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Research Article
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Investigation of genetic markers associated to type 2 diabetes mellitus in Santarém-Pará

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Abstract

Genetic, epigenetic and environmental factors play an important role in the genesis of Type 2 Diabetes Mellitus (T2D). In the genetic context, one of the strategies used to investigate possible associations with diabetes is the search for Single Nucleotide Polymorphisms (SNPs), involving the comparison of alelle frequencies, the phenotypic variations and other relevant factors, such as environmental influences and lifestyle choices, Thus, the aim of this study was to find the relationship of risk variants for T2D in SNPs (*rs4994*) in the *ADRB3* gene; (*rs1799854*) in the *ABCC8* gene; (*rs7901695* and *rs12255372*) in the *TCF7L2* gene; and (*rs8050136*) in the *FTO* gene in a sample of the population of the municipality of Santarém (PA), Brazilian Amazon, in the northern region of Brazil. *ABCC8* (*rs1799854* C>T) showed a statistically significant association with T2D. Each chosen gene and SNP has been previously implicated in T2D risk according to existing scientific literature, owing to their roles in glucose regulation and body fat.

Keywords: Genetic polymorphism, population genetics, diabetes mellitus, single nucleotides polymorphisms.

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Introduction

Diabetes Mellitus (DM) is a metabolic disease characterized by persistent hyperglycemia resulting from defects in insulin secretion by pancreatic beta cells or decreased sensitivity to insulin by other cells in the body (Lyra *et al*., 2006). With regard to the etiopathogenesis of diabetes, the disease can be classified as type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes (GDM), and other types of diabetes (SBD, 2022). T2D is responsible for 90-95% of diabetes cases. Furthermore, due to its complexity, this disease represents one of the biggest challenges for Primary Health Care with regard to prevention and treatment, which aims to prevent systemic complications related to the underlying inflammatory process in individuals with the disease (Leal *et al*., 2017; Romanciuc, 2017; Manoel *et al*., 2021).

The etiology of T2DM is mainly related to excessive consumption of carbohydrates and fats, excessive weight, sedentary behavior, a family history of diabetes, and insulin resistance. Thus, the disease depends on environmental and genetic factors. Among the risk factors for the development of the disease, some are highlighted, such as overweight (BMI>25) and central obesity, hypertriglyceridemia, hypertension, HDL < 40 mg/dL, age over 45 years, and family history (Tavares *et al*., 2010). The influence of age, although not fully understood, has an intrinsic relationship with environmental and genetic factors. With regard to environmental factors, it is worth stressing that a change in lifestyle to a more sedentary behavior, reinforced by metabolic changes in old age, makes the group of older people more vulnerable to the development of T2DM (Malafaia and Buglia, 2019).

Genetic, epigenetic, and environmental factors play an important role in the development of the disease. As for genetic factors, Genome-Wide Association studies (GWAs) have identified T2D susceptible in various populations, these studies have shown over 400 genetic risk variants at 250 loci for T2D (Carlson *et al*., 2013; Dziewulska *et al*., 2018). Other studies have also shown the contribution of genetic factors to the development of T2D in families with diabetic individuals, with concordance of 70% for monozygotic twins and only 20-30% for dizygotic twins (Newman *et al*., 1987; Kaprio *et al*., 1992). Another important observation is that the risk of T2D is about 40% when one of the parents is affected and 70% when both are affected (Köbberling and Tillil, 1982; Groop *et al*., 1996).

In this genetic context, one of the strategies used to investigate possible genetic associations with diabetes is the research of Single Nucleotide Polymorphisms (SNPs). As

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observed in some studies, the presence of SNPs *rs1799854* in the ATP Binding Cassette Subfamily C Member 8 (*ABCC8*) gene; *rs4994* in the β3-Adrenergic Receptors (*ADRB3*) gene; and *rs8050136* in the Fat Mass And Obesity Associated/ Alpha-Ketoglutarate Dependent Dioxygenase (*FTO*) gene have a direct association with obesity, one of the risk factors for T2D, as it is one of the main causes of insulin-related disorders (Horikoshi *et al*., 2007; Cruz *et al*., 2010; Diniz *et al*., 2022). Furthermore, the study by Guzmán *et al*. (2010) shows that overexpression of the Transcription Factor 7-like 2 (*TCF7L2*) gene may be related to decreased insulin secretion, which leads to hyperglycemia.

Santarém is a city located in the west of the state of Pará, at the confluence of the Tapajós and Amazon rivers. It is one of the oldest cities in the Brazilian Amazon, and has a strong history of miscegenation, since it was founded and colonized by Portuguese explorers, but also has a significant presence of African and Indigenous people. This contributes to the genetic risk factors, because there is a higher likelihood of inheriting a combination of genetic variants associated with diabetes. In addition to this, the unique lifestyle and dietary habits prevalent in this population increase the risk of diabetes (Santos, 2004; Sousa *et al.*, 2020).

In this sense, this study aims to investigate the association of risk variants in SNPs (*rs4994*) in the *ADRB3* gene; (*1799854*) in the *ABCC8* gene; (*rs7901695* and *rs12255372*) in the *TCF7L2* gene, and (*rs8050136*) in the *FTO* gene with type 2 diabetes (T2D) in a sample of the population of Santarém, in the state of Pará, in the North Region of Brazil, known as the Amazon Region, where the rates of T2D are high. This research aims to improve our understanding on the molecular mechanisms underlying chronic hyperglycemia and to identify individuals at risk of developing the disease early on. These findings could lead to effective hygiene-dietary interventions or medical treatments, potentially preventing and reversing the metabolic state of T2D in these diverse populations.

Subjects and Methods

Type of research

This is a descriptive cross-sectional epidemiological research with a quantitative approach, consisting of a case-control study (Pereira, 1995; Fontelles, 2010). The project was submitted to the Ethics Committee in Research of the João de Barros Barreto University Hospital, according to the approval term of protocol no. 2137/2010.

The study was conducted in Santarém-Pará-Brazil with a total of 410 individuals. Of these, 209 patients had T2D and 201 were controls (individuals with age equal to or greater than 40 years, without symptoms and/or previous diabetes diagnosis, without first-degree relatives with diabetes). Diabetic patients were registered in Hiperdia, a program for the care of hypertensive and diabetic patients within the Unified Health System (SUS), and were using medications provided by SUS (Metformin 500 mg; Simvastatin 20 mg and 40 mg). For this reason, the clinical data of these patients may show variations due to glycemic and cholesterol control and may be underestimated when compared to diabetic patients without treatment.

A sociodemographic interview was conducted with the participants using a questionnaire. Anthropometric evaluation was then carried out using Body Mass Index (BMI) and waist circumference (WC). Blood pressure (BP) was measured using a digital monitor and participants were then instructed to collect blood samples for biochemical and genetic analysis.

After a 12-hour fast, blood samples (5 mL) were collected from the patients through venipuncture. The concentration of triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL-cholesterol) and glucose were determined using an enzymatic-colorimetric method, according to the manufacturer's instructions. Hemoglobin A1c (HbA1c) was measured using the modified Trivelli microchromatographic-colorimetric method and low-density lipoprotein (LDL-cholesterol) was calculated using the Friedewald formula.

Genotyping of Single Nucleotide Polymorphisms (SNPs)

About 1 mL of peripheral blood was obtained from each patient and each control individual for genomic DNA extraction, using the conventional phenol-chloroform extraction method (Old and Higgs, 1993) with some modifications.

The polymorphisms of the *ADRB3* (*rs4994*), *ABCC8* (*rs1799854*), *FTO* (*rs8050136*), and *TCF7L2* (*rs7901695* and *rs12255372*) genes were identified using Real-Time Polymerase Chain Reaction (RT-PCR), with a commercial assay developed by Applied BioSystems – TaqMan®, with adaptations in the genotyping standard protocol. Genotyping was done using a TaqMan SNP genotyping assay (Applied BioSystems, Foster City, CA, USA) according to the manufacturer's instructions. Pre-designed probes were ordered for genotyping analysis. Approximately 10-50 ng of DNA was amplified with 5 µl of 2X TaqMan Universal PCR master mix, 0.5 µl of 40X primer and TaqMan probe mix. The cycles were 10 min at 95 °C, followed by 40 cycles of 15 s at 92 °C and 1 min at 60 °C. Allelic discrimination was performed on an Applied Biosystems RT-PCR system – Realtime (PCR). The genes were selected for this study based on previous studies that have shown the association of variants with obesity and/or type 2 diabetes in continental populations.

Statistical analyses

For comparison between continuous biological variables between the T2D and control groups, a Student's t-test was performed for variables with a normal distribution, Mann-Whitney's U-test for variables that did not have a normal distribution ($p<0.05$), and Pearson's $\chi 2$ test for categorical variables. For the comparison between the two groups (control and T2D), the Bonferroni correction was applied. Logistic regression analysis was used to verify possible associations between the SNPs investigated and T2D, under a priori genotypic models (dominant, codominant, and recessive) performed to calculate specific allele risk probabilities. The odds ratio (OR) was calculated with a 95% confidence interval to estimate the relative risk and strength of association, with an OR above 1 associated with an increased chance of a given characteristic occurring and an OR less than 1 the opposite. The p-values, after adjusted for sex and age, were calculated. These analyses were performed using the Statistical Package

for the Social Sciences (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study sample

The biochemical and anthropometric characteristics of patients with T2D and of the healthy control group are presented in Table 1. As expected, individuals with T2D, compared to the control group, had significantly higher values for BMI, glucose, and triglycerides (p=0.000), while exhibiting lower levels of HDL-cholesterol (p=0.001).

Polymorphisms in *ABCC8*, *ADRB3*, *FTO*, and *TCF7L2* genes

The observed allelic frequencies for the five genetic variants in the genes investigated in the present study and related to T2D in the population of Santarém, along with the frequencies found in continental populations of Africans, Americans, Europeans, Southeast Asians, and Eastern Asians ("1000 Genomes Project") (Table 2). For the *rs1799854* C>T variant in the *ABCC8* gene, it was observed that the mutant allele (allele T) had a high allelic frequency, above 40%, similar the allelic frequency observed in European, American, and East Asian populations. The highest allelic frequency for the *ABCC8 rs1799854* C>T variant was observed in the American population at 53.6% (allele T). The American subset of the "1000 Genomes Project" is composed of mixed populations from Puerto Rico, Colombia, and Mexico.

Genotype-phenotype association

ABCC8 (*rs1799854* C>T) showed a statistically significant association with T2D. A difference in the distribution pattern of the TT genotype frequencies was observed between individuals with T2D and controls (28.7% vs 23.9% [P= 0.042]). Furthermore, allele T significantly increased the risk for T2D compared to allele C OR=1.34 95% CI 1.02-1.76 P= 0.036. Significant associations were also observed for the co-dominant models (P=0.042 and 0.034, CC vs CT, TT) and dominant (P= 0.029, CC vs CT+TT) models, but not for the recessive model (P= 0.251. CC+CT vs TT) (Table 3). For the other variants *ADRB3* (*rs4994*), *FTO* (*rs 8050136*), TCFL7 (*rs7901695* and *rs12255372*-G>T), no statistically significant differences were observed.

Table 1 – Clinical characteristics of the studied population in Santarém/Pará.

Characteristics	Diabetes (209) (Minimum-maximum)	Control (201) (Minimum-maximum))	P
AGE	62.33 ± 10.7(33-86)	61.40 ± 12.5(36-97)	0.256
Gender %(N) F/M	76.1%(159)/23.9%(50)	68.7%(138)/31.3%(63)	0.093
BMI	27.03± 4.1(17.45-37.48)	26.25 ± 4.93(17.12-49.31)	**0.032**
Glycemia	200.54 ± 93.2(59-530)	87.46 ± 8.0(65-107)	**0.000**
SBP	145.16 ± 24.3(70-258)	143.18 ± 23.8(90- 232)	0.364
DBP	90.32 ± 15.8(45-160)	91.56 ± 14.8(50- 132)	0.195
Triglycerides	255.11 ± 180.2(55-375)	188.75 ± 130.8(30- 750)	**0.000**
Total cholesterol	196.85 ± 56.0(73-375)	194.77 ± 43.7(76-338)	0.936
HDL	41.36±9.6	45.43±11.7	**0.001**
LDL	110.15±46.1	113.37±40.4	0.392
Classification by weight			
Normal weight	33%	42.8%	
Overweight	44%	39.8%	0.101
Obese	23%	17.4%	
Fasting blood glucose			
Normal	7.7%	99.5%	
Altered	15.3%	0.5%	**0.000**
Elevated	77%	0.0	
Blood pressure			
Normal	27.3%	35.8%	0.062
Elevated	72.7%	64.2%	
Triglyceride			
Desirable	45%	67.7%	**0.000**
Elevated	55%	32.3%	
Cholesterol			
Desirable	81.3%	87.6%	0.083
Increased	18.7%	12.4%	

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Dyastolic blood pressure.

Table 2 – Allelic frequencies of the 5 single nucleotide polymorphisms (SNPs) related to diabetes in Amazon/PA population and in continental populations from the 1000 Genomes Project (%).

		Populations						
Gene/SNP	Allele	SANTARÉM	AFR	AMR	EUR	EAS	SAS	ALL
ABCC8 *rs1799854*	C	0.505	0.862	0.464	0.580	0.449	0.681	0.632
	T	0.495	0.138	0.536	0.420	0.551	0.319	0.368
ADRB3 *rs4994*	T	0.830	0.905	0.880	0.918	0.869	0.843	0.885
	C	0.169	0.095	0.120	0.082	0.131	0.157	0.115
FTO *rs8050136*	C	0.710	0.567	0.745	0.586	0.834	0.711	0.678
	A	0.286	0.433	0.255	0.414	0.166	0.289	0.322
TCF7L2 *rs7901695*	T	0.721	0.564	0.744	0.656	0.977	0.704	0.718
	C	0.278	0.436	0.256	0.344	0.023	0.296	0.282
TCF7L2 *rs12255372*	G	0.801	0.698	0.781	0.708	0.990	0.779	0.786
	T	0.198	0.302	0.219	0.292	0.010	0.221	0.214

Santarém-Amazônia/Pará; EUR, European; AFR, African; AMR, American; EAS, East Asian; SAS, Southeast Asian.

Table 3 – Comparison of the adjusted odds ratio for three genetic models for genetic polymorphisms related to diabetes in the population of Santarém/PA.

Gene (rsId)	Genotype/all	Case N(209)	Control N(201)	Allelic OR (95% IC)	Co-dominant OR (95% IC)	Dominant OR (95% IC)	Recessive OR (95% IC)
				C vs T	CC vs CT. TT	CC vs CT+TT	CC+CT vs TT
	CC	47 (22.5%)	65(32.3%)	**1.34** (1.02-1.76)	1.0	1.0	1.0
Gene *ABCC8* *rs1799854*	CT	102(48.8)	88(43.8%)	**P=0.036**	**1.65**(1.01-2.68)	**1.65**(1.05-0.55)	1.28(0.83-2.02)
	TT	60(28.7%)	48(23.9%)		**P=0.042**	**P=0.029**	P=0.251
	ALLELE C	196(46.9)	218(54.2%)		**1.80**(1.04-3.12)		
	ALLELET	222(53.1)	184(45.8%)		**P=0.034**		
				T vs C	TT vs TC. CC	TT vs TC+CC	TT+TC vs CC
	TT	145(69.4%)	144(71.6%)	0.88(0.61-1.24)	1.0	1.0	1.0
Gene *ADRB3* *rs4994*	TC	53(25.4%)	50(24.9%)	P=0.440	0.99(0.62-1.57)	1.06(0.69-0.64)	1.58(0.59-4.26)
	CC	11(5.3%)	7(3.5%)		P=0.980	P=0.775	P=0.359
	ALLELE T	343(82.1%)	338(84.1%)		1.48(0.54-4.03)		
	ALLELE C	75(17.9%)	64(15.9%)		P=0.439		
				C vs A	CC vs CA. AA	CC vs CA+AA	CC+CA vs AA
	CC	106(50.7%)	106(52.7%)	0.92(0.68-1.25)	1.0	1.0	1.0
Gene *FTO* *rs8050136*	CA	83(39.7%)	78(38.8%)	P=0.620	1.41(0.74-1.73)	1.11(0.75-1.64)	1.49(0.57-2.27)
	AA	20(9.6%)	17(8.5%)		P=0.540	P=0.591	P=0.692
	ALLELE C	295(70.6%)	290(72.1)%		1.12(0.54-2.30)		
	ALLELE A	123(29.4%)	112(27.9%)		P=0.749		
				T vs C	TT vs TC. CC	TT vs TC+CC	TT+TC vs CC
	TT	110(52.6%)	110(54.7%)	0.93(0.68-1.26)	1.0	1.0	1.0
Gene *TCF7L2* *rs7901695*	TC	79(37.8%)	73(36.3%)	P=0.665	1.04(0.68-1.26)	1.07(0.66-1.71)	1.09(0.53-2.15)
	CC	20(9.6%)	18(9.0%)		P>0.05	P=0.778	P=0.809
	ALLELE T	299(71.5%)	293(72.9%)		1.13(0.49-2.61		
	ALLELE C	119(28.5%)	109(27.1%)		P=0.760		
				G vs T	GG vs GT. TT	GG vs GT+TT	GG+CT vs TT
	GG	138(66.0%)	134(66.7%)	0.97(0.69-1.37)	1	1	1
Gene TCFL2 *rs12255372*	GT	58(27.8%)	55(27.4%)	P=0.873	1.01(0.63-1.61)	0.99(0.60-1.64)	0.98(0.40-4.37)
	TT	13(6.2%)	12(6.0%)		P=0.946	P=0.989	P=0.971
	ALLELE G	334(79.9%)	323(80.3%)		1.04(0.41-2.60)		
	ALLELE T	84(20.1%)	79(19.7%)		P=0.929		

P < 0.05 and OR with corresponding 95% CI > 1 are represented in bold. Odds ratios (OR) and corresponding 95% confidence interval (CI) adjusted for age, sex, and BMI as variables.

Discussion

In this study, the association of SNPs *ADRB3* (*rs4994*), *ABCC8* (*rs1799854*), *FTO* (*rs8050136*), and *TCF7L2* (*rs7901695* and *rs12255372*) with T2D was investigated in an admixed Amazonian population from the interior of the North Region of the state of Pará, in the city of Santarém. The research of polymorphisms associated with T2D had never been investigated in this population, making this the first study of its kind.

Among the 5 Brazilian regions, the North Region leads in the prevalence of obesity cases, especially due to poor nutrition and sedentary lifestyle (Malveira *et al*., 2021), a condition that contributes significantly to the development of T2D diabetes through disorders in insulin metabolism. In this study, weight did not express a statistically significant value (p=0.101), but if we observe the BMI, we will see a statistically significant difference (p=0.032), a variable that can be influenced by sex hormones in metabolism (Satler *et al*., 2021).

In this sample, it was possible to identify the presence of biochemical and anthropometric characteristics typical of T2D, showing metabolic changes due to the disease, which may be related to unbalanced diets and the social and economic conditions of the sample (Malveira *et al*., 2021). Elevated levels of triglycerides, blood glucose, BMI, and low levels of HDL-cholesterol were observed, and the prevalence of hypertriglyceridemia was higher in the T2D patient group (55%) compared to the estimated rate for adult Brazilians (31.2%) (Schmidt *et al*., 2015). Hypertriglyceridemia is the most common lipid disorder in patients with diabetes and is associated with an increased risk of cardiovascular disease (Hokanson and Austin, 1996; Miller *et al*., 1998; Rosenson *et al*., 2002). Additionally, there were elevated mean values of systolic and diastolic blood pressure in the study population, showing a considerable number of hypertensive individuals. The coexistence of hypertension and T2D double the risks of cardiovascular events in diabetic individuals, when compared to non-diabetic individuals (Curb, 1996; Penalva, 2008; Cryer *et al*., 2016).

Allelic frequencies in the Santarém population

Genetic variants associated with diabetes and obesity phenotypes were predominantly demonstrated in European and Asian populations (Wang *et al*., 2009); however, such allelic variants have also shown distribution in populations from other continents, such as the American population (Carlson *et al*., 2013).

Compared to the allelic frequencies of the populations of the "1000 Genomes Project," the data obtained showed similarity in the distribution of the allelic frequencies of the polymorphisms investigated in studies of continental populations with the samples of this study. Therefore, from the four genes investigated in this study, the *rs1799854* variant in the *ABCC8* gene, related to T2D (Wang *et al*., 2009), had the highest frequency (49.5%) of the risk allele (T). It is worth mentioning that the T allele of this variant also had a high distribution in the American and Asian populations at 53.6% and 55.1%, respectively. Diniz *et al*. (2022) also showed high frequencies of this same variant in indigenous populations of the state of Pará (53.3%). The similarity between these results denotes the importance of investigating this genetic variant as a susceptibility factor for T2D in mixed and native populations.

The gene frequency in the Santarém sample can be explained by the mixed character of its population, predominantly represented by Portuguese, Africans, and indigenous people (Santos, 2004), whose proportions were estimated as 39%, 28%, 33%, respectively (Santos *et al.*, 1996). For this reason, it is possible to point out that the contribution of the genetic risk factor has a great relevance in these populations and the result of the impact of these factors can be compared with other studies carried out with American and European populations (Rodrigues, 2018).

Genotype-phenotype relationship

This study found an association between the risk allele (T) and T2D only for variant *rs1799854* in the *ABCC8* gene. This gene encodes the sulfonylurea 1 receptor protein, which participates along with K+ channels, expressed by pancreatic beta cells, in the regulation and secretion of insulin in response to glucose at beta cells.

The intronic polymorphism *rs1799854*, as also observed in other studies, is associated with hyperglycemia observed in the population with T2D, in addition to abdominal obesity, body fat and high BMI, characteristics also observed in a study performed with indigenous populations of the Brazilian Amazon and in a Polish study (Pietrzak-Nowacka *et al*., 2012; Rodríguez-Rivera *et al*., 2019; Diniz *et al*., 2022). The same variant was also associated with T2D in Japanese (Sakamoto *et al*., 2007), Caucasian (Florez *et al*., 2004) and Chinese populations (Zhou *et al*., 2009).

The present study showed that the T allele and CT and TT genotypes, in the dominant model of the *rs1799854* variant in the *ABCC8* gene, were significantly associated with the risk of developing T2D. This association observed in the group of patients with T2D has also been demonstrated in other studies (Meirhaeghe *et al*., 2001; Niu *et al*., 2005; Yokoi *et al.*, 2006; Gonen *et al*., 2012).

On the other hand, some studies about the influence of SNP *rs1799854* did not demonstrate association between this variant and T2D susceptibility in Asian and Caucasian populations (Lv *et al*., 2011; Venkatesan *et al.*, 2014). However, the effect of this genetic variant on specific, unidentified subgroups with T2D cannot be excluded. Additionally, a number of environmental, genetic and statistical factors may be subject to variations in the results observed in these different populations.

Other variants were studied such as *ADRB3* (*rs4994*), *FTO* (*rs8050136*), and *TCF7L2* (*rs7901695* and *rs12255372*). Collins *et al*. (1994) e Yamakita *et al*. (2010) showed that decreased expression of *ADRB3* in adipose tissue may contribute to the obesity phenotype with insulin resistance; and Diniz *et al*. (2022) found statistically significant results that demonstrate this relation in native populations of the Amazon. Studies show that some *FTO* gene polymorphisms are related with obesity in different ethnic groups, such as Caucasians (Hunt *et al*., 2008), and asians (Chang *et al*., 2008; Hotta *et al*., 2008; Yajnik *et al*., 2009). As for the *TCFL72* variants, studies show that it is a strong marker associated

with T2D and have been robustly reported by GWA studies and consistently replicated in multiple populations of different genetic origins.

However, these variants have not shown statistically significant correlation with T2D in the population of this study. Nevertheless, this does not completely excludes the association between these variants and the increase in BMI and body fat (factors that are directly connected with obesity and T2D) in Santarém population.

The findings in this study may suggest the influence of different epigenetic and environmental factors in different population groups. In order to clarify the multi-factorial contributions related to T2D in mixed populations of the Brazilian Amazon, additional studies of gene-gene and gene-environment interactions are necessary (Bosque-Plata *et al*., 2021).

Conclusion

This study investigated the association of SNPs *ADRB3* (*rs4994*), *ABCC8* (*rs1799854*), *FTO* (*rs8050136*) and *TCF7L2* (*rs7901695* and *rs12255372*) with type 2 diabetes in a sample of the Amazonian population. It was observed that the T allele and CT and TT genotypes, in the dominant model of the *rs1799854* variant in the *ABCC8* gene, were significantly associated with the risk for developing type 2 diabetes and could be considered a good genetic marker in studies related to type 2 diabetes, both in admixed populations of the Brazilian Amazon and in native populations. Therefore, it is crucial that further studies be conducted to arrive at this conclusion more robustly.

The combination of multiple genetic and environmental factors contributes to the pathogenesis of type 2 diabetes (T2D), so the association between this polymorphism and T2D can be used as a risk marker for the disease and its complications. However, the precise mechanism of development and progression is not well understood. There is a need for further studies to identify individuals with T2D carrying these variants, as well as to understand the mechanisms by which these polymorphisms affect metabolic characteristics associated with the disease, as well as the gene-environment interaction in the predisposition to T2D, contributing to the elucidation of the potential biological role in the pathogenesis of T2D.

Knowledge of the individual genetic predisposition profile for type 2 diabetes (T2D) and associated comorbidities may contribute to the effective prevention of T2D and its complications, through the use of differentiated prevention and control strategies according to the needs of each group, considering the profile of patients through self-care orientation programs, physical activity, nutritional guidance, monitoring of glycemic control and lipid profile in Health Units, contributing to the reduction of morbidity and mortality related to diabetes, as well as the costs of treating the disease and its complications.

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Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Author Contributions

AESS, IGD, APAG, DSS and ANLMS performed the experiments, the investigation, methodology and data curation; AESS, CHSS, RCSO, HSSO, FAPLF wrote the paper and performed the formal analysis; FAPLF, GLCC, JFG administered and oriented the project. All authors read and approved the final version.

References

Bosque-Plata L, Martínez-Martínez E, Espinoza-Camacho MÁ and Gragnoli C (2021) The role of *TCF7L2* in type 2 diabetes. Diabetes 70:1220-1228.

Carlson CS, Matise TC, North KE, Haiman CA, Fesinmeyer MD, Buyske S, Schumacher FR, Peters U, Franceschini N, Ritchie MD *et al*. (2013) PAGE Consortium. Generalization and dilution of association results from European GWAS in populations of non-European ancestry: The PAGE study. PLoS Biol 11:e1001661.

Chang YC, Liu PH, Lee WJ, Chang TJ, Jiang YD, Li HY, Kuo SS, Lee KC and Chuang LM (2008) Common variations in the *FTO* gene confers risk of obesity and modulates body mass index in the Chinese population. Diabetes 57:2245-2252.

Collins S, Daniel KW, Rohlfs EM, Rankumar V, Taylor IL and Gettys TW (1994) Impaired expression and functional activity of the β3 and β1-adrenergic recptors in adipose tissue of congenitally obese (C57BL/6Job/ob) mice. Mol Endocrinol 8:518-527.

Cruz M, Valladares-Salgado A, Garcia-Mena J, Ross K, Edwards M, Angeles-Martinez J, Ortega-Camarillo C, de la Peña JE, Burguete-Garcia AI, Wacher-Rodarte N *et al*. (2010) Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico City. Diabetes Metab Res Rev 26:261-270

Cryer MJ, Horani T and DiPette DJ (2016) Diabetes and hypertension: A comparative review of current guidelines. J Clin Hypertens (Greenwich) 18:95-100.

Curb JD (1996) Effect of diuretic-based antihypertensive treatment on cardiovascular disease risk in older diabetic patients with isolated hypertension. Systolic Hypertension in the Elderly Cooperative Research Group. JAMA 276:1886-1892.

Diniz IG, Noce RRD, Pereira AP, Silva ANLM da, Sacuena ERP, Lemes RB, Costa GLC, Araújo GS, Machado JLP, Figueiredo FAPL *et al.* (2022) Common BMI and diabetes-related genetic variants: A pilot study among indigenous people in the Brazilian Amazon. Genet Mol Biol 45:e20210153.

Dziewulska A, Dobosz AM and Dobrzyn A (2018) High-throughput approaches onto uncover (Epi) genomic architecture of type 2 diabetes. Genes 9:374.

Fontelles MJ (2010) Bioestatística aplicada à pesquisa experimental. LF Editorial, Belém, vol. I and II, 234 p.

Florez JC, Burtt N, de Bakker PI, Almgren P, Tuomi T, Holmkvist J, Gaudet D, Hudson TJ, Schaffner SF, Daly MJ *et al*. (2004) Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 53:1360-1368.

Gonen MS, Arikoglu H, Erkoc Kaya D, Ozdemir H, Ipekci SH, Arslan A, Kayis SA and Gogebakan B (2012) Effects of single nucleotide polymorphisms in KATP channel genes on type 2 diabetes in a Turkish population. Arch Med Res 43:317-323.

Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissén M, Ehrnström BO, Forsén B, Isomaa B, Snickars B *et al.* (1996) Metabolic consequences of a family history of NIDDM (the Botnia Study): Evidence for sex-specific parental effects. Diabetes 45:1585-1593.

Guzmán JR, Lyra R, Aguilar-Salinas CA, Cavalcanti S, Escaño F, Tambasia M and Duarte E (2010) Treatment of type 2 diabetes in Latin America: A consensus statement by the medical associations of 17 Latin American countries. Rev Panam Salud Publica 28:463-471.

Hokanson JE and Austin MA (1996) Plasma triglicerydе level in a risk factor cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. J Cardiovascular Risk 3:213-219.

Horikoshi M, Hara K, Ito C, Shojima N, Nagai R, Ueki K, Froguel P and Kadowaki T (2007) Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. Diabetologia 50:2461-2466.

Hotta K, Nakata Y, Matsuo T, Kamohara S, Kotani K, Komatsu R, Itoh N, Mineo I, Wada J, Masuzaki H *et al*. (2008) Variations in the *FTO* gene are associated with severe obesity in the Japanese. J Hum Genet 53:546-553.

Hunt SC, Stone S, Xin Y, Scherer CA, Magness CL, Iadonato SP, Hopkins PN and Adams TD (2008) Association of the *FTO* gene with BMI. Obesity (Silver Spring) 16:902-904.

Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengård J and Kesäniemi YA (1992) Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 35:1060-1067.

Köbberling J and Tillil H (1982) Empirical risk figures for first-degree relatives of non-insulin dependent diabetics. In: Köbberling J and Tattersall R (eds) The genetics of diabetes mellitus. Academic Press, London, pp 201-209.

Leal J, Ahrabian D, Davies MJ, Gray LJ, Khunti K, Yates T and Gray AM (2017) Cost-effectiveness of a pragmatic structured education intervention for the prevention of type 2 diabetes: Economic evaluation of data from the Let's Prevent Diabetes cluster-randomised controlled trial. BMJ Open 7:e013592.

Lv Y, Cui L, Dehwah MAS, Gao M, Wen J and Huang Q (2011) Notice of retraction: *ABCC8* and type 2 diabetes: A global meta-analysis. In: 5th International Conference on Bioinformatics and Biomedical Engineering, Wuhan, China, pp 1-4.

Lyra R, Oliveira M, Lins D and Cavalcanti N (2006) Prevention of type 2 diabetes mellitus. Arq Bras Endocrinol Metab 50:239-249.

Malafaia FL and Buglia S (2019) Prescribing physical activity in the elderly: It is never too late to fight a sedentary lifestyle. Rev DERC 25:14-18.

Malveira AS, Santos RD, Mesquita JLS and Guedine CRC (2021) Prevalence of obesity in Brazilian regions. Braz J Health Rev 4:4164-4173.

Manoel LMO, Ribeiro LG, Oliveira REM and Ueta JM (2021) Collective actions for the control of type 2 diabetes mellitus in primary healthcare: An experience report. RSD 10:e49910817485.

Meirhaeghe A, Helbecque N, Cottel D, Arveiler D, Ruidavets JB, Haas B, Ferrières J, Tauber JP, Bingham A and Amouyel P (2001) Impact of sulfonylurea receptor 1 genetic variability on non-insulin-dependent diabetes mellitus prevalence and treatment: A population study. Am J Med Genet 101:4-8.

Miller M, Seidler A, Moalemi A and Pearson TA (1998) Normal triglyceride levels and coronary artery disease events: The Baltimore Coronary observational Long-Term Study. J Am Cool Cardiol 31:1252-1257.

Newman B, Selby JV, King MC, Slemenda C, Fabsitz R and Friedman GD (1987) Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia 30:763-768.

Niu XM, Yang H, Zhang HY, Li NJ, Qi XM, Chang Y, Chang Z and Zhang Y (2005) Study on association between gestational diabetes mellitus and sulfonylurea receptor-1 gene polymorphism. Zhonghua fu chan ke za zhi 40:159-163.

Old JM and Higgs DR (1993) Gene analysis. In: Weatherall DJ (ed) The Thalassemias: Methods in hematology. Churchill Livingstone, Edinburg, pp 74-102.

Penalva DQF (2008) Metabolic syndrome: Diagnosis and treatment. Rev Med (São Paulo) 87:245-250.

Pereira MG (1995) Epidemiology: Theory and practice. Guanabara Koogan, Rio de Janeiro, 583 p.

Pietrzak-Nowacka M, Safranow K, Bińczak-Kuleta A, Rózański J, Ciechanowski K and Ciechanowicz A (2012) Association of C49620T *ABCC8* polymorphism with anthropometric and metabolic parameters in patients with autosomal dominant polycystic kidney disease: A preliminary study. Nefrologia 32:153-159.

Rodrigues MMO (2018) Molecular analysis of blood group variants in indigenous populations. M. Sc. Thesis, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre.

Rodríguez-Rivera NS, Cuautle-Rodríguez P, Ortiz-López MG, Granados-Silvestre MÁ, Menjívar-Iraheta M and Molina-Guarneros J (2019) E23K (KCNJ11), and R1273R (*ABCC8*) polymorphism frequency in diabetes mellitus type 2 patients: Glycemic control association in patients undergoing oral hypoglycemic treatment. Rev Hosp Juá Méx 86:17-25.

Romanciuc M (2017) Type 2 diabetes mellitus as an inflammatory disease: Anatomy, physiopathology, and therapeutics. D. Sc. Thesis. Universidade do Algarve, Faro.

Rosenson RS, Shott S and Tangney CC (2002) Hypertriglyceridemia is associated with an elevated blood viscosity Rosenson: Triglycerides and blood viscosity. Atherosclerosis 161:433-439.

Sakamoto Y, Inoue H, Keshavars P, Miyawaki K, Yamagushi Y, Moritani M, Kunika K, Nakamura N, Yoshikawa T, Yasui N *et al.* (2007) SNPs in the KCNJ11-*ABCC8* gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. J Hum Genet 52:781-793.

Santos EJM, Ribeiro-dos-Santos AKC, Guerreiro JF, Aguiar GFS and Santos SEB (1996) Migration and change of ethinic structure in a hybrid population from Amazon Region (Santarém-Pará). Genet Mol Biol 19:511-515.

Satler L, de Souza J, de Oliveira G, Nunes J, Fagundes D and Mendes R (2021) Factors associated with the prevalence of type 2 diabetes mellitus: A literature review. In: Anais do Seminário Científico do UNIFACIG, 6, pp 1-10.

Schmidt MI, Duncan BB, Mill JG, Lotufo PA, Chor D, Barreto SM, Aquino EM, Passos VM, Matos SM, Molina MC *et al*. (2015) Cohort profile: Longitudinal study of adult health (ELSA-Brasil). Int J Epidemiol 44:68-75.

SBD – Sociedade Brasileira de Diabetes (2022) Diretrizes da Sociedade Brasileira de Diabetes. São Paulo.

Sousa RC, Gama IB, Costa, JDF, Macedo LAR, Sousa YS and Nascimento CC (2020) Prevalence and risk factors of diabetes mellitus in public school employees in Santarém-Pará. Braz J Develop 6:49932-49944.

Tavares DMS, Reis NA, Dias FA and Lopes FAM (2010) Diabetes mellitus: Occurrence of risk factors and care among nursing workers. Acta Paul Enferm 23:671-676.

Venkatesan R, Bodhini D, Narayani N and Mohan V (2014) Association study of the *ABCC8* gene variants with type 2 diabetes in south Indians. Indian J Hum Genet 20:37-42.

Yajnik CS, Janipalli CS, Bhaskar S, Kulkarni SR, Freathy RM, Prakash S, Mani KR, Weedon MN, Kale SD, Deshpande J *et al*. (2009) *FTO* gene variants are strongly associated with type 2 diabetes in South Asian Indians. Diabetologia 52:247-252.

Yamakita M, Ando D, Tang S and Yamagata Z (2010) The Trp64Arg polymorphism of the b 3-adrenergic receptor gene is associated with weight changes in obese Japanese men: A 4-year follow-up study. J Physiol Anthropol 29:133-139.

Yokoi N, Kanamori M, Horikawa Y, Takeda J, Sanke T, Furuta H, Nanjo K, Mori H, Kasuga M, Hara K *et al.* (2006) Association studies of variants in the genes involved in pancreatic beta-cell function in type 2 diabetes in Japanese subjects. Diabetes 55:2379-2386.

Wang F, Han XY, Ren Q, Zhang XY, Han LC, Luo YY, Zhou XH and Ji LN (2009) Effect of genetic variants in KCNJ11, *ABCC8*, PPARG and HNF4A loci on the susceptibility of type 2 diabetes in Chinese Han population. Chin Med J (Engl) 122:2477-2482.

Zhou D, Zhang D, Liu Y, Zhao T, Chen Z, Liu Z, Yu L, Zhang Z, Xu H and He L (2009) The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population. J Hum Genet 54:433-443.

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