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Aryl hydrocarbon receptor interacting protein and syndromic gene variants detected in Turkish isolated pituitary adenoma families by whole exome sequencing

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Genetic causes of familial isolated pituitary adenomas (FIPAs) remain mostly elusive. A cohort of 20 FIPA cases from 12 different geographical regions of Türkiye was included to characterize clinical and genetic features. Whole exome sequencing (WES) was performed on genomic DNA of index cases, followed by confirmation through Sanger sequencing utilizing indexes and their relatives to interpret disease associated variants. Index cases among homogeneous ($n=10$) and heterogeneous ($n=10$) FIPA groups (45% female /55% male), age at diagnosis was 36.3 ± 11.98 years, median follow-up was 103 months. GH-secreting adenomas dominated homogeneous group (60% vs. 30% of heterogeneous group). Two predefined *AIP* variants [p.(Arg304Ter) and p.(Arg81Ter)] and a novel *AIP* variant at splice acceptor site [(c.646-1G > C)] were detected in three families (15%). Syndromic heterozygous novel *NF1* [p.(Thr1295Ala)], *TSC1* [p.(Arg517Gln)], *SDHB* [p.(Glu176Gly)] and *CDH23* [p.(Ala765Val)] variants were detected in four FIPA families, along with novel candidate genes in the remaining patients of the cohort. Among all detected variants, three [p.(Arg81Ter) and (c.646-1G > C) in *AIP*, and p.(Glu216GlysfsTer61) in *TINF2*] were classified as pathogenic according to ACMG. *AIP* mutation frequency was 15% in our cohort. A novel *AIP* variant, and novel variations in syndromic genes were identified, along with the introduction of candidate genes. WES method is a crucial approach to identify new rare genetic variants in familial settings, and it will pave the way for future studies on targeted therapies.

Keywords FIPAs, Whole exome sequencing, *AIP*, Novel variants, Syndromic genes

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Pituitary neuroendocrine tumours (PitNETs) occur sporadically, with 5% are believed to be of familial origin. Familial PitNETs may present as a part of isolated familial pituitary adenomas (FIPA) or may be a component of various syndromic diseases, such as classical MEN1 syndrome or Carney complex or hereditary paraganglioma syndromes (3PAs), MEN4, Tuberous Sclerosis-TCS, Neurofibromatosis type 1, DICER1 syndrome, Lynch syndrome, CDH23 syndrome[1–5]. FIPA is an autosomal-dominant disease defined by the presence of pituitary adenoma (PA) in two or more relatives. FIPA families can be heterogeneous (when more than one type of PA is present in the same family) or homogeneous. Most *AIP* mutation-positive FIPA patients present with somatotrophinomas or somatolactotrophinomas, while some exhibit lactotrophinomas, and non-functioning adenomas and corticotropinomas are rare[6,7]. FIPA related tumors are generally large, aggressive, occur at younger ages, and are resistant to treatment with first-generation somatostatin receptor ligands (SRLs)[6]. Although germline *AIP* mutations account for ~20% of all FIPA cases, the genetic profile remains unknown in many instances[8–13]. Identifying potential new genetic variants across different geographical regions of the same country is essential for uncovering the hereditary causes of FIPAs. Therefore, we aimed to investigate the etiopathogenesis of PAs utilizing whole exome sequencing (WES) in affected individuals from FIPA families in different geographical regions of Türkiye.

Methods

In this multicenter study, clinical data and medical history of 45 FIPA families were followed-up for 2 to 276 months in 12 pituitary clinics located in different geographical regions of Turkiye, which were re-evaluated from the records by two senior endocrinologists. Functional PA diagnosis was based on clinical signs and symptoms, laboratory tests demonstrating elevated basal hormone levels and/or abnormal dynamic tests. Results were interpreted in accordance with the Endocrine Society's current guidelines[14–16]. In patients with GH-secreting PA, acromegaly is diagnosed if typical clinical acral features are accompanied by IGF-1 levels above the upper normal range of age-sex matched range (14). In PRL-secreting adenomas, serum PRL levels are usually above 250 ng/ml and the presence of galactorrhea and sexual dysfunction makes the diagnosis of prolactinoma (15). Definitive diagnosis of Cushing's disease due to hypercortisolemia caused by ACTH-secreting adenoma is made by abnormal circadian rhythm with failure to suppress hypercortisolemia with overnight 1 mg dexamethasone suppression test (DST) and low-dose 2-day dexamethasone suppression test (LDDT), and elevation of 24-hour urinary free cortisol (UFC) (16).

Pituitary MRI reports were provided by experienced neuroradiologists. The degree of cavernous sinus invasion of PAs was determined by Knosp classification, where grades 0–2 are considered non-invasive, and grades 3 and 4 indicated invasive characteristics[17]. The diagnosis was supported histopathologically in operated GH- and ACTH-secreting and nonfunctional PAs.

Among the 45 families meeting the FIPA criteria recruited from various centers, 20 index cases were identified as eligible for the study. These cases fulfilled the clinical, laboratory, and radiological criteria for inclusion and had histopathological diagnosis reports documented in their medical records. Of these families, 20 index cases and 15 relatives (alive and reachable) with proven history of pituitary adenoma were included in the study.

This human study was conducted in compliance with the principles in Declaration of Helsinki and was performed upon the approval of Clinical Ethics Committee of Baskent University Faculty of Medicine (project #KA21/247). All procedures strictly adhered to current guidelines and regulations. Peripheral blood samples were obtained following written informed consents of the participants.

Genetic analyses

WES was performed on DNA obtained from the peripheral blood of 20 index cases at the service of Medical Genetic Diagnostic Center in Izmir, Türkiye that utilized llumina NextSeq 550 system[18]. Variant annotations and subsequent filtering were achieved using NGS Cloud (www.ngscloud.com; Pairend Biotechnology LLC, Manisa, Türkiye), which is a cloud-based genetic data analysis platform supported through artificial intelligence[19]. Sequence reads were visualized through IGV 2.9.4 program, where minor allele frequencies (MAFs) were obtained from GnomAD, dbSNP, 1000 Genomes Project, Exome Sequencing Project, TopMED, Greater Middle East Variome Project and *in house* allele frequency of the NGS Cloud.

WES data was initially filtered for PA-related genetic variants using a gene panel developed from the literature. This panel comprised a comprehensive list of genes that are relevant to the pathogenesis of PAs and associated symptoms. It also included key molecular pathways, genes involved in epigenetic mechanisms, and additional genes identified through bioinformatics analyses (Supplementary Table S1). Variant interpretation and pathogenicity scores were achieved under the provisions of American College of Medical Genetics (ACMG) guidelines along with ClinVar database for PA-associated genes. This was followed by filtering for rare variants ($MAF \leq 0.01$), in which in silico prediction tools have been a guide for plausible impact of the variant. The prioritization among novel genetic variants was achieved through functional relevance of the genes to disease pathogenesis. In this way, an initial draft of candidate genes was compiled and validated through Sanger sequencing, first in the index cases, and then in their relatives, in an effort to eliminate unrelated genes in PA pathogenesis. Sanger sequences were analysed by CLC Main Workbench. A list of primers utilized both in PCR amplification and Sanger sequencing are listed in Supplementary Table S2.

Results

Among 20 index cases, 9 were females (45%), 11 were males (55%). There were 10 homogeneous FIPA families; 6 somatotrophinomas/ 4 prolactinomas. Index cases of 10 heterogeneous FIPA families were those with somatotrophinomas ($n=3$), prolactinomas ($n=4$) and corticotropinomas ($n=3$). The median age at diagnosis of index cases was 36.3 ± 11.98 years and median follow-up period was 103 months (range 2-276).

The frequency of GH-secreting adenomas in the homogeneous FIPA group was twice as high (60%) as in the heterogeneous group (30%). The mean delay in diagnosis of index cases with acromegaly was 24 months (range 24–132). 25% of index cases had micro (≤ 10 mm), 65% had macro (> 10 mm), and 10% had giant (≥ 40 mm) adenomas. Adenomas were classified as 65% Knosp 0–2 and 35% Knops 3–4 according to their degree of invasion.

Among relatives; there were 3 GH- and 4 PRL-secreting adenomas in homogeneous group ($n = 7/20$) and 2 PRL-, 3 GH-secreting and 3 apparently non-functional (clinically and hormonally inactive) adenomas in heterogeneous group ($n = 8/10$).

Table 1 summarizes the clinical findings of index cases with genetic variants confirmed in cases and available affected relatives. Figure 1 shows family pedigrees, highlighting the affected members and available clinical histories, as well as indicating which patients provided genetic materials. Accordingly, genetic material from the relatives of five index cases (2, 7, 10, 11 and 15) were not available. In 3 FIPA families, two known [p.(Arg304Ter) and p.(Arg81Ter)] and one novel [splice acceptor site (c.646-1G > C)] *AIP* variants were detected (15%). Moreover, (c.646-1G > C) and p.(Arg81Ter) *AIP* variants in cases 12 and 13, respectively, along with p.(Glu216GlysfsTer61) in *TINF2* in case 2 were determined as pathogenic by ACMG. In addition, variants in *RUNX2* p.(Gln68_Gln71dup) in case 9 and *NF1* p.(Thr1295Ala) in case 17 were reported as "conflicting classifications of pathogenicity" in the ClinVar database. All these variants, except for the one in *TINF2*, were shared among the relatives in pertinent families.

Clinical characteristics and genetic variation(s) of both index cases and relatives with *AIP* mutations are summarized in Table 2. Among the *AIP* positive families, Case 12 and his daughter had macroadenoma with cavernous sinus invasion. Despite these tumour characteristics, the father is in drug-free remission following surgery and SRL treatments. Excess GH was detected in daughter diagnosed with prolactinoma. Her father had acromegaly and upon inspection her nose felt slightly enlarged, although she reported no complaints about it. Nevertheless, the additional diagnosis of clinically silent acromegaly was not overlooked in her. She initially preferred DA treatment, however, the adenoma did not shrink and medication could not be discontinued, despite clinical and hormonal response to the treatment. In the other heterogeneous FIPA family (Case 13 and his sister), who carried no genetic variant other than the known *AIP* p.(Arg81Ter) mutation, adenoma was larger and more invasive in younger affected member. The brother with acromegaly had his disease activity controlled through three surgeries and a combination of two medications. In contrast, his sister, who previously suffered from apoplexy, achieved remission from prolactinoma, and her DA treatment was discontinued one year later.

Although other components of NF1, TCS, 3PAs and Usher syndromes were absent, heterozygous novel *NF1* [p.(Thr1295Ala)], *TSC1* [p.(Arg517Gln)], *SDHB* [p.(Glu176Gly)] and *CDH23* [p.(Ala765Val)] variants were detected in 4 FIPA families (Table 3). Candidate genes other than *AIP* and syndromic genes in our 13 FIPA families are summarized in Table 4.

Discussion

This study presents the first genetic analysis of a large cohort of FIPA families from various geographical regions of Turkiye, utilizing WES. We identified three *AIP* variants in three different families. Two pathogenic *AIP* variants previously described in the literature p.(Arg304Ter) and p.(Arg81Ter) were identified in two independent families from Western Black Sea Region, whereas the novel *AIP* variant (c.646-1G), located in intron 4–5 that potentially affects the splice acceptor site, was identified in a family from Eastern Anatolia region. Consistent with the literature, we determined *AIP* mutation frequency as 15% in our FIPA cohort[6–8,20].

Among limited number of studies screening *AIP* variations in sporadic young-onset somatotropinomas from Turkiye, prevalence of *AIP* mutation was found to be 1%, 2.2% or 2.1%, where p.(Arg304Ter) has been the prominent mutation[21–23]. In this respect, Arg304Ter was identified by Sanger sequencing in 7 acromegaly patients in a large FIPA family[24]. However, in our previous study, we identified only two homozygous missense SNPs (rs641081 [Q228K] and rs4930195 [Q307R]) in *AIP* by Sanger sequencing 14 different FIPA families[25]. The Arg304Ter mutation, located in the hotspot region of *AIP*, was most frequently reported in FIPAs from Ireland, Romania, Britain, Italy, USA, India and Mexico, while Arg81Ter mutation had been reported in families from Brazil, USA, India and the UK[10,12,26–29].

Interestingly, in homogeneous FIPA group, acromegalic index Case 6, who carried both pathogenic *AIP* p.(Arg304Ter) mutation and a novel variant p.(Gly334del) in *Sequestosome 1* (*SQSTM1*) gene, responded positively to first-generation SRL and her adenoma shrank by 50%. This contradicts the findings that state acromegaly patients harbouring *AIP* variations are unresponsive to first-generation SRLs[8,11,27,30]. This provoked the hypothesis of a possible impact of accompanying novel variant in treatment response. Accordingly, Tulipano et al. reported that in rat pituitary GH3 tumor cells, octreotide treatment enhanced autophagic flux through SQSTM1/p62 protein downregulation[31]. Further studies are needed to clarify the impact of this novel variant in SQSTM1/p62 protein expression. Unfortunately, we did not have the chance to support the index case's positive response to first-generation SRL, as no data could be obtained from the acromegalic sibling, who participated only with a blood sample and shared the same variations with the index.

In a heterogeneous FIPA family, we described a novel *AIP* (c.646-1G > C) variant for the first time in the literature. This novel *AIP* variant was accompanied by two different novel genetic variations as p.(Pro281Leu) in *SUFU* and p.(Ile240Thr) in *LGALS3*. Recently, it has been reported that PKA/SUFU/GLI1 signalling pathway, known as the Hedgehog signalling, is activated in primary PA cells, as well as in surgical PA samples, leading to inhibition of apoptosis and promotion of the cell cycle[32]. *LGALS3* encodes Galectin-3, which has an important role in pituitary cell proliferation and tumor progression[33]. Galectin-3 is a well-recognized biomarker for the aggressive behaviour of PRL-secreting adenomas and their prognosis[34]. The co-occurrence of these variations may result in unexpected outcomes, which merits functional evaluation.

Index number /FIPA type	Sex (F/M)/ Age (yrs)	Adenoma type/ size (mm)	Gene	Chr Position GRCh37 - hg19	Variant	Consequence	Pathogenicity	In Silico Tools				
								GERP++	DANN	SIFT	LIST-S2	ALPHA MISSENSE
1/ HO	F/23	PRL-secreting/9	*NR5A1 (SF1)*	Chr9:127262707 C > T	NM_004959.5:c.532 G > A	p.(Gly178Arg)	ACMG: VUS ClinVar: Not reported	4.84	D (0.9989)	T (0.56)	T (0.8883)	A (0.378)
			PKD1	Chr16:2150248 C > T	NM_001009944.3:c.9631 G > A	p.(Ala3211Thr)	ACMG: VUS ClinVar: VUS	4.69	T (0.7633)	T (0.56)	T (0.3465)	LB (0.057)
2/HO	M/22	GH-secreting/15	*TINF2*	Chr14:24710039 T > TC	NM_001099274.3: c.646dup	p.(Glu216GlyfsTer61)	ACMG: **Pathogenic** ClinVar: Not reported	NA	NA	NA	NA	NA
			ATP2B3	ChrX:152830460 G > C	NM_001001344.3:c.3241 G > C	p.(Glu1081Gln)	ACMG: VUS ClinVar: Not reported	4.63	D (0.9977)	T (0.07)	D (0.9383)	LB (0.114)
3/HO	M/33	GH-secreting/14	*RUNX2*	Chr6:45630999 C > T	NM_001024630.4:c.1169 C > T	p.(Pro390Leu)	ACMG: VUS ClinVar: Not reported	5.31	D (0.9950)	T (0.14)	T (0.4790)	LP (0.626)
4/HO	M/59	PRL-secreting/22	*LGALS3*	Chr14:55604881 C > G	NM_002306.3 :c.137 C > G	p.(Pro46Arg)	ACMG: VUS ClinVar: Not reported	5.34	T (0.9829)	D (0)	D (0.8980)	A (0.5)
			DRD4	Chr11:640307 G > A	NM_000797.4: c.1057 + 1 G > A	splice donor	ACMG: VUS ClinVar: Not reported	NA	NA	NA	NA	NA
5/HO	F/27	PRL-secreting/6	*SYTL3*	Chr6:159129392 G > T	NM_001242395.1:c.485 G > T	p.(Ser162Ile)	ACMG: LB ClinVar: Not reported	4.59	T (0.9879)	D (0.16)	NA	LB (0.152)
6/HO	F/43	GH-secreting/14	*SQSTM1*	Chr5:179260612 CAGG > C	NM_003900.4:c.1001_1003del	p.(Gly334del)	ACMG: VUS ClinVar: VUS	NA	NA	NA	NA	NA
			AIP	Chr11:67258381 C > T	NM_003977.2:c.910 C > T	p.(Arg304Ter)	ACGM: VUS ClinVar: Pathogenic	5.39	D (0.9964)	NA	NA	NA
7/HO	M/32	GH-secreting/60	*ATP2B3*	ChrX:152818590 G > A	NM_001001344.2:c.1921 G > A	p.(Asp641Asn)	ACMG: VUS ClinVar: Not reported	5.44	D (0.9986)	T (0.23)	D (0.9018)	LB (0.102)
			TSC1	Chr9:135781415 C > T	NM_001162427.1: c.1550 G > A	p.(Arg517Gln)	ACGM: VUS ClinVar: LB	6.05	D (0.991)	T (0.23)	NA	LB (0.084)
			SSTR4	Chr20:23017082 T > C	NM_001052.2:c.962 T > C	p.(Phe321Ser)	ACMG: VUS ClinVar: Not reported	3.65	T (0.7532)	T (1)	T (0.5168)	LB (0.099)
			FGFR2	Chr10:123260394 T > G	NM_022970.3:c.1510 A > C	p.(Ile504Leu)	ACGM : VUS ClinVar: Not reported	5.76	T (0.9756)	T (0.36)	T (0.6349)	LB (0.105)
8/HO	F/38	GH-secreting/18	*BRAF*	Chr7:140434597 G > GA	NM 004333.4:c.2128-28dup	Intronic	ACGM: VUS ClinVar: VUS	NA	NA	NA	NA	NA
9/HO	M/51	PRL-secreting/15	*RUNX2*	Chr6:45390433 G > GCAG CAGCAGCAA	NM_001024630.4:c.174_185dup	p.(Gln68_Gln71dup)	ACGM: VUS ClinVar: Conflicting classifications of pathogenicity	NA	NA	NA	NA	NA
10/HO	M/32	GH-secreting/7	*APC*	Chr5:112173317 A > G	NM_000038.5:c.2026 A > G	p.(Ile676Val)	ACGM : VUS ClinVar: VUS	5.99	D (0.9966)	T (0)	NA	LB (0.232)
11/HE	F/47	GH-secreting/12	*RARB*	Chr3:25215908 C > T	NM_001290216.3:c.20 C > T	p.(Ala7Val)	ACMG : VUS ClinVar: Not reported	5.88	D (0.9972)	D (0.03)	T (0.5300)	LB (0.075)
			SYTL3	Chr6:159086521 G > A	NM_001242395.2:c.205 G > A	p.(Val69Met)	ACGM: LB ClinVar: Not reported	5.8	T (0.9575)	T (0.05)	NA	LB (0.102)
12/HE	M/37	GH-secreting/23	***AIP***	Chr11:67257786 G > C	NM_003977.2:c.646-1 G > C	Splice acceptor site (intron 4–5)	ACMG: **Pathogenic** ClinVar: Not reported	NA	NA	NA	NA	NA
			LGALS3	Chr14:55611955 T > C	NM_002306.3:c.719 T > C	p.(Ile240Thr)	ACMG : VUS ClinVar: Not reported	5.77	D (0.9971)	D (0)	D (0.8707)	LP (0.658)
			SUFU	Chr10:104356982 C > T	NM_016169.3:c.842 C > T	p.(Pro281Leu)	ACGM : VUS ClinVar: VUS	6.03	D (0.9952)	D (0.3)	D (0.9881)	LB (0.14)
13/HE	M/27	GH-secreting/40	***AIP***	Chr11:67254618 C > T	NM_003977.2:c.241 C > T	p.(Arg81Ter)	ACMG: **Pathogenic** ClinVar: Pathogenic	5.22	D (0.9978)	NA	NA	NA
14/HE	M/30	PRL-secreting/29	*SDHB*	Chr1:17354257 T > C	NM_003000.2:c.527 A > G	p.(Glu176Gly)	ACGM : VUS ClinVar: VUS	5.76	D (0.9978)	D (0)	D (0.9671)	A (0.388)

Continued

Index number /FIPA type	Sex (F/M)/ Age (yrs)	Adenoma type/ size (mm)	Gene	Chr Position GRCh37 - hg19	Variant	Consequence	Pathogenicity	In Silico Tools				
								GERP++	DANN	SIFT	LIST-S2	ALPHA MISSENSE
15/HE	F/42	ACTH-secreting/12	*RARB*	Chr3:25216009 C > A	NM_001290216.3:c.121 C > A	p.(Leu41Ile)	ACMG : VUS ClinVar: Not reported	5.88	D (0.9935)	T (0.04)	T (0.3260)	LB (0.071)
16/HE	M/60	PRL-secreting/45	*DRD3*	Chr3:113890746 A > G	NM_000796.3:c.94 T > C	p.(Tyr32His)	ACGM : VUS ClinVar: Not reported	5	D (0.9982)	D (0)	D (0.8829)	LB (0.203)
17/HE	F/22	PRL-secreting/12	*NF1*	Chr17:29562948 A > G	NM_000267.3:c.3883 A > G	p.(Thr1295Ala)	ACGM: VUS ClinVar: Conflicting classifications of pathogenicity	5.94	T (0.9850)	T (0.43)	T (0.7980)	LB (0.055)
18/HE	F/19	PRL-secreting/18	*NPR2*	Chr9:35800044 A > G	NM_003995.3:c.1013 A > G	p.(Tyr338Cys)	ACGM: VUS ClinVar: VUS	5.69	D (0.9984)	D (0)	D (0.9622)	LP (0.656)
19/HE	M/36	ACTH-secreting/6	*PTTG1*	Chr5:159851328 A > G	NM_004219.2:c.361 A > G	p.(Asn121Asp)	ACGM : VUS ClinVar: Not reported	5.17	T (0.9866)	T (0.73)	NA	LB (0.106)
20/HE	F/46	ACTH-secreting/5	*CDH23*	Chr10:73570263 C > T	NM_001171934.1:c.2294 C > T	p.(Ala765Val)	ACGM : VUS ClinVar: VUS	5.67	D (0.9973)	(0.02)	D (0.9498)	A (0.516)
			KCNQ1	Chr11:2683253 G > A	NM_181798.1:c.1456 G > A	p.(Ala486Thr)	ACGM: VUS ClinVar: VUS	4.59	T (0.9082)	T (0.19)	T (0.8037)	LB (0.078)

Table 1. Clinical features and genetic variants confirmed in index cases and available affected relatives. FIPA, Familial Isolated Pituitary Adenoma; HO, Homogeneous; HE, Heterogeneous; Age: At diagnosis (years); F, Female; M, Male; PRL, prolactin hormone; GH, growth hormone; ACTH, adrenocorticotropic hormone; Chr, chromosomal; ACMG, American College of Medical Genetics classification of variants; VUS, Variant of Uncertain Significance; LB, Likely benign; NA, Not Available. D, Damaging, T, Tolerated; GERP++, measure of evolutionary conservation (score ≥ 2 considered constrained); DANN, Pathogenicity scored 0–1 (higher values indicative of deleteriousness); SIFT, deleteriousness based on amino acid substitutions; LIST-S2, predicts the deleteriousness of amino acid mutations in proteomes; A, Ambiguous. All genetic variants detected were heterozygous except for variations in *BRAF* and *PKD1* genes.

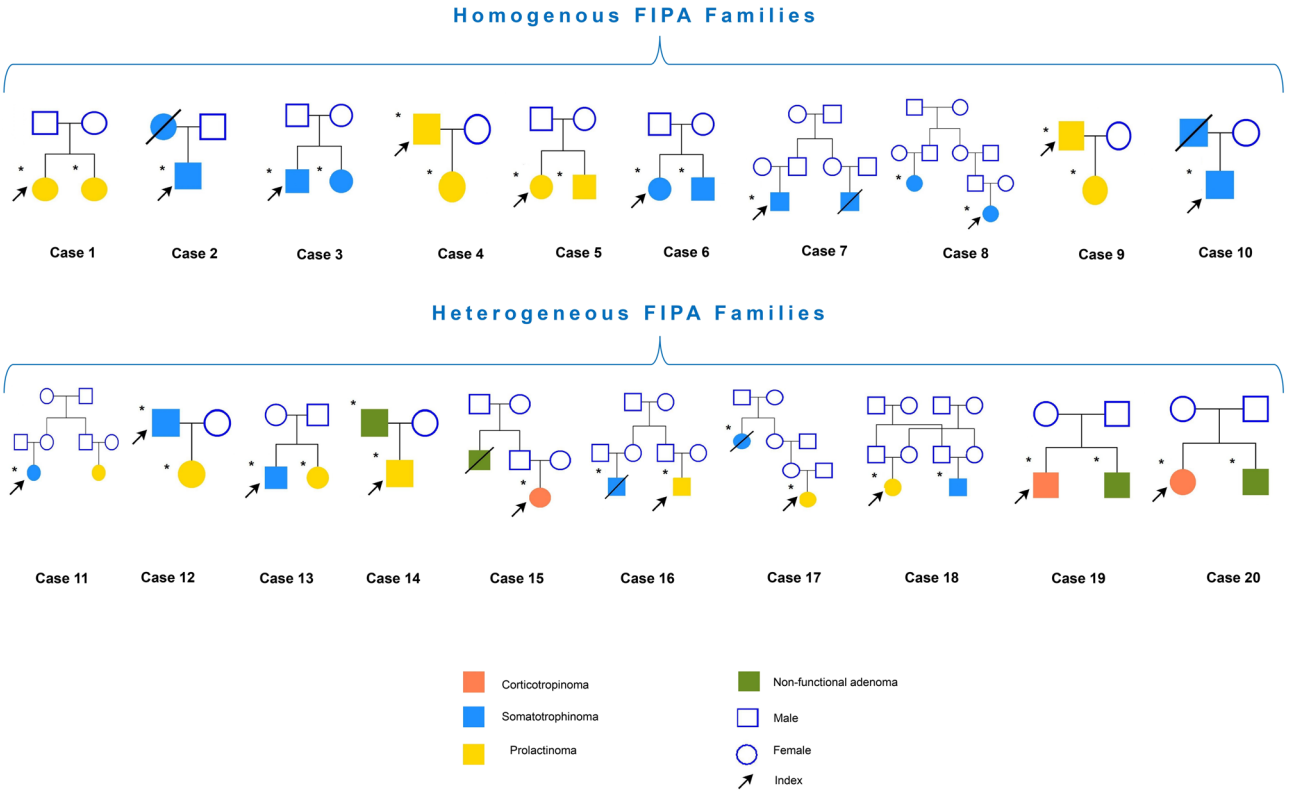

Fig. 1. Family Pedigrees Depicted is the pedigrees for 20 index cases evaluated. Different colors indicate distinct PA types to illustrate the disease history in the families. The star sign indicates individuals who consented to provide DNA material and the arrows indicate the index cases.

In the other heterogeneous FIPA family (Case 13 and his sister), carrying *AIP* p.(Arg81Ter) mutation, prognosis of the cases were consistent with the literature stating that tumor apoplexy is more common in individuals with the *AIP* mutation compared to those without it[8]. Additionally, familial pituitary apoplexy has also been described in *AIP* mutation-positive families[8,35]. Although prolactinomas with *AIP* mutations are generally reported to be large, aggressive, and to manifest at younger ages, they have also been reported not to cause resistance to DA, as seen in our case of prolactinoma[36,37].

Syndromic germline alterations in our FIPA cohort

Apart from the well-recognized FIPA-related *AIP* variations, we have detected heterozygous novel syndromic germline *SDHB*, *NF1*, *TSC1* and *CDH23* variations (Table 4). The "3PA" syndrome, which combines PAs with pheochromocytomas/paragangliomas, is associated with germline mutations in the SDHx genes, which are linked to the development of PAs[38]. Mutations in *SDHB* and *SDHD* have frequently been associated with PRL-secreting, GH-secreting and non-functioning adenomas in origin, which tend to be more aggressive, more resistant to SRLs, and often require surgical intervention[20,39]. In contrast to the literature, our cases carrying the same germline heterozygous *SDHB* variant showed distinct outcomes: the macroprolactinoma in Case 14 responded positively to CAB, while the macro-NFA in his father, who refused surgery, did not show an aggressive course.

Neurofibromatosis type-1 (*NF1*) gene encodes a large protein called neurofibromin, which functions as a negative regulator of the RAS proto-oncogene and mutations in *NF1* are responsible for NF1 syndrome[40]. Rarely, NF1 patients develop acromegaly due to autonomic GH hypersecretion resulting solely from optic pathway gliomas[41,42]. While true PAs are extremely rare, Hozumi et al.[43] reported for the first time a case of NF1 syndrome with acromegaly resulted from GH-secreting adenoma due to somatic *GNAS* p.(R201C) mutation. Recently, Hong et al.[44] described two cases of PAs with rare somatic *NF1* variants. In our study, within a FIPA family carrying a heterozygous germline *NF1* variant, the young index patient (Case 17) was unable to discontinue DA treatment despite her macroprolactinoma disappearing under therapy. In contrast, her acromegalic relative achieved full recovery following surgery. This germline variant was thought to be a factor determining the tendency to develop PA in our FIPA family.

Tuberous sclerosis is an autosomal dominant neurocutaneous syndrome, caused by mutations in one of the two tumor suppressor genes, *TCS1* and *TCS2*[45]. A few case reports have documented PAs in patients with TSC; including one GH-secreting adenoma, two ACTH-secreting adenomas, and one silent gonadotroph tumor. However, the existence of genotype–phenotype correlations remains a subject of debate[46–48]. In our young acromegaly patient (Case 7), who presented with invasive macroadenoma and carried heterozygous germline

FIPA type (index number)	Homogeneous (6)		Heterogeneous (12)		Heterogeneous (13)	
Cases	Index	Relative (Brother)	Index	Relative (Daughter)	Index	Relative (Sister)
Sex (F/M) / Age at diagnosis (yrs)	F/43	M/35	M/37	F/29	M/27	F/21
Complaint(s)	Hand-foot enlargement	Hand-foot enlargement	Headache	Secondary amenorrhea, Galactorrhea	Hand-foot enlargement	Severe headache (apoplectic event)
Phenotype	Acral	Acral	Acral	Only the nose looks a little wide ***	Acral	No feature
GH (ng/ml) IGF-1 (ng/ml, range) PRL (ng/ml)	4.2 454 (93–345) 6.4	NA	6.4 800 (94–210) 21.7	7.2 374 (117–329) 162	3.6 997 (232–385) 1350	- - 222*
Size of adenoma (milimeters) Knops	14 Grade 3	NA	23 Grade 4	12 Grade 3	40 Grade 4	25 (hemorrhagic adenoma) Grade 3
Diagnosis	Acromegaly	Acromegaly	Acromegaly	Prolactinoma, Clinically silent acromegaly	Acromegaly (mix adenoma)	Prolactinoma
Treatment modalities Surgery Medical (duration)	Refused SRL + DA (continues)	NA	TCS SRL (156 months)	Refused DA (continues)	2TSS + TCS SRL + DA (continues)	TSS (apoplexia) DA (12 months)
Pathology IHCS	-	NA	GH (+) PRL (-) TSH (-) FSH/LH : (-) ACTH (+)		GH 40% (+) PRL 75% (+) TSH (-) FSH/LH : (-) ACTH (-)	IHCS could not be performed due to hemorrhagic necrotic elements
Follow-up period (months)	132	NA	216	23	98	192
Latest hormonal status	Controlled under medication (SRL + DA)	NA	In remission (since 60 months)	DA could not be discontinued due to symptomatic PRL elevation, but IGF1 returned to normal (307 ng/ml)	Controlled under medication (SRL + DA)	In remission (since 180 months)
Latest residue tissue size (milimeters)	7	NA	9	Size did not change	18	0
Genetic variant(s)						
AIP	c.910 C > T p.(Arg304Ter)	c.910 C > T p.(Arg304Ter)	Splice acceptor site c.646-1G > C**	Splice acceptor site c.646-1G > C **	c.241 C > T p.(Arg81Ter)	c.241 C > T p.(Arg81Ter)
SQSTM1	c.1001_1003del p.(Gly334del)	c.1001_1003del p.(Gly334del)	-	-	-	-
LGALS3	-	-	c.719T > C p.(Ile240Thr)	c.719T > C p.(Ile240Thr)	-	-
SUFU	-	-	c. 842 C > T p.(Pro281Leu)	c. 842 C > T p.(Pro281Leu)	-	-

Table 2. Characteristics and genetic variation(s) of both index cases and relatives with *AIP* mutations. (*) Post-apoplexy level; IHCS: Immunohistochemical staining, (**) Novel *AIP* variant; (***) She complained only of symptoms of hyperprolactinemia, but her father had acromegaly; TSS, Transsphenoidal surgery; TCS, Transcranial surgery; SRL, Somatostatin receptor ligand; DA, Dopamine agonist; PEG, Pegvisomant; RT, Radiotherapy; NA: Not available (only a blood sample was provided) .

TSC1 p.(Arg517Gln) variant, disease activity was ultimately controlled only after two surgeries, radiotherapy and a triple drug combination. In addition to novel *TSC1* variant, Fibroblast Growth Factor Receptor-2 [*FGFR2* p.(Ile504Leu)], Somatostatin Receptor Type 4 [*SSTR4* p.(Phe321Ser)] and ATPase Plasma Membrane Ca2 + Transporting 3 [*ATP2B3* p.(Asp641Asn)] gene variants were identified in this patient. Unfortunately, we could not compare the clinical data of the index case with his deceased cousin, who had acromegaly.

Cadherin-related 23 (*CDH23*) is a member of the cadherin superfamily, which comprises calcium-dependent cell-cell adhesion glycoproteins[49]. Germline mutations in *CDH23* have been identified in people with Usher syndrome and nonsyndromic autosomal-recessive deafness[50]. Recently, mutations in *CDH23*, which is involved in cAMP-related pathways, are linked to familial and sporadic PAs and proposed to play important roles in PA pathogenesis[51,52]. Zhang et al.[51] identified a heterozygous missense p.(Arg1379Leu) mutation in *CHD23* in 33% of familial PAs and reported this gene as a risk factor for FIPAs. Our index patient (Case 20) and her brother harboured a heterozygous novel *CDH23* p.(Ala765Val) variant in one of the 20 FIPA families (5%). We additionally identified a novel variant p.(Ala486Thr) in *KCNQ1* in the affected individuals of this family. Both of these genes have been described to have roles in PA development[51,53]. The PA in our index case was ACTH-secreting type. To date, while many new mutations have been identified, approximately 12–28% of ACTH-secreting adenomas still have no known mutations[54]. The new variants in these cases may likely have triggered the onset of PAs, but they have not yet led to a relapse after surgery.

Other germline alterations in the remaining FIPA families

WES revealed additional candidate genes among which *SQSTM1*, *LGALS3* and *SUFU* accompanied *AIP* variations; *ATP2B3*, *SSTR4* and *FGFR2* accompanied *TSC1*, and *KCNQ1* accompanied *CDH23*. Our findings in four different Cases (3, 4, 9 and 12) have demonstrated the strength of our study, highlighting the interactions

Index case number	7	17	14	20
Syndromic gene-variant	***TSC1*** p.(Arg517Gln)	***NF1*** p.(Thr1295Ala)	***SDHB*** p.(Glu176Gly)	***CDH23*** p.(Ala765Val)
FIPA type	Homogeneous	Heterogeneous	Heterogeneous	Heterogeneous
Sex (F/M) / Age at diagnosis (yrs)	M/32	F/22	M/30	F/46
Adenoma Type / Size (mm) Knops classification	GH-secreting/60 Grade 4	PRL-secreting/12 Grade 1	PRL-secreting/29 Grade 2	ACTH-secreting/7 Grade 0
Treatment modality				
Surgery	2TSS	-	-	TSS
Medical	SRL ± DA + PEGV	DA	DA	-
Radiotherapy	Conventional	-	-	-
Total follow-up time (months)	216	204	48	4
Latest clinical status	Controlled under medical therapy	Controlled under medical therapy	Controlled under medical threapy	Remission
Additional genetic variants	*ATP2B3*, *SSTR4*, *FGFR2*	-	-	*KCNQ1*
Characteristics of relatives Sex (F/M)/Proximity/Age at diagnosis(yrs) Adenoma type /size (mm) Treatment modalities Latest status	M/ Cousin/35 GH-secreting/NA TSS Deceased (at 55 years)	F/Maternal aunt/43 GH-secreting/ NA TSS Postoperative remission for 44 years. NF1 syndromic characteristics are not observed.	M/Father/60 NFA*/21 Refused surgery due to no vision complaints. Receiving thyroid, steroid and gonad replacements for hypopituitarism for 24 months. The adenoma size remains stable.	M/Brother/54 NFA*/7 No treatment Being monitored. The adenoma is stable. Hormonal hyperactivity has not been observed for 12 months.

Table 3. Characteristics of both index cases and affected relatives of 4 FIPA families harboring syndromic gene variants. *TSC1*, Tuberosclerosis Complex Subunit 1 gene; *NF1*, Neurofibromatosis type 1 gene; *SDHB*, Succinate Dehydrogenase B gene; *CDH23*; Cadherin Related 23 gene; NA, Not available; TSS, Transsphenoidal surgery; SRL, Somatostatin receptor ligand; DA, Dopamine agonist; PEGV, Pegvisomant; RT, Radiotherapy; FU, Follow-up; NFA*, Apparently NFA (no pathology and immunohistochemical staining).

Index case number	Sex (F/M)/ Age at diagnosis (yrs)	Adenoma type/size (mm)	Treatment modality(s)	Follow-up period (months)	Latest status (remaining adenoma size, mm)	Confirmed genetic variant(s)
(1) **Index** **Relative**	F/23 Sister/20	PRL-secreting/9 PRL-secreting/9	DA DA	2 24	Controlled by CAB Controlled by CAB (4 mm)	*NR5A1(SF1)* *PKD1*
(2) **Index** **Relative**	M/22 Mother/43	GH-secreting/15 GH-secreting/20	3TSS + RT + Medical 3TSS + RT + Medical	192 178	Controlled with combination of SRL + DA + PEGV (no rest tissue) Controlled with combination of SRL + DA Thyroid carcinoma Deceased from Covid-19 infection	*TINF2* *ATP2B3*
(3) **Index** **Relative**	M/33 Sister/35	GH-secreting/14 GH-secreting/10	TSS TSS	120 132	Postoperative remission (no rest tissue) Postoperative remission (no rest tissue)	*RUNX2*
(4) **Index** **Relative**	M/59 Daughter/41	PRL-secreting/22 PRL-secreting/15	DA DA	90 24	Remission since 62 months (no rest tissue) Controlled by CAB (10 mm)	*LGALS3* *DRD4*
(5) **Index** **Relative**	F/27 Brother/29	PRL-secreting/6 PRL-secreting/37	DA DA	48 36	She has been breastfeeding her second baby for 12 months without any problems. Lost for follow-up.	*SYTL3*
(8) Index **Relative**	F/38 F-Cousin/63	GH-secreting/18 GH-secreting/15	TSS + SRL TSS	108 144	Drug-free remission for 12 months Postoperative remission (no tumor)	*BRAF*
(9) Index **Relative**	M/51 Daughter	PRL-secreting/15 PRL-secreting/15	DA DA	84 48	Controlled by DA (2 mm) Controlled by DA (6 mm)	*RUNX2*
(10) Index **Relative**	M/32 Father/45	GH-secreting/6 GH-secreting/18	Medical (DA, SRL) TSS	60 228	Controlled with SRL (no tumor) Postoperative blindness, hypopituitarism, Thyroid carcinoma Deceased from Covid-19 infection	*APC*
(11) Index **Relative**	F/47 F- Cousin/40	GH-secreting/12 PRL-secreting/11	Medical (SRL) Medical (DA)	12 72	Under control with SRL; DA was discontinued at menopause	*RARB* *SYTL3*
(15) Index **Relative**	F/42 Uncle/70	ACTH-secreting/12 NFA*/NA	TSS TCS	120 NA	Postoperative remission (no tumor) Deceased	*RARB*
(16) Index **Relative**	M/60 M-Cousin/49	PRL-secreting/45 GH-secreting/25	DA TSS + RT + Medical	96 60	Under control with DA (10 mm) Under control with DA + SRL (17 mm)	*DRD3*
(18) Index **Relative**	F/19 M-Cousin/37	PRL-secreting/18 GH-secreting/14	DA TSS	252 108	Drug-free remission for 204 months (no tumor) Postoperative remission (no tumor)	*NPR2*
(19) Index **Relative**	M/36 Brother/55	ACTH-secreting/6 Gonadotrophinoma/40	TSS TSS + RT	132 84	Postoperative remission (no tumor) Central hypothyroidism (20 mm stable residual adenoma)	*PTTG1*

Table 4. Characteristics and rare genetic alterations of affected members from the other FIPA families. DA, Dopamine agonist; TCS, Transcranial surgery; TSS, transsphenoidal surgery; SRL, Somatostatin receptor ligand; PEGV, Pegvisomant; RT, Radiotherapy.

between *SUFU*, *LGALS3* and *RUNX2* genes. The protein-protein interactions were established between SUFU and Galectin 3, both by an in vivo study in mice and in STING and Cytoscape based analyses utilizing human microarray data. This interaction was postulated to mediate global mRNA maturation[55]. Moreover, Galectin 3 was also determined to interact with RUNX2 protein, in which RUNX2 as a transcription factor was depicted to upregulate the expression of Galectin 3 in human pituitary tumors through initiating tumor progression[56]. Moreover, shared genes among index cases and relatives, as well as the presence of the same genes in different families prioritized them as candidates. In this respect, *NR5A1(SF1)*, *PKD1*, *NPR2*, *BRAF*, *PTTG1* were implicated to play roles in PA development[57–61]. Since other genes identified in this study were common in different cases in our cohort, we can emphasize that they are involved in PA pathogenesis as new genes that need to be replicated by independent studies.

In this context, studies utilizing WES on human subjects face challenges in variant interpretation, as each individual's data may accommodate around 20,000 variants. While WES is effective for identifying novel and rare variants, many are not documented in literature or genetic databases, necessitating the use of in silico prediction tools to estimate the potential impact of these variants on protein function and their conservation across species. However, varying algorithms can yield conflicting results, making it crucial to correlate these findings with existing gene function information to validate candidate genes. In our study, we addressed these challenges by leveraging familial cases. After filtering for candidate variants from WES data, we performed familial segregation analysis with Sanger sequencing, which, along with the presence of novel genes across cases, highlighted their potential role in PA pathogenesis. Thus, our research suggested novel variants and candidate genes within a cohort of FIPA families that received definitive diagnoses.

In conclusion, our study highlights the potential of WES in identifying possible common candidate genes other than *AIP* among affected relatives in FIPAs. In this respect, among the detected variants, p.(Arg81Ter) and (c.646-1G>C) in *AIP*, along with p.(Glu216GlysfsTer61) in *TINF2*, were reported as pathogenic in accordance with ACMG guidelines. Moreover, using in silico prediction tools GERP++, DANN, SIFT, LIST-S2 and AlphaMissense (a new machine-learning based tool), several novel variants were identified as potentially pathogenic. These findings were supported by AlphaMissense and at least one other tool. In this respect, *NR5A1* p.(Gly178Arg), *RUNX2* p.(Pro390Leu), *LGALS3* p.(Pro46Arg) and p.(Ile240Thr), *SDHB* p.(Glu176Gly), *NPR2* p.(Tyr338Cys) and *CDH23* p.(Ala765Val) variants were determined as strong candidates involved in PA pathogenesis. The advancement of next-generation sequencing will increase knowledge about the pathogenesis, invasiveness, recurrence, and prognosis of FIPAs and will contribute significantly to the development of targeted therapies.

Data availability

The data that support the findings of this study are available in Zenodo repository [https://doi.org/10.5281/zenodo.15697716 and https://doi.org/10.5281/zenodo.15704233], but restrictions apply to the availability of these data due to the Personal Data Protection Law (KVKK) in Turkiye. Data are however available from the authors upon reasonable request through the repository.

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Author contributions

S.Y., M.E.E. designed the study, collected data from centers, reviewed the eligibility of relevant data, performed literature search and wrote the paper. F.N.T. led the laboratory work and interpreted and wrote the genetic part of the paper. S.A. contributed to laboratory work and performed literature search S.C., S.T., O.S.S.,O.T., M.E., P.K., B.C.,C.S., Z.P.,G.G.O.,B.C. provided relevant data from their centers.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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