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N-acetyltransferase 2 (*NAT2*) and Cytochrome 2C9 (*CYP2C9*) Genes Polymorphisms in Type 2 Diabetes Mellitus Patients in Yaoundé, Cameroon

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ABSTRACT: *Although several environmental factors influence the onset of type 2 Diabetes Mellitus (T2DM), genetic factors contribute to an individual vulnerability to this disease. This study was aimed at studying CYP2C9*3 single nucleotide polymorphism (SNP) and NAT2 gene polymorphisms, and their correlation, if any, in the susceptibility to type 2 diabetes in Yaoundé, Cameroon. This was a case-control study involving 70 participants living in Yaoundé, Cameroon. DNA was extracted by Chelex 100 method. Polymorphisms of NAT2 gene and CYP2C9*3 SNP were assessed using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP). NAT2 gene characterization revealed the predominance of NAT2*5 alleles (35%) and slow metabolizing phenotype (72.9%). CYP2C9 gene characterization revealed the predominance of the wild-type allele (54%) and intermediate metabolizing phenotype (91%). Individuals with the "NAT2 slow metabolizer" phenotype were more likely to have T2DM while those with "intermediate metabolizer" phenotype were less likely to develop this disease (OR = 3.9740, P = 0.0009 and OR = 0.1406, P = 0.0044, respectively). CYP2C9*3 had no discernable predisposition to T2DM (OR= 0.1765, P= 0.1981). This study demonstrates that the NAT2 slow metabolizer phenotype could be associated with the development of T2DM in Yaoundé, Cameroon.*

KEYWORDS: Type 2 diabetes; susceptibility; *NAT2* gene; *CYP2C9* gene; PCR-RFLP; Cameroon.

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INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (WHO, 2017). It is a real public health problem around the world. In 2019, the International Diabetes Federation (IDF) estimated the world's diabetic population to be 463 million and 9.3% were adults between 20-97 years. The number is predicted to increase to 700 million by 2045 if these trends continue. The World Health Organization (WHO) predicts that diabetes will be the seventh leading cause of death in 2030 (WHO, 2017). This represents a significant public health concern in Sub-Saharan Africa which had an estimated diabetic population of 19.4 million in 2019 (Forouzanfar et al., 2016). In Cameroon, the prevalence of diabetes in adults in urban areas is currently estimated at 7.2% (Forouzanfar et al., 2016). Drugs commonly used in Cameroon to treat type 2 diabetes are oral antidiabetic drugs which include metformin and hypoglycemic sulfonamides. These drugs, once in the body, are absorbed, distributed, metabolized and finally excreted. The metabolism phase is subdivided into two main phases: phase one, the functionalization phase, where the main acting enzyme is Cytochrome P450 family 2 subfamily C member 9 (CYP2C9), and phase two, the conjugation phase, where the main acting enzyme is N-acetyltransferase type 2 (NAT2).

Family studies have revealed that first degree relatives of individuals with T2DM are about 3 times more likely to develop the disease than individuals without a positive family history of the disease (Florez, Hirschhorn, & Altshuler, 2003; Stumvoll, 2004). Thus, it is clear that T2DM has a strong genetic component. One approach that is used to identify disease susceptibility genes is based on the identification of candidate genes (Barroso et al., 2003; Stumvoll, 2004). Candidate genes are selected because they are thought to be involved in pancreatic ß cell function, insulin action/glucose metabolism, or other metabolic conditions that increase T2DM risk. To date, over 100 candidate genes for T2DM have been studied in various populations worldwide (Marullo, Gaulton, & Eicher; Zhao et al., 2017). Gene coding for the synthesis of drugs metabolizing enzymes such as NAT2, CYP2R1, CYP3A4, CYP2C9, Glutathione S-transferase has shown to be linked with the susceptibility to Type 2 diabetes mellitus (Al-Shaqha, Alkharfy, Al-Daghri, & Mohammed, 2015; Alexey, 2018; Nesa, Rahman, Kabir, & Rupam, 2014; Rabiee, Marjani, Khajeniazi, & Mojerloo, 2018; Semiz et al., 2011; Wang et al., 2018; Yalin et al., 2007).

N-acetyltransferase 2 is a gene that encodes an enzyme (EC 2.3.1.5) that both activates and deactivates arylamine and hydrazine drugs as well as carcinogens; it has two exons but the coding region, spanning 870 bp is located in exon 2. Polymorphisms in this gene are responsible for the N-acetylation polymorphism in which human populations segregate into rapid, intermediate, and slow acetylator phenotypes.[16] Seven major single nucleotide polymorphisms that occur isolated or combined have been described in the *NAT2* gene. These affect the positions 191, 282, 341, 481, 590, 803 and 857. In addition, rare SNPs affecting the positions 111, 190, 364, 411, 434, 499, 795, 845 and 859 have been described although their frequencies are unknown (EG-M., 2009). Critical gene variants leading to slow acetylation capacity contains mutations at positions 191, 341, 590 or 857. Since some genotypes can be due to the presence of different combinations of haplotypes leading to ambiguous phenotype prediction, haplotype reconstruction is often necessary to clarify ambiguous genotype data (Figueiredo Teixeira RL de; Pires Lopes MQ, 2013).

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CYP2C9 on the other hand is the most abundant CYP2C subfamily enzyme in the human liver and the most important contributor from this subfamily to drug metabolism. It metabolizes tolbutamide drugs. Polymorphisms resulting in decreased enzyme activity are common in the *CYP2C9* gene (Daly, Rettie, Fowler, & Miners, 2018). *CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile359Leu) are the two most widely studied genetic variants. Both variants are mainly present in Caucasians with allele frequencies of 10–15% (*2) and 4– 10% (*3). The *CYP2C9*2* variant appears absent in Asians and Africans. Interestingly, both *CYP2C9*2* (4–7%) and *CYP2C9*3* (4%) are present in Asian-Indians (Rathore, Agarwal, Pande, Mittal, & Mittal, 2010). Pharmacokinetics of glyburide depended significantly on *CYP2C9* genotypes. In homozygous carriers of genotype *3/*3, total oral clearance was less than half that of the wild-type genotype *1/*(Agarwal, 2015).

The question then is asked, to what extent these side effects and susceptibility to T2DM could have been avoided if pharmacogenomics had been taken into account? The *NAT2* and *CYP2C9* genes polymorphisms analysis will allow us to determine the group of individuals who are genetically prone to T2DM susceptibility, to determine how to adjust the drug dose and finally to come to personalized medicine where the choice of treatment is based on the genetic status of the patient. We, therefore, undertook to study the polymorphisms of *CYP2C9* and *NAT2* genes and their correlation if any in the susceptibility to type 2 diabetes in Yaoundé, Cameroon.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Cameroonian National Ethics Committee for Research in Human Health (NECRHH) under the ethical clearance document number 163/CNE/SE/09. The primary purpose of this data collection was to improve access to HbA1c measurement in Sub-Saharan Africa. All participants consented to additional pathophysiological investigations deemed important by the Principal Investigators.

Study population

This was a case-control study involving 70 participants living in Yaoundé; 35 T2DM patients and 35 non-diabetic healthy controls of Cameroonian origin; aged 40 years old and above. T2DM patients were recruited from the outpatient clinic of the National Obesity Center of the Yaoundé Central Hospital, and non-diabetic healthy controls from the general population. All were retained according to the inclusion criteria which included: age, 40 years and above, having a fasting plasma glucose level between 0.7 and 1.09 g/l for the control group and giving informed consent.

Sample collection and DNA extraction

Participants retained presented themselves early in the morning by appointment at the National Obesity Centre of the Yaoundé Central Hospital, after taking an overnight fast of at least 8 hours prior to the day of the appointment. Upon arrival, each of them was submitted to a study questionnaire containing the gender, weight, date of birth and feeding habits. Anthropometric parameters and fasting plasma glucose levels were gotten. Blood samples collected from participants were used for the deposition of spots on filter papers for genomic analysis. The

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dried blood spots on filter papers were excised with a sterile pair of surgical scissors. DNA was then extracted from the dried blood spots on filter papers by the Chelex 100 method.[21] The resultant DNA extracts were stored at -20 °C for PCR analysis.

Molecular Genotyping

The *NAT2*; G857A, C481GT, A803G and G590A gene polymorphisms and *CYP2C9*3* SNP were investigated. The primers used to amplify the *NAT2* gene were: F 5'-CCAATAAAAGTAGAAGCGA-3' and R 5'- CTCTTCCAGGACCTCCA-3' and those used to amplify *CYP2C9* were: F 5'- CTGAATTGCTACAACAAATGTGCCA-3' and R 5'-AGGCTGGTGGGGAGAAGGTCC-3'. Each PCR was carried out in a total volume of 25 µl and the reaction medium was composed of PCR water, buffer (10X thermopol buffer), 10mM dNTPs (200 µM of each deoxyribonucleotide), 0.8 µM of each primer, 1.25 U/µl of Taq DNA polymerase and 3ng of DNA. The amplification programs previously recorded in the thermocycler (T3 thermal cycler, Biometra, UK) proceeded as follows for the *NAT2* gene amplification: the pre-denaturation at 95°C for 5 minutes followed by 30 amplification cycles of which each cycle is a succession of denaturation at 95°C for 30 seconds, annealing at 55°C for 30s, elongation at 72°C for 30 seconds. The final extension which comes at the end of these cycles was carried out at 72°C for 10 minutes; for the *CYP2C9* gene amplification: the pre-denaturation at 94°C for 5 minutes followed by 30 amplification cycles of which each cycle is a succession of denaturation at 94°C for 30 seconds, annealing at 64.5°C for 30s, elongation at 72°C for 30 seconds. The final extension which comes at the end of these cycles was carried out at 72°C for 10 minutes. *NAT2* gene polymorphisms and *CYP2C9*3* SNP were determined by restriction fragment length polymorphism (RFLP) following amplification as described by Kengne et al (2016), for the *NAT2* gene polymorphisms using KpnI, BamHI and Taq I (New England Biolabs, USA) restriction enzymes and in Ref. [23] for the *CYP2C9*3* SNP using BstNI (New England, USA). The RFLP reaction conditions for KpnI and BamHI (New England Biolabs, USA) were set to digest at 37 ºC for 16 h while that of Taq I was digested at 65 ºC for 16 h and BstNI at 60°C for 16h. This was followed by inactivation at 80 ºC for 20 min. Digested and undigested fragments of each sample were separated on a 2% agarose gel stained with ethidium bromide.

Classification of acetylator genotypes

The *CYP2C9* acetylator phenotypes were distinguished as fast metabolizers (*CYP2C9*1/CYP2C9*1*), intermediate metabolizers (*CYP2C9*1/CYP2C9*3*) or slow metabolizers (*CYP2C9*3*). The *NAT2* acetylator phenotypes were established according to previously published data [22]. Homozygotes (*NAT2*4/NAT2*4*) were classified as fast acetylator phenotypes, heterozygotes (*NAT2*4/NAT2*5, NAT2*4/NAT2*6* and *NAT2*4/NAT2*7* combinations) as intermediate acetylator phenotypes, while homozygotes of the mutant alleles (*NAT2*5, NAT2*6* and *NAT2*7*) were classified as slow acetylator phenotypes (Table 1).

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Table 1. Interpretation of RFLP digestion of *NAT2* and *CYP2C9* genes

Genes	PCR products sizes (bp)	Restriction enzymes	Products size after digestion (bp)	Allele/Genotype
NAT2	535	KpnI	483 and 52	*NAT2*4*
			535	*NAT2*5*
		Taq α-1	205; 170 and 160	*NAT2*4*
			330 and 205	*NAT2*6*
		Bam HI	428 and 107	*NAT2*4*
			535	*NAT2*7*
CYP2C9	202	BstNI	127 and 75	*CYP2C9*1/1*
			105; 75 and 22	*CYP2C9*1/3*
			127; 105; 75 and 22	*CYP2C9*3/3*

RFLP: Restriction Fragment Length Polymorphisms; PCR: Polymerase Chain Reaction; *NAT2*: N-Acetyl Transferase 2; *CYP2C9*: Cytochrome 2C9; *Kpn I: Klebsiella pneumoniae* restriction enzyme 1; Taq α-1: *Thermus aquaticus alpha 1* restriction enzyme; Bam HI: *Bacillus amyloliquefaciens* restriction enzyme; BstNI (Mva I): *Micrococcus* variants RFL 19 restriction enzyme.

Data analysis

All data were entered on SPSS version 25.0 (SPSS Inc., USA) statistical software. The frequency of the *NAT2* and *CYP2C9* alleles and acetylators genotypes in the study population were performed by descriptive statistics. The association between *NAT2, CYP2C9* genes phenotypes and the susceptibility to type 2 diabetes mellitus in the study population was assessed by chi-square analysis. The odd ratios (ORs) and 95% confidence interval (CIs) were also calculated and a P-value < 0.05 was considered as statistically significant.

RESULTS

Characteristics of the Population

Of the 70 individuals who were enrolled in the study, 42 (60.0%) of them were females and 28 (40.0%) were males. Nineteen of the 42 women and 16 of the 28 men were Type 2 Diabetes patients. There was no significant difference (P = 0.1705) in mean age distribution between the T2D patients' group ([mean 56.00 ± (SD 6.25)]) and the normoglycemic healthy control group ([mean 53.00± (SD 5.34]). Fasting plasma glucose was significantly higher in the T2D patients' group than in the control group ([mean 1.630 ± (SD 0.7107) g/l vs. 0.92 ± (SD 0.091) g/l; p<0.0001]). The comparison of anthropometric measurements between the T2D patients' group and the normoglycemic healthy control group revealed that there was no significant difference in systolic blood pressure and body mass index between the T2D patients' group and the normoglycemic healthy control group (all p>0.05). Waist-to-hip ratio was significantly higher in the T2D patients' group than in the normoglycemic healthy control group ([mean 0.96 ± (SD 0.083) vs. 0.87 ± (SD 0.071); p=0.0003]) but diastolic blood pressure was significantly higher in the normoglycemic healthy control group than in T2D patients' group

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([mean 89.00 ± (SD 15.33) mmHg vs. 78.00 ± (SD 12.48) mmHg; p=0.0121]) respectively (Table 2).

Table 2. Characteristics of the study population

Characteristics	Type 2 diabetes patients n=35	Healthy controls n=35	p-value
Demographic			
Male/Female (ratio)	16/19 (0.84)	12/23 (0.52)	0.136
Age (Years)	56 (51-60)	53 (49-57)	0.1705
Clinical			
WHR	0.96 (0.88-0.98)	0.87 (0.83-0.92)	0.0003
SBP (mmHg)	128 (117-148)	141 (122-166)	0.0929
DBP (mmHg)	78 (71-87)	89 (76-118)	0.0121
BMI (kg/m^2)	29.00 (25.24-34.81)	28.31 (26.49-31.77)	0.6385
Biological			
FPG (g/L)	1.630 (1.27-2.19)	0.92 (0.84-1.00)	< 0.0001

FPG: Fasting Plasma Glucose, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, BMI: Body Mass Index, WHR: Waist to Hip Ratio.

NAT2 gene characterization results

The NAT2*4, NAT2*5, NAT2*6 and NAT2*7 alleles were found in our study population with NAT2*5 being predominant (35.0%). We determined a total of 9 genotypes in our study population with the predominance of the heterozygote NAT2*5/7 (30%). The fast, intermediate and slow metabolizers' phenotypes were found in our study population, with slow metabolizers being predominant (72.9%) (Table 3).

Table 3. *NAT2* and *CYP2C9* genes phenotypic, genotypic and allelic distribution

Genes	Phenotypes	Genotypes	Alleles
NAT2	Fast metabolizer	*NAT2*4/4* (2.9%)	*NAT2*4* (15.0%)
	Intermediate metabolizer	*NAT2*4/5* (10.0%)	*NAT2*5* (35.0%)
		*NAT2*4/6* (5.7%)	*NAT2*6* (25.0%)
		*NAT2*4/7* (8.6%)	*NAT2*7* (25.0%)
	Slow metabolizer	*NAT2*5/5* (1.4%)	
		*NAT2*5/6* (27.1%)	
		*NAT2*5/7* (30.0%)	
		*NAT2*6/6* (2.9%)	
		*NAT2*6/7* (11.4%)	
CYP2C9	Fast metabolizer	*CYP2C9*1/1* (9.0%)	*CYP2C9*1* (54.0%)
	Intermediate metabolizer	*CYP2C9*1/3* (91.0%)	*CYP2C9*3* (46.0%)

NAT2: N-Acetyl Transferase 2, *CYP2C9:* Cytochrome 2C9.

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The fast metabolizer phenotype was present among the non-diabetic participants and absent among the diabetic participants. On the other hand, slow metabolizer phenotype frequency was significantly higher (91.4%) while the intermediate phenotype frequency was significantly lower (8.6%) in the diabetic group as compared to the non-diabetic group (p=0.0009 and p=0.0044 respectively) (Table 4).

Table 4. Association between *NAT2* phenotypes and Type 2 diabetes mellitus

Phenotypes	Type 2 diabetes participants	Non-diabetes participants	Odds Ratio	95% CI	P
Fast	0 (0%)	2 (5.7%)	1.8870	0.0087-4.080	0.4928
Intermediate	3 (8.6%)	14 (40.0%)	0.1406	0.0360-0.5497	0.0044
Slow	32 (91.4%)	19 (54.3%)	3.9740	1.3770-11.470	0.0009
Total	35 (100%)	35 (100%)	/	/	/

NAT2: N-Acetyl Transferase 2; CI: Confidence interval; P: P-value.

CYP2C9 Characterization Results

We identified 2 alleles in our study population with the predominance of the wild type *CYP2C9*1* allele (54%). In our study population, we obtained the fast metabolizer and intermediate metabolizer phenotypes, with a predominance of intermediate metabolizer phenotype (91%) (Table 3). No significant difference of both phenotypes frequencies was observed between the two groups (Table 5).

Table 5. Association between *CYP2C9* genotypes (phenotypes) and susceptibility to T2DM

Genotype (Phenotypes)	Type 2 diabetes participants	Non-diabetes participants	Odds Ratio	95% CI	P
*CYP2C9*1/1* (Fast)	5 (14%)	1 (3%)	1.778	1.142-2.7680	0.1981
*CYP2C9*1/3* (Intermediate)	30 (86%)	34 (97.0%)	0.1765	0.0195-1.597	0.1981
*CYP2C9*3/3* (Slow)	0 (0%)	0 (0%)	/	/	/
Total	35 (100%)	35 (100%)	/	/	/

CYP2C9: Cytochrome 2C9 ; *NAT2* : N-Acetyl Transferase 2 ; T2DM : type 2 diabetes mellitus ; CI : Confidence interval ; P : P-value.

Correlation results between NAT2, CYP2C9*3 and T2DM

For the correlation between NAT2 phenotype and T2DM, individuals with the "slow metabolizer" phenotype are more likely to develop T2DM, while those with "intermediate metabolizer" phenotype are less likely to develop this disease (OR = 3.9740, P = 0.0009 and OR = 0.1406, P = 0.0044, respectively) (Table 4), contrary to the CYP2C9, unrelated to susceptibility to T2DM (Table 5).

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DISCUSSION

Data and information gotten on the distribution of genetic polymorphism in the population is essential to understand inter-individual differences in drug disposition and disease risk. The profiles of the *NAT2* and *CYP2C9* genotypes/phenotypes were assessed and their association with the onset of type 2 diabetes mellitus in Yaoundé, Cameroon.

*NAT2*4, NAT2*5, NAT2*6* and *NAT2*7* alleles were found in the study population with NAT2*5 being predominant (35%), these findings are similar to those obtained in Egypt in 2003 and Jordan in 2010, whereby the *NAT2*5* was also predominant, notably 50% among healthy Egyptians (Hamdy et al., 2003) and 37.3% among unrelated Jordanian volunteers (Jarrar, Ismail, & Irshaid, 2010). The most dominant genotype was *NAT2*5/7* (30.0%), similar to results obtained in Bangolan, North West region of Cameroon (18%) (Achonduh et al., 2013), but differed from the results obtained in Jordanian volunteers whose main genotype was shown to be *NAT2*5/6* (29.3%) and in the North and South-West regions of Cameroon whose main genotype was *NAT2*4/6* (19%) (Kengne et al., 2016). These findings can be explained by the fact that, in as much as individuals are found in a given location, they could be of different ethnic origins (Sabbagh, Darlu, Crouau-Roy, & Poloni, 2011). With respect to *NAT2* gene phenotypes, slow metabolizers were dominant (72.9%). These results were similar to those found previously in North and South-West regions of Cameroon, Bangolan and in Egypt (Achonduh et al., 2013; Hamdy et al., 2003; Kengne et al., 2016). This is in accordance with findings showing that Africans are predominantly slow metabolizers (Sabbagh et al., 2011). Our data demonstrated that fast metabolizers were present among the non-diabetic group and absent in the diabetic group. It also came out that the slow metabolizer phenotype frequency was significantly higher (P-value = 0.0009), whereas the intermediate metabolizer phenotype frequency was significantly lower (P-value = 0.0044) in diabetic patients as compared to non-diabetic controls. Strikingly, our results indicated that subjects with slow metabolizer phenotype have a higher risk for developing T2DM, whereas individuals with intermediate phenotype have a lower chance to develop this disease. This is in accordance with a study done in Turkey where the slow acetylator phenotype was found to be an important genetic determinant for DM (Yalin et al., 2007). Furthermore, another study performed in a Canadian Oji-Cree population that has a high prevalence of T2DM did not find this association (Hegele et al., 2000). Interestingly, Hegele et al. indicated that *NAT2* polymorphism affected plasma glucose levels in non-diabetic subjects (Hegele et al., 2000). The similarity and difference in the acetylator status of *NAT2* suggest that *NAT2* genetic polymorphisms could be variable in the same race but of different geographic areas. It is possible that dietary, environmental factors and/or genetic polymorphisms in xenobiotic-metabolizing enzymes may contribute to the development of the disease. NAT2 is an important xenobiotic-metabolizing enzyme and the nonacetylated xenobiotics may induce the DM (Yalin et al., 2007). Previous studies (El Desoky et al., 2005; FILIADIS et al., 1999; Leff, Fretland, Doll, & Hein, 1999; Okkels, Sigsgaard, Wolf, & Autrup, 1997) suggest that *NAT2* slow acetylator phenotype is not homogeneous and that the mechanism for this phenotype probably differs among the *NAT2* alleles, which is defined by various SNPs and haplotypes. Therefore, different polymorphisms may result in multiple slow acetylation phenotypes and consequent variations that modulate disease risk (Hein, 2006). Furthermore, the NAT2 genotype/phenotype relationship is not fully understood and, as described recently, one of the important factors that may contribute to a lack of agreement between genotype and phenotype is the inter-and intra-ethnic variability in both SNP frequency and the linkage among various SNPs (Garcia-Martin, 2008).

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The *CYP2C9* gene has several allelic forms known to be involved in drug metabolism. *CYP2C9*1* being the wild type and *CYP2C9*3* the mutated form. In this study, the wild type and the mutated alleles were found with wild type *CYP2C9*1* predominating (54%). From our results, we were able to distinguish the different *CYP2C9* genotypes (phenotypes) present in Yaoundé, Cameroon. The most dominant genotype was the heterozygote *CYP2C9*1/3* (91%). Data presented herein demonstrated that there were no significant differences in both genotypes (phenotype) frequencies among the diabetic and the nondiabetic control groups. This result is in accordance with a study done in Bosnia and Herzegovina which indicated no relationship between *CYP2C9* genotype and diabetes susceptibility in the Bosnian population (Semiz et al., 2010).

CONCLUSIONS

This study demonstrates that the *NAT2*5* allele, *NAT2*5/7* genotype and slow metabolizer phenotype were predominant in the study population. Individuals with the "slow metabolizer" phenotype are more likely to develop T2DM, while those with the "intermediate metabolizer" phenotype are less likely to develop this disease. With regard to the *CYP2C9* gene, *CYP2C9*1* (wild type) allele and *CYP2C9*1/3* genotype (intermediate metabolizer phenotype) were predominating in the study population with no relationship with the susceptibility to T2DM.

Author Contributions

Conceptualization, W.F.M., J.C.M., A.M.N., J.P.K.C. and H.C.D.; methodology, W.F.M., A.M.N., E.M.A., M.G.F., J.P.K.C., and H.C.D.; software, A.M.N., C.M.M., and H.C.D.; validation, W.F.M., J.C.M., E.S., M.G.F., and J.P.K.C.; formal analysis, H.C.D., N.L.N., and C.T.F.; investigation, M.G.F., and E.S.; resources, W.F.M., J.C.M., and E.S.; data curation, A.M.N., J.P.K.C., E.M.A. and H.C.D.; writing—original draft preparation, A.M.N., J.P.K.C., H.C.D., C.M.M., M.G.F., and C.P.B.; All authors contributed in the revision of the manuscript and approved the final version of the manuscript prior to submission; visualization, W.F.M.; supervision, W.F.M.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Cameroonian National Ethics Committee for Research in Human Health (NECRHH) under the ethical clearance document number 163/CNE/SE/09 on the 22 December 2009.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. The primary purpose of this data collection was to improve access to HbA1c measurement in Sub-Saharan Africa. All participants consented to additional pathophysiological investigations deemed important by the Principal Investigators.

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