

***CRY2* (rs11605924) and *G6PC2* (rs560887) single nucleotide polymorphisms increase risk of type 2 diabetes mellitus**

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Abstract. Single nucleotide polymorphisms (SNPs) are genetic risk factors for type 2 diabetes (T2D). SNPs of the cryptochrome circadian regulator 2 (*CRY2*), hepatocyte nuclear factor 1 α , prospero homeobox 1 and glucose-6-phosphatase catalytic subunit (*G6PC2*) genes are associated with T2D. To the best of our knowledge, however, the association is unclear in African patients and the burden of T2D is growing rapidly in Africa. The present study aimed to investigate SNPs of cryptochrome circadian regulator 2 (*CRY2*), hepatocyte nuclear factor 1 α , prospero homeobox 1 and glucose-6-phosphatase catalytic subunit (*G6PC2*) genes in T2D in a South African population. The study included 310 participants with T2D and 310 healthy controls. Demographic and lifestyle characteristics were self-reported, while anthropometric and biochemical measurements were determined using standard methods. Plasma glucose and insulin were measured by enzymatic hexokinase method and paramagnetic particle chemiluminescence assay, respectively. Highly sensitive c-reactive protein (hs-CRP) was measured by ELISA. iPLEX SNP genotyping was used for SNP analysis and results were confirmed by Sanger sequencing. Insulin resistance measured

using the homeostasis model assessment of insulin resistance (HOMA-IR) formula was significantly higher in carriers of the recessive rs11605924 ($P=0.049$), fasting plasma glucose was significantly lower in carriers of the recessive rs560887 ($P=0.015$) and highly sensitive c-reactive protein levels were significantly lower in carriers of the recessive rs1169288 ($P=0.018$). The risk of T2D was significantly higher in participants with the recessive rs560887 [odds ratio (OR)=1.58, 95% CI, 1.02-2.44, $P=0.041$] and rs11605924 (OR=1.82, 95% CI, 1.03-3.23, $P=0.041$) compared with the dominant alleles. *CRY2* and *G6PC2* SNPs were associated with an increased risk of T2D and may partly contribute to high prevalence of T2D in the mixed ancestry South African population. Further studies are warranted to validate these findings in other African populations and explore downstream pathways.

Introduction

Type 2 diabetes (T2D) is a multifactorial disorder characterised by high levels of glucose in the blood as a result of defects in insulin secretion and/or action. It is a global health challenge due to a continuous increase in its incidence and prevalence. As recorded in the 10th edition of the International Diabetes Federation Atlas, the global prevalence of T2D in 2021 was 10.5% (537 million) in adults aged 20-79 years and is projected to rise to 12.2% (783 million) by 2045 (1). A total of ~50% of individuals with T2D (239.7 million) are unaware of their condition, with the highest proportion (87.5%) of undiagnosed cases of T2D in low and medium-income countries (1). This results in chronic complications such as diabetic neuropathy, nephropathy and retinopathy, impacting the quality of life (2).

Non-communicable diseases constitute the leading cause of natural death in South Africa, with diabetes, hypertension and cerebrovascular disease among the top six causes of death since 2017 (3). Moreover, as of 2021, the prevalence of T2D in South Africa was the highest on the African continent (11.3%).

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Socio-economic (income and level of education), demographic (age, sex and ethnicity) and environmental factors (diet, alcohol consumption and physical inactivity) have contributed to the rise in the prevalence of prediabetes and diabetes (4). Over half of South African adults are overweight or obese and up to 50% are hypertensive (5,6), further exacerbating the risk of diabetes. While these factors alone cannot account for the increase in the prevalence of T2D, their impact in the presence of a favourable genetic background is more pronounced. This has been shown in studies where individuals with similar lifestyle and environmental exposures had varied T2D susceptibility, suggesting the possible role of genetic differences (7-9). Twin studies have reported that the risk of T2D is higher in individuals with one or both parents with T2D, compared with individuals whose parents do not have diabetes (8,9). Family studies have shown that the risk of T2D is three times higher in those with first-degree relatives with diabetes compared with individuals without a positive family history (10,11).

Associations between single nucleotide polymorphisms (SNPs) of transcription factor 7-like 2 (rs7903146 and rs12255372), hematopoietically expressed homeobox (rs1111875) and peroxisome proliferator-activated receptor γ (rs17036314 and rs1801282) genes and T2D have been extensively reported across different ethnicities (12-15). Novel gene variants associated with T2D include rs11605924, rs1169288, rs340874 and rs560887 (16-20). rs11605924 is a SNP of cryptochrome circadian regulator 2 (*CRY2*) gene encoding a flavine adenine dinucleotide-binding protein, which is a key component of the circadian clock, necessary for stability of the circadian rhythm (16). Dysregulation of circadian rhythm leads to sleep disruption, which is a risk factor for metabolic diseases including T2D (21). The A-allele of rs11605924 SNP is moderately associated with impaired fasting glucose and T2D in a Chinese population (22). rs1169288 is a SNP variant of the hepatocyte nuclear factor 1 α gene, whose protein is involved in development and function of pancreatic β cells (17). This variant is a well-known risk factor for maturity-onset diabetes of the young (23,24) and is associated with T2D in normal weight individuals of Japanese ancestry (25). A large-scale meta-analysis reported an association between rs340874 SNP of prospero homeobox 1 (*PROX1*) gene and T2D (18,19). The *PROX1* gene encodes a transcription factor that plays a role in development of the pancreas (26) and the C-allele of the rs340874 SNP is associated with insulin resistance, β cell dysfunction and hyperglycemia (20,27,28). rs560887 is a SNP of the glucose metabolism gene glucose-6-phosphatase catalytic subunit 2 and is associated with increased fasting plasma glucose (FPG) levels and pancreatic β cell function (20,27,28).

Investigations into the genetics of diabetes mellitus have potential clinical implications. The management of T2D increasingly requires a personalised approach, as glycaemic response to treatments such as metformin varies across individuals. The detection of SNPs that are associated with therapy efficacy may facilitate the adoption of personalized interventions with higher success rates compared with blanket approaches for genetically diverse individuals. For example, a study in a South African population demonstrated an interaction between rs889299 and rs2162145 SNPs, which affected metformin treatment outcomes (29). In another study, variants (rs2727528 and rs1105842) of protein kinase AMP-activated non-catalytic

subunit $\gamma 2$ (*PRKAG2*) gene are associated with metformin response in a cohort of patients with T2D from China (30). While T2D susceptibility genes have been identified in various populations (Caucasian, Asian and African-American), to the best of our knowledge, such studies are scarce in Africa (13,31), including South Africa, which is experiencing a rising prevalence of prediabetes and T2D. The present study aimed to investigate the association between well-described genetic risk factors and T2D in a mixed ancestry South African population characterized by a high prevalence of T2D.

Materials and methods

Study design and population. The present case-control study comprised 122 male and 498 female participants (310 with T2D and 310 normo-glucose tolerant) matched for age (median age, 61 years for cases and 59 years for control), sex and body mass index (BMI). The participants were selected from a larger cohort of 1,989 mixed ancestry participants recruited from April 2014 to August 2016 at the Bellville South community in Cape Town, South Africa into the ongoing Cape Town Vascular and Metabolic Health study (32). The Bellville South community is an urban suburb in the north of Cape Town, with a predominantly mixed ancestry South African population in which a high prevalence of diabetes, hypertension and obesity has been previously reported (33,34). The mixed-ancestry South African population is a well-defined, multiracial ethnic group that comes from ethnic backgrounds including indigenous South Africans, Caucasians, Griquas and Asians (33). Participants of mixed ancestry origin aged between 20 to 79 years and residing in Bellville South Community were included in the study, while pregnant patients and those suffering from acute illnesses were excluded.

Data collection and sampling. Following an overnight fast of ≥ 8 h, study participants were transported to a clinic area where trained fieldworkers using a standardized questionnaire collected demographic information and personal and family medical history. Using the semi-automated, digital Omron M6 (Omron Healthcare) comfort-preformed cuff blood pressure monitor, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in triplicate; the lowest SBP and corresponding DBP was used in the analysis. After taking off their shoes and socks, weight was measured with a BF511 Body Composition Monitor (Omron Healthcare, Japan), whilst a stadiometer was used to measure height as they stood on a flat surface. Following anthropometric measurements, ~ 10 ml blood samples were collected into EDTA tubes for SNP genotyping and tubes without anti-coagulant for biochemical analysis.

Biochemical analysis. The analysis of biochemical parameters was performed at an International Organization for Standardization (ISO) 15189 accredited pathology practice (PathCare, Reference Laboratory, Cape Town, South Africa). Plasma glucose and insulin were measured by enzymatic hexokinase method and paramagnetic particle chemiluminescence assay (both Beckman Coulter, Inc.) respectively. Highly sensitive c-reactive protein (hs-CRP) was measured by

Table I. General characteristics of individuals with and without diabetes.

Variable	Cases (n=310)	Controls (n=310)	P-value
Age, years (range)	61.0 (54.0-67.0)	59.0 (52.0-66.0)	0.377
Sex (%)			>0.999
Male	61.0 (19.7)	61.0 (19.7)	
Female	249.0 (80.3)	249.0 (80.3)	
Weight, kg (range)	82.7 (65.7-90.3)	77 (66.3-90.7)	0.183
Height, cm (range)	155.5 (152.5-163.0)	158.0 (153.5-163.0)	>0.999
BMI, kg/m ² (range)	31.5 (27.3-37.1)	31.3 (26.7-36.9)	0.936
WC, cm (range)	101.7 (93.0-111.8)	97.7 (88.5-108.8)	0.003
HC, cm (range)	109.0 (98.8-121.0)	108.6 (100.5-118.7)	0.904
WHR (range)	0.9 (0.9-1.0)	0.9 (0.8-0.9)	<0.001
SBP, mmHg (range)	133.0 (119.0-151.0)	130.0 (118.0-144.0)	0.099
DBP, mmHg (range)	84.0 (76.0-92.0)	84.0 (75.0-92.0)	0.355
Heart rate, bpm (range)	71.0 (63.0-83.0)	67.0 (60.0-75.0)	<0.001
FBG, mmol/l (range)	6.7 (5.6-8.5)	4.9 (4.6-5.2)	<0.001
HbA1c, % (range)	8.0 (6.7-9.0)	5.8 (5.5-6.0)	<0.001
Insulin levels, μ IU/ml (range)	10.3 (5.7-18.4)	7.1 (4.6-10.1)	<0.001
HOMA-IR (range)	3.0 (1.7-5.8)	1.5 (1.0-2.3)	<0.001
HOMA-B (range)	71.5 (50.0-111.6)	98.9 (69.3-150.0)	<0.001
Total cholesterol levels, mmol/l (range)	5.3 (4.5-6.5)	5.5 (4.8-6.1)	0.127
HDL cholesterol levels, mmol/l (range)	1.2 (1.1-1.4)	1.3 (1.1-1.5)	0.004
Triglyceride levels, mmol/l (range)	1.4 (1.1-2.2)	1.3 (1.0-1.7)	<0.001
LDL cholesterol levels, mmol/l (range)	3.5 (2.7-4.2)	3.4 (2.9-4.1)	0.465
hs-CRP levels, mg/l (range)	5.2 (3.3-14.0)	4.4 (2.2-8.4)	0.084
γ GT levels, IU/l (range)	35.0 (22.0-59.5)	28.0 (20.0-40.0)	0.006
Cotinine levels, ng/ml (range)	10.0 (10.0-142.0)	10.0 (10.0-224.0)	0.005

Sex is reported as count (percentage) and all other variables are reported as median (25th-75th) percentile. BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; IR, insulin resistance; HOMA-B, homeostasis model assessment of β cell dysfunction; hs-CRP, highly sensitive C-reactive protein; GT, glutamyl transferase.

Table II. Genotype and allele frequency of single nucleotide polymorphisms.

Gene	Allele (dom/rec)	Single nucleotide polymorphism	Dom	Het	Rec	MiAF	HWE P-value
<i>CRY2</i>	A/C	rs11605924 (n=614)	296	263	55	0.300	0.952
<i>PROX1</i>	T/C	rs340874 (n=601)	354	210	37	0.236	0.738
<i>G6PC2</i>	C/T	rs560887 (n=616)	517	95	4	0.084	0.987
<i>HNF1A</i>	A/C	rs1169288 (n=614)	360	217	37	0.237	0.854

MiAF, minor allele frequency; dom, dominant; het, heterozygous; rec, recessive; HWE, Hardy Weinberg equilibrium; *CRY2*, cryptochrome circadian regulator 2; *PROX1*, prospero homeobox 1; *G6PC2*, glucose-6-phosphatase catalytic subunit; *HNF1A*, hepatocyte nuclear factor 1 α .

ELISA (Biomatik) according to the manufacturer's protocol. Total cholesterol (TC) and HDL cholesterol (HDL-C) were measured by enzymatic immune inhibition, triglycerides (TG) by glycerol phosphate oxidase-peroxidase and low-density lipoprotein cholesterol (LDL-C) by enzymatic selective protection-End Point (Beckman Coulter, Inc.) A modified Jaffe-Kinetic method was used to measure serum creatinine (Beckman Coulter, Inc.).

DNA extraction and genotyping. The salt extraction method (35) was used to extract DNA from 5 ml blood samples stored in EDTA tubes and the Nanodrop™ One (Thermo Fisher Scientific, Inc.) was used to determine concentration and purity of the extracted DNA. Only samples with a minimum concentration of 25 ng/ μ l and 260/280 nm optical density ratios between 1.7 and 2.2 were used for SNP genotyping, which was outsourced and performed by

Table III. Association between SNP genotype and clinical characteristics.

CMD Trait	rs11605924				rs340874				rs560887				rs1169288			
	A/A	A/C	C/C	P-value	T/T	T/C	C/C	P-value	C/C	C/T	T/T	P-value	A/A	A/C	C/C	P-value
SBP	132 (118- 148)	133 (118- 148)	136 (125- 149)	0.575	133 (120- 149)	123 (117- 148)	131 (120- 152)	0.816	132 (118- 147)	134 (118- 153)	137 (127- 144)	0.524	132 (118- 147)	134 (119- 150)	132 (121- 149)	0.493
DBP	84 (76- 93)	83 (76- 92)	82 (74- 94)	0.388	85 (76- 92)	82 (77- 92)	84 (72- 93)	0.094	84 (76- 92)	82 (76- 91)	91 (79- 100)	0.642	83 (76- 92)	83 (76- 93)	84 (76- 91)	0.964
FPG	5.3 (4.8- 8.0)	5.4 (4.8- 7.8)	6.7 (4.9- 11.2)	0.184	5.4 (4.8- 7.7)	5.4 (4.8- 8.3)	5.5 (5.0- 10.7)	0.703	5.4 (4.8- 7.7)	6.0 (4.9- 11.1)	4.9 (4.8- 5.0)	0.015*	5.4 (4.8- 8.2)	5.4 (4.8- 7.8)	5.8 (5.0- 8.6)	0.362
Insulin	8.1 (5.3- 13.5)	7.8 (5.0- 12.3)	9.1 (5.4- 12.6)	0.502	7.8 (5.1- 12.9)	8.7 (5.4- 13.1)	8.7 (5.7- 16.8)	0.370	8.1 (5.2- 13.0)	7.3 (5.2- 13.5)	10.6 (7.7- 13.0)	0.590	8.1 (5.2- 13.0)	7.5 (5.1- 12.3)	9.8 (7.1- 15.4)	0.060
HOMA-IR	2.17 (4.71- 1.28)	2.14 (1.35- 4.16)	3.29 (1.39- 4.77)	0.049	2.11 (1.22- 4.21)	2.28 (1.39- 4.49)	3.05 (1.48- 6.06)	0.174	2.13 (1.28- 4.46)	2.59 (1.47- 4.61)	2.17 (1.61- 2.83)	0.349	2.15 (1.30- 4.15)	2.16 (1.23- 3.91)	3.11 (1.98- 5.47)	0.175
HOMA-B	78.3 (42.4- 118.7)	78.1 (33.8- 120.0)	70.0 (25.0- 107.9)	0.620	80.0 (40.0- 117.9)	75.7 (38.9- 119.6)	80.5 (25.2- 144.3)	0.725	80 (40- 120)	58.3 (26.3- 102.9)	192.5 (120.5- 192.6)	0.118	77.4 (37.7- 123.8)	76.0 (36.6- 113.3)	74.7 (42.0- 118.7)	0.780
HbA1c	6.2 (5.7- 7.8)	6.1 (5.7- 8.0)	6.5 (5.8- 9.3)	0.646	6.2 (5.7- 7.9)	6.2 (5.7- 8.2)	6.4 (5.7- 8.4)	0.923	6.1 (5.7- 7.8)	6.9 (5.8- 9.9)	5.8 (5.4- 6.2)	0.119	6.2 (5.7- 8.0)	6.1 (5.8- 7.9)	6.6 (5.8- 9.0)	0.635
hs-CRP	5.2 (2.5- 10.3)	4.7 (2.3- 9.9)	4.7 (2.7- 11.8)	0.644	5.6 (2.7- 11.3)	4.7 (2.1- 10.5)	4.7 (2.8- 7.5)	0.274	5.0 (2.4- 10.5)	4.8 (2.5- 9.6)	10.9 (3.2- 18.3)	0.951	5.8 (2.6- 12.6)	4.7 (2.3- 8.4)	4.6 (1.9- 9.3)	0.018

CMD, cardiometabolic diseases; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; IR, insulin resistance; HOMA-B, homeostasis model assessment of β cell dysfunction; hs-CRP, highly sensitive C-reactive protein; SNP, single nucleotide polymorphism.

Table IV. SNPs associated with type 2 diabetes mellitus.

SNP	Model (reference genotype)	Genotype	Odds ratio (95% CI)	P-value
rs11605924	Additive (A/A)	A/A vs. A/C vs. C/C	1.14 (0.89-1.46)	0.301
	Dominant (A/A)	A/A vs. A/C + CC	1.03 (0.75-1.41)	0.876
	Recessive (A/A + A/C)	A/A + A/C vs. CC	1.82 (1.03-3.23)	0.041
rs340874	Additive (T/T)	T/T vs. T/C vs. C/C	1.09 (0.85-1.43)	0.484
	Dominant (T/T)	T/T vs. T/C + C/C	1.14 (0.82-1.58)	0.435
	Recessive (T/T + T/C)	T/T + T/C vs. C/C	1.06 (0.55-2.07)	0.857
rs560887	Additive (C/C)	C/C vs. T/C vs. T/T	1.50 (0.99-2.26)	0.055
	Dominant (C/C)	C/C vs. C/T + T/T	1.58 (1.02-2.44)	0.041
	Recessive (C/C + C/T)	C/C + C/T vs. T/T	0.99 (0.14-7.10)	0.995
rs1169288	Additive (A/A)	A/A vs. A/C vs. C/C	1.12 (0.86-1.46)	0.389
	Dominant (A/A)	A/A vs. A/C + CC	1.08 (0.79-1.50)	0.623
	Recessive (A/A + A/C)	A/A + A/C vs. CC	1.50 (0.76-2.96)	0.238

Additive, dominant/heterozygous/recessive; dominant, dominant/heterozygous + recessive; recessive, dominant + heterozygous/recessive; SNP, single nucleotide polymorphism.

Inqaba Biotech using the iPLEX SNP genotyping protocol. Briefly, PCR Master Mix containing 10X PCR buffer with 20 mM MgCl₂ (0.5 μ l), 0.4 μ l 25 mM MgCl₂, 0.1 μ l dNTP mix (25 mM each), 1.0 mM primer mix (500 nM each), 0.2 μ l PCR enzyme (5 U/ μ l) and 1.8 μ l ddH₂O was made. Thereafter, 4 μ l Master Mix was pipetted into each well of a 96-well PCR plate followed by 1 μ l DNA sample (25-50 ng). The plate was centrifuged at 1,000 x g for 2 min at 4°C and PCR reaction was run using the following conditions: Pre-denaturation at 94°C for 2 min, followed by 45 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 60 sec and final extension at 72°C for 5 min with samples kept on hold at 4°C. Following amplification, the samples were treated with shrimp alkaline phosphatase [1.53 water, 0.17 10X buffer and 0.3 μ l enzyme (1.7 U/ μ l)] to remove non-incorporated dNTPs from amplification products. iPLEX-single base extension Master Mix was used for extension of the PCR products. A second clean-up step was conducted wherein a slurry of resin was added directly to the primer extension reaction products to remove Na⁺, K⁺, and Mg²⁺ ions. The extended/desalted analyte products from microtiter plates were arrayed on SpectroCHIPS and detected by mass spectrometry. SNP genotyping results were confirmed by Sanger dideoxy sequencing. The detailed methodology is outlined in Data S1 and Table SI.

Definitions and calculations. Body mass index (BMI) was calculated as weight (kg)/[height (m)]² and used to categorize participants as normal weight (BMI<25 kg/m²), overweight (25 kg/m²≤ BMI<30 kg/m²) and obese (BMI≥30 kg/m²). Waist-to-hip ratio (WHR) was calculated as waist circumference/hip circumference. Central obesity was determined as WC >94 in males and >80 cm in females. Hypertension was defined as SBP≥140 or DBP≥90 mmHg or known hypertension on anti-hypertensive treatment (36). Dyslipidemia was defined as TC>5 mmol/l, triglycerides >1.5 mmol/l, HDL-C<1.2 mmol/l, LDL-C>3.0 mmol/l and non-HDL-C>3.37 mmol/l or taking anti-lipid agents (37).

Diabetes was defined as FPG≥7.0 mmol/l and/or 2-h post glucose load ≥11.1 mmol/l, previous diagnosis or taking antidiabetic medications (38). Insulin resistance (IR) was calculated based on the homeostasis model assessment (HOMA) as follows: HOMA-IR=fasting insulin (μ U/l) x fasting glucose (nmol/l)/22.5. β cell function was determined by HOMA- β using the formula HOMA- β =fasting insulin x 20/fasting glucose -3.5.

Statistical analysis. IBM SPSS version 29 software (IBM Corp.) was used for data analysis. Data are presented as the mean $\pm$ SD or median and interquartile range. Continuous variables were skewed, hence differences were determined using the median test. Hardy-Weinberg equilibrium (HWE) was tested for all the SNPs and those in HWE were used for further analysis. Logistic regression analyses were performed to test the interactions between T2D and the genotypes of the SNPs using the dominant, recessive and additive models. All analyses were performed at 95% confidence interval. P<0.05 was considered to indicate a statistically significant difference.

Results

General characteristics of participants. Amongst the 620 participants, 80.3% were female, 46.4% had hypertension and 56.5% were obese. Individuals with T2D had a significantly higher waist circumference, WHR, heart rate, triglyceride, HDL cholesterol and γ glutamyl transferase levels and lower serum cotinine levels when compared with individuals without T2D (Table I).

Genotypic and allelic frequencies of SNPs. DNA samples from all 620 participants passed quality and concentration checks and were used for genotyping; all four SNPs (rs11605924, rs340874, rs560887 and rs1169288), were in HWE (Table II). All three genotypes of each of the SNPs (dominant, heterozygous and recessive) were detected for all SNPs, although the genotyping failure rate ranged from 0 to 3%.

Association between SNP genotypes and clinical characteristics. A total of three of the four SNPs in HWE were associated with FPG, HOMA-IR and CRP (Table III), while there was no significant association with markers of dyslipidemia (total, HDL and LDL cholesterol and triglyceride levels) and adiposity (WC and WHR). Carriers of the recessive C/C genotype of rs11605924 SNP had significantly higher HOMA-IR values when compared with carriers of the dominant A/A genotype. Fasting plasma glucose was significantly lower in carriers of the recessive T/T genotype of rs560887 SNP when compared with the heterozygous C/T genotype. Analysis of the rs1169288 SNP showed that carriers of the recessive C/C genotype had significantly lower CRP levels when compared with carriers of the dominant A/A genotype.

Association between SNPs and T2D. Binary logistic regression was used to assess the risk of T2D in carriers of SNP genotypes using the dominant, recessive and additive models (Table IV). Recessive C/C genotype of the rs11605924 SNP was associated with a higher risk of T2D in the recessive model (OR, 1.82; 95% CI, 1.03-3.23). Moreover, the recessive TT genotype of rs560887 SNP was associated with a higher risk of T2D in the dominant model (OR, 1.58; 95% CI, 1.02-2.44).

Discussion

The present study sought to investigate the relationship between four SNPs and T2D in a mixed-ancestry South African population. While all four SNPs were in HWE, only three were associated with either FPG, HOMA-IR, hs-CRP or T2D. When compared with carriers of the dominant A/A genotype, those with recessive C/C genotype of rs11605924 SNP had significantly higher HOMA-IR levels. Moreover, carriers of C/C genotype were at higher risk of developing T2D when compared with carriers of the A/A and A/C genotypes. The recessive T/T genotype of rs560887 SNP was associated with high FPG and T allele (C/T and T/T) was associated with a 1.5-fold increased risk of T2D. While there was no association between rs1169288 and rs340874 SNPs and T2D, the recessive genotype of the rs1169288 SNP was associated with lower CRP levels.

The association between the rs11605924 SNP of the *CRY2* gene and gestational diabetes mellitus (GDM) and T2D have been previously investigated, with the recessive allele associated with decreased and increased risk of GDM in Indian and Swedish patients, respectively (39), and increased risk of combined IFG/T2D in a Chinese population (22). Unlike in India, there is a seasonal variation in circadian rhythm in Sweden, which may underlie the difference in the association of the *CRY2* gene and GDM between both populations. The *CRY2* gene regulates circadian rhythm, which when disturbed, negatively impacts glucose metabolism by impairing the regulation of energy intake and expenditure (40), leading to development of IR and T2D (41,42). While disruption of the circadian rhythm has been reported to impair pancreatic β cell function and insulin sensitivity (22), the present study did not show significant differences in HOMA-B levels. However, high HOMA-IR levels in individuals with the minor CC genotype compared to those with the major AA genotype

of the rs11605924 SNP suggests that the minor genotype may contribute to T2D by impairing the insulin action pathway without affecting the insulin secretion pathway. However, functional studies in murine models of T2D are required to confirm these possibilities. SNPs of glucose metabolism genes, including *G6PC2*, are associated with the risk of T2D (13-15,20). While studies have consistently associated the rs560887 SNP (dominant G allele) of the *G6PC2* gene with elevated FPG, the association with T2D has been inconsistent (43-46). A meta-analysis showed that this allele has no association with T2D overall and in Asian populations but was significantly associated with decreased risk of T2D in Caucasians under the additive model (46). The present results are consistent with findings from other studies, reporting significantly higher FPG in carriers of the dominant allele when compared with the recessive allele (20,43,44). However, the association with T2D in the present study was observed with the dominant model, indicating that rs560887 may be a genetic risk factor for T2D.

While SNPs of the *CRY2* gene (rs11605924) and *G6PC2* gene (rs560887) were associated with T2D in the present study, SNPs of the *HNF1A* (rs1169288) and *PROX1* (rs340874) genes did not show any association with T2D and CMD. The rs340874 SNP of the *PROX1* gene has been consistently associated with diabetes, with C allele reported as glucose-raising, displaying a higher risk of T2D due to its association with reduced insulin secretion and sensitivity (27,28). On the other hand, the rs1169288 SNP of the *HNF1A* gene has been reported to be associated with maturity-onset diabetes of the young (24) and T2D in non-obese individuals (25,47). The present findings therefore show that rs1169288 and rs340874 SNPs may not constitute genetic risk factors of T2D or that the risk might be minimal and undetected based on the present sample size.

To the best of our knowledge, the present study is the first to identify novel genetic loci as risk factors for T2D in South Africa. However, the findings are not generalizable to wider populations as only one ethnic group was investigated.

In conclusion, minor alleles of rs11605924 SNP of the *CRY2* gene and rs560887 SNP of the *G6PC2* gene were genetic determinants of T2D in a mixed ancestry South African population. These alleles/genotypes increased the risk of T2D by potentially affecting the insulin sensitivity pathway. rs1169288 SNP of the *HNF1A* gene and the rs340874 SNP of the *PROX1* gene may not constitute a risk factor for T2D in the present South African population. Further studies to replicate the present findings in the same and other populations are warranted to elucidate a complete genetic risk profile for T2D. Moreover, investigating downstream insulin signalling pathways may contribute to unravelling the pathophysiology of T2D as well as establishing therapeutic targets.

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Availability of data and materials

The data generated in the present study may be found in the BankIt under accession numbers PV068680, PV068681, PV068682, PV068683, PV068684, PV068685, PV068686, PV068687, PV068688, PV068689, PV068690, PV068691 or at the following URL: ncbi.nlm.nih.gov/WebSub/?tool=genbank&form=history.

Author's contributions

TEM, APK, GMD and RTE conceived and designed the study. NEN and DMM confirm the authenticity of all the raw data, analyzed the data and wrote the manuscript. GMD, TEM, APK, and RTE revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Cape Peninsula University of Technology Health and Wellness Sciences Research Ethics Committee (approval no. National Health Research Ethics Council : REC-230 408-014) and Stellenbosch University Health Research Ethics Committee (approval no. N14/01/003). The study was conducted following the principles of the Declaration of Helsinki. All participants provided written informed consent to participate.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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