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Genetic Polymorphisms in Cytokine Genes and their Association with Susceptibility to Type 2 Diabetes Mellitus in Yaoundé, Cameroon

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Abstract

Major advances have been made in understanding the mechanisms that are involved in the pathogenesis of type 2 diabetes (T2DM). It has been hypothesised that inflammation has a role in the pathogenesis of T2DM. Cytokines are immunomodulatory proteins or glycoproteins which control or modulate the activities of cells within the immune system. Therefore, the genetic polymorphisms that regulate the expression levels of these cytokines might have an essential impact on the interindividual differences in T2DM. The purpose of the present study was to investigate the possible association of IL-1β-511C/T and IL-22 rs1179251G/C polymorphisms with T2DM amongst individuals attending the Yaounde Central Hospital, Cameroon. Totally, 100 patients with T2DM and 100 healthy controls were studied. The genetic polymorphisms for IL-1β-511C/Tand IL-22 rs1179251G/C were analyzed by PCR-RFLP method. The results were analyzed with SPSS software using χ^2 test and a p-value < 0.05 was considered statistically significant. The GC genotypes of the IL-22 rs1179251 polymorphism showed a decreased risk of T2DM (OR= 0.479, P= 0.015, 95% CI= 0.272-0.845). However, no significant difference was found for the IL-1β -511C/T polymorphism between the two groups. Our findings indicated that IL-22 rs1179251 intron 4 polymorphism affects the risk of T2DM in our study and this polymorphism might be involved in the pathogenesis of this disease.

Keywords: IL-1β, IL-22, Gene polymorphism, Type 2 diabetes, Cameroon.

Introduction

Diabetes mellitus, more simply called diabetes, is a serious, long-term (or "chronic") condition that occurs when raised levels of blood glucose occur because the body cannot produce any or enough of the hormone insulin or cannot effectively use the insulin it produces (1). WHO guidelines classify diabetes in three main groups namely: Type 1 diabetes which is as a result of the body's failure to produce enough insulin and requires daily administration of insulin, Type 2 Diabetes is the most common type of diabetes which is as a result of the body's ineffective use of insulin, and gestational diabetes is hyperglycemia, which occurs when blood glucose levels are above normal but below those that are indicative of diabetes (1–3). In 2021, the International Diabetes Federation (IDF) estimated that 537 million people, representing 10.5% of the global population, were living with diabetes. This figure is expected to rise to 643 million by 2030 and 783 million by 2045. Additionally, the IDF reported that approximately 24 million adults aged 20-79 years in the African region had

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diabetes in 2021, corresponding to a regional prevalence of 4.5%. In Cameroon, the age-adjusted prevalence of diabetes among adults in the same age group was 5.5% in 2021 (1,3). Globally, the prevalence of type 2 diabetes is high and rising across all regions. This rise is driven by population ageing, economic development and increasing urbanisation, leading to more sedentary lifestyles and greater consumption of unhealthy foods linked with obesity (1,4).

Major advances have been made in understanding the mechanisms that are involved in the pathogenesis of type 2 diabetes. It has been hypothesised that inflammation has a role in the pathogenesis of T2DM (2,5–7). Cross-sectional and prospective studies have described elevated circulating levels of acute-phase proteins (such as C-reactive protein (CRP), haptoglobin, fibrinogen, plasminogen activator inhibitor and serum amyloid A) and sialic acid, as well as cytokines and chemokines, in patients with T2DM (5,8–10). Cytokines are immunomodulatory proteins or glycoproteins which control or modulate the activities of cells within the immune system. The production of many cytokines is under genetic control and polymorphisms have been identified within a large number of these genes (11). Pro and anti-inflammatory cytokines play a key role in identifying patients at elevated risk for several diseases (11,12). Interleukin-1 beta (IL-1β) and Interleukin-22 (IL-22) are cytokines involved in immune regulation and inflammation. Therefore, the genetic polymorphisms that regulate the expression levels of these cytokines might have an essential impact on the interindividual differences in T2DM.

IL-1β, a pro-inflammatory cytokine, has been underlined as a strong driver of β -cell damage. β-cell macrophages are the main contributors to IL-1β production (13). IL-1β encompasses various functions in regulating inflammatory responses and metabolism; it can regulate insulin secretion and promote β cell apoptosis which can eventually lead to T2DM (13–15). A previous observational study demonstrated that a combined elevation of IL-1β and IL-6 was associated with a roughly threefold increased risk of T2DM, and IL-1β might induce insulin resistance via activating the IκB kinaseβ (9,16). Research has shown that IL-1β, in conjunction with TGF-β and IL-6, promotes the differentiation of Th17 cells. These Th17 cells subsequently produce IL-17A, IL-17F, and IL-22, which are key cytokines involved in inflammatory responses (17,18). IL-22, a novel protein, was discovered for the first time by Dumoutier *et al.* in the year 2000 (19,20). Initially, IL-22 was called Interleukin-10-related T cell-derived inducible factor due to its significant structural resemblance (homology of 22%) to mouse interleukin-10 (19). IL-22 is a class II cytokine and has been categorized in the IL-10 family of cytokines due to its biochemical and functional characteristics, along with other interleukins including IL-10, IL-28, IL-26, IL-29, IL-24, and IL-20 (21). In humans, chromosome 12 contains the gene responsible for encoding IL-22 (22). IL-22 is a cytokine produced by immune cells and is important for the regulation of tissue cell responses during inflammation and infection (20).

IL-22 exhibits many pro-inflammatory effects in order to generate an immune response (22) and also anti-inflammatory responses, depending upon tissue microenvironment including cytokine milieu (22,23). The polymorphisms in cytokine genes lead to interindividual differences in their production, leading to variations in immune responses (24). Many genetic association studies have been performed to estimate the relationship of the IL-1β (-511C/T) and IL-22 (rs1179251) polymorphisms with T2DM risk and other inflammatory diseases (16,20,25). Thus, there is lot of evidence showing the involvement of genetic determinants in the etiology of T2DM. The aim of the present study was to investigate the association of polymorphisms of IL-1β (-511C/T) and IL-22 (rs1179251) genes to the susceptibility of Type 2 Diabetes Mellitus in Yaoundé, Cameroon.

Materials and Methods

Study location and setting

This study was carried out in Yaoundé, the capital of the Centre Region in Cameroon (3°51′ N 11°29′ E) the second largest city of Cameroon with a population of more than 4 million. It contains people from all works of life and people from all other 10 regions of the country. It has a surface area of about 180 km² and it lies in the Centre Region of the nation at an elevation of about 800 meters above sea level and surrounded by many hills with an average annual rainfall of 1628.3mm per year. The city is located within the Congo-Guinean phytogeographic zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November (3,26).

Study Population and Sampling

A total of 200 participants were recruited for the study with 100 cases and 100 controls as a case control study. The study, along with its potential benefits, was thoroughly explained to the participants, who were then invited to take part using an information sheet. After signing the informed consent form, participants were officially enrolled in the study. An appointment was scheduled with the enrolled participants at the diabetic unit at the Etoug-Ebe Baptist Hospital. Participants were instructed to fast for 8 hours overnight before their scheduled appointment. During the visit, anthropometric measurements and fasting plasma glucose levels were collected, averaging the results from two readings. The values collected were recorded on our data collection sheet. Inclusion Criteria included being a Cameroonian, must have Type 2 Diabetes (fasting blood glucose ≥ 1.26 g/L for the diabetic group and a confirmed

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Nkenfou-Tchinda CN, et al., Fortune J Health Sci 2026
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HbA1c >7%), must be non-diabetic (fasting blood glucose between 0.7 and 1.09 g/L, control group), must be 40 years old or older and willing and able to provide informed consent. Exclusion criteria included pregnant women, individuals diagnosed with Type 1 Diabetes Mellitus or gestational diabetes, any patient with gross or microscopic proteinuria, with renal affection related or not related to DM, with systemic or blood disease that may affect the kidneys as SLE, leukemia and lymphoma and cases with heavy urinary tract infection. Diagnosis of microalbuminuria was done by testing the first fasting mid-stream urine sample with Micral-2 test strips which is specific semi-quantitative test for detection of micro albuminuria (11). Venus blood samples were also collected from study participants, from which dried blood spots were made using the Whartman N° 3-filter paper, air dried, labelled, sealed in sterile individual zip lock bags and stored at room temperature for further molecular analysis.

DNA extraction and genotyping

Human genomic DNA was extracted from dried blood spot on filter papers using Chelex boiling method as previously described (2,20). Genotyping of the IL-1β (-511) and IL-22 (rs1179251) was done using the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR–RFLP) technique. The amplification of the IL-1β (-511) and IL-22 (rs1179251) genes respectively was performed using a T3 Thermocycler (Biometra, UK), as previously described and adapted (20,27). The primer sequences used to amplify the genes were IL-1βF:5′-TGGCATTGATCTGGTTCATC-3′ and IL-1βR: 5′-GTTTAGGAATTCTCCCACTT-3′, IL–22F:5′-CAGAAATTAGCCCTATATGC-3′ and IL-22R: 5′-GAAAAAGGTAGGTAGGACTGATAAC-3′, respectively. For the amplification of IL-1β (-511), a total reaction mixture of 20µl, consisting of 7 µl of nuclease free water, 10 µl of One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 0.5 µl of each primer (10 pmol) and 2µl of DNA template made up the master mix. The PCR protocol was as follows: an initial denaturation step at 94°C for 5 min, 40 cycles including 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 7 min. Digestion of 8 µl of each PCR amplification product was carried out with AvaI restriction enzyme (New England Biolabs) at 37°C for 16 h according to manufacturer's instructions as previously described (27). Products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light with the undigested being the mutant type producing bands at 305bp (Table 1). The IL-22 (rs1179251) was amplified in a total reaction mix of 20µl, consisting of 6 µl of nuclease free water, 10 µl of One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 1 µl of each primer (10 pmol) and 2µl of DNA template made up the master mix. The PCR protocol was as follows: an initial denaturation step at 95°C for 5 min, 35 cycles including 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 7 min. Digestion of 8 µl of each PCR amplification product was carried out with AlwNI restriction enzyme (New England Biolabs) at 37°C for 16 h according to manufacturer's instructions as previously described (20). Products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light with the undigested being the wild type producing bands at 560bp (Table 1).

Statistical Analysis

Data were analyzed using the IBM SPSS biostatistics version 20.0 software (SPSS, Chicago, IL). Descriptive statistics, percentage rate and frequencies were used to describe the socio demographic and clinical data. Genotypic and allelic frequencies for individual polymorphisms were compared between cases and controls using the $\chi 2$ test. Where the number of expected observations was less than 5, the Fisher's test was used. A $p < 0.05$ was considered significant in all comparisons. The associations between alleles and genotype and disease risks were calculated by odds ratios (OR) with a 95% confidence interval (CI).

Ethical Considerations

The study was conducted according to the guidelines of the Declaration of Helsinki and received approval from the Regional Ethics Committee for Human Health Research (CRECRHH) under the ethical clearance document number CE:N°1310/CRERSH/2022. Prior to participant enrolment, written and signed consent from each participant was obtained. The potential risks and benefits risks and benefits as well as data privacy and confidentiality were explained to all participants. Only those who signed the informed consent form were included in the study.

Table 1: Restriction enzymes to digest IL-1β-511 promoter, IL-22 rs1179251 intron 4 SNPs

SNP	Amplicon size(bp)	Restriction enzyme	Digested Amplicon size (bp)	Alleles and Genotypes
IL-1β-511C>T	305	AvaI	T: 305	Alleles: C and T
			C: 190+115	Genotypes: CC, CT and TT
IL-22-rs1179251G>C	560	AlwNI	C:299+261	Alleles: G and C
			G: 560	Genotypes: GG, GC and CC

SNP= Single Nucleotide Polymorphism

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Results

Evaluation of Demographic and clinical characteristics of study Participants

Out of the 200 samples used in this study, 54(27.00%) were men and 146(73.00%) were women. In the T2DM group, 38 were male (38.00%) and 62 were female (62.00%) and on the other hand, in the control group 16 individuals were male (16.00%) and 84 were female (84.00%). The mean age of study participants was 54.79±12.13 years, that of the patients was 59.30±9.62 years, and controls were 50.28±12.74 years. As presented in Table 2, there was a significant difference between the mean age of diabetic patients and that of the controls (P=< 0.0001). Diabetic patients exhibited higher diastolic blood pressure (HDBP) and systolic blood pressure (HSBP) compared with the controls (85.40±14.60 vs. 79.74±14.31, P=0.006) and (138.28±25.33 vs. 130.52±23.31, P=0.025) respectively (Table 2).

Genotype and Allele Frequency of the IL-1β-511C/T and IL-22 rs1179251G/C Gene Poly morphisms

Results from genotyping were as follows: the IL-1β-511C/T single nucleotide polymorphism revealed the distribution of CC (18, 18.00%), CT (26, 26.00%) and TT (56, 56.00%) in the diabetic group and CC (14, 14.00%), CT (34, 34.00%) and TT (52, 52.00%) in the control group (Table 3). The IL-22 rs1179251 single nucleotide polymorphism revealed the distribution of GG (30, 30.00%), GC (36, 36.00%), and CC (34, 34.00%) in the diabetic group and GG (22, 22.00%), GC (54, 54.00%), and CC (24, 24.00%) in the controls group (Table 4). Allele frequencies for the IL-1β-511C/T SNP were 31.00% for the C allele and 61.00% for the T allele in both groups (Table 3). Also, allele frequencies for the IL-22 rs1179251 SNP were 48.00% for the G allele and 52.00% for the C allele the case group and 48.00% for the G allele and 52.00% for the C allele in the control group (Table 4).

Association between the IL-1β-511C/T and IL-22 rs1179251G/C Gene Polymorphisms with susceptibility to T2DM

Results from the association analysis (χ^2 test) for the IL-22 rs1179251 SNP, showed statistical significance between the T2DM group and control group. Individuals possessing the GC genotype were at reduced risk of developing T2DM (OR=0.479, P=0.015) (Table 4). Additionally, the results showed that Individuals possessing the GG and GC genotypes could be at risk to develop T2DM although no statistically significant difference was observed (OR=1.520 and OR=1.631, P=0.259 and P=0.160, respectively) (Table 4). No statistically significant difference was found between the genotypes and alleles of IL-1β-511C/T SNP and susceptibility to T2DM but individuals possessing the CT genotype were at reduced risk of developing this disease (OR=0.682, P=0.280) (Table 3).

Table 2: Demographic and clinical characteristics of study participants

Variables		Total Population N=200	T2DM N=100	Controls N=100	P-Value
Age (Mean±SD)		54.79±12.13	59.30±9.62	50.28±12.74	**< 0.0001**
Sex	Male	54(27.00%)	38(38.00%)	16(16.00%)	**0.0007**
	Female	146(73.00%)	62(62.00%)	84(84.00%)	
SBP (Mean±SD)		134.40±24.59	138.28±25.33	130.52±23.31	**0.025**
DBP (Mean±SD)		82.57±14.70	85.40±14.60	79.74±14.31	**0.006**
BMI (Mean±SD)		27.70±4.60	28.18±4.79	27.22±4.38	0.141
FBS (Mean±SD)		129.95±58.63	175.28±51.82	84.62±8.57	**< 0.0001**
Temperature (Mean±SD)		36.75±0.61	36.69±0.53	36.80±0.67	0.218
Pulse (Mean±SD)		82.74±14.94	82.68±13.98	82.80±15.91	0.955

P < 0.05 = Statistically significant, T2DM= Type 2 Diabetes mellitus, SD=Standard Deviation, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, BMI=Body Mass Index

Table 3: Frequencies of IL-1β-511(C/T) genotype and allelic polymorphisms among type 2 diabetic patients compared to control subjects

IL-1β-511	T2DM N=100	Controls N=100	OR	95%CI	P-Value
Genotype					
CC	18(18.00%)	14(14.00%)	1.348	0.630-2.887	0.563
CT	26(26.00%)	34(34.00%)	0.682	0.371-1.254	0.28
TT	56(56.00%)	52(52.00%)	1.175	0.673-2.050	0.67
Allele					
C	62(31.00%)	62(31.00%)	0.851	0.488-1.485	0.67
T	138(69.00%)	138(69.00%)	0.742	0.346-1.588	0.563

T2DM= Type 2 Diabetes mellitus, OR=Odds Ratio, CI=Confidence Interval

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Nkenfou-Tchinda CN, et al., Fortune J Health Sci 2026
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Table 4: Frequencies of IL-22 rs1179251 (G/C) genotype and allelic polymorphisms among type 2 diabetic patients compared to control subjects

IL-22 rs1179251	T2DM N=100	Controls N=100	OR	95%CI	P-Value
Genotype					
GG	30(30.00%)	22(22.00%)	1.52	0.803-2.875	0.259
GC	36(36.00%)	54(54.00%)	0.479	0.272-0.845	**0.015**
CC	34(34.00%)	24(24.00%)	1.631	0.879-3.026	0.16
Allele					
G	96(48.00%)	98(49.00%)	0.613	0.330-1.114	0.16
C	104(52.00%)	102(51.00%)	0.658	0.348-1.245	0.259

T2DM= Type 2 Diabetes mellitus, OR=Odds Ratio, CI=Confidence Interval

Discussion

In the current study, we investigated the association of polymorphisms in IL-1β-511C/T (promoter) and IL-22 rs1179251(intron 4) genes with Type 2 Diabetes mellitus (T2DM) in 200 participants (100 diabetics and 100 healthy controls) in Yaounde, Cameroon. Several studies have demonstrated significant association of SNPs present in immune genes with increased susceptibility to diseases and infections (7,16,28). Host genetic background is a crucial factor which regulates cytokine responses, and this may be linked with inflammation, viral clearance, or disease progression. Cytokines are proteins that help control inflammation in the body; they play principal roles in defense against infections (6,29). Polymorphisms in the regulatory region of a cytokine gene can alter its expression level related to immunologic reactions (27). Studies have shown that interleukins and other cytokines may be involved in susceptibility to develop T2DM (13,15). Because IL-1β and IL-22 may affect the risk of diabetes, we hypothesize that their gene polymorphisms may be associated with the pathogenesis of T2DM. In this study, IL-22 rs1179251 GC heterozygotes were significantly lower in the patient group, suggesting that the GC genotypes might have a protective effect in the development of T2DM (OR = 0.479, P = 0.015, 95 % CI = 0.272 – 0.845). Also, we found that individuals with the homozygous genotypes (GG and CC), had a greater risk of T2DM.

This is the first study in the Cameroonian population to show that a specific genotype of the IL-22 at rs1179251 GC is protective against T2DM. IL-22 gene is highly polymorphic and single nucleotide polymorphisms (SNPs) on IL-22 gene have been associated with several diseases. SNP in introns can introduce novel splice sites, activate novel promoters or introduce/eliminate enhancer activity (29). The single nucleotide polymorphism (SNP) rs1179251 in the interleukin-22 (IL-22) gene has been studied for its association with various diseases (20,30–32). However, specific studies directly linking the G allele of rs1179251 to increased IL-22 production are limited. One study investigated the association between IL-22 genetic polymorphisms and the risk of colon cancer. The findings suggested that individuals carrying the G allele of rs1179251 had an increased risk of developing colon cancer, with odds ratios of 1.46 for heterozygous (CG) and 2.10 for homozygous (GG) carriers. While this study did not directly measure IL-22 production levels, the association implies a potential link between the G allele and altered IL-22 activity (Thompson et al., 2010). We could infer that the CC genotype in the intron 4 region is associated with reduced IL-22 levels of the proinflammatory signals in response to stimulation. This is consistent with the findings of other studies carried out in Cameroon (20,29). Our findings do not align with those of Thompson *et al* (30), who evaluated the risk of colon cancer in the Kentucky population, and Song et al (31), who assessed the risk of autoimmune thyroid disease in the Chinese population. Interplay between IL-22 rs1179251 genotypes and some peripheral factors might underlie the discrepancies found among studied populations. Interestingly, the influence of some of the genetic factors may have a geographic distribution (33,34). In other words, one specific SNP may predispose individuals in a given ethnic group, whereas in a different group, it may neither predispose to nor protect against T2DM.

In our study, no differences were found in either genotype or allele frequencies of the IL-1β-511C/T polymorphism between patient and control groups. The result of the present study was not consistent with those of other studies on the IL-1β-511C/T polymorphism and the risk of T2DM. In that systematic review and meta-analysis carried out by Jaio *et al.* in 2021, stratification by ethnicity revealed that IL-1B (-511) was associated with a decreased risk of T2DM in the dominant model (OR=0.76, 95% CI [0.59–0.97], Phet = 0.218, Pz = 0.027) and codominant model (OR = 0.73, 95% CI [0.54–0.99], Phet = 0.141, Pz = 0.040) in the East Asian (EA) subgroup (16). Another study carried out by Stefandis *et al.* evaluating the association between IL-1β-511C/T polymorphism and the risk diabetic nephropathy in type 2 diabetes in a Caucasian population, found evidence that the IL-1β-511C/T variant might be associated with the pathogenesis of this disease

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Nkenfou-Tchinda CN, et al., Fortune J Health Sci 2026
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(35). The structural and functional characteristics of the IL-1 family of cytokines have been extensively studied by various research groups. However, genetic variations in cytokine genes across different populations have puzzled scientists worldwide (36). In recent years, there has been a growing focus on association studies examining genetic polymorphisms in IL-1 family genes and other inflammatory cytokines, both pro- and anti-inflammatory. These studies have the potential to identify significant haplotypes that may serve as susceptibility markers for T2DM in diverse ethnic populations (36).

Conclusion

In summary, the results of this study indicate that individuals with the IL-22 rs1179251 GC genotype have a protective effect against the development of T2DM. Interestingly, we also found that the IL-22 rs1179251 homozygous genotypes are associated with an increased risk of developing T2DM in our study population. Therefore, this SNP could serve as a useful marker, alongside other risk factors, for predicting susceptibility to T2DM.

Authors' Contributions

Carine Nguefeu Nkenfou-Tchinda: conceptualization; writing-original, writing-review and editing; Calvino Fomboh Tah: conceptualization; Sample collection; writing-original; formal analysis, writing-review and editing; Wilfred Fon Mbacham: conceptualization, review and editing; Celine Nguefeu Nkenfou: review and editing; Andrillene Laure Deutou Wondeu: review and editing, Wilfried Olivier Ngandjeu Tchamdjeu: review and editing; Gillian Njuo Ngeh: review and editing, formal analysis; Marina Lucie Ngo Gwed, Nora Kefeyin Kemei, Honore Awanakam, Jimmy Loic Keumo Takendong and Xaviera Jamieson Pouwawe Moumami; were all involved in formal analysis and editing and sample collection.

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Conflicts of Interest

The authors declare no conflicts of Interest

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