Identification of the First PAX4-MODY Family Reported in Brazil

Purpose: The aim of this study was to sequence the coding region of the PAX4 gene in a Brazilian cohort with clinical manifestations of monogenic diabetes.

Patients and Methods: This study included 31 patients with autosomal dominant history of diabetes, age at diagnosis ≤40 years, BMI <30 kg/m², and no mutations in GCK or HNF1A, HNF4A, and HNF1B. Screening of the PAX4 coding region was performed by Sanger sequencing. In silico algorithms were used to assess the potential impact of amino acid substitutions on protein structure and function. Additionally, PAX4-MODY family members and 158 control subjects without diabetes were analyzed for the identified mutation.

Results: The molecular analysis of PAX4 has detected one missense mutation, p.Arg164Gln (c.491G>A), segregating with diabetes in a large Brazilian family. The mutation was absent among the control group. The index case is a woman diagnosed at 32 years of age with polyneuropathy and treated with insulin. She did not present diabetic renal disease or retinopathy. Family members with the PAX4 p.Arg164Gln mutation have a heterogeneous clinical manifestation and treatment response, with age at diagnosis ranging from 24 years to 50 years.

Conclusion: To the best of our knowledge, this is the first study to report a PAX4-MODY family in Brazil. The age of PAX4-MODY diagnosis in the Brazilian family seems to be higher than the classical criteria for MODY. Our results reinforce the importance of screening large monogenic diabetes families for the understanding of the clinical manifestations of rare forms of diabetes for the specific and personalized treatment.

Keywords: diabetes mellitus, monogenic diabetes, MODY, PAX4, mutation

Introduction

In the past years, mutations in genes that disrupt the secretion and signaling of insulin have been recognized as causative factors for monogenic forms of diabetes mellitus (DM). Among these genes, there are critical transcription factors, such as HNF4A, HNF1A, HNF1B, PDX1, NEUROD1, KLF11, and PAX4. The Paired Box Gene 4 (PAX4; OMIM*167413), also known as MODY9 gene, encodes a transcription factor that plays an important role in the development of β-cells and δ-cells. PAX4 acts in the differentiation of β-cells and δ-cells precursors in the early pancreas and later maintaining β-cells in differentiated state. In vivo experiments demonstrated that newborn mice that are knockout for both Pax4 alleles exhibit growth retardation and dehydration, dying 3 days after birth. To date, several variants in the PAX4 gene have been associated with a number of DM types, including type 1 DM (T1D), type 2 DM (T2D), Ketosis-Prone Diabetes.
(KPD),\textsuperscript{11} as well as monogenic diabetes.\textsuperscript{7} Mutations associated with monogenic diabetes were first identified in two patients of Thai origin, who did not present mutations in the other known MODY genes.\textsuperscript{7} More than one decade after this initial report, the number of studies supporting the involvement of \textit{PAX4} mutations in monogenic diabetes remains limited to a few cases, and restricted to Asian populations.\textsuperscript{7,12,15} Due to its rarity, the clinical characteristics of PAX4-MODY remain unclear, compromising its diagnosis. In this context, the identification of new cases will be helpful to better understand the PAX4-MODY phenotype. This study aimed to screen the coding region of \textit{PAX4} gene in a sample of Brazilian patients with a clinical suspicion of monogenic diabetes. To the best of our knowledge, this is the first study to describe a \textit{PAX4} mutation in a large Brazilian family with autosomal dominant diabetes.

**Patients and Methods**

**Subjects**

In this cross-sectional observational study, 31 unrelated patients with DM (13 males and 18 females; average age at diagnosis: 19.7±10.9 years) were recruited from the Clementino Fraga Filho University Hospital and from the State Institute for Diabetes and Endocrinology Luiz Capriglione, Rio de Janeiro, Brazil. In this study, the inclusion criteria were as follows: 1) age at onset equal to or less than 40 years old; 2) a positive family history of diabetes in at least two generations; and 3) negative β-cells anti-GAD (Glutamic Acid Decarboxylase) and anti-IA-2 (Islet Antigen-2) autoantibodies. We excluded patients with T1D, obesity (Body Mass Index [BMI] ≥30 kg/m\textsuperscript{2} or ≥95th percentile for age at diagnosis), history of diabetic ketoacidosis at diabetes onset, clinical signs of insulin resistance, and the presence of secondary causes of diabetes. Clinical information was obtained through a review of the medical chart. All patients were previously screened for \textit{GCK} or \textit{HNF1A} (based on the clinical phenotype),\textsuperscript{16} \textit{HNF4A} and \textit{HNF1B} mutations and did not show mutations. Additionally, family members were screened for the novel variant, as well as 158 healthy controls (59 males and 99 females; average age: 32.03±8.41 years; BMI average: 22.48±1.40 kg/m\textsuperscript{2}). The control group inclusion criteria were as follows: 1) fasting plasma glucose (FPG) <100 mg/dL and glycated hemoglobin (HbA1c) <5.7%; 2) BMI ≤24.9 kg/m\textsuperscript{2}; and 3) Individuals without a family history of diabetes. The Ethics and Research Committee of the Clementino Fraga Filho University Hospital (CAAE n° 04232512.4.0000.5257) and of the State Institute for Diabetes and Endocrinology Luiz Capriglione (CAAE n° 04232512) approved this study protocol. All participants were informed about the aim of this study and provided verbal and written consent.

**Molecular Genetics**

Genomic DNA from the probands and nondiabetic controls were isolated from peripheral blood leukocytes using the QiAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The proband’s family had their genomic DNA extracted from buccal epithelial cells.\textsuperscript{17} Screening of the entire coding region and exon-intron boundaries of the \textit{PAX4} gene was done (Supplemental Table S1). PCR products were purified by ExoSAP-IT Reagent (Applied Biosystems, Vilnius, Lithuania). Sanger sequencing was performed using the Big Dye Terminator Kit v3.1 (Applied Biosystems, Austin, TX, USA), conducted on an ABI 3130 Automatic Genetic Analyzer (Applied Biosystems).

**Bioinformatic Analysis**

The \textit{PAX4} variants identified were checked against PubMed, Clinvar, dbSNP (https://www.ncbi.nlm.nih.gov/), Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/), ExAC Browser (http://exac.broadinstitute.org), 1000 Genomes project database (http://www.internationalgenome.org), and Online Archive of Brazilian Mutations (ABraOM; http://abraom.ib.usp.br/index.php),\textsuperscript{18} in order to investigate their previous occurrence in these public databases. To assess the potential impact of the missense mutations identified, in silico pathogenicity prediction algorithms were used, including SIFT,\textsuperscript{19} PolyPhen-2,\textsuperscript{20} PROVEAN,\textsuperscript{21} Revel,\textsuperscript{22} WS-SNPs&GO,\textsuperscript{23} MutPred,\textsuperscript{24} SNAP,\textsuperscript{25} Fathmm,\textsuperscript{26} M-CAP,\textsuperscript{27} CADD,\textsuperscript{28} Mutation assessor,\textsuperscript{29} Align-GVGD,\textsuperscript{30} PANTHER-PSEP,\textsuperscript{31} and Mutation Taster.\textsuperscript{32} The Ensembl reference transcript ENST00000341640.2 of \textit{PAX4} gene (Genome release GRCh37.p13) was used as reference (https://wwwensembl.org/index.html).

**Results**

In this study, we screened the entire coding region of the \textit{PAX4} gene in 31 unrelated probands from Brazil. The participants have clinical characteristics of monogenic diabetes. Our results showed a missense mutation p.Arg164Gln (c.491G>A) segregating with DM in a large Brazilian family. This variant was absent among the 158 normoglycemic controls analyzed and was not found in the ABraOM database. We

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also found the variant p.Arg133Trp (c.397C>T) in heterozygous state in three patients (9.67%) and in one homozygous patient (3.22%), and the common missense p.His321Pro (c.962A>C) variant in 28 probands (C allele frequency: 0.677). The synonymous p.Gln173Gln (c.519A>G) and p. Gly150Gly (c.450C>T) variants were found in five patients (16.12%) and in one patient (3.22%), respectively.

The arginine residue in position 164 of the PAX4 homeodomain is evolutionary conserved among several species (Figure 1). The change of the arginine amino acid to glutamine in the 164 position was predicted to be harmful by all 15 algorithms (Table 1). The arginine is an amino acid charged positively while glutamine belongs to uncharged polar side groups. This mutation was registered in dbSNP under the access number rs587780414; it was found with allele frequency of 0.00004119 in ExAC. However, we did not find any previously association of this mutation to DM (Table 2).

We identified the PAX4 p.Arg164Gln in the heterozygous state in a normal weight woman (BMI: 24.8 kg/m²; Current age: 45 years). She was diagnosed with diabetes during her second pregnancy at the age of 32 years (BMI at diagnosis: 21.68 kg/m²). She reported polyneuropathy and did not present diabetic renal disease or retinopathy until that moment. The patient was treated with insulin therapy since the diagnosis of DM. The family pedigree is shown in Figure 2 and clinical features are summarized in Table 3.

**Figure 1** Diagrammatic representation of PAX4 gene and protein domains. Pathogenic mutations described associated to diabetes mellitus are pointed in the figure and PAX4-MODY are show in bold. Electropherograms of PAX4 exon 4 wild type and p.Arg164Gln (c.491G>A) in the patient DM35. Alignment by Clustal W (1.81) of PAX4 gene across species are presented (below).
The index-case’s mother (II-4) was diagnosed with DM at 45 years of age and died at 73 years with chronic kidney disease. The patient also reported three deceased uncles (individuals II-2, II-3, and II-5), three deceased aunts (individuals II-1, II-6, and II-7), and a cousin (individual III-9) with diabetes and four sisters with hyperglycemia (individuals III-2, III-3, III-4, and III-6). Thirteen family members were available for genetic testing and eight of them were found to be carrying the p.Arg164Gln, of which four exhibited hyperglycemia.

The proband’s older sister (individual III-2) is an overweight woman of 56 years old (BMI = 29.48 kg/m²; FPG = 128 mg/dL; HbA1c = 11.3%) diagnosed with DM at 38 years. She carried the mutation p.Arg164Gln in a heterozygous state. She has been on oral antidiabetic agents (OAD) treatment for 8 years (Metformin 1500 mg/day; Gliclazide 60 mg/day) and has hypertension. The carrier proband’s sister (individual III-3) is 49 years, non-obese (BMI = 23.61 kg/m²), and was diagnosed at 49 years with FPG of 104 mg/dL, glucose 2 hours post dextrose of 142 mg/dL, and HbA1c 6%. She is on Metformin 1000 mg/day. Like her, the carrier sister (individual III-4) was diagnosed with impaired glucose tolerance (IGT) at the age of 50 years and has been managed with nutritional

| Table 1 | In silico Prediction of Missense Mutations Identified in PAX4 Gene |
|---------|---------------------------------------------------------------|
| **Prediction Tool** | **Output** | **PAX4 – p.Arg164Gln** | **PAX4 – p.Arg133Trp** |
| SIFT | <0.05 damaging/≥0.05 tolerated (score range: 0–1) | 0.01 | Damaging | 0.1 | Tolerated |
| PolyPhen-2_HVAR | Probably damaging, possibly damaging or benign (score range: 0 [benign] to 1 [damaging]) | 0.997 | Probably damaging | 0.123 | Benign |
| PolyPhen-2_HDIV | Probably damaging, possibly damaging, or benign (score range: 0 [benign] to 1 [damaging]) | 1.000 | Probably damaging | 0.829 | Possibly damaging |
| PROVEAN | <−2.5 deleterious/−2.5 neutral (default score threshold:−2.5) | −3.04 | Deleterious | 1.19 | Neutral |
| Revel | >0.50 likely disease causing/<0.50 likely benign (score range: 0 to 1) | 0.871 | Likely disease causing | 0.318 | Likely benign |
| WS-SNP&GO | >0.5 disease-associated (score range: 0 to 1) | 0.642 | Disease | 0.160 | Neutral |
| MutPred2 | General pathogenicity score: >0.50 (score range: 0 to 1) | 0.501 | Possibly pathogenic | 0.175 | Benign |
| SNAP | ≥1 effect (score range: −100 to 100) | 74 | Effect | 71 | Effect |
| Fathmm Pathogenicity threshold: <0 | −4.12 | Damaging | −3.40 | Damaging |
| M-CAP | Pathogenicity threshold: >0.025 | 0.337 | Possibly pathogenic | * | * |
| CADD | >30 likely deleterious/>30 likely benign | 31 | Likely deleterious | 22 | Likely benign |
| Mutation Assessor | Score cutoff: 0.8 neutral and low impact/1.9 low impact and medium impact/3.5 medium impact and high impact | 3.615 | High | 0 | Neutral |
| Align-GVGD | C0 most likely neutral to C65 most likely deleterious (classifiers: C0 to C65) | C35 | Likely deleterious | C65 | Likely deleterious |
| PANTHER-PSEP | Length of time: >450 my probably damaging/450 my>time>200 my possibly damaging/<200 my probably benign | 1038 | Probably damaging | 30 | Probably benign |
| Mutation Taster | A. Disease causing: probably deleterious/D. disease causing automatic: deleterious/N. polymorphism: probably harmless/P. polymorphism automatic: harmless | A | Disease causing | N | Polymorphism |
| Total prediction tools = 15 | 15 = predicted to be harmful | 4 = predicted to be harmful |

Notes: *M-CAP scores not available for some alleles at Ch7:127,254,351 chromosome position. SIFT: https://sift.bii.a-star.edu.sg/, Polyphen: http://genetics.bwh.harvard.edu/pph2/index.shtml, PROVEAN: http://provean.jcvi.org/, Revel: https://sites.google.com/site/revelgenomics/downloads, WS-SNP&GO: http://snps.biolfold.org/snps-and-go/, MutPred2: http://mutdb.org/mutpred, SNAP: http://www.rostlab.org/services/SNAP, Fathmm: http://fathmm.biocompute.org.uk/index.html, MCAP: http://bejerano.stanford.edu/mcap/, CADD: https://cadd.gs.washington.edu/snv, MutationAssessor: http://mutationassessor.org/r3/, Align GVGD: http://agvgd.hci.utah.edu/agvgd_input.php, PANTHER-PSEP: http://www.pantherdb.org/tools/csnpScore.do, MutationTaster: http://www.mutationtaster.org/. Abbreviation: my, millions of years.

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therapy (FPG = 93 mg/dL; HbA1c = 6.1%). The sister with DM (individual III-6) did not present the mutation. She received the diagnosis in her second gestation at the age of 25 years old. She has been treated with fast-acting insulin analog. The family reported that the proband’s older brother (individual III-1) had schizophrenia and died at 48 years old due to a heart attack, and DM was not reported.

The proband’s cousin with DM (individual III-9) also presented the mutation tested (FPG = 169 mg/dL; HbA1c = 7.4%) and received the diagnosis in her second gestation at 24 years. She has diabetic retinopathy. Her mother with DM (individual II-7) was diagnosed at 36 years in her second gestation and had been on dialysis before dying.

In the younger examined generation, all eight individuals do not have DM (individuals IV-3, IV-5, IV-6, IV-9, IV-12, IV-13, IV-14, and IV-15). Four of them presented the genetic variant, including three proband’s nieces (individuals IV-3, IV-5, and IV-6), of 27 years, 35 years, and 29 years old, respectively, and the proband’s younger daughter (individual IV-13), of 14 years old.

**Discussion**

Variants in the PAX4 gene have been associated to the risk of non-monogenic types of DM in the past years. However, a few mutations in this gene have also been described as the cause of monogenic diabetes (Table 4), and, to the best of our knowledge, this is the first monogenic diabetes case (PAX4-MODY) reported in a Brazilian family.

Shimajiri et al. described the p.Arg121Trp mutation in seven Japanese patients with T2D and absent among 161 controls (Table 4). One of these patients, a woman diagnosed at the age of 29 years, carried this variant in the homozygous state. The variant p.Arg121Trp segregated from her heterozygous parents, who were cousins, to the patient and to her heterozygous sister. Severe diabetes was presented only in the homozygous proband. In our sample, we identified the p.Arg133Trp in three patients in heterozygosis and in one patient in homozygosis. This variant was described as benign/risk factor by ClinVar and it was predicted to be benign by the majority of the in silico tools analyzed (Table 1). Mauvais-Jarvis et al. previously reported an

| Exon or Intron | Mutated Protein | Mutated DNA | Consequence | Access Number | Clinvar | Domain | Functional Studies | Ref. |
|----------------|----------------|-------------|-------------|--------------|---------|--------|-------------------|------|
| 1              | p.Arg31Leu     | c.92G>A     | Missense    | rs115887120  | Likely-benign | PD     | Decreased transcriptional repress promoter function and decreased binding activity | [14] |
| 2              | p.Arg37Trp     | c.109C>T    | Missense    | rs35155575  | Uncertain-significance, risk-factor | PD     | na                | [11,33] |
| 3              | p.Arg52Cys     | c.154C>T    | Missense    | rs770923465 | na       | PD     | na                | [15] |
| 3              | n.a            | c.374_412del| Sequence alteration | rs132588896 | na       | PD     | Loss of transcriptional repressor function | [12] |
| 3              | p.Arg121Trp    | c.361C>T    | Missense    | rs114202595 | Pathogenic | PD     | na                | [10] |
| 3              | p.Arg133Trp    | c.397C>T    | Missense    | rs2233578 | Benign/Likely benign, risk factor | Between PD and HD | Decreased transcriptional repress promoter function | [11] |
| 4              | p.Arg164Trp    | c.490C>T    | Missense    | rs121917718 | Pathogenic | HD     | Decreased transcriptional repress promoter function | [7] |
| 4              | p.Arg164Gln    | c.491G>A    | Missense    | rs587780414 | na       | HD     | na                | # |
| IVS7           | IVS7-1G>A (p. Gln250del) | c.748–1G>A | Splice acceptor variant | rs371715169 | Pathogenic | Between HD and RD | Decreased transcriptional repress promoter function | [7,13] |

**Note:** #Mutation identified in this study.

**Abbreviations:** Ref, reference; PD, paired domain; HD, homeodomain; RD, repressor domain; na, not available.

**Table 2** Characterization of Mutations in PAX4 Gene Associated to Diabetes Mellitus

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association of the p.Arg133Trp in homozygous state to keto-
sis-prone diabetes (KPD), a rare form of T2D. In vivo and in
vitro studies showed that this variant alters the protein func-
tion (Table 2). They also observed the p.Arg37Trp mutation
in a patient from Cameroon. This variant was later described
cosegregating in a heterozygous form with BLK p.
Phe112Ser (c.335C>T) in a Nigerian woman with KPD.33
Further case-control studies should be carried out to evaluate
the association of these variants with different forms of
diabetes.

Plengvidhya et al7 described in Thai families the first
association of mutations in PAX4 to MODY diabetes. They
observed one missense mutation, p.Arg164Trp, in the PAX4
homeodomain in a female patient diagnosed at the age of 20
years and treated with OAD. In vitro analysis showed that p.
Arg164Trp decreased PAX4 repression activity. They also
found an intronic variant (IVS7-1G>A) in one women with
DM diagnosed at 44 years of age7 and after, in her daughter
who was diagnosed at 30 years of age with gestational DM
and required insulin treatment.13 Another four non-tested

Table 3 Clinical Features and Laboratory Parameters of the Family 35 Members

| Patient | II-1 | II-2 | II-3 | II-4 | II-5 | II-6 | II-7 | II-8* | II-9 | II-10 | II-11 | II-12 | II-13 | II-14 | II-15 |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Gender  | Female | Female | Female | Female | Female | Female | Female | Female | Female | Female | Female | Female | Female | Female | Male |
| BMI (kg/m²) | 29.48 | 23.61 | 24.93 | 27.97 | 24.8 | 30.62 | 25.73 | 25.55 | na | 20.38 | na | na | 23.23 | 28.84 |
| Current age (years) | 56 | 54 | 53 | 52 | 45 | 39 | 27 | 29 | 35 | 24 | 32 | 27 | 35 | 29 |
| AAD (years) | 38 | 49 | 50 | 25 | 32 | 24 | – | – | – | – | – | – | – | – |
| FPG (mg/dl) | 128 | 101 | 93 | 256 | na | 255 | 249 | na | na | na | na | 255 | na | na |
| HbA1c (%) | 11.3 | 6 | 6.1 | 9.6 | na | 7.4 | 5.3 | 5.5 | na | 5.1 | na | na | 5.4 | 5.4 |
| Treatment | OAD | OAD | Diet | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin | Insulin |
| Genotype | MN | MN | MN | NN | MN | MN | MN | MN | MN | MN | MN | MN | MN | MN |

Note: *Proband.
Abbre va tions: BMI, body mass index (at admission); AAD, age at diagnosis; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; OAD, oral antidiabetic agent; Genotypes are expressed by normal allele (N) and mutated allele (M); na, not available; –, not applicable.
members from this family showed several complications, such as diabetic renal disease and retinopathy, and three of them died of end-stage renal failure.\(^7,13\) Similarly, two non-tested members from the Brazilian family reported in our study had diabetic end-stage renal disease (\textit{Figure 2}; individuals II-4 and II-7); and one mutated patient (\textit{Figure 2}; individual III-9) had diabetic retinopathy 15 years after disease onset. The guanine to adenine change in the last nucleotide of intron 7 (IVS7-1G>A) disrupts mRNA splicing and results in an in-frame deletion p.Gln250del (exon 8). Similar to p.Arg164Trp, the \(PAX4\) p.Gln250del have its repressor activity of glucagon and insulin promoter impaired. Studies in vitro showed that this mutation increased susceptibility to apoptosis within high glucose condition.\(^13\)

Jo et al\(^{12}\) found a frameshift deletion (c.374_412del) in a 15-year-old Japanese proband on insulin treatment. His father was diagnosed at 30 years old with T2D and had his glucose controlled only by nutritional therapy. This deletion leads to the loss of \(PAX4\) homeodomain, decreasing its repression activity. Another two missense mutations, p.Arg31Leu\(^{14}\) and p.Arg52Cys\(^{15}\) were found in an Indian and in a Malay patient, respectively. Both exhibited clinical hallmarks of monogenic diabetes.

Here, we report a rare missense mutation in the \(PAX4\) gene, p.Arg164Gln, in a large Brazilian family. Interestingly, this mutation is located in the same residue of the first mutation described associated to \(PAX4\)-MODY in a Thai family by Plengvidhya et al.\(^7\) The age at diagnosis of the hyperglycemic

### Table 4 Clinical Characteristics of Patients with DM with Mutations in \(PAX4\) Gene

| P | Sex | AAD | BMI | HbA1c | Treat. | Mutation | Segregation Study | N° P (He; Ho) | N° C (He; Ho) | Ethnic Group | DM Type | Ref. |
|---|-----|-----|-----|-------|-------|----------|-------------------|--------------|--------------|-------------|---------|------|
| 1 | F   | 43  | 29.4| 7     | Diet  | p.Arg121Trp | na                | 200 (6:1)    | 161 (0:0)    | Japanese    | T2 DM   | [10] |
| 2 | M   | 49  | 26.7| 6.1   | Diet  | p.Arg121Trp | na                |              |              |             |         |      |
| 3 | M   | 49  | 17.8| 8.1   | OAD   | p.Arg121Trp | na                |              |              |             |         |      |
| 4 | F   | 47  | 32.4| 6.8   | OAD   | p.Arg121Trp | na                |              |              |             |         |      |
| 5 | M   | 32  | 22  | 8.8   | OAD   | p.Arg121Trp | na                |              |              |             |         |      |
| 6 | F   | 25  | 21.8| 8.2   | Ins   | p.Arg121Trp | na                |              |              |             |         |      |
| 7 | F   | 29  | 22.2| 7.3   | Ins   | p.Arg121Trp\(^*\) | 1MN/2MN         |              |              |             |         |      |
| 8 | M   | 47  | 26.5| 13.8  | OAD   | p.Arg133Trp\(^*\) | na                | 101 (27:4)   | 355 (69:0)   | West African | KPD     | [11] |
| 9 | M   | 22  | 16.2| 12.2  | OAD   | p.Arg133Trp\(^*\) | na                |              |              |             |         |      |
| 10| M   | 38  | 25.4| 14.1  | OAD   | p.Arg133Trp\(^*\) | na                |              |              |             |         |      |
| 11| M   | 20  | 21.6| 12.5  | OAD   | p.Arg133Trp\(^*\) | na                |              |              |             |         |      |
| 12| M   | 39  | 28.7| 11.6  | Ins   | p.Arg37Trp | na                | 101 (1:0)    | 255 (0:0)    | West African | KPD     | [11] |
| 13| F   | 20  | na  | na    | OAD   | p.Arg164Trp | 2 NN, 3 MN.       | 46 (1:0)     | 344 (0:0)    | Thai        | MODY    | [7]  |
| 14| F   | 44  | na  | na    | na    | IVS7-1G>A | 1 MN/1NN         | 46 (1:0)     | 344 (0:0)    | Thai        | MODY    | [7,13]| |
| 15| M   | 15  | 18.2| 14.5  | Ins   | c.374_412del | 1 MN.            | 1 (1:0)      | 150 (0:0)    | Japanese    | MODY    | [12] |
| 16| M   | 14  | 23  | na    | Ins, OAD | p.Arg31Leu | na                | 56 (1:0)     | 60 (0:0)     | Indian      | MODY    | [14] |
| 17| F   | 38  | 28.4| 14    | Ins   | p.Arg37Trp | na                | 1 (1:0)      | 0            | African     | KPD     | [33] |
| 18| M   | 35  | 28.1| 9.2   | OAD   | p.Arg52Cys | na                | 84 (1:0)     | 0            | Malay       | MODY    | [15] |
| 19| F   | 32  | 21.6| na    | Ins   | p.Arg164Gln | 1 NN, 5MN-4 NN, 4 MN | 31 (1:0) | 158 (0:0) | Brazilian MODY | [10,11] |

**Notes:** *Mutation in homozygous state; \(^*\)Mutation identified in this study; DM includes patients with impaired glucose tolerance.*

**Abbreviations:** P, patient; AAD, age at diagnosis (in years); BMI, body mass index (kg/m\(^2\)); HbA1c, glycated hemoglobin (%); Treat., treatment; DM, diabetes mellitus; NDM, non-diabetic subjects; N°, Number; He, mutation in heterozygous; Ho, mutation in homozygous; C, controls; Ref., reference; F, female; M, male; OAD, oral antidiabetic agents; Ins, insulin; na, not available; NM, genotype mutated in heterozygous state; NN, genotype homozygous normal; T2 DM, type 2 diabetes mellitus; KPD, ketosis-prone diabetes; MODY, maturity-onset diabetes of the young.
members from the family described here ranged from 24 years to 50 years. Whereas in the Thai family described, members were treated with OAD or diet, in the Brazilian family the treatment was variable (Diet: 1; OAD: 2; Insulin: 3). In addition, two proband’s sisters presented impaired glucose tolerance; the same was observed in the proband’s brother from a Thai family. It seems that phenotypes can vary between affected members from the same family, from severe to mild clinical presentations, as also observed by other studies of PAX4-MODY families, imposing a challenge for establishing a clinical pattern for PAX4-MODY. The age at diagnosis observed in the patients with the p.Arg164Gln mutation from the Brazilian family was remarkably high. Among the five mutated patients from the third generation, three presented diabetes symptoms after 35 years of age; the age at diagnosis was higher than that expected for MODY most common forms. This late development could explain the absence of DM in the younger carrier individuals of this family. Unexpectedly, one sister with DM (Figure 2; individual III-6) did not show the mutation p.Arg164Gln. She reported weight gain at the time of diagnosis, which could represent a phenocopy of diabetes. This is similar to the two sisters described in the Thai family, who presented impaired glucose tolerance and did not carry the mutation.7

Our study has some limitations; the proband’s biochemical exams were not available and she abandoned treatment and medical care. We did not have access to the two brothers (Figure 2; individuals III-5 and III-7) and the cousin (Figure 2; individual III-10) without DM, which could reinforce the role of PAX4 p.Arg164Gln as the cause of DM in this family.

Until now, PAX4-MODY had been described only in families with Asian origins. To our knowledge, this is the first study to report a PAX4-MODY in a family in South America. Functional studies are needed to better understand the role of PAX4 p.Arg164Gln mutation in the cause of monogenic diabetes and its contribution to the clinical profile of PAX4-MODY patients.

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Disclosure

The authors declare no conflict of interest.

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