Article

Association between Transcription Factor 7-like-2 Polymorphisms and Type 2 Diabetes Mellitus in a Ghanaian Population

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Abstract: Type 2 diabetes mellitus (T2DM) has been strongly associated with single nucleotide polymorphisms (SNPs) in the TCF7L2 gene. This study investigated the association between rs12255372, rs7903146 in the TCF7L2 gene and T2DM in a Ghanaian population. A case-control study design was used for this study. A total of 106 T2DM patients and 110 control participants were selected. Basic data collected included body mass index, blood pressure and socio-demographics. Fasting blood samples were collected and processed for: serum lipid analysis, plasma glucose estimation and plasma HbA1c estimation. Parts of the whole blood samples were used for DNA extraction using a modified salting-out method. Common and allele-specific primers were designed for genotyping using the Modified Tetra-Primer Amplification assay. Associations were evaluated using logistic regression models. The rs7903146 risk variant was significantly associated with 2.16 vs. 4.06 increased odds for T2DM in patients <60 years vs. ≥60 years. Both rs7903146 and rs12255372 were significantly associated with increased odds of T2DM in women, overweight/obese, T2DM negative family history (T2DM-NFH) and low-HDL-C. In a multivariate model, rs7903146 but not rs12255372 was significantly associated with 2.18, 5.01 and 2.25 increased odds of T2DM, under the codominant, recessive and additive model, respectively ($p < 0.05$). The association between rs7903146 and rs12255372 with T2DM is more highly associated in a subgroup—women and those with T2DM-NFH, yet who have cardiometabolic risk.

Keywords: TCF7L2; type 2 diabetes mellitus; cardiometabolic risk factors; single nucleotide polymorphisms

1. Background

Diabetes continues to rise in prevalence across the globe. Alarmingly, four out of five people with diabetes live in low-middle-income countries (LMIC), where adequate resources for diabetes management are scarce [1]. According to the International Diabetes Federation (IDF) 2017, it is estimated that nearly half a billion of the world's population is living with diabetes [1]. Khan et al. [2] reported that 462 million individuals are affected by diabetes, corresponding to 6.28% of the world's population. This figure is projected to reach 7079 individuals per 100,000 by 2030, reflecting a continued rise across all regions of the world [2]. Due to the continued increase in diabetes worldwide, the 2030 projected rate is likely to be exceeded [2,3]. This leads to high mortality from diabetic complications including cardiovascular disease [4]. The alarming situation is that 174.8 million (45.8%) of all diabetic cases, in adults, are undiagnosed with the most affected individuals from LMIC [5]. Thus, T2DM remains a threat to the world's population, even though there is increasing life expectancy, which requires both public health and clinical care to prevent what is already a worldwide health crisis.

In Ghana, T2DM is present among 6.46% [6] of the population, which is significantly greater than the 2017 International Diabetes Federation (IDF) estimation [1]. A number of factors including physical inactivity, poor diet, rapid urbanisation and ageing contribute to the high incidence of diabetes in Ghana [7]. Besides these factors, T2DM has a strong genetic predisposition, taking the combination of inequalities across different populations, familial aggregation, and concordance among monozygotic twins [8,9]. Specifically, in T2DM, these factors are closely related to the metabolic abnormalities that underline the conditions of impaired insulin action, obesity, increased endogenous glucose output, and insulin secretory dysfunction [10]. It is well known that genes control and regulate glucose and metabolic homeostasis, which influence the digestion, absorption, and metabolic processes of lipids and carbohydrates can initiate the major mechanisms in T2DM [11].

Among these genetic risk variants, the transcription factor 7-like 2 (TCF7L2), represent the most robust and promising T2DM candidate gene to date [12]. Variants in this gene have been identified with the strongest association with T2DM [12–17]. Since a common microsatellite in the TCF7L2 gene region, DG10S478, (containing SNPs rs7903146 and rs12255372) was found to be associated with T2DM [18], the finding has been convincingly replicated in different population groups. Using non-coding SNPs in strong linkage disequilibrium with DG10S478 [14,19,20], an estimated population attributable risk of 17–28% has been established.

TCF7L2 encodes a high mobility group of box-containing transcription factors, which is involved in the Wnt signalling pathway [15]. The Wnt signalling pathway represents one of the pancreatic beta cell's important developmental and regulatory mechanisms, which is critical in determining its fate, proliferation, polarity, and apoptosis during embryonic development and adult tissue homeostasis [18]. Risk alleles of TCF7L2 have been strongly associated with impaired insulin secretion/beta-cell function and T2DM [21–23]. Likewise, TCF7L2 has been shown to mediate regulation of glucagon-like peptide-1 expression, which represents a mechanism by which alteration of this gene influences susceptibility to T2DM [22]. There has been only a limited number of studies within the African Context, which makes it difficult to pool the effects and homogeneity of variants associated with T2DM [24]. Thus, replication studies are needed to understand the effect of these gene variants on T2DM. This study investigated the association between TCF7L2 gene polymorphisms and T2DM in a Ghanaian population.

2. Results

2.1. Characteristics of the Study Participants

Table 1 shows the socio-demographic characteristics of the study participants. The proportion of participants aged 60 and above was significantly higher in the cases compared to the controls (31.1% vs. 17.3%, $p = 0.017$). Most of the T2DM participants had a T2DM positive family history (T2DM-PFH) than the control group (42.5% vs. 11.8%, $p < 0.001$).

Aside from LDL-C (p = 0.288), the mean TC, TG, and HDL-C significantly differed between the cases and control participants (p < 0.05). The proportion of cases vs. controls with high TC (12.3% vs. 5.6%, p = 0.077), overweight (40.6% vs. 35.5%, p = 0.441) and obese (27.4% vs. 19.1%, p = 0.149) did not differ significantly. High BP, atherogenic dyslipidaemia and metabolic syndrome (MetS) were higher in the cases than the controls (p-value < 0.001).

Table 1. Comparison of demographic variables, anthropometric measures and biochemical profiles between the cases and controls.

Variables	Cases (N = 106)	Controls (N = 110)	p-Value
Sex			
Male	46 (43.4)	37 (33.6)	0.140
Female	60 (56.6)	73 (66.4)	
Family history of T2DM			
No	61 (57.7)	97 (88.2)	
Yes	45 (42.5)	13 (11.8)	<0.001
Age (years)	53.90 ± 12.31	45.80 ± 12.14	<0.001
<60 years	73 (68.9)	91 (82.7)	
≥60 years	33 (31.1)	19 (17.3)	0.017
Anthropometric variables [#]			
BMI (kg/m^2)	28.18 ± 5.53	26.10 ± 4.41	0.002
Systolic BP (mmHg)	133.35 ± 19.76	121.25 ± 18.41	<0.001
Diastolic BP (mmHg)	83.68 ± 11.74	76.22 ± 11.17	<0.001
Biochemical parameters [#]			
Triglycerides (mmol/L)	1.69 ± 0.64	1.29 ± 0.50	<0.001
T. Chol (mmol/L)	5.25 ± 1.03	4.65 ± 1.06	<0.001
HDL-C(mmol/L)	0.99 ± 0.24	1.23 ± 0.27	<0.001
LDL-C (mmol/L)	3.44 ± 0.95	3.32 ± 0.72	0.288
FPG (mmol/L)	8.72 ± 4.66	4.85 ± 0.91	<0.001
HbA1c (%)	6.99 ± 1.87	4.83 ± 0.60	<0.001
Cardiometabolic factors			
High BP	36 (34.0)	10 (9.1)	<0.001
High TC	13 (12.3)	6 (5.5)	0.077
High TG	61 (57.5)	16 (14.5%)	<0.001
Low HDL-C	81 (76.4)	52 (47.3)	<0.001
High LDL-C	64 (60.4)	36 (32.7)	<0.001
Atherogenic dyslipidaemia	44 (41.6)	5 (4.5)	<0.001
Overweight	43 (40.6)	39 (35.5)	0.441
Obesity	29 (27.4)	21 (19.1)	0.149
MetS	70 (66.0)	14 (12.7)	<0.001

All values are presented as frequency and percentages unless stated. [#] Values are presented as the mean and standard deviations. p-values highlighted are statistically significant (<0.05).

Genotype and Allele Frequencies of TCF7L2 Gene Polymorphism

The genotype and minor allele frequencies (MAF) of rs7903146 and rs12255372 of the TCF7L2 gene are shown in Table 2. The MAF of rs7903146 was 50.5% and 33.3% among cases and control participants, with statistically significant differences (p = 0.011). The homozygote recessive allele (TT) of rs7903146, was more prevalent among cases than control participants (13.2% and 4.5%, p = 0.024). The MAF of rs12255372 was 24.1% among cases, which is significantly higher (p = 0.013) than that of controls (14.2%). The GG genotype (p = 0.013) and GT genotype (p = 0.023) but not the TT genotype (p = 0.501) of rs12255372 was disproportionately distributed between the cases vs. control participants.

Table 2. Distribution of *TCF7L2* gene polymorphism in cases and control participants.

SNP	Controls (N = 110)			Cases (N = 106)			*p*-Value
	%	Allele Frequency		N (%)	Allele Frequency		
		C	T		C	T	
rs7903146		0.667	0.333		0.495	0.505	0.011
CC	49 (44.5)			26 (24.5)			0.002
CT	56 (50.9)			66 (62.3)			0.092
TT	5 (4.5)			14 (13.2)			0.024
rs12255372		G	T		G	T	
		0.857	0.143		0.759	0.241	0.068
GG	81 (73.6)			61 (57.5)			0.013
GT	24 (21.8)			38 (35.8)			0.023
TT	5 (4.5)			7 (6.6)			0.501

Highlighted *p*-values are statistically significant.

2.2. Linkage Disequilibrium between rs7903146 and rs12255372

The linkage disequilibrium (LD) and pairwise LD coefficients r^2 and D′ between rs12255372 and rs7903146 demonstrated that the alleles for the rs7903146 (C > T) and rs12255372 (G > T) SNPs are weakly associated (D′ = 0.021; r^2 = 0.0001).

2.3. Association between TCF7L2 Polymorphisms and T2DM

There is an association between rs12255372 and rs7903146 with T2DM (Table 3). Here rs7903146 was significantly associated with increased risk of T2DM in the codominant model (OR = 2.22, *p* = 0.008), recessive model (OR = 5.28, *p* = 0.004) and additive model (OR = 2.47, *p* = 0.002). After adjusting the model for age, sex, family history, high cholesterol and overweight/obesity, rs7903146 was still associated with T2DM; additive (OR = 2.25, *p* = 0.003), recessive (OR = 4.92, *p* = 0.011) and codominant (OR = 2.29, *p* = 0.019). rs12255372 was significantly associated with T2DM under the codominant (OR = 2.10, *p* = 0.017) and additive model (OR = 1.70, *p* = 0.026) but not the recessive model (OR = 1.86, *p* = 0.309). After adjusting for confounders, no association was found between rs12255372 and T2DM (*p* > 0.05). In a multivariate model, rs7903146 but not rs12255372 was significantly associated with 2.18, 5.01 and 2.25 increased odds of T2DM under the codominant, recessive and additive model, respectively (*p* < 0.05).

Table 3. Association between rs12255372 and rs7903146 and T2DM.

SNPs	Crude Model		Adjusted Model		Multivariate Model	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
rs7903146						
Codominant	2.22 (1.23–4.02)	0.008	2.29 (1.14–4.58)	0.019	2.18 (1.08–4.39)	0.030
Recessive	5.28 (1.71–16.27)	0.004	4.92 (1.45–16.72)	0.011	5.01 (1.44–17.44)	0.011
Additive	2.47 (1.38–4.42)	0.002	2.25 (1.32–3.83)	0.003	2.25 (1.31–3.84)	0.003
rs12255372						
Codominant	2.10 (1.14–3.87)	0.017	1.80 (0.92–3.52)	0.085	1.72 (0.86–3.43)	0.124
Recessive	1.86 (0.56–6.14)	0.309	1.13 (0.29–4.43)	0.859	1.26 (0.32–5.044)	0.742
Additive	1.70 (1.06–2.71)	0.026	1.40 (0.83–2.36)	0.206	1.39 (0.82–2.38)	0.222

OR—Odds ratio; CI—confidence interval; highlighted *p*-values are statistically significant.

Table 4 demonstrates an association between rs12255372 and rs7903146 polymorphisms and T2DM within subcategories. The rs7903146 polymorphism (C > T) was significantly associated with 2.16 vs. 4.06 increased odds ratio of T2DM in persons aged below 60 years vs. persons aged above 60-years. The rs7903146 (C > T) was significantly associated with increased odds ratio of T2DM in both normal weight (OR = 2.52, 95% CI: 1.02–6.26) and overweight/obese (OR = 2.15, 95% CI: 1.01–4.56) individuals; normal BP

(OR = 2.93, 95% CI: 1.51–5.66); T2DM negative family history (T2DM-NFH) (OR = 2.71,95% CI: 1.35–5.45); females (2.71, 95% CI: 1.30–5.65); elevated TG (OR = 3.87, 95% CI: 1.2112.41) and low HDL-C (OR = 2.95, 95% CI: 1.40–6.24). On the other hand, rs12255372 (G > T) was significantly associated with excess risk of T2DM among patients aged 60 and above (OR = 52.45, 95% CI: 2.92–942.04); increased odds of T2DM among patients with low HDL-C (OR = 2.35, 95% CI: 1.10–5.00), overweight/obese (OR = 2.43, 95% CI: 1.19–4.98), T2DM-NFH (OR = 2.07, 95% CI: 1.04–4.12) and females (OR = 2.34, 95% CI: 1.11–4.95).

Table 4. Association between rs12255372 and rs7903146 polymorphisms and T2DM whiles keeping other metabolic risk factors as fixed.

Risk Factors	rs7903146	rs12255372
	OR (95% CI)	OR (95%CI)
Age		
<60 years	2.16 (1.11–4.22) *	1.18 (0.62–2.26)
60 and above	4.06 (1.25–13.19) **	52.45 (2.92–942.04) **
Family history		
NFH	2.71 (1.35–5.45) **	2.07 (1.04–4.12) *
PFH	1.00 (0.25–3.98)	1.01 (0.31–3.36)
Sex		
Male	1.90 (0.75–4.84)	1.53 (0.64–3.68)
Female	2.71 (1.30–5.65) **	2.34 (1.11–4.95) **
BMI Status		
Normal weight	2.52 (1.02–6.26) *	1.28 (0.47–3.45)
Overweight/obese	2.15 (1.01–4.56) *	2.43 (1.19–4.98) *
BP status		
Normal	2.93 (1.51–5.66) **	1.55 (0.81–2.95)
Hypertensive	1.35 (0.32–5.66)	2.81 (0.67–11.80)
Dyslipidaemia		
Normal TG	1.45 (0.70–2.99)	1.64 (0.76–3.55)
Elevated TG	3.87 (1.21–12.41) **	1.23 (0.42–3.61)
Normal HDL-C	1.57 (0.60–4.13)	1.48 (0.56–3.95)
Low HDL-C	2.95 (1.40–6.24) **	2.35 (1.10–5.00) *

OR—odds ratios; *p*-values are statistically significant. * *p*-value < 0.05; ** *p*-values < 0.01.

Individuals with the rs7903146 polymorphism comparatively had higher mean TG ($p = 0.048$) and high mean LDL-C in both the controls ($p = 0.001$) and cases ($p = 0.046$). Among the cases, rs7903146 SNP was associated with significantly higher mean systolic BP ($p = 0.015$) (Table S2). In Table S2, those with rs12255372 SNPs, among the control group, had higher mean TG ($p = 0.003$), TC ($p = 0.048$), LDL-C ($p = 0.001$), FPG ($p = 0.005$) and HbA1c ($p < 0.001$). Among the cases, rs12255372 polymorphism was associated with higher BMI ($p = 0.044$) and systolic BP ($p = 0.003$).

In Table S3, rs7903146 was significantly associated with increased odds of high LDL among the controls (OR = 2.98, $p = 0.017$). Among the case group, individuals with the minor allele of rs7903146 were significantly associated with an increased odds ratio of high TG (OR = 2.95, $p = 0.023$) (Table S3). rs12255372 SNP was significantly associated with an increased odds ratio of high cholesterol (OR = 14.0, $p = 0.023$) and elevated LDC-C (OR = 3.12, $p = 0.027$) among the control group (Table S3). Among the case group, rs12255372 was significantly associated with increased odds of high BP (OR = 3.01, $p = 0.013$) and overweight/obesity (OR = 3.33, $p = 0.012$).

3. Discussion

Variants of the TCFL2 gene have been consistently associated with T2DM in several studies among multiple ethnic groups [14,19,20]. This study demonstrated the transferability of the TCF7L2 gene polymorphisms (rs12255372 and rs7903146) and their association with T2DM among a Ghanaian population. However, the associations were observed to be relevant only in a subgroup- women, patients that do not have a family history of

diabetes and those with dyslipidaemia with or without an elevated blood pressure and increased BMI.

This study observed that rs7903146 and rs12255372 polymorphisms have a significant effect on T2DM, however, the effect was mediated by other diabetes risk factors. In a systematic review and meta-analysis among a Sub-Saharan Africa (SSA) population [24], the effect of rs7903146 was 1.75 in a recessive model and 2.27 in an additive model, which is similar to our present findings. These effects are also consistent among Caucasian, East Asian, South Asian and other ethnicities populations [19]. To the best of our knowledge, this study is currently among the few studies in SSA that have demonstrated the transferability of rs12255372 [14,25]. In contrast, studies by Yako et al. [26] and Mandour et al. [27] found no significant association between rs12255372 and T2DM in a South African mixed-ancestry and Egyptian population, respectively.

A meta-analysis by Wang et al. [20] showed that among different ethnic groups, there are significant differences in the LD structure between rs12255372 and rs7903146. From the findings of this study, weak interaction between rs12255372 and rs7903146 for T2DM was reported (in both male and female participants). This result is similar to that observed among the Yoruba population (D′ = 0.075, r^2 = 0.001) but unlike that of the Europeans (D′ = 0.948, r^2 = 0.746) and Chinese (D′ = 0.479, r^2 = 0.114) [20].

The MAF of rs7903146 observed in this study is similar to the findings of Danquah et al. [13] among a Ghanaian population, where the MAF of rs7903146 was 30% in the control population and 36% in the T2DM population. Among other SSA populations, the MAF of rs7903146 among T2DM vs. controls has been reported at 9.5% vs. 0% in the Cameroonian population [28] and 32.2% vs. 24.3% in South African mixed-ancestry [26]. Furthermore, from the Allele Frequency Aggregator (ALFA) HapMap and 1000 Genomes project [29], the MAF of rs7903146 were 29.2%, 24.1% and 22.8%, respectively, which is consistent with our findings. The race-specific risk allele frequency of rs7903146 is lower among the Asian population (range: 3.1–4.7%) and higher among Europeans (29.2%), Africans (29.5%), African Americans (29.7%), Latin Americans (34.2%) and South Asians (31.8%) [29]. A wide population difference in the minor allele frequency of rs12255372 (Asians = 0.000 to 0.029, Europeans = 0.217–0.50 and SSA = 0.267–0.274) has been reported [15,27,30]. Moreover, from the results of the 1000 genomes project, the MAF of rs12255372 is 21.4% globally, 30.2% in Africa, 29.2% among Europeans and the Americans 21.9%, similar to our study.

From the findings of this study and related literature, the association between rs12255372 and rs7903146 with T2DM are similar among West African populations, Asians, Caucasians [31,32] and other population groups [14]. This affirms that the association of rs7903146 and rs12255372 with T2DM is transferable in multiple ethnicities which are likely to project a vital contribution to understanding common pathways and genetic involvement in T2DM.

It appears that rs12255372 and rs7903146 are not interdependently associated with T2DM among West African populations (Ghanaians and Yoruba), unlike other population groups. This finding is essential in understanding the different types and aetiology of T2DM present in SSA [33], and the wider population differences in T2DM prevalence. For example, as indicated in this study, the association between rs12255372 with T2DM was relevant among women, obese/overweight individuals, individuals with negative family history, low HDL-C and individuals aged above 60 years. Similarly, rs7903146 was relevant among females, individuals with negative family history, elevated TG and low HDL-C but normal blood pressure. This indicated that TCF7L2 risk variants may be relevant to a subgroup of individuals with single or multiple diabetes risk factors, other than those with a positive family history [34]. It is therefore recommended that an age- and sex-matched case-control study that seeks to evaluate the association between rs7903146, rs12255372 and diabetes with or without family history will be necessary understand these observations

This study observed that the risk allele rs7903146 was associated with elevated FPG and HbA1c in both the diabetic and controls. In addition to FPG and HbA1c, the risk alleles

of rs12255372 were associated with elevated BMI, blood pressure, TG and reduced LDL in T2DM and controls. This finding reflects the pleiotropic role TCF7L2 polymorphisms play in T2DM (adipogenesis, incretin and insulin expression as well as beta-cell growth and development). The rs7903146 risk alleles are associated with reduced proportional control of β-cell function among newly diagnosed T2DM patients [35]. Loos et al. [36] reported that the T-allele of rs7903146 was associated with higher HbA1c concentrations and reduced β-cell function, assessed by the homeostasis model assessment of β-cell function. TCF7L2 protein was also found to be increased during adipogenesis in 3T3-L1 cells and primary adipocyte stem cells [37]. Shu et al. found that high expression of TCF7L2 mRNA was identified with reduced TCF7L2 protein levels in individuals with the T-allele of rs7903146, which is associated with a downregulation of GIP- and GLP-1 receptors and impaired beta-cell function [38].

These findings suggest that TCF7L2 polymorphisms rs7903146 and rs12255372 may confer T2DM susceptibility through the expression of adipocyte-insulin resistance, delayed insulin secretion (resulting from beta-cell dysfunction) and reduced intracellular energy homeostasis. Therefore, individuals with the risk alleles of TCF7L2 (rs7903146 and rs12255372) may present with obesity, elevated TG and reduced HDL-C. Moreover, the findings suggest that among individuals with multiple diabetes risk factors, those who do develop T2DM may have a genetic predisposition to beta-cell dysfunction which includes the SNPs rs12255372 and rs7903146 [16].

Some limitations of the study are worth mentioning when interpreting the study's findings. The differences in population substructures may result in a different interpretation of risk alleles in candidate gene association studies. In addition, the number of genetic roots underlying T2DM is still being investigated and could be numerous given its multifaceted nature. Therefore, the effect of the variants among the small population considered in this study may be unpredictably low, when we consider it from a larger population. Besides this limitation, the findings of this study are consistent with the recommended approach for conducting a candidate gene association study and present consistent findings with similar studies among other population groups.

4. Materials and Methods

This case-control study was conducted at the Begoro District Hospital (BDH) and two communities (Bosuso and Begoro) in the Eastern Region of Ghana. The study included participants aged 30–79 years. Cases were defined as clinically diagnosed T2DM patients attending the diabetic clinic at the BDH. Controls were defined as non-diabetic patients with normal fasting plasma glucose (FPG) and/or normal HbA1C that voluntarily participated in a health screening program conducted as part of the study in Bosuso or Begoro. Participants willing to have their body mass index (BMI), FPG, HbA1c and lipid profile checked and provided written informed consent during the health screening were recruited as controls. Control participants with a self-reported, previous or current, history of renal, liver, thyroid disorders or other endocrine diseases, chronic diseases, on hormone replacement therapy or corticosteroid therapy were excluded from the study. T2DM patients who self-managed or being managed for micro-vascular or macrovascular complications were excluded from the study.

4.1. Sample Size Calculation

Taking the minor allele frequency (MAF) of the lead SNP rs7903146 from a previous study [15], at a patient-to-control ratio of 1:1, at 95% CI, and 6% proportional differences ($p * = (0.36 + 0.30)/2 = 0.33$; (P1-P2) = (0.36–0.30) = 0.06, the minimum recommended sample size at 80% power replication under the Kelsey method [39] was 100 cases and 100 controls. Overall, 106 T2DM and 110 non-diabetic controls met the inclusion criteria.

4.2. Recruitment Process

The control participants were recruited during a health screening program conducted in the two communities. Additional controls were sorted through community members that were informed via the various religious organizations. Initially, the intent, preparation and benefits of the health screening program were announced in all the religious-based organisations. The health screening process took place every morning (Monday, Wednesday and Friday) from 8:00 am to 11:30 am from May 2019 to November 2019. T2DM patients were recruited using a total sampling method. All patients that visited the BDH hospital during the study period and met the inclusion criteria were considered as a sample.

Blood pressure measurement was taken using an automated sphygmomanometer (Omron BP7200, Omron Healthcare, Inc., Chicago, IL, USA). Three different measurements were taken at 5 min intervals and the average was used as the final reading. All blood pressure recordings were carried out in mmHg.

The Omron B511 (Omron Healthcare, Inc., Chicago, IL, USA) was used for weight measurement, following the manufacturer's instructions. Briefly, participants were asked to stand upright on the main unit of the scale and without shoes. Portable Height Rod Stadiometers (QuickMedical, Warwick, UK) were used for body height; the subject stood upright, with feet together and flat on the ground. BMI was calculated as the ratio of weight to height-squared (kg/m^2).

Six millilitres (mL) of venous blood were taken in a fasting state and two millilitres each was placed into sterile ethylene diamine tetraacetic acid (EDTA) (BD Vacutainer®, Franklin Lakes, NJ, USA), gel separator and sodium fluoride/calcium oxalate tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). Fasting was defined as no caloric intake for at least 8 h, overnight. The EDTA samples were used for HbA1c analysis using the standard A1cCare System (SD BIOSENSOR, Kyonggi-do, Korea). The remaining EDTA sample was used for DNA extraction and consequent SNPs genotyping. The gel separator tubes samples were processed by centrifugation at 3000 rpm for 10 min and the serum was collected into 1.5 mL Eppendorf tubes. The obtained sera were used for lipid profiling. The plasma obtained from the sodium fluoride/calcium oxalate tube samples was used for the determination of fasting plasma glucose (FPG).

4.3. Biochemical Measurements and Definition of Cardiometabolic Risk Factors

The Lipid and FPG analysis were conducted at the Eastern Regional Hospital's Clinical Chemistry laboratory, using the SELECTRAPRO M chemistry analyser (EliTech Group B.V, Netherlands). All analysis was recorded in mmol/L. The lipid profile included HDL-C, low-density lipoprotein cholesterol (LDL-C) and TG and total cholesterol (TC)

Dyslipidaemia was defined as follows: high TC (>5.0 mmol/L), high LDL-C (>3.0 mmol/L), high TG (>1.7 mmol/L), low HDL-C (<1.0mml/L for men and <1.2 mmol/L for women) [40]. Atherogenic dyslipidaemia was defined as high TG levels, low HDL cholesterol levels and an increase in small dense LDL [41]. BMI was categorized into four groups according to the conventional WHO classification [42]: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). Metabolic syndrome (MetS) was defined according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), without waist circumference [43]; BP over 130/85 mmHg, TG > 1.7 mmol/L, HDL-C levels less than 1.03 mmol/L (men) or 1.29 mmol/L (women) and fasting blood sugar over 5.5 mmol/L (Table 5).

4.4. Genomic DNA Extraction

Genomic DNA was extracted using a modified, simplified non-enzymatic salting-out method (details are shown in supplementary material). Briefly, 300 ul of whole blood was used from each of the 24 samples resulting in good quality DNA with the concentration ranging from 60 to >100 ng/μL being extracted. The results of 1% (*w/v*) Agarose gel and Thermo Scientific™ NanoDrop 2000 full spectrum of the product are presented in Figures S1 and S2.

Table 5. Definition of metabolic syndrome.

Component	Standard Criteria [43]	Criteria Used in the Present Study
Elevated waist circumference	Population- and country-specific definitions	BMI $\geq$ 30 kg/m^2
Elevated TG	TG $\geq$ 150 mg/dL (1.7 mmol/L), or drug treatment	Fasting TG $\geq$ 150 mg/dL (1.7 mmol/L)
Reduced HDL-C	HDL-C < 40mg/dL (1.0 mmol/L) in males; HDL-C < 50 mg/dL (1.3 mmol/L) in females; or drug treatment	HDL-C < 40 mg/dL (1.0mmol/L) in males HDL-C < 50 mg/dL (1.3 mmol/L) in females
Elevated blood pressure	Systolic $\geq$ 130 and/or diastolic $\geq$ 85 mmHg, or drug treatment	Systolic $\geq$ 130 and/or diastolic $\geq$ 85 mmHg
Elevated glucose	$\geq$100 mg/dL in plasma, or drug treatment	Fasting blood glucose $\geq$ 100 mg/dL (5.5 mmol/L) and/or T2DM based on hospital records

4.5. Primer Design and Primer Information

Based on the GenBank sequence of human TCF7L2, the sequence surrounding the SNPs rs7903146 (NG_012631.1:g.53341) and rs12255372 (NG_012631.1:g.10389) was obtained. Two sets of primers for the modified tetra-primer amplification assay (MTPA) PCR were designed using web-based software (http://sci.ui.ac.ir/*rahgozar; accessed on 6 June 2020). Several pairs of primers were identified and further refined using Primer-BLAST (http://www.ncbi.nlm.nih.gov/blast; accessed on 6 June 2020) and Oligo-Analyzer 3.1 IDT (https://www.idtdna.com/calc/analyzer; accessed on 6 June 2020). The primers used are provided in Table 6.

Table 6. Primers and concentration of reagents.

SNP	Primer Name	Primer Sequence (5′->3′)	Melting Temp (°C)	Product (BP)
rs7903146	FIP (T allele)	TAGAGAGCTAAGCACTTTTTAGA<u>GA</u>T	57.5	395
rs7903146	RIP (C allele)	GCCTCATACGGCAATTAAATTATA<u>GA</u>G	58.3	285
rs7903146	FOP	AAAACCTTTCCAATTTTTTCAC<u>C</u>TG	57.5	628
rs7903146	ROP	ATCCATCCACAAGCTAAC<u>C</u>CT	59.1	628
rs12255372	FIP (T allele)	CCAGGAATATCCAGGCAAGA<u>TT</u>G	55.8	171
rs12255372	RIP (C allele)	GGCCTGAGTAATTATCAGAATATG<u>C</u>TG	58.3	106
rs12255372	FOP	GTCCCTTGAGGTGTACTG<u>C</u>AA	56.7	228
rs12255372	ROP	TATTTGGCATTCAAATGGA<u>C</u>GC	55.0	228

FIP—forward inner primer; RIP—reverse inner primer; FOP; forward outer primer; ROP: reverse outer primer; Tm—melting temperature; Ta—annealing temperature.

4.6. Genotyping of TCF7L2 rs7903146 and rs12255372

Each 25 μL reaction mixture contained 1X of OneTaq® 2X Master Mix (New England Biolabs), 0.2 μM each of forward and reverse primers and >50 ng of DNA. Incubation of the 25 μL reaction mixture was completed at 94 °C for 5 min, followed by 30 cycles of 30-s denaturation (94 °C), 1 min annealing (57.7 °C for rs7903146 or 55.0 °C for rs12255372), 30 s extension (72 °C) and additional 10 min extension at 72 °C at the end of the 30 cycles. The final product was visualised with 1.5% (*w/v*) of agarose gel electrophoresis alongside a 100 base pair (bp) marker with ethidium bromide stain (Figures S3 and S4). For quality control and troubleshooting, see Supplementary Material (Table S1).

4.7. Data Analysis

Analysis of the data was conducted using SPSS version 25. The normality of continuous data was checked using the Kolmogorov–Simonov test. Descriptive statistics were reported using frequencies for categorical data and mean for continuous data. Chi-square test was used to evaluate the independence of the categorical data and *t*-test for the contin-

ues data between cases and controls. To test for associations between T2DM phenotype and all SNPs, logistic regression models were fitted, in which each SNP was presented as a predictor variable whose values were equal to the number of copies of the minor allele (0, 1, 2) in an additive model, or presence of at least one copy of the minor allele (0, 1) in a codominant model or presence of two copies of the minor allele (0, 1) in a recessive model. Sex, age and family history were included as covariates in the fitted model. The structure of the model was represented as:

$$\text{Logit [pr (D = 1)]} = \alpha + \beta_1 G + \beta_2 \text{sex}_{(male)} + \beta_3 \text{age}_{(\geq 60 \text{ years})} + \beta_{\text{positive family history}} + \beta_{\text{high cholesterol}} \beta_{\text{overweight/obesity}}$$

To examine the associations between T2DM phenotype and various allelic configurations of rs7903146 and rs12255372, haplotype association analysis was performed. The statistical analyses for linkage disequilibrium (LD), haplotype frequencies, and multiple test corrections per 1000 random permutations were performed using the Haploview 4.2 software [44,45]. The haplotype blocks were defined by the four-gamete test [46,47] as implemented in Haploview. A weak interaction between rs7903146 and rs12255372 was observed (data not shown). Univariate models adjusted for age and gender were used to evaluate the association between the SNPs and cardiometabolic markers. *p*-values < 0.05 were considered statistically significant.

5. Conclusions

There is a relatively high prevalence of the risk alleles of TCF7L2 (rs7903146 and rs12255372) among the Ghanaian population with a modest association with T2DM. The association between rs7903146 and rs12255372 with T2DM may be relevant in a subgroup of individuals (women), particularly those with no family history of diabetes and those with cardiometabolic risk with or without elevated blood pressure. Therefore, genotyping of these SNPs is essential for future multi-ethnic association studies and clinically significant for classifying high-risk patients.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/sci3040040/s1, Figure S1: 1% agarose gel electrophoresis of genomic DNA extracted from MSO method. Figure S2: A typical Nanodrop curve of absorbance against the path of light travel (wavelength) of DNA samples. Figure S3: 2% agarose gel electrophoresis of TCF7L2- rs7903146. Figure S4: 2% agarose gel electrophoresis of *TCF7L2*- rs12255372. Table S1: Master mix recipe for genotyping quality control. Table S2: Association of rs7903146 with Anthropometric and biochemical markers among the control and case groups. Table S3: Association of rs7903146 with cardiometabolic risk factors among the control and case groups. Table S4: Association of rs12255372 with Anthropometric and biochemical markers among the control and case groups. Table S5: Association of rs12255372 with cardiometabolic risk factors among the control and case groups.

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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 Committee of Human Research Publication and Ethics (CHRPE), School of Medicine and Dentistry (SMD), Kwame Nkrumah University of Science and Technology (protocol code CHRPE/AP/034/20 and date of approval: 20 May 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data supporting the conclusions of this study are summarized in Tables and supplementary materials. The raw data is available from the corresponding author upon reasonable request.

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Abbreviations

DNA—Deoxyribonucleic acid; GIP—Gastric inhibitory polypeptide; GLP-1—Glucagon-like peptide 1; HbA1c—Glycated haemoglobin; LD—Linkage disequilibrium; MAF—Minor allele frequency; MTPA—Modified tetra-primer amplification; SNP—Single nucleotide polymorphism; SSA—sub-Saharan Africa; T2DM—Type 2 diabetes mellitus; TCF7L2—Transcription factor 7 like 2.

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