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Genetics of Primary Adrenal Insufficiency Beyond CAH in Saudi Arabian Population

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ABSTRACT

Background: The use of exome sequencing (ES) has helped in detecting many variants and genes that cause primary adrenal insufficiency (PAI). The diagnosis of PAI is difficult and can be life-threatening if not treated urgently. Consanguinity can impact the detection of recessively inherited genes. Here, we report families with PAI in a consanguineous population of Saudi Arabia.

Materials and Methods: A cohort of 47 PAI patients (41 males and six females) representing 30 families was recruited. The cohort excluded congenital adrenal hyperplasia (CAH) cases and had a known consanguinity of 70%. Using ES, molecular genetic causes of PAI were investigated.

Results: In 30 unrelated families with PAI, pathogenic/likely pathogenic variants were detected in 27 families with a diagnostic yield of (90%). Clinically associated variants of uncertain significance (VUS) were identified in a further two PAI families (7%). Hemizygous variants in *ABCD1* were the most common cause of PAI in this cohort (16 families) leading to adrenoleukodystrophy. A total of six novel variants were detected, of which four were predicted to be pathogenic (P) / likely pathogenic (LP) and two were VUS. Four pathogenic variants in *ABCD1*, *NR0B1*, and *MC2R* were detected in 10 families suggesting founder mutations.

Conclusion: In this cohort, ES detected a diagnostic molecular abnormality in 90% of patients with PAI phenotypes. X-linked inheritance is the most common cause of PAI and founder mutations likely contributed to a high diagnostic yield.

1 | Introduction

Primary adrenal insufficiency (PAI) is defined by the impaired synthesis and release of cortisol. PAI is a critical and life-threatening condition that requires accurate diagnosis and urgent management (Guran et al. 2016). Generally, it requires urgent treatment with glucocorticoid and mineralocorticoid replacement therapy (Bornstein et al. 2016). Acquired causes are common in adults while genetic causes are the most common in children (Buonocore et al. 2020; Fluck 2017).

A large proportion of patients with PAI have congenital adrenal hyperplasia (CAH), most of which is caused by enzymatic defects in cortisol biosynthesis, such as deficiencies in 21-hydroxylase and 3β-hydroxysteroid dehydrogenase.

In adult patients with PAI, 80% of cases are due to autoimmune causes, and in pediatric cases, 80% are due to genetic defects (Roucher-Boulez et al. 2018). Genetic causes of inherited PAI can be categorized into five groups: (I) impaired steroidogenesis associated with pathogenic variants in *STAR* and

CYP21A2; (II) adrenal hypoplasia associated with variants in *NR0B1*, *NR5A1*, *CDKN1C*, and *SAMD9*; (III) adrenocorticotrophic hormone (ACTH) resistance or familial glucocorticoid deficiency (FGD) associated with variants in *MC2R*, *MRAP*, *AAAS*, *NNT*, and *TXNRD2*; (IV) complex lipid metabolism associated with variants in *ABCD1*, *PEX1*, *LIPA*; and (V) autoimmune destruction associated with variants in *CLTA-4*, *HLA-DR3*, *HLA-DR4*, *HLA-B8*, and *BACH2* (Roucher-Boulez et al. 2018). Genetic causes of PAI vary from one region to another (Guran et al. 2016; Amano et al. 2017; Chan et al. 2015; Tsai et al. 2016).

Saudi Arabia has reported a high incidence of CAH with one in 7908 newborns (Alfadhel et al. 2017). The data on other causes of PAI in our population are rare, although they could be higher due to consanguineous marriages that could reach 56% (El-Mouzan et al. 2007).

Recently, PAI gene studies have helped with the diagnosis of 85% of the cases (Chan et al. 2015; Buonocore et al. 2021). Population studies were conducted to identify the genetic causes of PAI. A Turkish study reported that the commonly affected genes are *MC2R*, then *NR0B1*, after the exclusion of syndromic, ALD, and autoimmune cases. The Japanese study reported *NR0B1* and *STAR*, respectively (Guran et al. 2016; Chan et al. 2015; Tsai et al. 2016).

Even though cortisone has saved lives since its discovery in 1949, there were high variabilities in phenotype and biochemical findings that made the diagnosis of those patients more challenging (Guran et al. 2016; Alfadhel et al. 2017). Besides, some genes lead to both adrenal insufficiency as a gain of function, like *CDKN1C*, or loss of function (Bornstein et al. 2016). Mutations in *NNT*, *SPGL1*, and *SAMD9* genes were reported for the first time in 2012 and 2017 (Chang et al. 2021). PAI is more common than expected, but it might be underdiagnosed and nowadays research is reporting novel gene mutations with the advent of whole-exome sequencing (WES).

In the Middle East, few case reports were found on PAI. The population of the Kingdom of Saudi Arabia (KSA) has a tribal structure, and the consanguineous marriage rate is more than 55% (El-Mouzan et al. 2007). Over one-third of autosomal recessive disorders in the Saudi population are estimated to result from founder mutations, and the estimated percentage of disease-causing founder mutations is 42% (Monies et al. 2017).

In this study, we used exome sequencing in clinically diagnosed PAI patients from Saudi Arabia to identify underlying molecular causes and to report common features.

2 | Materials and Methods

2.1 | Human Subjects

The cohort study consisted of 47 PAI patients (41 males and six females) representing 30 families followed at pediatric endocrinology clinics at King Faisal Specialist Hospital and Research Center (KFSH&RC), Riyadh, Saudi Arabia, for the period between 2018 and 2021. We excluded patients with CAH. These cases were not reported or investigated previously and were not compared to another population control.

The study included PAI patients presenting with high ACTH and early-morning low cortisol with or without hyperkalemia, hyponatremia, high renin, and low aldosterone at initial presentation Table 1 and Table S1. Patients consented, and DNA was extracted from the peripheral blood samples. The study adhered to the Declaration of Helsinki and was performed with the approval of the Ethics Committee of the KFSH&RC (RAC#2181257).

Exclusion criteria included patients with CAH (21α-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase type-2, 17α-hydroxylase, or cytochrome P450 reductase deficiencies), autoimmune adrenal failure.

Data collection of patients included patient age, weight, height, sex, consanguinity, age of presentation, presence of electrolyte imbalance, presence of family history, serum ACTH, cortisol, aldosterone, and 17-OH progesterone at diagnosis. Following informed consent, DNA was extracted from the peripheral blood cells of PAI patients and available family members using the Gentra Systems PUREGENE DNA Isolation kit (Qiagen, Valencia, California, USA).

2.2 | Exome Sequencing and Variant Interpretation

We used the same protocol for exome sequencing as described previously (Al-Hamed et al. 2022). In brief, DNA libraries were constructed using an Agilent Sureselect All Exons V6 (50 Mb) capture kit and sequenced on an Illumina HiSeq2500 platform with an average target depth of 80×. Exome data were mapped to the human reference genome (NCBI build 37.1, UCSC hg19). Data were analyzed using QIAGEN Clinical Insight (QCI) Interpret that include copy number variation (CNV) detection in addition to an in-house variant interpretation pipeline. The in-house databases include collections of known disease-causing variants in the KSA population and aggregation of the variants produced by the samples in the Center for Genomic Medicine (CGM-DB). We used the ACMG guidelines for variant interpretation (Richards et al. 2015).

2.3 | Database Screening and In Silico Analysis

The Human Gene Mutation Database (HGMD), ClinVar, VarSome, and our in-house database were screened for detected variants. To predict the pathogenicity of novel variants, in silico analysis tools including Provean, SIFT, MutationTaster, PolyPhen, and Combined Annotation Dependent Depletion (CADD score) were used.

2.4 | Sanger Sequencing and Familial Segregation

Sanger sequencing was performed to validate novel variants detected and confirm segregation in families. Oligonucleotide primers for PCR amplification of targeted variants were

TABLE 1 | PAI cases with detected genes variants.

FAM #	Sex	Age (years)	Consanguinity	Type	Electrolyte imbalance (yes/no)	Treatment	Gene	Genotype	Zygosity	Inheritance	ACMG classification	Reference
FAM1-1	M	9	No	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.796G>A (p.Gly266Arg)	Hemi	XLR	P	ClinVar: RCV000012051
FAM1-2	M	16	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.796G>A (p.Gly266Arg)	Hemi	XLR	P	ClinVar: RCV000012051
FAM2-1	M	17	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1202G>A (p.Arg401Gln)	Hemi	XLR	P	ClinVar: RCV000012052
FAM2-2	M	14	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1202G>A (p.Arg401Gln)	Hemi	XLR	P	ClinVar: RCV000012052
FAM3	M	18	No	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1454C>A (p.Ser485*)	Hemi	XLR	P	Novel
FAM4-1	M	8	Yes	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1233G>C (p.Glu411Asp)	Hemi	XLR	LP	Novel
FAM4-2	M	11	Yes	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1233G>C (p.Glu411Asp)	Hemi	XLR	LP	Novel
FAM5-1	M	8	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM5-2	M	6	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM5-3	M	12	No	ALD	No	Hydrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM6-1	M	6	No	AHC	Yes	Hydrocortisone & fludrocortisone	*NR0B1*	NM_000475.5: c.919G>T (p.Glu307*)	Hemi	XLR	P	ClinVar: RCV000722070
FAM6-2	M	20	No	AHC	Yes	Hydrocortisone & fludrocortisone	*NR0B1*	NM_000475.5: c.919G>T (p.Glu307*)	Hemi	XLR	P	ClinVar: RCV000722070
FAM7	M	12	No	AHC	Yes	Hydrocortisone & fludrocortisone	*NR0B1*	NM_000475.5: c.919G>T (p.Glu307*)	Hemi	XLR	P	ClinVar: RCV000722070

(Continues)

TABLE 1 | (Continued)

FAM #	Sex	Age (years)	Consanguinity	Type	Electrolyte imbalance (yes/no)	Treatment	Gene	Genotype	Zygosity	Inheritance	ACMG classification	Reference
FAM8-1	M	17	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.459dupC (p.Ile154Hisfs*95)	Homo	AR	P	ClinVar: RCV000512930
FAM8-2	M	19	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.459dupC (p.Ile154Hisfs*95)	Homo	AR	P	ClinVar: RCV000512930
FAM9	M	11	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.107T>A (p.Ile36Asn)	Homo	AR	VUS	Novel
FAM10-1	M	3	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.459dupC (p.Ile154Hisfs*95)	Homo	AR	P	ClinVar: RCV000512930
FAM10-2	M	8	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.459dupC (p.Ile154Hisfs*95)	Homo	AR	P	ClinVar: RCV000512930
FAM10-3	M	1.3	Yes	FGD	No	Hydrocortisone	*MC2R*	NM_000529.2: c.459dupC (p.Ile154Hisfs*95)	Homo	AR	P	ClinVar: RCV000512930
FAM11	M	8	Yes	FGD	Yes	Hydrocortisone	*MRAP*	NM_178817.3: c.106+1G>C	Homo	AR	P	ClinVar: RCV000001911
FAM12-1	M	7	Yes	FGD	Yes	Hydrocortisone	*NNT*	NM_182977.3: c.1025T>C (p.Val342Ala)	Homo	AR	VUS	Novel
FAM12-2	F	9	Yes	FGD	Yes	Hydrocortisone	*NNT*	NM_182977.3: c.1025T>C (p.Val342Ala)	Homo	AR	VUS	Novel
FAM13	M	13	Yes	FGD	No	Hydrocortisone	*NNT*	NM_182977.3: c.93dupT (p.Leu33Phefs*13)	Homo	AR	P	ClinVar: RCV000985151
FAM14-1	F	27	Yes	Syndromic	No	Hydrocortisone	*AAAS*	NM_015665.6: c.885G>A (p.Trp295*)	Homo	AR	P	ClinVar: RCV000493433
FAM14-2	F	25	Yes	Syndromic	No	Hydrocortisone	*AAAS*	NM_015665.6: c.885G>A (p.Trp295*)	Homo	AR	P	ClinVar: RCV000493434
FAM15	M	14	Yes	IMAGe syndrome	Yes	Hydrocortisone & fludrocortisone	*CDKN1C*	NM_001122630.1: c.820G>A (p.Glu274Lys)	Het	AD	P	Novel
FAM16	M	4	Yes	Wolman syndrome	Yes	Hydrocortisone & fludrocortisone	*LIPA*	NM_000235.4:c.652C>T (p.Arg218*)	Homo	AR	P	ClinVar: RCV001951298

(Continues)

TABLE 1 | (Continued)

FAM #	Sex	Age (years)	Consanguinity	Type	Electrolyte imbalance (yes/no)	Treatment	Gene	Genotype	Zygosity	Inheritance	ACMG classification	Reference
FAM17	M	9	Yes	Syndromic	No	Hydrocortisone	*SGPL1*	NM_003901.3: c.665G>A (p.Arg222Gln)	Homo	AR	P	ClinVar: RCV000495961
FAM18-1	F	13	No	FGD	No	Hydrocortisone	N/A	N/A	N/A	N/A	N/A	Negative
FAM18-2	F	2	No	FGD	No	Hydrocortisone	N/A	N/A	N/A	N/A	N/A	Negative
FAM19-1	M	24	Yes	FGD	No	Hydrocortisone	*MRAP*	NM_178817.3: c.-151+125C>T	Homo	AR	LP	rs1380139859
FAM19-2	M	25	Yes	FGD	No	Hydrocortisone	*MRAP*	NM_178817.3: c.-151+125C>T	Homo	AR	LP	rs1380139859
FAM19-3	F	13	Yes	FGD	No	Hydrocortisone	*MRAP*	NM_178817.3: c.-151+125C>T	Homo	AR	LP	rs1380139859
FAM20-1	M	17	No	ALD	No	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM20-2	M	18	No	ALD	No	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM21-1	M	24	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4:c.1415_1416del (p.Gln472Argfs*83)	Hemi	XLR	P	ClinVar: RCV000077955
FAM21-2	M	16	Yes	ALD	No	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4:c.1415_1416del (p.Gln472Argfs*83)	Hemi	XLR	P	ClinVar: RCV000077955
FAM22	M	20	No	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4:c.1553G>A (p.Arg518Gln)	Hemi	XLR	P	ClinVar: RCV000723954
FAM23	M	11	Yes	ALD	No	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952
FAM24	M	19	Yes	ALD	No	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1202G>A (p.Arg401Gln)	Hemi	XLR	P	ClinVar: RCV000012052
FAM25	M	9	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1825G>A (p.Glu609Lys)	Hemi	XLR	P	ClinVar: RCV000723952

(Continues)

TABLE 1 | (Continued)

FAM #	Sex	Age (years)	Consanguinity	Type	Electrolyte imbalance (yes/no)	Treatment	Gene	Genotype	Zygosity	Inheritance	ACMG classification	Reference
FAM26	M	12	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	ABCD1	NM_000033.4:c.1780G>A(P.(G ly594Ser)	Hemi	XLR	LP	Novel
FAM27	M	15	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1679C>T (p.Pro560Leu)	Hemi	XLR	P	ClinVar: RCV000723625
FAM28	M	20	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1816T>C (p.Ser606Pro)	Hemi	XLR	P	ClinVar: RCV001781608
FAM29	M	13	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4: c.1816T>C (p.Ser606Pro)	Hemi	XLR	P	ClinVar: RCV001781608
FAM30-1	M	8	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4:c.818C>A (p.Ala273Glu)	Hemi	XLR	LP	rs868992338
FAM30-2	M	18	Yes	ALD	Yes	Hydrocortisone & fludrocortisone	*ABCD1*	NM_000033.4:c.818C>A (p.Ala273Glu)	Hemi	XLR	LP	rs868992338

Abbreviations: AD, autosomal dominant; ALD, adrenoleukodystrophy; Age, current age of patient; AR, autosomal recessive; FGD, familial glucocorticoid deficiency; Homo, homozygous; Het, heterozygous; Hemi, hemizygous; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; XLR, X-linked recessive.

designed using Primer3 software (http://frodo.wi.mit.edu/) and synthesized in-house. The amplified PCR products were then sequenced using an ABI 3730xl capillary sequencer (Applied Biosystems, CA, USA) and sequences were analyzed using Mutation Surveyor software V.3.24 (SoftGenetics LLC, State College, Pennsylvania, USA).

3 | Results

A total of 47 patients (41 males and six females) representing 30 families were investigated (Table 1 and Figure 1). Twenty-one families were reported consanguineous, and nine were not. Exome sequencing provided clinically relevant results in 29 families. Pathogenic/likely pathogenic (P/LP) variants were detected in 27 families with a diagnostic yield of (90%), whereas clinically relevant variants of uncertain significance (VUS) were found in 2 families (7%). One family was reported with negative results.

As shown in Figure 2, nine genes were found to be associated with PAI in our cohort. Hemizygous variants in *ABCD1* are the most common cause of PAI in the cohort (16 families) leading to the adrenoleukodystrophy (ALD) type. FGD type is the second in the cohort and caused by variants in *the MC2R*, *MRAP*, and *NNT* genes.

Twenty-one sequence variants were detected in the study. Loss-of-function (LoF) variants were identified in 10 of the 27 molecularly genetically solved families with P/LP variants, whereas the previously reported missense variants were detected in 14 families. There were a total of six novel variants, four predicted P/LP alleles and two VUS.

The following variants were detected in more than one family: the variant in *ABCD1* (NM_000033.4: c.1825G>A, p.Glu609Lys) was detected in four families, and the other variant (NM_000033.4: c.1202G>A, p.Arg401Gln) was detected in two families. The *NR0B1* variant (NM_000475.5: c.919G>T, p.Glu307*) was detected in two families, and the *MC2R* variant (NM_000529.2: c.459dupC, p.Ile154Hisfs*95) in two families.

Usually, autosomal recessive inheritance is the expected mode of inheritance in a consanguineous population; however, X-linked inheritance was the major mode of inheritance, although consanguinity was reported in 70% of cases. This is due to the inheritance of variants in *ABCD1*, where 16 families (53%) were found to harbor alterations in the *ABCD1* gene located on the X chromosome. Autosomal recessive inheritance here only represents 33% of families, and therefore, the contribution of consanguinity in causing PAI in our cohort is limited. Male patients (41 cases) accounted for almost 87% of cases, and this may be due to X-linked inheritance of *ABCD1* variants.

The age of PAI presentation is widely variable even within the family, and 37% of cases started to have symptoms below 1 year, and 40% presented symptoms at the age of 5 years and above. Despite symptom variability at presentation, hyperpigmentation is the hallmark ($n = 41$) followed by vomiting ($n = 11$) and lethargy ($n = 11$). The association of electrolyte imbalance was reported in 44% of the patients, including 10 cases with *ABCD1* variants.

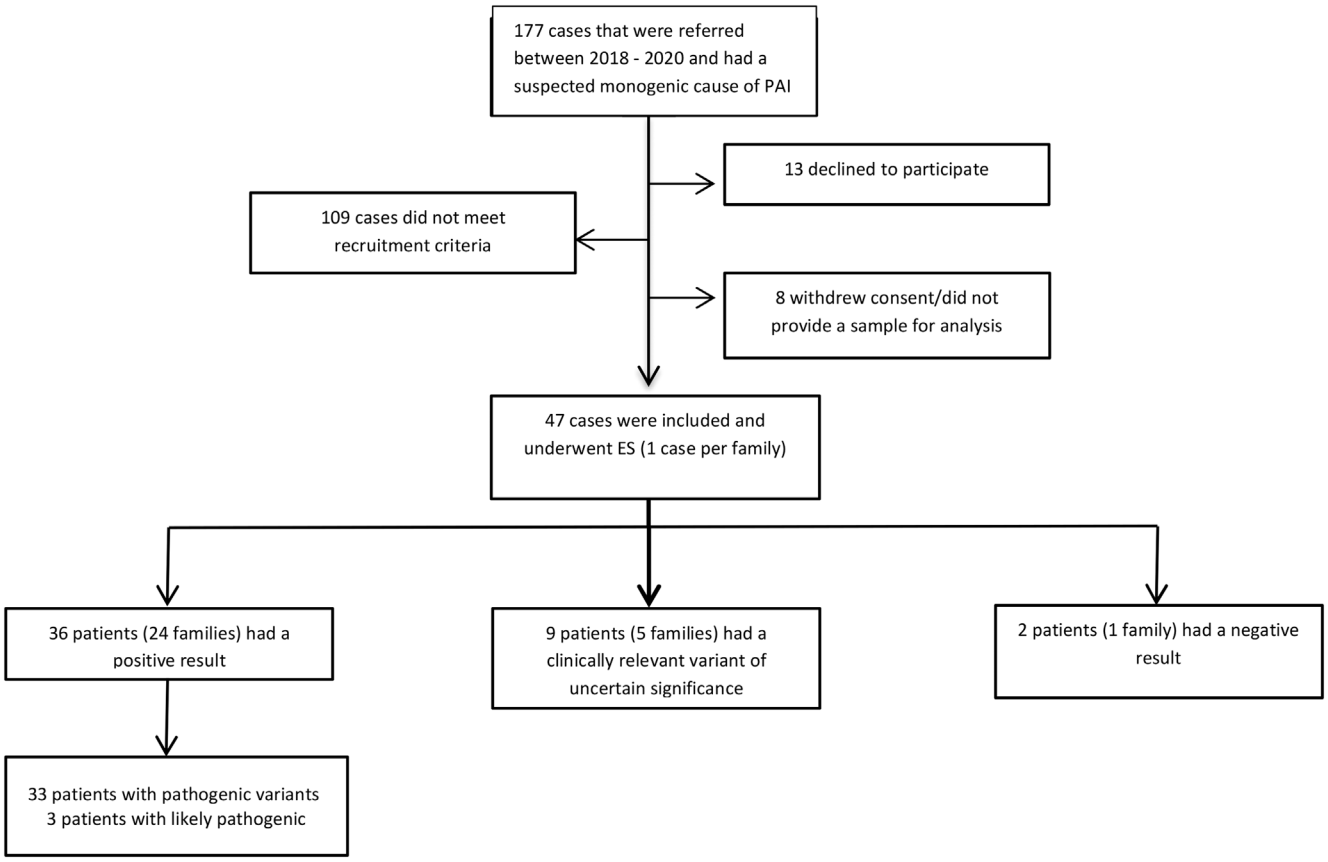

FIGURE 1 | Flowchart of recruitment and results. ES: Exome sequencing.

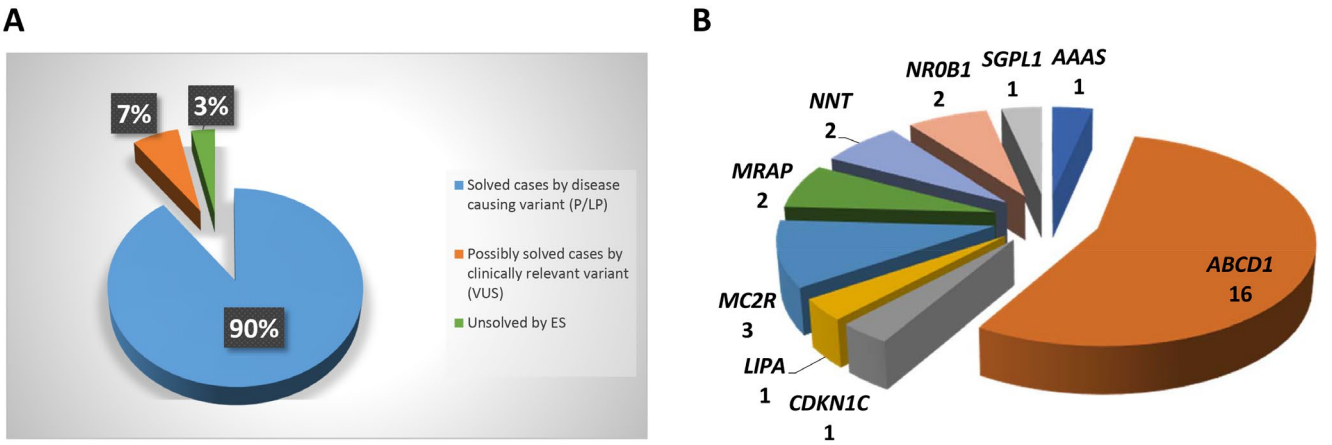

FIGURE 2 | Exome sequencing of a cohort of primary adrenal insufficiency (PAI) patients. (A) Within the 30-family PAI cohort, ES provided genetically solved cases (P/LP) in 80%, possibly solved cases (VUS) in 17%, and unsolved cases in 3% by ES. (B) ES detected nine genes associated with the PAI phenotype, including variants of uncertain significance.

4 | Discussion

Molecular genetic diagnosis for PAI was established in 27 of 30 families (90%) with P/LP variants. Two families (7%) had VUS associated with the phenotype. Variations in the genetic diagnosis of PAI have been reported to be 75% in the UK cohort (Buonocore et al. 2021), 81% in the Turkish cohort (Guran et al. 2016), 85% in the Japanese cohort (Amano et al. 2017), and more than 40% in the European cohort (Chan et al. 2015). These variations can be attributed to the study population and test modality. We identified *ABCD1* variants as the main cause of PAI in our cohort, while other population studies reported variants in other genes, that is, *MC2R* in the Turkish study, *STAR* in the Japanese study, and *CYP11A1* in the European study. The *ABCD1* and *NR0B1* genes are on the X chromosome, and X-linked inheritance is the leading pattern in the cohort. The effect of consanguinity was relatively less important in our cohort, although 70% of families are consanguineous.

Many forms of PAI do not have diagnostic phenotypes or biochemical parameters, so genetic analysis is the only way to establish a precise diagnosis (Buonocore et al. 2021). Recent molecular genetic methods have revealed novel genes and variants underlying PAI and have enhanced our understanding of the pathophysiology of these disorders (Malikova and Fluck 2014). Next-generation sequencing (NGS) technology is likely to enhance the diagnostic yield in patients with PAI. The use of NGS allowed characterizing of clinical cases with apparently similar phenotypes but different genetic alterations (Guzzetti et al. 2018).

Nowadays, almost 20 genes are associated with CAH (https://panelapp.genomicsengland.co.uk/panels/145/). At present, with such a limited number of genes, a gene panel would be an appropriate tool to detect variants in these genes with high sequence depth and >100× coverage for accurate variant calling (Contini et al. 2015). Using WES or whole genome sequencing (WGS) may help in the identification of genes associated with PAI or related syndromes. In this study, variants in nine genes were detected as being associated with PAI. We would recommend using a gene panel as a first tier, followed by WGS bearing in mind that more than 5% of pediatric PAI cases are still undetermined (Settas et al. 2019) in addition to the diversity of pathogenic mechanisms causing PAI. As recommended previously, all pediatric PAI cases should undergo genetic investigation after the exclusion of CAH, metabolic, or autoimmune disorders (Almeida 2021). Genetic diagnosis of PAI can have important implications for counseling and management (Buonocore and Achermann 2020). PAI cases could be underdiagnosed secondary to early death and misdiagnosis due to vague and nonspecific symptoms (Pintaldi et al. 2019). In this study, we observed a predominance of males over females (14 vs. 6). Interestingly, this finding resembles a Turkish study, despite the removal of X-linked adrenal hypoplasia congenita patients in their study (Guran et al. 2016; Buonocore et al. 2021). In addition, we observed that hyperpigmentation is the most common presenting symptom, followed by vomiting and lethargy. In addition, almost 43% of our cases had electrolyte imbalances. For ALD cases with *ABCD1* variants, central nervous system (CNS) manifestation was the earliest sign, with a mean age of symptom onset at 7 years. Progressive neurodegeneration and abnormal accumulation of very long-chain fatty acids (VLCFAs) are the main associated features. We recommend VLCFA screening first for all boys suspected of having ALD, followed by genetic testing to confirm and identify the molecular cause.

Clinical presentation of FGD cases in the cohort started at the age of 1 year or below. Variant alterations in *MC2R* are the main cause of FGD in our cohort with tall stature presentation, followed by variants in *MRAP* and *NNT* that are associated with obesity. The *MC2R* gene codes for the melanocortin-2 receptor (more commonly the ACTH receptor), which is found primarily in the adrenal glands.

The *MRAP* gene codes a protein called melanocortin-2 receptor accessory protein (MRAP). This protein transports the melanocortin-2 receptor from the endoplasmic reticulum (ER), which is involved in protein processing and transport, to the cell membrane so that the receptor can function. Fifty percent of FGD cases are caused by mutations in one of the three genes: *MC2R*, *MRAP*, or *STAR* genes. Defects in these proteins, MC2R and MRAP, lead to a defect in the ACTH signaling-steroidogenic pathway (Roucher-Boulez et al. 2018). The *NNT* gene codes

for nicotinamide nucleotide transhydrogenase enzyme that is found embedded in the mitochondria. *NNT* gene mutations account for approximately 10% of cases of FGD due to oxidative stress (Meimaridou et al. 2012; Roucher-Boulez et al. 2016). Hyperpigmentation is the initial presentation for all FGD cases, and seizures or loss of consciousness secondary to severe hypoglycemia may develop later with mild presentation. Patients with *MC2R* mutations in our cohort presented with hypoglycemia and without mineralocorticoid effect consistent with previous observations (Abuduxikuer et al. 2019; Mohammed, Haris, and Hussain 2022).

Usually, patients with PAI require glucocorticoid replacement with or without mineralocorticoid replacement. In our cohort, 50% of patients were on isolated glucocorticoid replacement; we noted that patients with *MC2R*, triple A syndrome, and *SGPL1* needed only glucocorticoid replacement.

In conclusion, for a cohort of patients with PAI, in whom there was a high rate of consanguinity, we provide evidence for using ES in endocrinology clinics to define a precise molecular genetic diagnosis.

Author Contributions

M.H.A.-H., A.Q., and A.F.-S. conceived the study and participated in its design, coordination, and drafted the manuscript. A.F.-S., A.S., Z.H., M.H.-O., and L.R. participated in the clinical diagnosis of the cases. M.H.A.-H. performed exome analysis. A.F.-O. carried out all technical aspects of molecular diagnosis. All authors read and approved the final manuscript.

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Ethics Statement

The study was approved by the Research Advisory Council at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated during this study are included in this published article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.