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ORIGINAL ARTICLE

Genetic carrier screening for disorders included in newborn screening in the Saudi population

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ABSTRACT

Background: Inborn errors of metabolism (IEM) are prevalent autosomal recessive disorders in Saudi Arabia. Socio-economic factors, such as consanguineous marriages, play a role in the high rate of diseases. The government of Saudi Arabia created a newborn screening program (NBS) for the most prevalent disorders to facilitate early intervention and the prevention of severe complications. The study aimed to determine the carried pathogenic allele of the diseases included in the NBS and the most frequently carried phenotype in the Saudi population.

Methods: We performed targeted genetic screening for the genes associated with the IEM in the NBS. We used the results of the whole exome sequencing of 1,314 affected and unaffected individuals from 650 families. The results constitute the King Abdullah International Medical Research Center Genomic Database.

Results: According to the data set, four diseases occurred most frequently in the Saudi population: adrenal hyperplasia, propionic acidemia, phenylketonuria, and maple syrup urine disease. In total, 12 pathogenic variants occurred frequently.

Conclusion: This study generated an updated list of the most pathogenic variants in the Saudi population, based on the National Guard Hospital dataset. Additional research with larger data sets from the different regions will provide valuable information about the allele distribution in the Saudi population, creating a carrier screening program.

Keywords: Carrier screening, variants, Saudi population, exome sequencing, common genetic variation.

Introduction

Consanguineous marriages (CM) is the preferred way of partnering in the Middle East and North Africa (MENA) region to assure the continuation of the marriage, strengthen the relationship, and social ties. The MENA region has the highest CM rate globally (1). Economic factors also play a role in CM as it reduces the cost of the marriage compared to marriage outside the family or clan (2). In Saudi Arabia, the estimates vary per region, but approximately 50% of the Saudi population are involved in a CM, of which 50% are married to a first-cousin (3). The social-economic and demographic factors have a significant effect on the partnering choice (1), which increases the rate of autosomal recessive (AR) genetic disorders (4-6). The mortality rate is 3.5% higher in first-cousin children than non-consanguineous children (1).

Due to CM, the Saudi population has the highest AR birth rate globally, with 40% of the founder mutations of the total mutation pool (7,8), 81% of the Mendelian phenotypes reported as AR, and 97% in a homozygous form (9,10). One of the most prevalent Mendelian monogenic phenotypes in Saudi Arabia is inborn errors of metabolism (IEM) (Table 1) (3). The genetic burden

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of recessive single-gene disorders can be reduced by changing marriage patterns, using screening programs (SP) and public health education (1,11). Hundreds of IEMs have been linked to a rare genetic variation found in the protein-coding regions, disrupting the function of enzymes known to process certain metabolites (12,13). The global prevalence varies per region. In Saudi Arabia, the frequency in the Eastern Province is 1 in 667, and in the Central Region, according to Alfadhel et al., one in 591 (12,14). SP introduce a number of advantages to prevent the most prevalent disorders in populations through early detection and preventing future complications (15,16).

The Kingdom of Saudi Arabia established a National transformation program in 2016 to fulfill Vision 2030, which included several programs to ensure healthcare transformation. Led by the Ministry of Health (MOH), programs were launched in healthcare to control and prevent diseases, including the newborn screening program (NBS) (17,18). The NBS focus on the IEM in Table 1, which supports early case detection (19). After years of implementation, there is a notable reduction in IEM, indicating that the program succeeded in raising awareness and afford the proposed partners an option to proceed with the marriage or not (20).

However, SP targeting cases are not appropriate to detect carriers, which is important for securing healthy progeny (21). Population carrier SP can deliver significant public health benefits for asymptomatic individuals or persons at risk of partnering with another carrier (22). Up until now, no SP is available in our region to detect the carrier status. Although the MOH proposes to expand the tests to more diseases, such a program will be used for case confirmation on a diagnostic level, not carrier detection. In this paper, we are estimating the carrier frequency of diseases included in the NBS.

Subjects and Methods

We perform targeted genetic screening for the gene list in Table 1 to identify the most frequently carried allele in the Saudi population. We used whole-exome sequencing, done for diagnostic purposes and informed consent for research use. The sequencing was performed in College of American Pathologists-accredited genetic laboratories in King Abdulaziz Medical City or a sequencing service. The results are stored in the King Abdullah International Medical Research Center (KAIMRC) Genomic Database (KGD) in variant calling files. The data have been recruited since 2014 up to date and realized as 650 families, with 1,314 affected and unaffected individuals (23). In total, 2,173,863 filtered variants, only 2,181 variants are classified as pathogenic or likely pathogenic variants, based on the American College of Medical Genetics and Genomics scoring system (24) or previously observed in ClinVar (25). One member of each family was included to avoid bias. We eliminated autosomal dominant variants, including AR and X-linked variants. The pipeline used for the filtration comprised of a read depth of more than 15× and a minor allele frequency of less than 1% (Figure 1), using the local KGD database and the Saudi human genome project database (SHGP db), other multi-ethnic databases, the genome aggregation database (GenomAD) and dbSNP/1,000 (26,27). The approval for this study was obtained from the Institutional Research Board of KAIMRC, #RC19/315/R.

Results

For the gene list in Table 1, 12 pathogenic variants were identified. CAH (OMIM:201910) was the most frequently carried disorder in the Saudi population, based on the KGD (Table 2). The variant creates a stop-gain of function

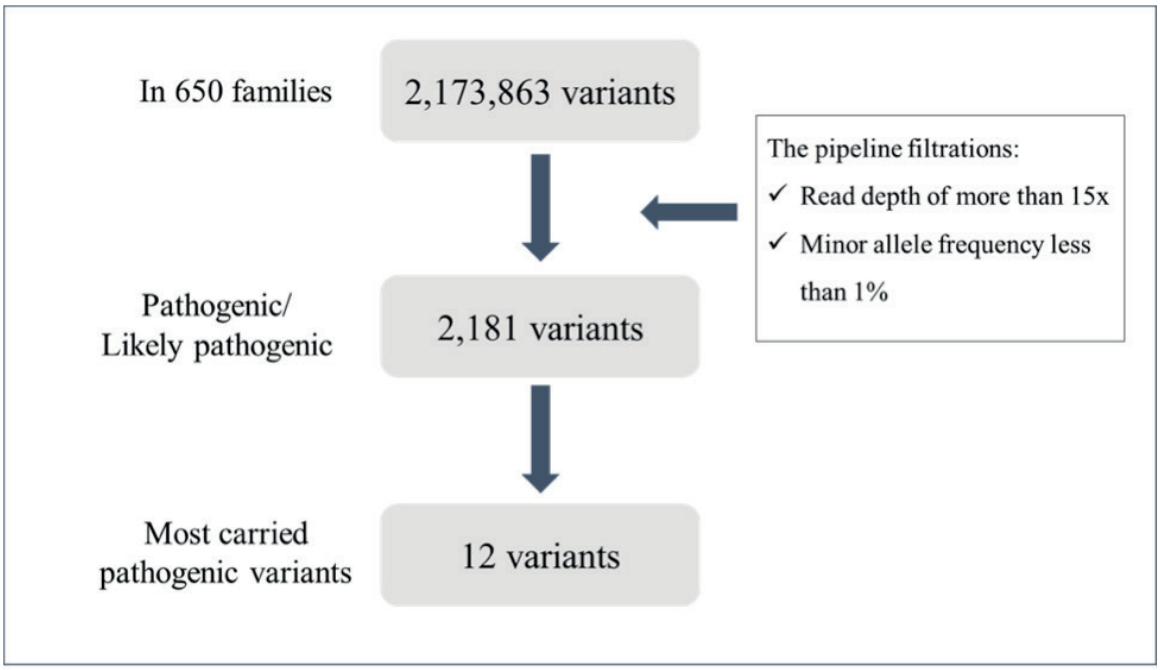

Figure 1. *Study summary.*

Table 1. *The diseases included in the Saudi neonatal SP.*

Metabolite	Disorder	Abbreviations	Gene/Locus
Aminoacidopathies	Phenylketonuria	PKU	*PAH*
	Argininosuccinate lyase deficiency	ASL	*ASL*
	Maple syrup urine disease	MSUD	*DBT, BCKDHB, BCKDHA, DLD*
	Citrullinaemia	CIT	*ASS1*
Organic acid disorders	Propionic acidaemia	PPA	*PCCB, PCCA*
	Methylmalonic acidaemia	MMA	*MUT, C2orf25, MMAA, MMAB, MMACHC, PRDX1, CD320, LMBRD1, ACSF3, ABCD4, SUCLA2, HCFC*
	Glutaric acidaemia type-I	GA-I	*GCDH*
	Isovaleric acidaemia	IVA	*IVD*
	3-methylcrotonyl-CoA carboxylase deficiency	(3-MCC)	*MCCC1*
Fatty acid oxidation defect	Medium-chain acyl CoA dehydrogenase deficiency	MCAD	*ACADM*
	Very long-chain acyl CoA dehydrogenase deficiency	VLCAD	*ACADVL*
Ketogenesis and ketolysis defects	3-hydroxy-3-Methylglutaryl-Co A lyase deficiency	HMG	*HMGCL*
	Beta-ketothiolase deficiency	BKT	*ACAT1*
Carbohydrate disorder	Galactosaemia	GALT	*GALT*
Endocrine disorders	Congenital hypothyroidism	CH	*DUOX2, TG, TPO, TSHR, TTF1, TTF2, PAX8, NKX2-5, GNAS, THRA, TSHB, IYD, SLC5A5*
	Congenital adrenal hyperplasia	CAH	*CYP21A2*
Vitamin responsive disorder	Biotinidase deficiency	BTD	*BTD*

in chromosome 6 in 32008198, which changes cytosine to thiamin. The change was only observed in the KGD and not in the GenomAD or SHGP. Five variants were also found in the *CYP21A2* gene previously reported in the Saudi population. A variant in chr13:100809551G>A, associated PPA (OMIM:606054), was found in six individuals in the KGD (0.46%) and the SHGP (0.24%) but not in the GenomAD. Four missense variants were found in the *PAH* gene, causing PKU (OMIM:261600), previously reported in the Saudi population. Finally, a pathogenic variant was reported previously in MSUD, also known as Dihydrolipoamide dehydrogenase deficiency (OMIM:246900). The incidence rate in the data was compared to AlFadhel et al. studying the Saudi population (Table 3).

Discussion

The diseases in Table 1 are known as the most frequent IEM in the Saudi population. The SP, implemented for a number of years, supports prevention, and early intervention. Of the diseases included in the NBS program, the most frequently occurring allele causes adrenal hyperplasia. Due to the mode of inheritance in CAH as compound heterozygotes, it is normal to find a number of variants in the same gene (28-30). The most frequent variation in this gene has been observed in the SHGP. The variant in chromosome 6 position 32008198 C>T (c.955C > T), found in 24 individuals in the KGD, does not appear in the SHGP. Individuals with this variant usually have a duplication of the *CYP21A2* gene, and they are normal with no phenotype if this changes c.955C > T on the duplicated allele (31). Although there are many pathogenic variants in this gene, CAH is an AR disease due to pathogenic homozygous or compound heterozygous variants. A single pathogenic variant does not cause the disease without other cis or trans compound heterozygous variants. While it is the most frequently carried disease, due to the increased number of pathogenic variants according to our data, it may not be the most prevalent IEM.

PPA is the second in line with an incidence of 1 per 12,500 births (32). According to Al-Hamed et al. (32), c.425 G>A is the most frequent variant causing PPA in the Saudi population, reported by AlFadhel et al. Another disease from the NBS disease list is PKU; the number of variants observed is shown in Table 2. PKU has been reported in different populations (33), though the allele distribution varies in populations. Finally, a variant has been found in the *DLD* gene associated with MSUD, although other studies with the Saudi population also indicate other genes (Table 1), with variations related to MSUD (34). As shown in Table 3, the incidence varied

Table 2. *Most carried pathogenic variants of the diseases included in the Saudi SP data extracted from the KGD.*

GENE	Genomic coordinates	c.DNA	Amino acid	Transcript	Impact	RS ID	Variant count	KGD_MAF	GenomAD	SHGP	Disease
CYP21A2	chr6:32008198C>T[a]	c.955C>T	p.Gln319Ter	NM_000500.9	stop gained	rs7755898	24	1.85%	NA	NA	Adrenal hyperplasia
	chr6:32008870C>T	c.1447C>T	p.Pro483Ser	NM_000500.9	missense variant	rs776989258	11	0.85%	0.0524%	1.11%	
	chr6:32007893A>G	c.850A>G	p.Met284Val	NM_000500.9	missense variant	rs770199817	9	0.69%	0.0043%	0.51%	
	chr6:32007887G>T	c.844G>T	p.Val282Leu	NM_000500.9	missense variant	rs6471	8	0.62%	0.5515%	0.24%	
	chr6:32006858C>G	c.293-13C>G	NA	NM_000500.9	5 prime UTR variant	rs6467	3	0.23%	0.2205%	0.24%	
	chr6:32008783C>T	c.1360C>T	p.Pro454Ser	NM_000500.9	missense variant	rs6445	1	0.08%	0.4530%	0.06%	
PCCA	chr13:100809551G>A	c.425G>A	p.Gly142Asp	NM_000282.4	5 prime UTR variant missense variant	rs796052019	6	0.46%	NA	0.24%	Propionic acidemia
PAH	chr12:103245479C>A	c.898G>T	p.Ala300Ser	NM_000277.3	missense variant	rs5030853	4	0.31%	0.0538%	0.09%	Phenylketonuria
	chr12:103237484G>A	c.1139C>T	p.Thr380Met	NM_000277.3	missense variant	rs62642937	2	0.15%	0.0418%	0.38%	
	chr12:103260377C>T	c.506G>A	p.Arg169His	NM_000277.3	missense variant	rs199475679	1	0.08%	0.0273%	0.06%	
	chr12:103288554G>T	c.311C>A	p.Ala104Asp	NM_000277.3	missense variant	rs62642929	1	0.08%	0.0052%	NA	
DLD	chr7:107555951G>T	c.685G>T	p.Gly229Cys	NM_000108.5	missense variant	rs121964990	3	0.23%	0.0308%	0.15%	Dihydrolipoamide dehydrogenase deficiency

*Variant found in chr6:32008198C>T,c.550C>T, p.Gln184Ter causing a stop gained found in 24 in the database never been reported and known to be associated with certain haplotype not necessarily disease-related.

Table 3. *Comparing carrier frequency in two studies, AlFadhel et al. and KGD, excluding adrenal hyperplasia (not in AlFadhel et al.) and the numbers of variants involved in the different haplotypes.*

	AlFadhel et al.			KGD			
Disease	Numbers of carriers	Total screened	Incidence	Numbers of carriers	Total screened	Carriers	Incidence
Propionic acidemia	9	110,601	1:12,289	6	680	113.3	1:51,347.56
Phenylketonuria	5	110,601	1:22,120	8	680	85	1:28,900
Maple syrup urine disease	5	110,601	1:22,120	3	680	226.7	1:205,571.56

between the current study and AlFadhel et al. This is due to the sample size difference and AlFadhel et al. observed disease incidence. Still, in the current study, the incidence is calculated from the carrier frequencies.

Conclusion

In this study, we determined the most frequently carried alleles in the Saudi population. The allele distribution of the diseases screened in the NBS improves understanding of the most frequent allele for each disease and the distribution in families or regions. Diseases, such as CAH with compound heterozygous variations increasing the carrier status, will support other programs, including pre-marital screening or carrier screening, to facilitate detection before pregnancy. The prevalence of these alleles facilitates the design of regional carrier SP. Unfortunately, the data only presents the KGD population. There is an urgent requirement to determine the diseases or allele distributions for the different regions and the need for larger sample size for a more accurate estimation of the carrier frequencies. Additional research will add value in understanding the allele distribution in the Saudi population.

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Author contribution
MAI, and AA designed the study, interpreted the clinical data, and wrote the article. MAI, TA, and LA collected samples, genotyped the cases and helped in statistical analysis. AA, WE, FAM, FA, and MA, contributed in samples collection, clinical correlation and manuscript revision. All authors have read and approved the final manuscripts.

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Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
Ethical approval was granted by the Institutional Research Board of KAIMRC via reference number #RC19/315/R dated 18/12/2019.

Consent to participate
Informed consent was obtained from the patients.

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