A Pilot Mitochondrial Genome-Wide Association on Migraine Among Saudi Arabians

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**Background:** Mitochondrial DNA (mtDNA) mutations have been reported in multiple neurological diseases and helped to explain the pathophysiology of these diseases. Similarly, variations in mtDNA might exist in migraine and can explain the effect of low ATP production in the neurons on the initiation of migraine attack. Therefore, in the current study we aim to explore the association of mtDNA mutations on migraine in the Saudi population.

**Subjects and Methods:** Over 1950 young Saudi female students were screened for migraine, among that a total of 103 satisfied the ICHD-3 criteria. However, 20 migraine cases confirmed in the neurology clinic and gave consent to participate in the study. Another 20 age-matched healthy controls were also recruited. Mitochondrial sequence variations were filtered from exome sequencing using NCBI GenBank Reference Sequence: NC_012920.1 and analysed using MITOMAP. Genes with significant single nucleotide polymorphisms (SNPs) were investigated by the gene functional classification tool DAVID and functional enrichment analysis of protein–protein interaction networks through STRING 11.5 for the most significant associated genes.

**Results:** Genome wide analysis of the mitochondrial sequence variations between the patients with migraine and control revealed the association of 30 SNPs (p < 0.05) in the mitochondrial genome. The highest significance (p = 0.001033) was observed in a coding SNP (rs1603225278) in the CYTB gene and rs386829281 in the region of origin of replication. Twenty-four significant SNPs were in the coding region of nine (ND5, ND4, COX2, COX1, ND3, CYTB, COX3, ND2 and ND1) genes.

**Conclusion:** This is the first study to demonstrate the association of mtDNA variations with migraine in the Saudi population. The current findings will help to highlight the significance of mtDNA mutations to migraine pathophysiology and will serve as a reference data for larger national and international studies.

**Keywords:** mitochondrial DNA, Saudi Arabia, mtDNA variations, migraine, CYTB gene

**Introduction**

Mammalian mitochondrion is found to own a specific deoxyribonucleic acid (DNA) in its matrix. Mitochondrion DNA (mtDNA) is responsible for the transcription of essential proteins for the mitochondrial function.1 As the paternal mtDNA is degraded upon fertilization, mtDNA is purely inherited from the maternal side.2 Although mtDNA has double strands it is different in structure from the nuclear DNA.3 Interestingly, it was found that mutations can occur in mtDNA as they do in the nuclear DNA and culminate in diseases. Furthermore, mtDNA is more vulnerable to mutations due to the lack of histone and the high exposure to oxygen free radicals in the mitochondria.1 Mutations in mtDNA can lead to disturbances in the oxidative phosphorylation process and can affect the energy production process.4 The first description of mtDNA mutation was reported in 1988 in patients with mitochondrial myopathy.5 Later, multiple mutations were discovered and linked to specific diseases. Most mtDNA mutations are manifested by neurological symptoms such as...
myopathies, optic neuropathy, ophthalmoplegia, and myoclonic epilepsy which is seen in diseases such as Leber hereditary optic neuropathy (LHON) and Kearns–Sayre syndrome (KSS). Later, the study and identification mtDNA mutations helped to identify the pathophysiology of Alzheimer, Parkinson’s, diabetes, and cancer.\(^6\)

Migraine with its multiple types was proposed to be one of the neurological diseases that could be explained by mtDNA mutations. This is supported by the possible role of increased neuronal excitability, failure of the ATP synthesis and cerebral vascular abnormalities related to mitochondrial dysfunction on the pathophysiology of migraine.\(^7\) In addition, the prominent maternal inheritance of migraine might also support the involvement of mtDNA mutations.\(^8\) Studies that explore the pathophysiology of migraine with aura revealed some evidence for the involvement of mitochondrial dysfunction in the first stage of migraine attack. Similarly, a higher level of serum lactate was demonstrated in migraineurs during and between attacks that can further reflect the possible impairment of pyruvate metabolism in the Krebs cycle.\(^9\) Studies from Finland\(^10\) and Germany\(^11\) on migraine patients reported associations with mitochondrial DNA variant. The Nord-Trøndelag Health Study (HUNT) is a single population-based cohort study conducted in Norway’s Nord-Trøndelag County which revealed no significant association between the studied gene variations and migraine.\(^12\) However, it is worth mentioning that, the study by Børte et al utilised a microarray of known variants.\(^12\) Hence, a mitochondrial sequence-based study is needed to obtain all variants. Therefore, in the current study, we aim to identify the significant association of mitochondrial sequence variation using next generation-based sequencing analysis of mitochondrial sequence variation on migraine in the Saudi population and add a critical piece of information for studying migraine in humans.

**Materials and Methods**

The protocol of the current study was written in concordance with the ethical considerations of human studies of the Declaration of Helsinki and it was reviewed and authorized (IRB number: IRB-2021-01-250) by the Institutional Review Board of Imam Abdulrahman Bin Faisal University. Young female candidates (n = 1950), with an age range of 18–30 years, were screened for migraine, among which a total of 103 satisfied the ICHD-3 criteria. Twenty migraine cases among the females who satisfied the ICHD-3 criteria were confirmed in the neurology clinic of the university hospital and were recruited upon receiving the consent from migraineurs in the campus of Imam Abdulrahman Bin Faisal University (IAU), Dammam, Saudi Arabia. Twenty healthy volunteer candidates who were age matched were enrolled as controls upon receiving the consent and had no complaints of headache.

The participants were first interviewed and the history of the disease was collected, including the severity of the headache on a scale of 1–10, number of attacks per month, the presence or absence of aura, other associated symptoms, use of medication, and possible precipitating factors such as stress, sleep deprivation, fasting or missed meal, physical activity, loud sounds, changes of weather or temperature, strong smell or lights, specific food articles, phases of menstrual cycle, family history of migraine, and past history (other chronic diseases). The aim and objectives of the study were also explained to them and then they signed informed consents for their participation in the study.

**Analysis of Mitochondrial Sequence Variations**

The DNA was extracted (QIAamp DNA Blood Mini Kit, Qiagen, Germany) from blood and the purity of the DNA was assessed by nanodrop, and concentration of the DNA was examined by qubit fluorometer. Agarose gel electrophoresis was used to check the DNA integrity. All the samples were then sequenced using paired end whole exome sequencing followed by quality screening. Good quality was considered when the sample depth mean was ≥7.5, the variant call rate of sample was ≥0.5, and the genotype quality of sample mean was ≥28. For good quality variants filtering, the following criteria like raw read depth ≥10, phred score quality ≥30, and mapping quality ≥30 were considered. One python package was utilized for quality assessment of samples, genotypes and variants. Similarly, the python package-based Hail standard was implemented for the entire pipeline analysis of genome-wide association. The p-value <0.05 was considered as significant. Mitochondrial sequence variations were filtered for further analysis using NCBI GenBank Reference Sequence: NC_012920.1 (mitochondrion of *Homo sapiens*, complete genome). Moreover, the mitochondrial sequence variations were analyzed for the mitomap frequency and gnomAD (genome aggregation database) frequency using MITOMAP.\(^13\) Genes with significant single nucleotide polymorphisms (SNPs) were tested by the gene functional classification tool.
DAVID\textsuperscript{14} and functional enrichment analysis of protein-protein interaction networks using STRING 11.5.\textsuperscript{15} The top nine associated genes functional annotation was done by STRING 11.5 ($p$-value <0.05). For the pathway enrichment using the enrichR server for genes and pathway involvement were performed by KEGG search and reactome pathway through DAVID and STRING 11.5.

**Results**

Age matched migraineurs (22.10±3.63) and the controls (21.86±1.75) were selected for the whole exome sequencing to identify the mitochondrial sequence variations. The controls and cases were not significantly different in the baseline characteristic such as age ($p$-value = 0.818), body weight (kg) ($p$-value = 0.115) and BMI ($p$-value = 0.095). Frequency of the most common precipitating factors among the migraineurs are presented in Figure 1. Among the migraineurs of the study 57.9%, 50% and 55%, were with aura, using pain killer and have positive family history of migraine, respectively.

A total of 189 (Table S1) mitochondrial sequence variations were retrieved from the exome sequence data. Genome wide analysis of the mitochondrial sequence variations between the patients with migraine and control revealed association of 30 SNPs ($p$<0.05) in the mitochondrial genome (Table 1). The highest significant ($p = 0.001033$) observed in a coding SNP (rs1603225278) in the CYTB gene. Twenty-four significant SNPs are in the coding region of nine different genes (ND5, ND4, COX2, COX1, ND3, CYTB, COX3, ND2 and ND1) (Figure 2). A total of 7 SNPs (rs1603223919x, rs200044200, rs386829179, rs28359177, rs2854123, rs28359180 and rs193302971) were observed as significant in the ND5 gene.

Networks of protein-protein interaction analysis and the functional enrichment of the genes (ND5, ND4, COX2, COX1, ND3, CYTB, COX3, ND2 and ND1) with the significant SNPs revealed the most significant associated pathway, oxidative phosphorylation ($p = 2.55\times10^{-12}$) in the Kyoto encyclopedia of genes and genomes pathway analysis of the significant genes (Figure 3; Table 2). Enrichment analysis of mitochondrial genes with significant variations associated with migraine individuals revealed the association of nervous system disease ($p = 0.0083$), brain disease ($p = 0.0193$), central nervous system disease ($p = 0.0065$), and Leber hereditary optic neuropathy ($p = 4.46\times10^{-11}$) (Table 2).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Precipitating possible factors of migraine attack and frequency in the migraine participants from Saudi Arabia.}
\end{figure}
Discussion

The present study aimed to identify the significant association of mitochondrial gene variations and migraine in Saudi candidates using next generation-based whole sequencing analysis of mitochondrial DNA. Successfully, we were able to

| S. No | Locus Contig | Locus Position | Existing Variation (SNP ID) | Alleles | Associated Allele | Gene | p-value |
|-------|--------------|----------------|-----------------------------|---------|------------------|------|---------|
| 1     | chrM         | 15433          | rs1603225278                | ["C", "T"] | T                | CYTB | 0.001033 |
| 2     | chrM         | 16220          | rs386829281                 | ["A", "G"] | G                |      | 0.001033 |
| 3     | chrM         | 10217          | rs1556423786                | ["A", "G"] | G                | ND3  | 0.010229 |
| 4     | chrM         | 12834          | rs1603223919                | ["A", "G"] | G                | ND5  | 0.010229 |
| 5     | chrM         | 13135          | rs200044200                 | ["G", "A"] | A                | ND5  | 0.010229 |
| 6     | chrM         | 15317          | rs2853507                   | ["G", "A"] | A                | CYTB | 0.010229 |
| 7     | chrM         | 8152           | rs1603221312                | ["G", "A"] | A                | COX2 | 0.010229 |
| 8     | chrM         | 16301          | rs879194775                 | ["C", "T"] | T                |      | 0.026534 |
| 9     | chrM         | 10115          | rs3899188                   | ["T", "C"] | C                | ND3  | 0.03179  |
| 10    | chrM         | 1018           | rs2856982                   | ["G", "A"] | A                |      | 0.03179  |
| 11    | chrM         | 11386          | rs1556423940                | ["T", "C"] | C                | ND4  | 0.03179  |
| 12    | chrM         | 11944          | rs3087901                   | ["T", "C"] | C                | ND4  | 0.03179  |
| 13    | chrM         | 13395          | rs386829179                 | ["A", "G"] | G                | ND5  | 0.03179  |
| 14    | chrM         | 13590          | rs28359177                  | ["G", "A"] | A                | ND5  | 0.03179  |
| 15    | chrM         | 13650          | rs2854123                   | ["C", "T"] | T                | ND5  | 0.03179  |
| 16    | chrM         | 13803          | rs28359180                  | ["A", "G"] | G                | ND5  | 0.03179  |
| 17    | chrM         | 13934          | rs193302971                 | ["C", "T"] | T                | ND5  | 0.03179  |
| 18    | chrM         | 16209          | rs386829278                 | ["T", "C"] | C                |      | 0.03179  |
| 19    | chrM         | 16354          | rs878897391                 | ["C", "T"] | T                |      | 0.03179  |
| 20    | chrM         | 2789           | rs28358581                  | ["C", "T"] | T                |      | 0.03179  |
| 21    | chrM         | 3594           | rs193303025                 | ["C", "T"] | T                | ND1  | 0.03179  |
| 22    | chrM         | 5196           | rs1603219826                | ["T", "C"] | C                | ND2  | 0.03179  |
| 23    | chrM         | 7175           | rs28358874                  | ["T", "C"] | C                | COX1 | 0.03179  |
| 24    | chrM         | 7256           | rs1556423258                | ["C", "T"] | T                | COX1 | 0.03179  |
| 25    | chrM         | 7274           | rs879089638                 | ["C", "T"] | T                | COX1 | 0.03179  |
| 26    | chrM         | 7771           | rs366038563                 | ["A", "G"] | G                | COX2 | 0.03179  |
| 27    | chrM         | 8206           | rs28358883                  | ["G", "A"] | A                | COX2 | 0.03179  |
| 28    | chrM         | 9221           | rs367578507                 | ["A", "G"] | G                | COX3 | 0.03179  |
| 29    | chrM         | 9530           | rs879237361                 | ["T", "C"] | C                | COX3 | 0.03179  |
| 30    | chrM         | 11467          | rs2853493                   | ["A", "G"] | G                | ND4  | 0.041918 |
demonstrate a significant association of 30 different SNPs allocated mainly in nine genes (ND5, ND4, COX2, COX1, ND3, CYTB, COX3, ND2 and ND1). The most significant association was in gene: CYTB ($p = 0.001033$), while the highest number of associated SNPs was demonstrated in gene ND5. These mitochondrial genes are responsible for encoding important proteins in the respiratory electron transfer chain in the mitochondria. Mt-CYTB which is the most significantly associated gene is responsible for encoding cytochrome b which is part of complex III. Mt-ND genes including ND1, ND2, ND3, ND4, and ND5 are genes responsible for encoding essential components of complex I which is NADH dehydrogenase. Mt-COX1, mt-COX2 and mt-COX3 genes encode cytochrome c oxidase which is a subunit of complex IV. Mutations in these mitochondrial genes are reported earlier in multiple neurological and muscular diseases like MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), mitochondrial encephalomyopathy, mitochondrial myopathy, Leber hereditary optic neuropathy, and muscle weakness. The link between neurological and muscular symptoms and the mitochondrial mutations can be explained by the absolute reliance of these
tissues ie nervous and muscular tissue on the aerobic metabolism and the oxidative phosphorylation system of the mitochondria. Therefore, any disturbance in the machinery of energy production particularly in the respiratory electron transfer chain can be manifested by neurological and muscular symptoms.

Few studies in the literature have focused on studying the association of mtDNA mutations and migraine. One study has identified a mutation in the mitochondrial transfer RNA (m.3243A>G) in a group of subjects. Further analysis of the same cohort showed that the subjects of this mutation (m.3243A>G) have higher prevalence of migraine. Similarly, studies of diseases with mitochondrial mutations demonstrated a higher incidence of migraine in these patients. Studying the mitochondrial mutations, as is the case with other neurological diseases, can help in understanding the pathophysiology of migraine. Some studies proposed that the alteration of the energy production that resulted from abnormal oxidative phosphorylation and a deranged respiratory electron transfer chain can lower the level of ATP in the involved neurons. In migraine, particularly migraine with aura, a lower ATP level of the affected neurons can reduce the threshold for the cortical spreading depression (CSD) and can culminate in the initiation of the aura. Another supportive evidence is that imaging techniques in migraineur patients revealed disturbances in the mitochondrial function in certain areas of the brain. Mitochondrial DNA variations from Arab ancestries have reported for its association with obesity, however there were no studies from the Arab population, specifically from Saudis on the neurological diseases. On an international level the HUNT study in Norway’s Nord-Trøndelag County revealed no significant association using a microarray of known variants between these specific variants and migraine in the Norwegian population; furthermore, the study excluded samples not passing quality control for nuclear genotypes, in addition to samples with low call rate and closely maternally related. Hence, this study is the first to study the association of mtDNA mutations on migraine in Arab ancestries, specifically from the Saudi population and it adds a valuable piece of knowledge to the Saudi genome project. It further encourages large studies to confirm the current found mutations.
| Pathway ID | Pathway Description | Observed Number of Gene Count | Background Gene Count | Strength | False Discovery Rate | Matching List of Proteins in the Network |
|-----------|---------------------|-----------------------------|-----------------------|---------|---------------------|-----------------------------------|
| hsa00190  | Oxidative phosphorylation | 7                          | 130                   | 2.12    | 2.55E-12            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa04714  | Thermogenesis        | 7                          | 229                   | 1.87    | 6.09E-11            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa05012  | Parkinson disease    | 7                          | 240                   | 1.85    | 6.09E-11            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa05020  | Prion disease        | 7                          | 265                   | 1.81    | 8.31E-11            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa05016  | Huntington disease   | 7                          | 298                   | 1.76    | 1.49E-10            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa05010  | Alzheimer disease    | 7                          | 355                   | 1.68    | 3.93E-10            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa05014  | Amyotrophic lateral sclerosis | 7                        | 352                   | 1.69    | 3.93E-10            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa01100  | Metabolic pathways  | 8                          | 1447                  | 1.13    | 5.20E-08            | MT-CO1,MT-CYB,PTGS1,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| hsa04723  | Retrograde endocannabinoid signaling | 4                    | 145                   | 1.83    | 1.1E-05             | MT-ND1,MT-ND4,MT-ND2,MT-ND3 |
| hsa04260  | Cardiac muscle contraction | 3                      | 87                    | 1.93    | 0.00024             | MT-CO1,MT-CYB,MT-CO2 |
| hsa04932  | Non-alcoholic fatty liver disease | 3                       | 148                   | 1.7     | 0.001               | MT-CO1,MT-CYB,MT-CO2 |
| HSA-611105| Respiratory electron transport | 7                      | 101                   | 2.23    | 1.77E-11            | MT-CO1,MT-CYB,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |
| HSA-6799198| Complex I biogenesis | 4                          | 55                    | 2.25    | 2.24E-05            | MT-ND1,MT-ND4,MT-ND2,MT-ND3 |
| HSA-1430728| Metabolism          | 8                          | 2089                  | 0.97    | 5.96E-05            | MT-CO1,MT-CYB,PTGS1,MT-ND1,MT-CO2,MT-ND4,MT-ND2,MT-ND3 |

**Reactome Pathway**

| Pathway ID | Pathway Description | Observed Number of Gene Count | Background Gene Count | Strength | False Discovery Rate | Matching List of Proteins in the Network |
|------------|---------------------|-----------------------------|-----------------------|---------|---------------------|-----------------------------------|

**WikiPathways**

| Pathway ID | Pathway Description | Observed Number of Gene Count | Background Gene Count | Strength | False Discovery Rate | Matching List of Proteins in the Network |
|------------|---------------------|-----------------------------|-----------------------|---------|---------------------|-----------------------------------|

(Continued)
### Table 2 (Continued).

| Pathway ID | Pathway Description                          | Observed Number of Gene Count | Background Gene Count | Strength | False Discovery Rate | Matching List of Proteins in the Network |
|------------|---------------------------------------------|-------------------------------|-----------------------|----------|----------------------|------------------------------------------|
| WP4919     | Neuroinflammation                           | 2                             | 13                    | 2.58     | 0.0067               | MT-CO1, MT-CO2                           |
| WP4396     | Nonalcoholic fatty liver disease            | 3                             | 155                   | 1.68     | 0.0098               | MT-CO1, MT-CYB, MT-CO2                   |
| WP4922     | Mitochondrial complex IV assembly           | 2                             | 35                    | 2.15     | 0.0282               | MT-CO1, MT-CO2                           |
|            | **Disease gene association**                |                               |                       |          |                      |                                          |
| DOID:705   | Leber hereditary optic neuropathy          | 5                             | 11                    | 3.05     | 4.46E-11             | MT-CO1, MT-CYB, MT-ND1, MT-ND4, MT-ND2  |
| DOID:700   | Mitochondrial metabolism disease           | 7                             | 173                   | 2        | 8.57E-11             | MT-CO1, MT-CYB, MT-ND1, MT-CO2, MT-ND4, MT-ND2  |
| DOID:0060536 | Mitochondrial complex I deficiency       | 4                             | 41                    | 2.38     | 1.23E-06             | MT-ND1, MT-ND4, MT-ND2, MT-ND3          |
| DOID:1398  | Parasitic infectious disease                | 4                             | 62                    | 2.2      | 4.46E-06             | MT-CO1, MT-CYB, MT-ND1, MT-CO2          |
| DOID:3652  | Leih disease                                | 4                             | 72                    | 2.13     | 7.04E-06             | MT-ND1, MT-ND4, MT-ND2, MT-ND3          |
| DOID:883   | Parasitic helminthritis infectious disease  | 3                             | 31                    | 2.37     | 9.70E-05             | MT-CO1, MT-ND1, MT-CO2                   |
| DOID:0050251 | Coenurosis                        | 2                             | 2                     | 3.39     | 0.00029              | MT-CO1, MT-ND1                          |
| DOID:1495  | Cystic echinococcosis                      | 2                             | 4                     | 3.09     | 0.00068              | MT-CO1, MT-ND1                          |
| DOID:3687  | MELAS syndrome                             | 2                             | 4                     | 3.09     | 0.00068              | MT-ND1, MT-ND4                          |
| DOID:331   | Central nervous system disease             | 5                             | 1107                  | 1.04     | 0.0065               | MT-CO1, MT-ND1, MT-CO2, MT-ND4, MT-ND2  |
| DOID:3762  | Cytochrome-c oxidase deficiency disease     | 2                             | 22                    | 2.35     | 0.0083               | MT-CO1, MT-CO2                          |
| DOID:850   | Lung disease                                | 3                             | 172                   | 1.63     | 0.0083               | MT-CO1, MT-CYB, MT-ND1                  |
| DOID:863   | Nervous system disease                      | 6                             | 2132                  | 0.84     | 0.0083               | MT-CO1, MT-CYB, MT-ND1, MT-CO2, MT-ND4, MT-ND2  |
| DOID:10652 | Alzheimers disease                         | 2                             | 35                    | 2.15     | 0.0169               | MT-ND1, MT-ND2                          |
| DOID:0080000 | Muscular disease                       | 3                             | 254                   | 1.46     | 0.0193               | MT-CO1, MT-ND1, MT-ND4                  |
| DOID:114   | Heart disease                               | 3                             | 257                   | 1.46     | 0.0193               | MT-CO1, MT-CYB, MT-ND1                  |
| DOID:936   | Brain disease                               | 4                             | 739                   | 1.12     | 0.0193               | MT-CO1, MT-ND1, MT-CO2, MT-ND4          |

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Further studies among Arab ancestries with a large sample size and haplotyping analysis may confirm the possible association of the significant impact on the pathways of migraine development. Even though the small sample size in the study is one of the notable limitations, the current pilot study opens an avenue for large confirmatory studies.

**Conclusion**

Our present study demonstrated the significant association of 30 SNP variants in 9 genes: CYTB, COX1, COX2, ND1, ND2, ND3, ND4, ND5, and COX3 with Saudi migraineurs, which should be further confirmed. The most significant SNP variant was located in the CYTB gene, while the highest number of associated SNP variants with migraine were found in ND5. The mutated variants are responsible for encoding essential proteins that constitute important subunits in the respiratory electron transfer chain, and therefore play a significant role in the energy production and ATP synthesis by the mitochondria in the neurons. The current study's findings are the first in this population and require larger scale studies to confirm the association.

**Data Sharing Statement**

All data will be available on reasonable request from the corresponding author.

**Ethics Statement**

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) at Imam Abdulrahman Bin Faisal University. IRB approval number: IRB-2021-01-250.

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**Disclosure**

The authors declare no conflicts of interests in this work.

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