A Novel Variant in the PAX4 Gene Causes Maturity-Onset Diabetes of the Young (MODY), Type IX with Neurodevelopmental Disorder

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ABSTRACT

A genetically diverse condition, maturity-onset diabetes of the young (MODY), frequently develops before the age of 25. MODY is caused by disease-causing sequence variations in the PAX4 gene, which is found on chromosome 7q32.1. Additionally, it has also been observed that variants in PAX4 have also been associated with neurodevelopmental disability. Whole exome sequencing (WES) followed by Sanger sequencing was performed for all the available affected and unaffected members of the family. Data analysis revealed a novel heterozygous nonsense variant (c.61C>T; p.Gln21*) in the PAX4 gene in the affected individuals, which segregated perfectly with the disease phenotype. The present study adds to the PAX4 mutation spectrum and reports on the first case of MODY associated with neurodevelopmental disorders in humans.

KEYWORDS
MODY, PAX4, neurodevelopmental disorders, nonsense variant, paired domain, DNA sequencing, whole exome sequencing

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a genetically and clinically heterogeneous autosomal dominant monogenic diabetes type which mostly occurs before 25 years of age (Jo et al., 2011). MODY is the most common form of monogenic diabetes, accounting for an estimated 1-5% of diabetes cases in Europe (Firdous et al., 2018), often misdiagnosed as diabetes type 1 or type 2. However, its prevalence varies by ethnicity owing to less resources for its genetic testing. Recent studies have reported a MODY prevalence of 1/23,000 among children and 1/10,000 among adult population (Owen, 2014). MODY prevalence data in Asian countries are largely unknown (Nkonge et al., 2020). Pathogenic disease-causing variants have been identified in 14 different genes associated with MODY subtypes (MODY1-14) located on different human chromosomes (Table 1) (Stenson et al., 2017).

Heterozygous PAX4 (MIM 167413) variants have been studied to be associated with MODY type IX (MIM 612225) located on chromosome 7q32.1. The transcriptional factor (PAX4) act as a transcriptional repressor through two domains, a homeodomain (HD) and a paired domain (PD) (Brun and Gauthier, 2008). Studies have revealed the involvement of PAX4 in β-cell development. The Pax4 knockout mice, having a heterozygous variant, did not exhibit β-cell and δ-cell proliferation and differentiation suggesting its role in transporting progenitor cells to the different islet cell lineages (Sosa-Pineda, 2004; Napolitano et al., 2015). Earlier PAX4 variants were found to be associated with diabetes type 2 (Shimajiri et al., 2001); later, heterozygous PAX4 variants were reported in patients with MODY (Plengvidhya et al., 2007; Gao et al., 2021). The overlapping clinical features of MODY with the classical...
diabetes present a diagnostic challenge for the clinicians and require genetic testing for differentiation among 14 MODY subtypes (Chapla et al., 2015). Similarly, denovo variant in the \( \text{PAX4} \) gene has been associated with developmental disorders (Jin et al., 2017; Turner et al., 2019; Edwards et al., 2020).

This study aimed to screen the present family via whole exome sequencing (WES) for any variants associated with MODY pathogenesis. This is the first study to report a novel variant in the \( \text{PAX4} \) gene in a family with diabetes and neurodevelopmental disorder.

### METHODS

#### Ethics statement

The clinical and molecular examination of a nonconsanguineous family with MODY with neurodevelopmental disorder is described in the present study. In accordance with the Declaration of Helsinki, written informed consent was obtained from the patients and other family members for the publication of this work. In ethylenediaminetetraacetic acid (EDTA) tubes, blood samples were collected at the time of sampling (Fig. 1a). Standard techniques were used to extract and quantify DNA (Umair et al., 2016). All subjects gave their informed consent for inclusion in the study, and the study was approved by the Ethics Committee of King Saud Bin Abdulaziz University for Health Sciences.

#### Molecular analysis

Using DNA from the afflicted member (III-1) and conventional techniques, next-generation sequencing (NGS) procedures, including WES, were carried out. As previously mentioned (Hayat et al., 2020; Nøstvik et al., 2021), WES and variation filtering stages were carried out. Standard screening techniques were employed to look for previously described functional variations in several encephalopathy genes, such as nonsense, missense, indels, and splice sites. The filtered disease-causing variation was confirmed using standard Sanger sequencing (Ullah et al., 2018; Umair et al., 2020).

#### In silico analysis

The pathogenicity index of the detected sequence variations was estimated using Mutation Taster, Mutation Assessor, Varsome, and combined annotation dependent depletion (CADD). In addition, using 1000 genomes, ExAC Browser, and gnomAD, the frequency of the variant was evaluated in the general population.

#### Sanger sequencing

Using University of California, Santa Cruz (UCSC) genome database browser, the genomic sequence of the concerned gene was obtained. The primer-3 program was used to create the primer sequences for the PCR reaction. In both affected and unaffected family members, the region spanning the variant was Sanger sequenced using standard methods (Irfanullah et al., 2015; Shah et al., 2016). Purification of the PCR-amplified DNA was performed according to the manufacturer’s instructions. The PCR-amplified DNA was purified in accordance with the manufacturer’s recommendations. The BIOEDIT sequence alignment editor was used to find sequence variations and position of the variant.

#### PAX4—3D protein modeling

The AlphaFold 2 was used to model the 3D structure of PAX4 protein using standard methods (Nayab et al., 2021). After the structure was prepared and energy minimized with the mmff99s force field implemented in MOE, the mutant structure was modeled from the native pax4 truncation (Mahmood et al., 2023).

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**Table 1: List of genes involved causing MODY.**

<table>
<thead>
<tr>
<th>Type</th>
<th>OMIM</th>
<th>Gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODY1</td>
<td>125850</td>
<td>HNF4A; 600281</td>
<td>Chromosome 20q13.12</td>
</tr>
<tr>
<td>MODY2</td>
<td>125851</td>
<td>GCK; 138079</td>
<td>Chromosome 7p13</td>
</tr>
<tr>
<td>MODY3</td>
<td>600496</td>
<td>HNF1A; 142410</td>
<td>Chromosome 12q24.31</td>
</tr>
<tr>
<td>MODY4</td>
<td>606392</td>
<td>PDX1; 600733</td>
<td>Chromosome 13q12.2</td>
</tr>
<tr>
<td>MODY5</td>
<td>137920</td>
<td>TCP2; 189907</td>
<td>Chromosome 17q12</td>
</tr>
<tr>
<td>MODY6</td>
<td>606394</td>
<td>NEUROD1; 601724</td>
<td>Chromosome 2q31.3</td>
</tr>
<tr>
<td>MODY7</td>
<td>610508</td>
<td>KLF11; 603301</td>
<td>Chromosome 2p25.1</td>
</tr>
<tr>
<td>MODY8</td>
<td>609812</td>
<td>CEL; 114840</td>
<td>Chromosome 9q34.13</td>
</tr>
<tr>
<td>MODY9</td>
<td>612225</td>
<td>PAX4; 167413</td>
<td>Chromosome 7q32.1</td>
</tr>
<tr>
<td>MODY10</td>
<td>613370</td>
<td>INS; 176730</td>
<td>Chromosome 11p15.5</td>
</tr>
<tr>
<td>MODY11</td>
<td>613375</td>
<td>BLK; 191305</td>
<td>Chromosome 8p23.1</td>
</tr>
<tr>
<td>MODY13</td>
<td>616329</td>
<td>KCNJ11; 600937</td>
<td>Chromosome 11p15.1</td>
</tr>
<tr>
<td>MODY14</td>
<td>616511</td>
<td>APPL1; 604299</td>
<td>Chromosome 3p14.3</td>
</tr>
</tbody>
</table>

*Abbreviation: MODY, maturity-onset diabetes of the young.*
RESULTS

Clinical report

The age of the boy (III-1) at the time of study was 17 years. The affected boy (III-1) was nonobese with 164.8-cm height, 52.3-kg weight, and a normal blood pressure (114/65 mmHg). His urine ketone bodies were positive, having 147-mg/dl fasting glucose level. Other laboratory tests were also normal. Despite the higher blood glucose levels (252/357 ng/ml), the blood C-peptide levels both fasting and postprandial were low (1.05/1.21 ng/ml). Details of the clinical tests performed are presented in Table 2.

Brain MRI

The brain parenchyma exhibits diffuse atrophic alterations, including a deepening of the cortical sulci, gyri, and prominence of the ventricular system. The left corona radiata shows an oval-shaped region with an altered signal intensity of 1.1 cm × 0.3 cm. This region shows low-signal intensity on T1 and fluid-attenuated inversion recovery (FLAIR) and high-signal intensity on T2, with a rim of high-signal intensity on T1 and low-signal intensity on T2. Susceptibility artifacts are observed in this area’s dependent and peripheral regions on gradient echo sequences. There is no discernible aberrant postcontrast enhancement. The results most likely point to a previous vascular injury or hemorrhage (Fig. 1b).

Given the patient’s age, there is evidence of delayed myelination of the white matter. Without diffusion limitation, altered areas of low-signal intensity include the frontoparietal region, which looks low on T1 and high on T2 and is likely to represent an old ischemic injury. There is no evidence of an acute infarction. There is no evidence of hydrocephalus or a midline shift, pellucid septum cavum. The thalamus, basal ganglia, corpus callosum, brainstem, and cerebellar hemispheres are normal. On postcontrast

Table 2: Clinical diagnostic tests performed.

<table>
<thead>
<tr>
<th>Family member</th>
<th>Variant</th>
<th>Diabetes status</th>
<th>BMI (kg/m²)</th>
<th>Age at diagnosis</th>
<th>FPG (mmol/l)</th>
<th>PG 2 h after OGTT (mmol/l)</th>
<th>HbA1c (age) (%)</th>
<th>Current treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-2</td>
<td>No</td>
<td>NG</td>
<td>15.3</td>
<td>–</td>
<td>4.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>III-1</td>
<td>Yes</td>
<td>MODY</td>
<td>21.5</td>
<td>17</td>
<td>INS</td>
<td>–</td>
<td>7.1</td>
<td>INS</td>
</tr>
<tr>
<td>II-3</td>
<td>No</td>
<td>NG</td>
<td>17.2</td>
<td>–</td>
<td>5.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>III-2</td>
<td>No</td>
<td>NG</td>
<td>20</td>
<td>–</td>
<td>4.7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; INS, insulin; MODY, maturity-onset diabetes of the young; NA, not available; NG, normoglycemic; OGTT, oral glucose tolerance test; PG, plasma glucose; T2D, type 2 diabetes (nonautoimmune diabetes).
examination, no aberrant enhancing lesion is observed. An unintentional finding of asymmetric transverse sinus appearance in which the left transverse sinus is hypoplastic (normal variant) revealed a minor rise signal, most likely related to a low-velocity flow (Fig. 1b).

**Molecular analysis**

WES followed by Sanger sequencing revealed a novel nonsense variant (c.61C>T) in the \(\text{PAX4}\) gene [ENST00000341640.2; NM_006193.2] at chromosomal location 7q32.1 that resulted in a premature stop codon (p.Gln21*) (Fig. 2). Online available bioinformatics tools predicted the variant to be highly deleterious. The pathogenicity index was calculated using Mutation Taster and Varsome. The frequency of the variant in the general population was determined using ExAC/gnomAD and the variant was not observed in the public databases. According to American College of Medical Genetics and Genomics (ACMG), the identified variant is classified as likely pathogenic (Class-II; PVS1, PM2). To exclude the presence of variant as polymorphism, exon 1 of the \(\text{PAX4}\) gene was Sanger sequenced in 185 ethnically matched control individuals.

**Figure 2:** (a and b) Sanger sequencing electropherograms of the affected (heterozygous) and normal individuals (homozygous wild type) of the family. (c) Image showing that glutamine amino acid at position 21 is conserved across different species.

**Figure 3:** (a and b) 3D protein structure of PAX4 wild type and mutated PAX4 proteins.
DISCUSSION

PAX4 is a member of the Pax gene family, a group of highly conserved transcription factors that play a vital role in cell plasticity in adults and in embryonic organogenesis (Wang et al., 2008; Blake and Ziman, 2014). PAX4 is expressed in the pancreas where it helps to stabilize the cells that produce insulin during embryonic development. Moreover, it also acts as a master regulator in the adaptation processes during adulthood (Brun and Gauthier, 2008; Napolitano et al., 2015). The Pax family consists of nine members (PAX1-9) that share three conserved structural motifs. These are the highly conserved 127–128 amino acid PD, a HD, which interacts with DNA, and the octapeptide (OP) motif, which is positioned between the PD and the HD (Fig. 4a and b) (Lorenzo et al., 2017).

Studies have shown that mutations in any of these domains lead to structural variations which ultimately can lead to impaired β-cell function (Hosoe et al., 2021). PAX4 variants have been identified earlier in East Asian populations to be the cause of T2D (Napolitano et al., 2015; Suzuki et al., 2019). To date, only 33 variants have been reported in the PAX4 gene human gene mutation database (HGMD); while only 17 variants have been associated with MODY, 13 variants have been associated with diabetes (type 1, 2), and only 2 variants have been associated with neurodevelopmental disorder with conotruncal heart defect (Table 3). Here, we report a novel nonsense variant (c.61C>T) in the exon 1 of the PAX4 gene (Fig. 2a and b), which resulted in a premature stop codon (p.Gln21*). The mutation is located in a highly conserved PD and might result in complete LOF of the PAX4 protein (Fig. 4a and b). The glutamine amino acid at position 21 is highly conserved across different species (Fig. 2c).

The present study describes a family diagnosed with MODY and neural network-based (NDD). The affected boy (III-1) had no signs of obesity but he might need an insulin therapy. Severe features such as renal anomalies reported earlier were not observed in our patients (Lorenzo et al., 2017). In addition, the patient reported here had additional neurodevelopmental concerns that have been reported previously (Napolitano et al., 2015; Suzuki et al., 2019). However, conotruncal heart...
defect was not reported in the presented proband. Previously, MODY and neurodevelopmental disorder have not been associated in a single patient with PAX4 pathogenesis.

Taken together, the findings showed that PAX4 might be added to the growing list of genes having MODY-related neurodevelopmental disorders (Jin et al., 2017; Turner et al., 2019; Edwards et al., 2020). Additionally, the newborn screening program can be expanded to include the identification of novel candidate genes (Alfadhel et al., 2019), and parents who want to become pregnant again can use techniques like noninvasive prenatal testing (NIPT) and preimplantation genetic testing (PGT) (Alyafee et al., 2021a, b, 2022). Thus, in order to completely understand the cellular response to the PAX4 related depletion and how this impacts MODY and NDD in patients, additional in-depth studies are required leveraging current advancements in NGS-sequencing technology and functional characterization (Umair, 2023).

CONCLUSION

In conclusion, we have identified a novel heterozygous variant in the PAX4 gene associated with MODY and NDD. We suggest proper genetic and molecular testing for patients suffering from diabetes prior to treatment. PAX4 genetic regulations might help in the proper understanding of the protein function, designing therapy and improving the outcome for the patient.

FUNDING

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AUTHOR CONTRIBUTIONS

T.A. and M.U. designed the study, conceived the study, and analyzed the results. S.R., A.W., T.A. and S.A. conceived an initial part of the study, performed the experiment and histology, and helped in compiling the results. M.U. and S.R. performed the experiment. S.R., A.W., T.A., S.A. and M.U. helped in writing the results. S.A. and S.R. wrote the paper with input from all other authors. M.U., S.R., T.A. and A.W. made substantial contribution to the interpretation of data and revising the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in association with the present study.

ACKNOWLEDGEMENTS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All subjects gave their informed consent for inclusion in the study, and the study was approved by the Ethics Committee of King Saud Bin Abdulaziz University for Health Sciences. The study protocol was done in accordance with the principles of the Declaration of Helsinki.

AVAILABILITY OF DATA AND MATERIALS

All the data are provided in the manuscript.

REFERENCES


