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Research Article

Polymorphism of the rs12255372 Allele of the *TCF7L2* Gene Associated with Predisposition to Type 2 Diabete Using SNP Markers in a Population in the North of the Côte d'Ivoire

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Abstract

Background: Type 2 diabetes (T2D) is a chronic metabolic disease characterised by hyperglycaemia due to insulin resistance and impaired insulin secretion. Genetic and environmental factors can influence predisposition to this disease. Genetic predisposition plays a significant role in the risk of developing the disease, and the TCF7L2 (T-Cell Factor-Like 2) gene is one of the main genes associated with type 2 diabetes. *Objective:* The aim of this study was to investigate the prevalence of the rs12255372 (G/T) polymorphism in TCF7L2, a gene associated with the risk of type 2 diabete (T2D) in the Ivorian population in the north of Côte d'Ivoire. *Methodology*: We included a total of 75 participants, 50 with type 2 diabete and 25 healthy subjects, for various anthropometric, clinical and genetic parameters. Participants were recruited from the Korhogo Regional Hospital. After obtaining consent, a blood sample was taken from each participant for glycaemia measurement and confetti realization for molecular biology. Genomic DNA extracted from the confetti was used to perform TCF7L2 gene genotyping using allele-specific PCR. *Results*: Analysis of the prevalence of the T allele of the SNP rs12255372 showed a statistically significant association between type 2 diabetic patients and non-diabetics ($p \leq 0.05$). The analysis revealed a genotypic prevalence of the rs12255372 variant of the TT allele significantly more expressed in non-diabetics (52%) compared with diabetics (26%) ($p=0.03$, $z=2.23$). *Conclusion*: This study revealed a high prevalence of the rs12255372 genetic variant of the TCF7L2 gene in non-diabetic populations in the north of Côte d'Ivoire, suggesting a significant predisposition to types 2 diabetes and the involvement of other factors, such as environmental conditions, lifestyle habits and genetic interactions in the development of type 2 diabetes in healthy subjects carrying the TT allele of the SNP rs12255372 of the TCF7L2 gene.

Keywords

Type 2 Diabetes, TCF7L2 Gene Polymorphism, Genetic Predisposition

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1. Introduction

Type 2 diabete (T2D) is one of the most common metabolic disorders, caused by a combination of two main factors. A defective secretion of insulin by the β cells of the islets of Langerhans of the pancreas and the incapacity of insulin-sensitive tissues leading to chronic hyperglycaemia [1].

Type 2 diabete accounts for 90% of diabetes in the world, so high is its incidence that we are talking about a veritable epidemic, and forecasts for the coming years are very alarming. This increase in the prevalence of diabetes every year is due to a number of causes, including lifestyle, with a processed diet that is higher in sugar and a high risk of obesity [1].

In 2011, there were 366 million people with diabetes in the world, with forecasts of 552 million by 2030 [2]. Epidemiological forecasts estimate that the prevalence of diabetes will have increased by 98% by 2030 in sub-Saharan Africa [3]. In Côte d'Ivoire, this prevalence was estimated at 4.9% in 2014 [4].

The study conducted by Sassor et al (2017) [5], looking at the dietary practices of type 2 diabetics followed at the Abidjan Antidiabetic Centre in Côte d'Ivoire, revealed that the patients examined had an average age of 56.2 years and had had diabetes for an average of 8.6 years. The main comorbidities were overweight (56.8%) and high blood pressure (45.3%). The study conducted by Dago et al (2022) [6] at the Korhogo Regional Hospital Centre revealed a significant correlation between the growing prevalence of metabolic diseases such as diabetes and hypertensive disorders and age in the north of Côte d'Ivoire.

The *TCF7L2* gene is involved in the regulation of gene expression related to insulin secretion and pancreatic beta cell function [7]. It is the major gene involved in the expression of type 2 diabete and is the first locus to have been reported by genomic linkage and genome-wide association studies [8].

The human TCF7L2 gene spans 21,063 nucleotides and is located on chromosome 10 in the q25.3 region [7].

Genetic variations in this gene, such as SNP rs12255372, can affect the expression of TCF7L2 and therefore influence the pancreas' ability to produce insulin in response to high levels of glucose [9].

The SNP rs12255372 in the TCF7L2 gene is one of the most extensively studied genetic polymorphisms in relation to the risk of developing type 2 diabetes. It is located in the intronic region (intron 3) of TCF7L2 and contains a single G base to T transversion at position 293 [10]. Studies have shown that individuals carrying the T allele of rs1225372 have impaired beta cell function, which may contribute to insufficient insulin secretion leading to hyperglycaemia, thus increasing the risk of type 2 diabete [11-13]. Given the increasing number and dynamism of cases of type 2 diabete in the northern region of Côte d'Ivoire [6], we thought it would be useful to infer the distribution of the T allele of the rs1225372 genetic variant of the TCF7L2 gene in populations in this geographical area of the country.

2. Material and Methods

2.1. Site and Study Population

This experimental prospective study was conducted from February to April 2024 at the Korhogo Regional Hospital Centre (CHR) and the Pasteur Institute of Côte d'Ivoire. The study involved 75 patients, 50 with type 2 diabete and 25 without diabetes. The study involved all samples of diabetic (50) and non-diabetic (25) patients. The 50 diabetics were recruited in the diabetology department of the Korhogo Regional Hospital, while the 25 non-diabetics were recruited in the other consultation departments of the Regional Hospital.

2.2. Study Population

The study involved 150 patients, 100 with type 2 diabete and 50 healthy subjects (non- diabetics). These patients were recruited at the Korhogo Regional Hospital from February to April 2024 in the Diabetology and Biology Laboratory Departments of the Centre. Of the 150 patients included in the study, 100 had type 2 diabete and 50 were non-diabetic. The diagnosis of diabetes is currently based on criteria established by a committee of experts and adopted by the WHO. According to these criteria, there are three ways of diagnosing diabetes: firstly, fasting blood glucose levels of 1.26 g/l (7.0 mmol/l) or more on two occasions; secondly, blood glucose levels of 2 g/l (11,1 mmol/l) 2 hours after a 75g glucose load, and finally a blood glucose level greater than or equal to 2.00 g/l (11.1 mmol/l) at any time, associated with symptoms of diabetes (polyuria, polydipsia, unexplained weight loss, drowsiness and even coma).

2.3. Blood Sampling and Confetti Preparation

2.3.1. Blood Sampilng

After obtaining their consent to take part in the study, approximately 5 ml of blood was taken from each participant. Blood samples were taken by venipuncture from the elbow of subjects who had been fasting for at least 12 hours. Blood was collected in two different tubes, one containing the anticoagulant EDTA (Ethylene diamine tetra-acetic acid) and one containing sodium fluoride and calcium oxalate. Whole blood collected in tubes containing EDTA was used to make confetti for molecular biology.

2.3.2. Production of Confetti

Blood collected in tubes containing EDTA was used to make confetti. Approximately 50 µL of whole blood was deposited on Whatman 3 MM filter paper using a micropipette with filter cones. The paper containing the blood spots was dried for approximately 60 to 120 minutes at room temperature in a dust-free environment. Unused blood from the

EDTA tube was stored in cryotubes at -20 ℃ for possible future use.

2.3.3. Measurement of Anthropometric and Biochemical Parameters

Blood pressure was measured with an Omron M2 HEM-7121-E, China blood pressure monitor. The patient was at rest for 10 minutes before the blood pressure was taken.

Blood glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and glycated haemoglobin were measured on a COBAS C311 HITACHI spectrophotometer from Roche Diagnostics, France.

2.3.4. Extraction of Plasmodium Falciparum Genomic DNA

The genomic DNA was extracted using the resin column extraction method using a commercial kit (Spin-x viral DNA/RNA extraction kit from SD Biosensor) and following the manufacturer's instructions. Briefly, this method uses silica membrane technology (Spin column) for the isolation and purification of genomic DNA. Blood cells (red blood cells) were lysed by adding 400 µl of lysis buffer (AVL) to 20 µl of proteinase K (Protease K) followed by incubation at 70 ℃ for 10 minutes. The genomic DNA was then purified in a column by adding 500 µl of absolute ethanol to the lysate, which was briefly vortexed before centrifuging the tubes briefly (one minute) at 10,000 rpm. This operation was repeated in order to recover all the solution in a new collection tube. After washing the membrane (with buffers AW1 and AW2), the genomic DNA was eluted using AVE buffer (60µl added). The eluate (constituting the DNA extract) was collected in the Eppendorf tube.

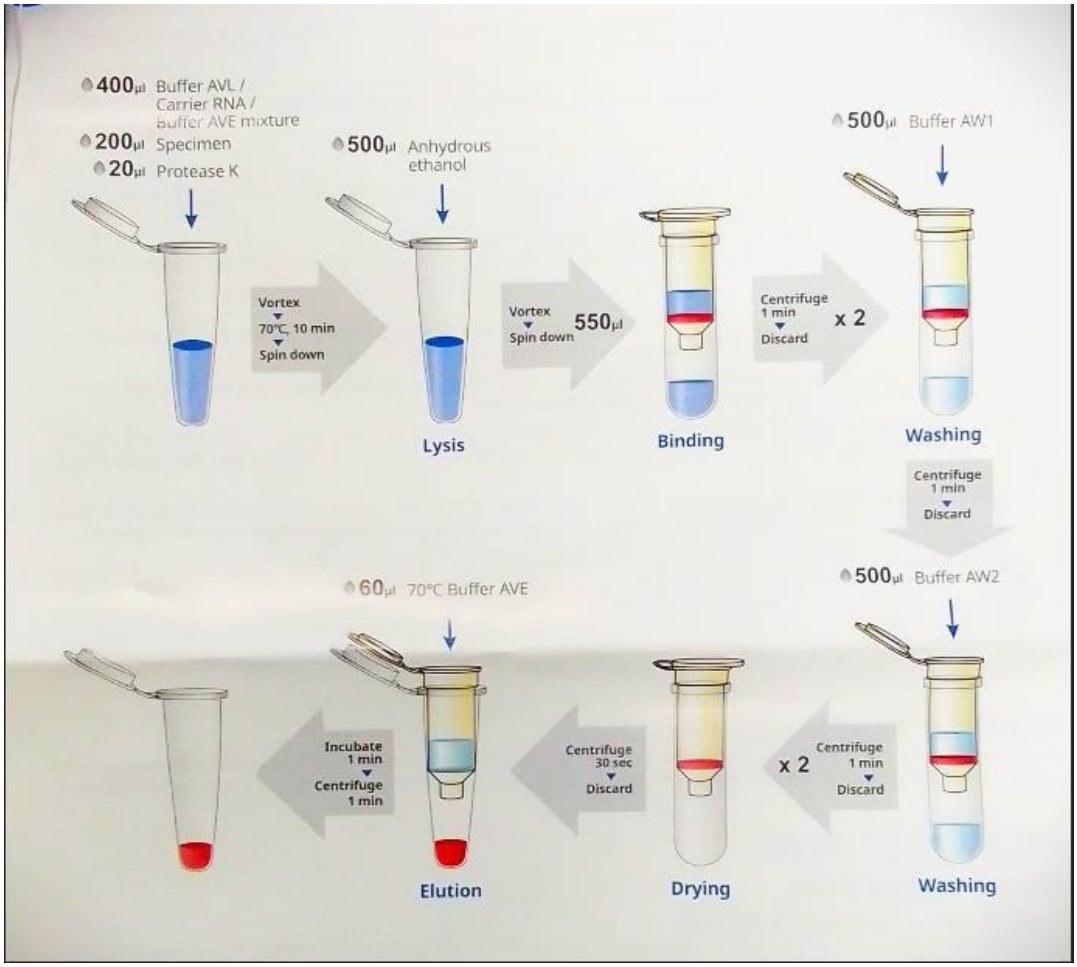


Figure 1. *The main DNA extraction steps according to supplier.*

2.3.5. Genotyping of the TCF7L2 Gene

The rs12255372 (G/T) polymorphism of the *TCF7L2* gene was genotyped by allele-specific PCR. Two PCRs were performed for each sample. Each PCR used a common sense primer (CFW) and one of two antisense primers (Rev G or Rev T) specific to the Poly T and Poly G alleles of the rs12255372 polymorphism (Table 1).

Table 1. Primers used for gene amplification.

Polymorphism	primers Nucleotide sequences
rs12255372 G/T	CFw: 5'CTGGAAACTAAGGCGTGAGGGA 3' Rev_G: 5'CAGAGGCCTGAGTAATTATCAGAATATGATC 3' Rev_T: 5'CAGAGGCCTGAGTAATTATCAGAATATGCTA 3'

The reaction mixes and programming for amplification were carried out according to the procedure for the commercial Firepol Master Mix kit from Solis Biodyne Master Mix kit from Solis Biodyne.

To detect the T (Poly T) and G (poly G) polymorphisms we prepared reaction mixes containing the primer pairs CFW and Rev T and CFW and Rev G as shown in Table 2. The same amplification program was used to perform the amplifications using an applied biosystems thermal cycler (Table 3).

Table 2. Preparation of reaction mixtures.

Mix for T polymorphism detection		Mix for G polymorphism detection	
Reagents	**Volume (µL)**	**Reagents**	**Volume (µL)**
H2O Bio- mol	15	H2O Bio- mol	15
5X Firepol Master Mix	4	5X Firepol Master Mix	4
CFW	0.5	CFW	0.5
Rev T	0.5	Rev G	0.5
Volume Mix (µL)	20	Volume Mix (µL)	20
DNA	5	DNA	5
Reaction volume (µL)	25	Reaction volume (µL)	25

Table 3. Amplification programming for the detection of T and G polymorphisms.

Steps	Temperatures (°C)	Time	Cycles
Initial denaturation	95	3 min	1X
Final denaturation	95	20 sec	
Hybridization	60	30 Sec	40X
Elongation	72	30 Sec	
Final elongation	72	5 min	1X

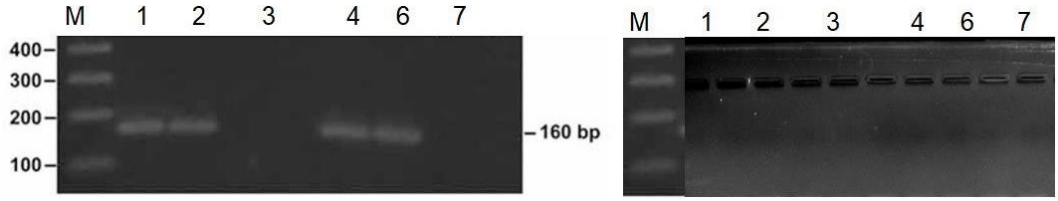
2.3.6. Detection and Analysis of PCR Products

PCR products were separated by electrophoresis on a 2% (W/V) agarose gel to detect specific alleles. After migration, the gel was recovered and observed under a UV lamp using the UV transluminator (Gel DocTM EZ Imager) to assess the presence or absence of bands.

The bands obtained were compared with those of the molecular weight marker to estimate the size of the DNA bands on the electrophoregram. The size of the gene (Poly T, Poly G) of interest in the amplicon is 160 bp (Figure 1).

For each sample, two PCR reactions were performed, each comprising a common forward primer and a reverse primer rs12255372 G (G reaction) or rs12255372 T (T reaction),

depending on the allele to be amplified. A DNA fragment of 160 bp indicated the presence of the allele, while amplification failure indicated the absence of the allele in the sample. In a 3% w/v agarose gel, M indicates the molecular size marker, G and T indicate the product of the G reaction and the T reaction respectively, amplified from a selected DNA sample of each possible genotype (GG, GT and TT).



Reaction product T Reaction product G

Figure 2. *Genotyping of the TCF7L2 rs12255372 (G/T) polymorphism using an allele- specific PCR assay.*

2.3.7. Statistical Analysis

Quantitative results were presented as percentages or mean ± standard deviation. Allelic and genotypic frequencies in diabetic and non-diabetic patients were estimated by direct counting. Fisher's statistical test was used to compare qualitative data and Wicolson's test was used to compare quantitative data. The relationships between the different groups, genotypes and alleles of the rs12255372 polymorphism were evaluated by logistic regression, calculating the 95% confidence interval. P-value values of less than 0.05 were considered significant. These analyses were performed using SPSS version 23.0 for Windows.

To determine the number of age groups, we used the Sturges rule (Sturges, 1926) which states that k≃ 1+3.22 * log10(n), where n is the total number of observations [14].

3. Results

Anthropomorphic and clinical characteristics of participants

The mean age of the participants in our study was 53.89 years [95% CI: 50.96-56.81]. Among the different age groups, the 40-60 age group was the most represented with 66 participants, i.e. 44.00% of participants [95% CI: 33.73%, 54.88%]. Conversely, the numbers in the 20-40 and 61-80 age groups were relatively low, with 32 (21.33%) and 52 (34.67%) participants respectively, representing 21.33% of the sample (Figure 3).

The profile of the participants' clinical parameters and lifestyle habits are presented in Table 4.

In fact, our results indicate that hypertension was observed in 54% (54/100) of diabetic participants, compared with only 4% (2/50) of non-diabetics. This difference was significant with a p-value = 0.00.

Participants' weights ranged from 38 to 118, with an average of 66.3kg for diabetics and 68.6kg for non-diabetics. The mean weights of the participants showed no significant difference between the diabetic and non-diabetic groups (p-value 0.559). Non-diabetics had a higher minimum weight (51kg) compared with diabetics (38kg), but the maximum weight was similar between the two groups.

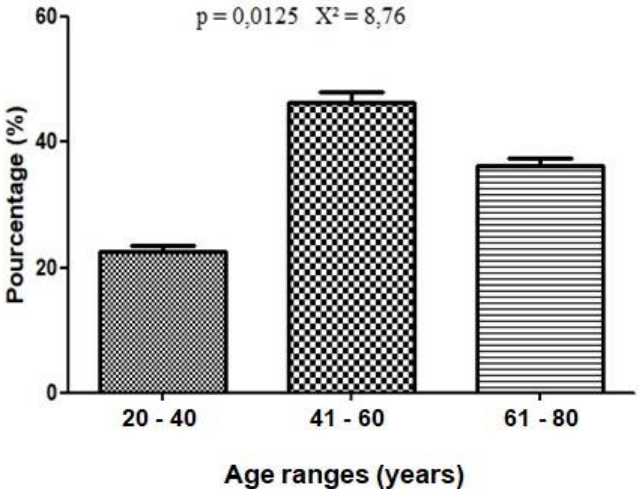


Figure 3. *Age distribution of the study population.*

The study also showed that very few diabetic participants (2%; i.e. 2/100) used tobacco while none of the non-diabetics did. No significant difference was observed between diabetics and non-diabetics who smoked (p-value = 1).

Sport was also observed in 34.6% (52/150) of participants. No significant difference was observed between diabetics (34%) and non-diabetics (36%), (p-value of 1.0).

No diabetic participant reported drinking alcohol, whereas 28% of non-diabetics did. This difference was statistically significant, with a p-value =0.00.

Table 4. Profile of clinical parameters and lifestyle habits of participants.

Clinical parameters and lifestyle habits	By-laws	Total population (n = 150)	Diabetics (n = 100)	Non- Diabetics (n = 150)	p-value
Hypertension	Yes	56 (37.33%)	54 (54%) a	2 (4%) b	0.0000104
	No	94 (62.66%)	46 (46%)	48 (96%)	
Blood glucose	Hypoglycaemia (< 0.70)	4 (2.67%) b	0 (0.0%) b	4 (8.0%) b	0.768
	Normal (0.70 - 1.26)	88 (58.67%)	42 (42.0%)	46 (92.0%)	0.000034
	Hyperglycaemia (> 1.26)	58 (38.67%)	58 (58.0%)	0 (0.0%)	0.000000116
Weight (Kg)	Mean ± sd	67.07 ± 14.84	66.3 ± 13,81	68.6 ± 16.92	0.559
	Min - Max	38 - 118	38 - 101	51 - 118	
Smoker	Yes	2 (1.33%)	2 (2%)	0 (0%)	1.0
	No	148 (98.67%)	98 (98%)	50 (100%)	
Practising sport	Yes	52 (34.67%)	34 (34%)	18 (36%)	1.0
	No	98 (65.33%)	66 (66%)	32 (64%)	
Alcohol consumption	Yes	14 (9.33%)	0 (0.0%)	14 (28.00%)	0.00024
	No	136 (90.67%)	100 (100%)	36 (72 %)	

The results are expressed as absolute values and percentages; as the mean ± sd Standard deviation; Min = minimum; Max = maximum; the value P<0.05 is considered significant.

Prevalence of the T allele in the study population according to sex.

The study showed that 34.67% (52/150) of participants were carriers of the T allele [95% CI: 24.88%, 45.95%]. Of the 52 T allele carriers, 20 (38.46%) were male [95% CI: 22.43%, 57.47%], while 32 (61.54%) were female [95% CI: 42.53%, 77.57%]. The proportion test revealed a p-value of 0.096 (Figure 4).

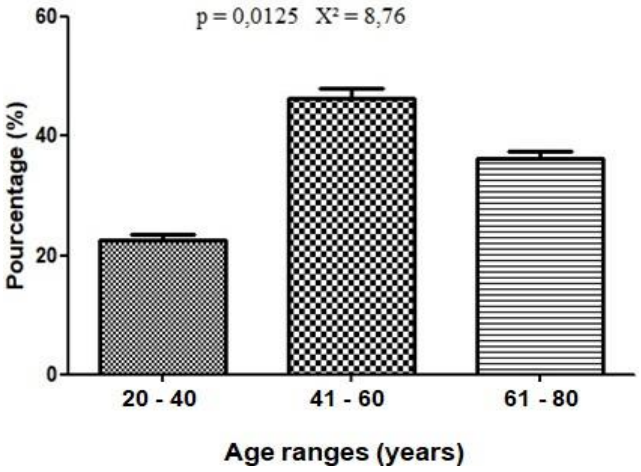


Figure 4. Prevalence of the T allele in the population, by sex.

Prevalence of the T allele in non-diabetic and diabetic populations

The results indicated that 34.66% (52/150) of the participants were carriers of the T allele, of whom 52.00% (26/50) were non-diabetics and 26% (26/100) were diabetics. The proportion test revealed a Z statistic = 2.23 with a p-value = 0.03. Statistical analysis showed that there was a significant difference between the proportions of diabetics carrying the TT allele of the rs12255372 genetic variant of the *TCF7L2* gene and non-diabetics carrying the same allele (p=0.00) (Figure 5).

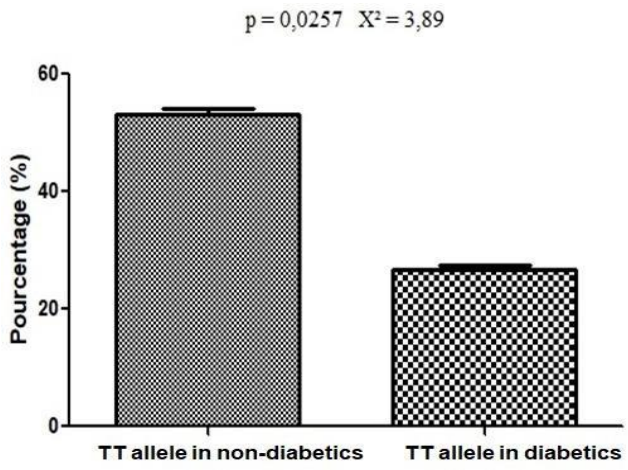


Figure 5. Prevalence of the T allele in non-diabetic and diabetic populations.

4. Discussion

Type 2 diabete (T2D) is a chronic multifactorial disease that develops when the pancreas does not produce enough insulin or the body does not use the insulin it does produce correctly. A number of genetic and environmental factors may be involved in the development of an abnormality in the function of insulin-secreting cells, leading to the development of insulin resistance and type 2 diabete [15]. The burden of T2D is enormous in sub-Saharan populations and epidemiological data on the disease are limited, particularly on the genetic determinants of the disease [16]. The aim of this study was to evaluate the rs12255372 (G/T) polymorphism of the *TCF7L2* gene in a population in the north of Côte d'Ivoire, where there is a growing incidence of metabolic diseases, i.e. type 2 diabete.

The results of the anthropomorphic profile (age and sex) showed that participants in the 41-60 age group were in the majority, with 44% of participants and a predominance of women (62.67%) compared with 37.33% of male participants. The average age of the participants was 53.89 years. Our results are consistent with those of Dago et *al* (2022), who clearly showed an increase in the frequency of type 2 diabete with age in the population of Korhogo in the north of Côte d'Ivoire [17]. These results could be explained by the fact that many people in this age group are confronted with stress, physical inactivity (sport) and an uncontrolled diet. The results are also similar to those of Yao et *al* (2023), who found a predominance of women in the national diabetes prevalence survey PREVADIA-CI 2017 [18, 19]. This female predominance could be explained by differences in interest in the subject of the study between the sexes or sociocultural constraints that influence male participation [18]. The results on the prevalence of the T allele of the *TCF7L2* gene in the participants indicate 34.67% of the participants carried the T allele of the *TCF7L2* gene including 30.77% male carriers versus 69.23% female carriers. Our results are in agreement with those of Saoud (2012) who found an estimated female predominance of 76.8% versus 23.32% male in a population of diabetics in the city of FES in Morocco [20].

Among participants carrying the T allele of the *TCF7L2* gene, 52% were non-diabetic compared with 26% of diabetic participants. Our study thus showed a significantly higher prevalence of the T allele of the *TCF7L2* gene in the non-diabetic population (52%) compared with the diabetic population (26%) (p-value=0.0257). Similar studies conducted in Turkey by Demirsoy *et al*, (2016) and in the islands of the province of Bali by Saraswati *et al*, (2017) showed similar results with a predominance of the T allele and TT genotype in non-diabetics compared to diabetics [21, 22]. This predominance was confirmed by a study conducted in Egypt by Mandour *et al*, (2018) where the T allele was observed in 51.7% of healthy controls (non-diabetics) compared to 39.2% in diabetic patients [23]. The presence of the T allele in non-diabetic participants could be explained by a predisposition of these participants to type 2 diabete [24, 25]. The *TCF7L2* gene codes for a transcription factor that plays an important role in regulating the function of pancreatic beta cells responsible for insulin secretion [26]. Several authors have also reported that the rs12255372 and rs7903146 polymorphisms in the *TCF7L2* gene are associated with an increased risk of developing type 2 diabete [27-29].

These results could be explained by the fact that these non-diabetic carriers of the T allele, although genetically predisposed, have not yet developed the disease or benefit from protective factors that delay its onset. It is also possible that in these specific populations, other genetic or environmental factors moderate the effect of the *TCF7L2* gene.

The limitations of our study are essentially linked to the small number of participants in the study (151 participants). In addition, our very limited financial resources did not allow us to extend the study to other polymorphisms of the *TCF7L2* gene (rs7903146, rs1196205 and rs45065665) which are strongly implicated in predisposition to type 2 diabete. Given the complexity of the relationship between the rs12255372 polymorphism of the T allele of the *TCF7L2* gene and type 2 diabetes, it would be interesting to continue this work to gain a better understanding of the mechanisms underlying this association in various populations and to consider research on the other polymorphisms (rs7903146, rs1196205 and rs 45065665) predisposing to diabetes2 in the Korhogo population with a larger number of participants.

5. Conclusion

The results of our study indicate that the relationship between the T allele of the *TCF7L2* gene and type 2 diabete is complex and potentially modulated by additional factors, both genetic and environmental. A higher prevalence of the T allele in non-diabetics may indicate a 'pre- diabetic' phase, where the effects of the gene have not yet fully manifested, or could reflect selection against carriers of the T allele in the diabetic population because of the increased risk of complications. Our study confirms the existence of a significant association between SNP rs12255372 of the *TCF7L2* gene and the risk of developing type 2 diabete in the population of northern Côte d'Ivoire.

Abbreviations

DNA	DeoxyriboNucleic Acid
EDTA	Ethylene Diamine Tetra-Acetic Acid
PCR	Polymerase Chain Reaction
PREVADIA-CI	Prevalence of Diabetes in Côte d'Ivoire
SNP	Single Nucleotide Polymorphism
TCF7L2	T-Cell Factor-Like 2
T2D	Type 2 Diabete

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Author Contributions

Dagnogo Olé fongo: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing

Dago Dougba Noel: Formal Analysis, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing

Yeo Tenedjoh Korotoum: Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing

Kouman Kouamé Bouatini Angédo: Formal Analysis, Investigation, Methodology, Software

Coulibaly N'golo David: Methodology, Supervision, Visualization

Djaman Allico Joseph: Formal Analysis, Supervision, Validation, Visualization

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki and approval was received from the National Ethics and Research Committee (CNER) of the Ministry of Health, Public Hygiene and Universal Health Coverage of Côte d'Ivoire. After appropriate information and explanations, the adult participants, parents or legal guardians of all children wishing to participate in the study gave their written consent prior to sampling.

Conflicts of Interest

The authors declare no conflicts of interest.

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