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ORIGINAL RESEARCH

Identification of the First PAX4-MODY Family Reported in Brazil

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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/m^2, 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,[1] HNF1A,[2] HNF1B,[3] PDX1,[4] NEUROD1,[5] KLF11,[6] and PAX4.[7] 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 latter maintaining β-cells in differentiated state.[8] In vivo experiments demonstrated that newborn mice that are knockout for both *Pax4* alleles exhibit growth retardation and dehydration, dying 3 days after birth.[8] To date, several variants in the *PAX4* gene have been associated with a number of DM types, including type 1 DM (T1D),[9] type 2 DM (T2D),[10] Ketosis-Prone Diabetes

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(KPD),[11] as well as monogenic diabetes.[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.[7] More than one decade after this initial report, the number of studies supporting the involvement of *PAX4* mutations in monogenic diabetes remains limited to a few cases, and restricted to Asian populations.[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 *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 *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^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 *GCK* or *HNF1A* (based on the clinical phenotype),[16] *HNF4A* and *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^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^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.[17] Screening of the entire coding region and exon-intron boundaries of the *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 *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),[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,[19] PolyPhen-2,[20] PROVEAN,[21] Revel,[22] WS-SNPs&GO,[23] MutPred,[24] SNAP,[25] Fathmm,[26] M-CAP,[27] CADD,[28] Mutation assessor,[29] Align-GVGD,[30] PANTHER-PSEP,[31] and Mutation Taster.[32] The Ensembl reference transcript ENST00000341640.2 of *PAX4* gene (Genome release GRCh37.p13) was used as reference (https://www.ensembl.org/index.html).

Results

In this study, we screened the entire coding region of the *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

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/m^2; 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/m^2). 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.

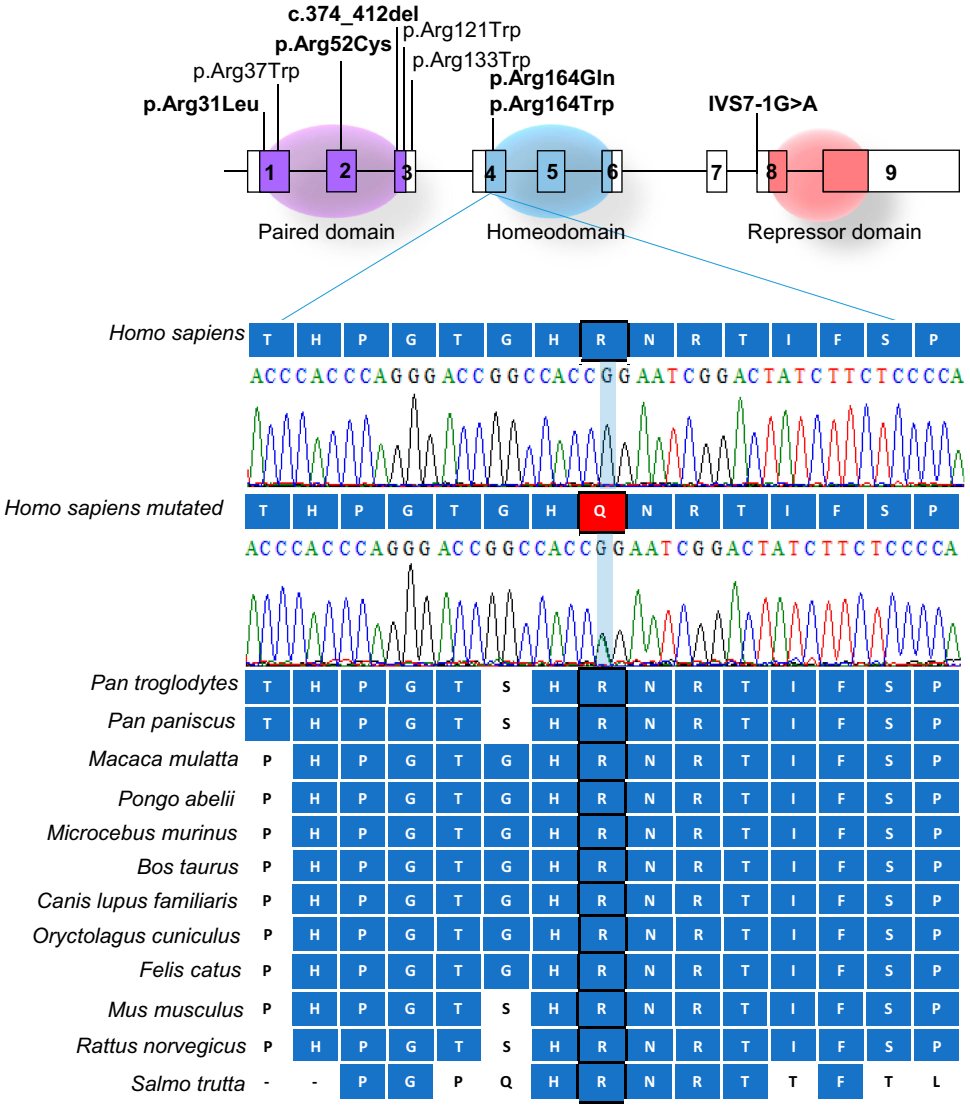

Figure 1 Diagrammatic representation of *PAX4* gene and protein domains. Pathogenic mutations described associated to diabetes mellitus are pointed in the figure and PAX4-MODY are show in bold. Electropherograms of *PAX4* exon 4 wild type and p.Arg164Gln (c.491G>A) in the patient DM35. Alignment by Clustal W (1.81) of *PAX4* gene across species are presented (below).

Table 1 In silico Prediction of Missense Mutations Identified in *PAX4* Gene

Prediction Tool	Output	PAX4 – p.Arg164Gln		PAX4 – p.Arg133Trp	
		Score	Prediction	Score	Prediction
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-SNPs&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 Ch7:127,254,551 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.biofold.org/snps-and-go/, MutPred2: http://mutdb.org/mutpred, SNAP: http://www.rostlab.org/services/SNAP, Fathnm: 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.

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/m^2; 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/m^2), 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 2 Characterization of Mutations in *PAX4* Gene Associated to Diabetes Mellitus

Exon or Intron	Mutated Protein	Mutated DNA	Consequence	Acess Number	Clinvar	Domain	Functional Studies	Ref.
1	p.Arg31Leu	c.92G>A	Missense	rs115887120	Likely-benign	PD	na	[14]
1	p.Arg37Trp	c.109C>T	Missense	rs35155575	Uncertain-significance, risk-factor	PD	Decreased transcriptional repress promoter function and decreased binding activity	[11,33]
2	p.Arg52Cys	c.154C>T	Missense	rs770923465	na	PD	na	[15]
3	n.a	c.374_412del	Sequence alteration	rs1325888696	na	PD	Loss of transcriptional repressor function	[12]
3	p. Arg121Trp	c.361C>T	Missense	rs114202595	Pathogenic	PD	Loss of transcriptional repressor function	[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.

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[10] 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[11] previously reported an

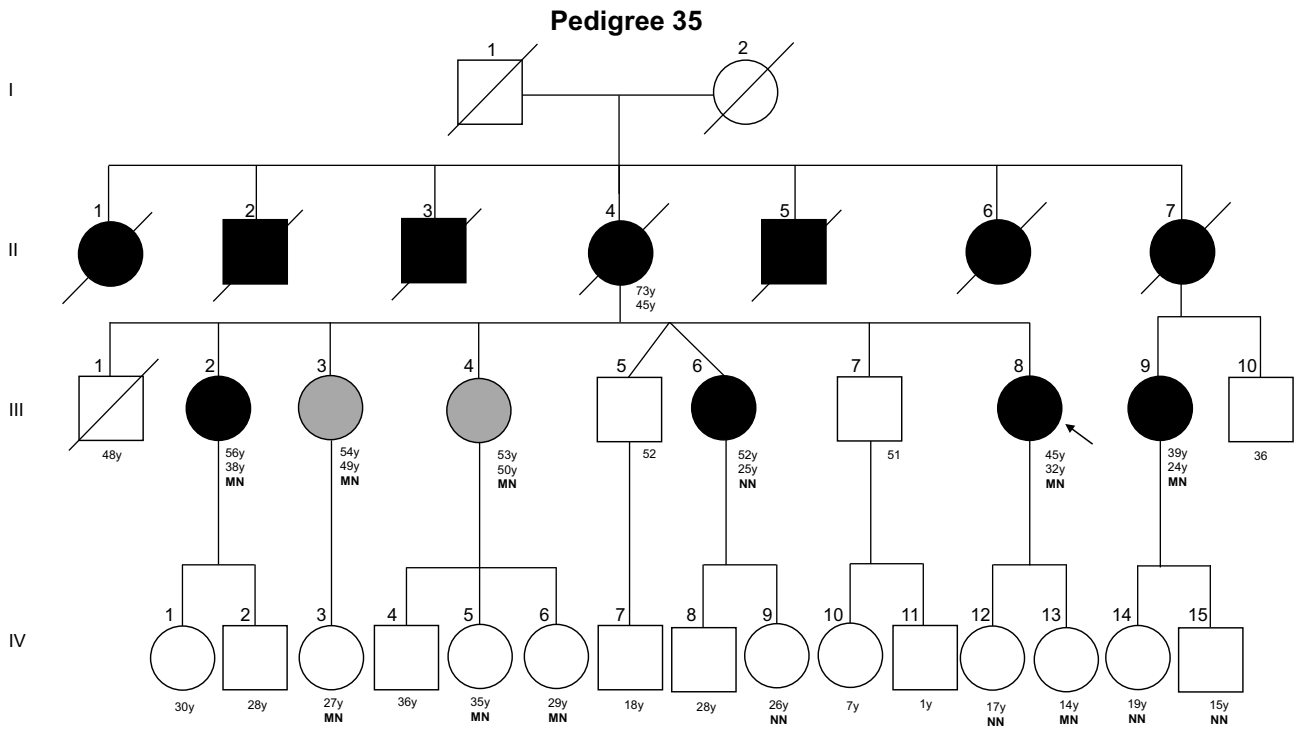

Figure 2 Pedigree of family 35. Filled black symbols, grey symbols, and empty symbols represent diabetic patients, impaired glucose tolerance individuals, and healthy individuals, respectively. The present age of the individuals are shown below the symbols in years (y), followed by age of diagnosis in years, and genotype. Genotypes are expressed by normal allele (N) and mutated allele (M); An arrow indicates the index case.

association of the p.Arg133Trp in homozygous state to ketosis-prone diabetes (KPD), a rare form of T2D. In vivo and in vitro studies showed that this variant alters the protein function (Table 2). They also observed the p.Arg37Trp mutation in a patient from Cameroon. This variant was later described co-segregating 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 al[7] 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 age[7] 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	III-2	III-3	III-4	III-6	III-8*	III-9	IV-3	IV-5	IV-6	IV-9	IV-12	IV-13	IV-14	IV-15
Gender	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Male
BMI (kg/m^2)	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	35	29	26	17	14	19	15
AAD (years)	38	49	50	25	32	24	–	–	–	–	–	–	–	–
FPG (mg/dl)	128	101	93	256	na	169	84	80	na	80	na	na	68	86
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	–	–	–	–	–	–	–	–
Genotype	MN	MN	MN	NN	MN	MN	MN	MN	MN	NN	NN	MN	NN	NN

Note: *Proband.

Abbreviations: 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.

Table 4 Clinical Characteristics of Patients with DM with Mutations in *PAX4* Gene

P	Sex	AAD	BMI	HbA1c	Treat.	Mutation	Segregation Study DM/NDM	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	#

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 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 (Figure 2; individuals II-4 and II-7); and one mutated patient (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

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,[7,12] 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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