagnosed with Cushing disease were sequenced for the *CDKN1B* gene. Variants were detected in five patients (2.3%). Because all of the cases with variants were younger than 18, sequence analysis for this gene was recommended for cases diagnosed at an early age. However, the mean age of Cushing's disease in our study was 44.1, and the youngest patient was 23 years old. This may explain the absence of variants in the *CDKN1B* gene in our study.

In another recently conducted study with 192 Cushing's disease cases, variants in the *DICER1* gene were detected in seven patients (3.6%) (19). However, all variants were reported as B, LB, or VUS in ClinVar and LB according to ACMG criteria. As a result, it was emphasized that more patients and studies are needed to show the relationship between *DICER1* gene variants and Cushing's disease. The absence of variants in the *DICER1* gene in eight cases with Cushing's diagnosis in our study supports this view.

In the literature, there are three publications on the possible relationship between hereditary nonpolyposis colorectal cancer syndrome (HNPCCS-Lynch syndrome) and PitNETs. The first detected PitNET in two cases and pituitary carcinoma in one case in the HNPCCS cohort of 910 patients (5). In another study, corticotroph macroadenoma was detected in a HNPCCS case (30). In the most recent study, corticotroph macroadenoma was detected in a patient who was not previously diagnosed with HNPCCS, but a pathogenic variant was found in the *MSH2* gene (18). These three studies show that the penetration of PitNETs is low in patients diagnosed with HNPCCS and that corticotroph macroadenomas are common in this group. In our study, although pathogenic variants were not detected in HNPCCS-related genes, seven VUS were detected. Only 2 of the 7 cases had corticotropinoma. All these results and the small number of patients show that more studies are required to clarify the relationship between HNPCCS and PitNETs, and the HNPCCS cases need to be followed up in terms of PitNETs.

This study has some limitations. Because the DNA obtained from the peripheral blood of the patients was studied, the impact of the detected variants at the tissue level could not be investigated. Although copy number variations (CNVs) in PitNET-related genes were not frequently reported, multiplex ligation-dependent probe amplification or array comparative genomic hybridization was planned only in the presence of clinical suspicion of a specific gene because CNVs could not be detected in the next-generation sequencing analysis. This may have led us to overlook the CNVs that can be detected in isolated cases.

CONCLUSION

To the best of our knowledge, this is the first genetic panel study in Turkish patients with PitNETs. Despite being the panel study that includes the largest number of genes associated with PitNETs in the literature, it failed to provide a significant genetic diagnosis contribution compared to other panel studies. This shows that genes other than those clearly associated with pituitary adenoma, such as *AIP* and *MEN1,* are rare factors in the etiology of pituitary adenoma. This finding also indicates that the genetic etiology in PitNETs has not been fully elucidated yet, and it is likely that new genes will be identified in the future.

Declarations

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AUTHORSHIP CONTRIBUTION

Study conception and design: CA, AIG
Data collection: OS, FB
Analysis and interpretation of results: CA, BBG
Draft manuscript preparation: CA
Critical revision of the article: BBG, FB, AIG
All authors (CA, OS, BBG, FB, AIG) reviewed the results and approved the final version of the manuscript.

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