Contribution of ENPP1, TCF7L2, and FTO polymorphisms to type 2 diabetes in mixed ancestry ethnic population of South Africa

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Abstract

Background: Transcription factor 7-like 2 gene (TCF7L2), fat mass and obesity-associated gene (FTO), and ectonucleotide pyrophosphatase/phosphodiesterase gene (ENPP1) are known risk loci for type 2 diabetes (T2DM) mostly in European populations.

Objectives: To assess the association of these genes with T2DM risk in a South African mixed-ancestry population.

Methods: Five hundred and sixty six participants were genotyped for ENPP1-rs997509 and -rs1044498, FTO-9941349 and -rs3751812, TCF7L2-rs12255372 and -rs7903146 polymorphisms using Taqman genotyping assays and validated by automated sequencing to assess the association of the polymorphisms with cardiometabolic traits.

Results: In logistic regression models adjusted for age, sex, body mass index (BMI) and insulin resistance, minor allele of rs997509 was associated with a higher risk of prevalent T2DM under a recessive model [odd ratio 4.60 (95% confidence interval: 1.07 to 19.86); p = 0.040].Under additive model, the rs7903146 [1.43 (1.00 to 2.04); p= 0.053] and rs9941349 [1.43 (1.00 to 2.04); p = 0.052] minor alleles showed marginally significant associations with a high risk of T2DM. However, only the rs7903146 alleles (p=0.011) and genotypes (p=0.025) distributions were statistically significantly different between diabetic and non-diabetic individuals.

Conclusion: Our findings demonstrate that ENPP1, TCF7L2, and FTO may predispose to T2DM in the mixed-ancestry population.

Keywords: Type 2 diabetes, genetics, ENPP1, TCF7L2, FTO, Africa

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Introduction

The completion of the human genome a decade ago has paved a way for a better understanding of the genetic basis of common and complex diseases. Through candidate gene and linkage analyses, and recently genome wide association studies (GWAS), several single nucleotide polymorphisms (SNPs) have been reported as risk loci for type 2 diabetes in European (39 loci) and Asian (19 loci) population groups[1,2]. The majority of these loci seem to influence beta-cell function and insulin sensitivity, although the mechanisms by which

they impair such functions are yet to be determined. Among these loci are transcription factor 7-like 2 gene (TCF7L2), fat mass and obesity-associated gene (FTO), and ectonucleotide pyrophosphatase/phosphodiesterase gene (ENPP1).

Transcription factor 7-like 2 gene is the first T2DM risk locus to be identified through large-scale association analyses in European populations[3]. The protein encoded by the TCF7L2 is a transcription factor of the Wnt signalling pathway, which plays a crucial role in beta-cell function, insulin secretion, and proglucagon gene expression[4, 5]. The first surge of T2DM GWA identified among others the fat mass and obesity-associated gene (FTO), which is the strongest obesity genetic risk factor reported so far[6,7]. It has been shown to confer susceptibility to T2DM in the presence[8,9] and absence of obesity[10,11]. The most widely studied SNP of the ENPP1, K121Q (rs1044498), has produced conflicting results in relation to T2DM susceptibility[12-14]. The ENPP1-encoded protein (also known as plasma cell-1, PC-1) is a class II transmembrane glycoprotein that interacts with insulin receptor and inhibits subsequent insulin-signalling through its impaired beta-subunit autophosphorylation, thus causing insulin resistance[15].

Although few number of T2DM susceptibility genes discovered through candidate gene, linkage analyses, and GWA studies elsewhere have been replicated in African studies[16-21], it must also be emphasized that many other association studies failed to show significant and replicable findings[22-24]. Therefore in this study we investigated three genes, namely, TCF7L2, FTO, and ENPP1 as risk factors for T2DM in a South African ethnic population group of Mixed-ancestry (Coloureds) with a unique genetic architecture. Structure analysis conducted in this population revealed that its origin is predominantly Khoesan (32-43%), Bantu-speaking African (20-36%), European (21-28%), and a small proportion Asian (9-11%)[25].

Materials and Methods
Ethical approval of the study
The study was approved by the Faculty of Health and Wellness Sciences Ethics Committee of the Cape Peninsula University of Technology (CPUT) NHREC: REC-230408-014, and was conducted according to the code of ethics of the World Medical Association (Declaration of Helsinki). All participants who were recruited for the study voluntarily signed written consent after the procedures had been fully explained in the language of their choice. Permission to conduct the study was granted by relevant authorities such as city and community authorities.

Study design and population
The present study was cross-sectional by design, involving participants from a mixed-ancestry ethnic population group residing in Bellville South township in Cape Town, South Africa. The population group has lived in Bellville South since late 1950s. A detailed description of the survey and procedures conducted in the study are available elsewhere[26,27].

Clinical data
Clinical data was collected in the form of a standardized questionnaire. During this time, physical examination was conducted with data collection on blood pressure according to World Health Organisation (WHO) guidelines[28] using a semi-automatic digital blood pressure monitor (Rossmax PA, USA) on the right arm in sitting position, and anthropometric measurements; body weight was measured to the nearest 0.1 kg with a Sunbeam EB710 digital bathroom scale, which was calibrated and standardized using a weight of known mass. Measurements were recorded with each subject wearing light clothing, without shoes and socks. Waist circumference was determined using a non-elastic tape at the level of the narrowest part of the torso, as seen from the anterior view. All anthropometric measurements were performed three times and their average used for analysis. Participants with no history of doctor diagnosed diabetes mellitus underwent a 75 g oral glucose tolerance test (OGTT) as recommended by the WHO[29].

Biochemical analysis
Blood samples were collected from participants after an overnight fast. Blood glucose level and glycated haemoglobin (HbA1c) were measured, respectively, by enzymatic hexokinase method and turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics, Germany). Insulin was determined by a microparticle enzyme immunoassay (Axsym, Abbot). High-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were estimated by enzymatic colorimetric methods (Cobas 6000, Roche Diagnostics). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedwald's formula.

Definitions and calculations

Body mass index (BMI) was calculated as weight per square meter (kg/m^2) and waist-hip-ratio (WHR) as waist/hip circumferences(cm). Diabetes was based on a history of doctor-diagnosis, fasting blood glucose concentration $\geq$ 7.0 mmol/L (or 126 mg/dL) and/or 2-hour post-OGTT plasma glucose $\geq$ 11.1mmol/L (or 200mg/dL). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: HOMA-IR= [fasting insulin concentration (mIU/L) x fasting plasma glucose (mmol/L]/ 22.5; while functional β-cells (HOMA-B %) were estimated using the formula: 20 × fasting insulin (µIU/ml)/fasting glucose (mmol/ml) − 3.5. The fasting insulin resistance index (FIRI) was calculated with the formula: [fasting insulin (µU/ml) × fasting glucose (mM)]/25 and the quantitative insulin-sensitivity check index (QUICKI) as: 1/ [log (fasting insulin (µU/ml) × log (fasting glucose (mg/dl)].

Genotyping

Genomic DNA was extracted from peripheral blood using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, white blood cells were lysed, thereafter cellular proteins were removed by salt precipitation, and high molecular weight genomic DNA left in solution was then concentrated and desalted by isopropanol precipitation. A total of 566 participants (11.7% males) who consented to genetic analyses were genotyped for six single nucleotide polymorphisms (SNPs) that have been shown to confer susceptibility to T2DM in previous studies elsewhere: ENPP1-rs997509 and -rs1044498, FTO-9941349 and -rs3751812, TCF7L2-rs12255372 and -rs7903146. The SNPs were genotyped using their corresponding Taqman genotyping assays (Applied Biosystems, USA) on a BioRad Optica (Biorad, USA) and validated by sequencing, giving 100% genotyping concordance for all samples that were repeated.

Statistical analysis

General characteristics of the study group are summarized as count and percentage for dichotomous traits, mean and standard deviation (SD) or median and 25th-75th percentiles for quantitative traits. Traits were log-transformed to approximate normality, where necessary, prior to analysis. SNPs were tested for departure from Hardy-Weindberg Equilibrium (HWE) expectation via a chi square goodness of fit test. Linkage disequilibrium (LD) was estimated using the D' statistic. Logistic regression models were used for the analysis of dichotomous traits, assuming both recessive and log-additive models for the SNPs. We investigated the association of each SNP with each trait, overall and tested for heterogeneity by major subgroups by adding the interaction term of major grouping variables and each SNP to a model that contained the main effects of grouping variable and the relevant SNP. Results corresponding to p-values below 5% are described as significant. Adjustment for multiple testing was conducted via Bonferroni methods. All analyses used the statistical software R (version 3.0.0 [2013-04-03], The R Foundation for statistical computing, Vienna, Austria). SNPs analyses used the packages 'genetic', 'gap', 'SNPassoc' and 'hapassoc'.

Results

Baseline characteristics

Of 566 participants that consented for genetic studies, 480 had complete data for analysis and their clinical characteristics are summarized in Table 1. One hundred and fifty eight (32%) individuals had T2DM. As expected, T2DM-related traits differed significantly according to diabetes status. For example, significant differences in the distribution of insulin resistance/sensitivity indicators were observed between the two groups (all p < 0.0001, except for glucose/insulin ratio (p = 0.009). Participants with diabetes compared to non-diabetics, were older (p < 0.0001), had higher body mass index (p= 0.023), higher waist circumference and waist-to-hip ratio (both p < 0.0001), higher systolic blood pressure (130 vs. 121 mmHg, p < 0.0001), and consisted of a higher proportion of individuals with hypertension (77.6% vs. 67.1%, p = 0.018). Furthermore, diabetic individuals had higher triglycerides (p < 0.0001).

Characteristics	No diabetes	Diabetes	p-value	Normal weight	overweight	obese	p-value	Overall
N	328	152		118	129	233		480
Gender, male n (%)	18 (5.5)	34 (22.4)	<0.0001	16 (13.6)	17 (13.2)	19 (8.1)	0.185	52 (10.8)
Mean Age, year (SD)	53.2 (13.5)	60.1 (11.8)	<0.0001	54.7 (15.0)	55.9 (12.8)	55.5 (12.9)	0.793	55.4 (13.4)
Mean Systolic blood pressure, mmHg (SD)	121 (18)	130 (22)	<0.0001	119 (22)	122 (17)	127 (20)	0.0007	124 (20)
Mean Diastolic blood pressure, mmHg (SD)	74 (12)	77 (16)	0.076	73 (18)	74 (11)	77 (12)	0.016	75 (14)
Hypertension, n (%)	220 (67.1)	118 (77.6)	0.018	64 (54.2)	93 (72.1)	181 (77.7)	<0.0001	338 (70.4)
Mean Body mass index, kg/m^2 (SD)	30.1 (7.2)	31.9 (7.2)	0.012	22.0 (2.3)	27.7 (1.4)	36.5 (5.8)	<0.0001	30.6 (7.4)
Mean Waist circumference, cm (SD)	96 (15)	103 (14)	<0.0001	81 (10)	93 (6)	109 (11)	<0.0001	98 (15)
Mean Hip circumference, cm (SD)	111 (15)	112 (16)	0.558	96 (9)	106 (6)	122 (13)	<0.0001	111 (15)
Mean Waist/hip ratio, (SD)	0.86 (0.08)	0.92 (0.08)	<0.0001	0.84 (0.07)	0.89 (0.07)	0.89(0.08)	<0.0001	0.88 (0.08)
Mean HbA1c, % (SD)	5.7 (0.4)	7.8 (1.9)	<0.0001	5.8 (0.9)	6.6 (1.8)	6.4 (1.3)	<0.0001	6.3 (1.4)
Mean Fasting blood glucose, mmol/l (SD)	5.3 (0.7)	9.5 (3.8)	<0.0001	5.9 (2.1)	7.0 (3.8)	6.8 (2.8)	0.007	6.6 (3.0)
Mean 2h glucose, mmol/l (SD)	6.8 (1.7)	13.4 (4.7)	<0.0001	7.2 (2.5)	7.7 (3.6)	8.7 (3.9)	0.0008	8.1 (3.5)
Mean Triglycerides, mmol/l (SD)	1.4 (1.0)	1.8 (0.9)	<0.0001	1.2 (1.0)	1.6 (1.1)	1.6 (0.8)		
Mean HDL cholesterol, mmol/l (SD)	1.3 (0.3)	1.2 (0.3)	0.0001	1.4 (0.4)	1.3 (0.3)	1.2 (0.3)	<0.0001	1.3 (0.3)
Mean LDL cholesterol, mmol/l (SD)	3.8 (1.0)	3.8 (1.1)	0.984	3.6 (0.9)	3.8 (1.0)	3.8 (1.0)	0.075	3.8 (1.0)
Mean Total cholesterol, mmol/l (SD)	5.7 (1.2)	5.8 (1.2)	0.582	5.5 (1.1)	5.8 (1.2)	5.8 (1.2)	0.084	5.7 (1.2)
Median Insulin mmol/l(25th-75th percentiles)	6.9 [2.9-12.2]	8.7 [2.8-14.8]	0.043	3.7 [1.5-8.0]	7.0 [3.2-11.1]	9.1 [4.9-15.0]	<0.0001	7.4 [2.9-13.2]
Median 2h insulin mmol/l(25th-75th percentiles)	39.5 [21.7-67.4]	54.9 [21.8-116.1]	0.026	28.8 [17.1-45.2]	36.7 [22.7-61.0]	56.5 [31.0-106.4]	<0.0001	
Median Glucose/insulin (25th-75th percentiles)	0.75 [0.44-1.74]	1.03 [0.53-2.71]	0.025	1.37 [0.66-3.63]	0.81 [0.53-1.95]	0.67 [0.40-1.49]	<0.0001	0.78[0.48-1.95]
Median HOMA-IR (25th-75th percentiles)	1.56 [0.60-2.91]	3.11 [1.21-5.89]	<0.0001	0.95 [0.35-2.11]	1.90 [0.80-3.35]	2.62 [1.20-4.69]	<0.0001	1.85 [0.76-3.71]
Median HOMA-B% (25th-75th percentiles)	75.4 [35.6-149.0]	34.8 [9.4-71.9]	<0.0001	43.0 [15.2-97.1]	56.2 [15.1-96.8]	75.4 [31.2-142.9]	0.001	60.0 [20.0-117.5]
Median QUICKI (25th-75th percentiles)	0.15 [0.14-0.18]	0.14[0.13-0.16]	<0.0001	0.17 [0.15-0.20]	0.15 [0.14-0.17]	0.14 [0.13-0.16]	<0.0001	0.15 [0.14-0.17]
Median FIRI (25th-75th percentiles)	1.40 [0.54-2.62]	2.80 [1.09-5.30]	<0.0001	0.85 [0.31-1.90]	1.71 [0.72-3.01]	2.36 [1.08-4.22]	<0.0001	1.66 [0.68-3.34]

Abbreviations: **CRP,** C-reactive protein; **eGFR,** estimated glomerularfiltration rate; **FIRI,** fasting *insulin* resistance index; **HbA1c,** glycated haemoglobin; **HDL** ,High Density Lipoproteins; **HOMA-B%,** functional β-cells; **GGT,** γ-glutamyltransferase; **LDL,** Low Density Lipoproteins; **HOMA-IR,** homeostatic model assessment of insulin resistance; **QUICKI,** the quantitative *insulin*-sensitivity check index; **SD,** standard deviation.

Allele distribution and association test

Table 2 shows the allele and genotype distribution of SNPs investigated in the present study. None of the SNPs investigated deviated from Hardy Weinberg Equilibrium (HWE) in the overall sample (p ≥ 0.103).
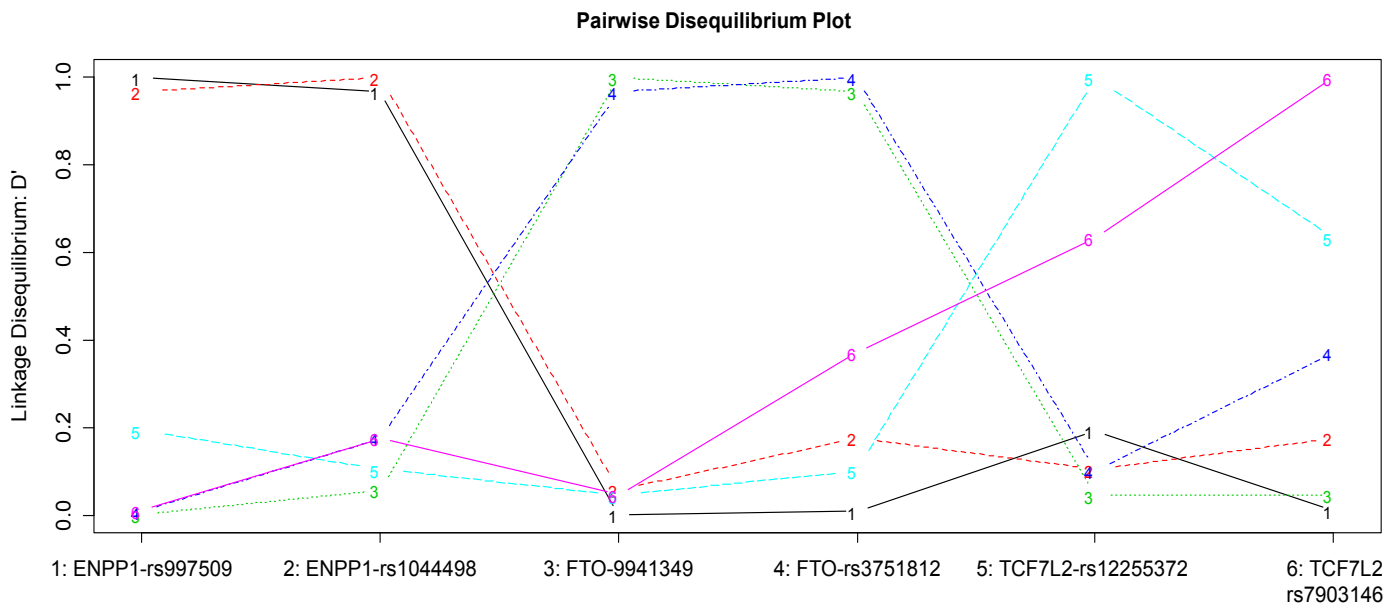
Table 2- Genotype distributions, minor allele frequencies, and unadjusted p-values for comparing genotype distributions according to diabetes and BMI status

	No diabetes	Diabetes	p-value	Normal weight	overweight	Obese	p-value	Overall
N	328	152		118	129	233		532
ENPP1-rs997509								
C/C, n (%)	254 (77.4)	121 (79.6)	0.186	97 (82.2)	94 (72.9)	184 (79.0)	0.342	375 (78.1)
C/T, n (%)	70 (21.3)	26 (17.1)		28 (15.2)	33 (25.6)	45 (19.3)		96 (20.0)
T/T, n (%)	4 (1.2)	5 (3.3)		3 (2.5)	2 (1.5)	4 (1.7)		9 (1.9)
T, n (%)	78 (11.9)	36 (11.8)	0.920	34 (14.4)	37 (14.3)	53 (11.4)	0.383	114 (11.9)
HWE (p-value)	>0.999	0.038		0.095	>0.999	0.511		0.379
ENPP1-rs1044498								
C/C, n (%)	81 (24.7)	41 (27.0)	0.548	33 (28.0)	35 (25.6)	56 (24.0)	0.852	122 (25.0)
C/A, n (%)	175 (53.4)	73 (48.0)		62 (52.5)	66 (51.2)	120 (51.5)		248 (51.7)
A/A, n (%)	72 (21.9)	38 (25.0)		23 (19.5)	30 (23.3)	57 (24.5)		110 (22.9)
A, n (%)	319 (48.6)	149 (49.0)	>0.999	108 (45.8)	126 (48.8)	234 (50.2)	0.538	468 (48.8)
HWE (p-value)	0.269	0.629		0.582	0.861	0.695		0.522
FTO-9941349								
C/C, n (%)	187 (57.0)	73 (48.0)	0.185	63 (53.4)	64 (49.6)	133 (57.1)	0.595	260 (54.2)
C/T, n (%)	118 (36.0)	66 (43.4)		44 (37.3)	56 (43.4)	84 (36.0)		184 (38.3)
T/T, n (%)	23 (7.0)	13 (8.5)		11 (9.3)	9 (7.0)	16 (6.9)		36 (7.5)
T, n (%)	164 (25.0)	92 (30.3)	0.102	66 (28.0)	74 (28.7)	116 (24.9)	0.475	256 (26.7)
HWE (p-value)	0.463	0.848		0.492	0.666	0.599		0.642
FTO-rs3751812								
G/G, n (%)	218 (66.5)	93 (61.2)	0.516	75 (63.6)	79 (61.2)	157 (67.4)	0.761	311 (64.8)
G/T, n (%)	100 (30.5)	53 (34.9)		38 (32.2)	46 (35.7)	69 (29.6)		153 (31.9)
T/T, n (%)	10 (3.0)	6 (3.9)		5 (4.2)	4 (3.1)	7 (3.0)		16 (3.3)
T, n (%)	120 (18.3)	65 (21.4)	0.299	48 (20.3)	54 (20.9)	83 (17.8)	0.530	185 (19.3)
HWE (p-value)	0.854	0.810		>0.999	0.593	>0.999		0.662
TCF7L2-rs12255372								
G/G, n (%)	221 (67.4)	92 (60.5)	0.305	72 (61.0)	88 (68.2)	153 (65.7)	0.623	313 (65.2)
G/T, n (%)	92 (28.0)	50 (32.9)		37 (31.6)	36 (27.9)	69 (29.6)		142 (29.6)
T/T, n (%)	15 (4.6)	10 (6.6)		9 (7.6)	5 (3.9)	11 (4.7)		25 (5.2)
T, n (%)	122 (18.6)	70 (23.0)	0.131	55 (23.3)	46 (17.8)	91 (19.5)	0.295	192 (20.0)
HWE (p-value)	0.200	0.364		0.197	0.552	0.403		0.115
TCF7L2-rs7903146								
C/C, n (%)	184 (56.1)	66 (43.4)	0.025	57 (48.3)	66 (51.2)	127 (54.5)	0.330	250 (52.1)
C/T, n (%)	129 (39.3)	74 (48.7)		52 (44.0)	53 (41.1)	98 (42.1)		203 (42.3)
T/T, n (%)	15 (4.6)	12 (7.9)		9 (7.6)	10 (7.7)	8 (3.4)		27 (5.6)
T, n (%)	159 (24.2)	98 (32.2)	0.011	70 (29.7)	73 (28.3)	114 (24.5)	0.275	257 (26.8)
HWE (p-value)	0.231	0.196		0.661	>0.999	0.050		0.103

Abbreviation: **ENPP1,** ectonucleotide pyrophosphatase/phosphodiesterase gene; **FTO,** fat mass and obesity-associated gene; **TCF7L2,** transcription factor 7-like 2 gene; **HWE,** Hardy-Weindberg Equilibrium (HWE p-values are from exact tests**)**

Within each gene, the SNPs were in linkage disequilibrium (LD) in all sub-groups of the study population (D' ≥ 0.614) (online Figure 1). The SNPs investigated showed no differences in the distribution of alleles and genotypes between the study population sub-groups according to T2DM and BMI status, except rs7903146. The TCF7L2-rs7903146 minor allele was prevalent in diabetic compared to non-diabetic individuals (32.2% vs. 24.2 %, p = 0.011).

Online Figure 1 – linkage disequilibrium plot (upper panel) and linkage disequilibrium table (lower panel) for the six SNP in 480 participants. *ENPP1*-**rs997509** is in linkage disequilibrium (LD) with *ENPP1*-**rs1044498** overall (D'=0.968), in participants without diabetes (D'=0.950), in those with diabetes (D'=0.999), in normal weight (D'=0.998), overweight (D'=0.999) and obese participants (D'=0.939). *FTO*-**9941349** is in linkage disequilibrium (LD) with *FTO*-**rs3751812** overall (D'=0.968), in participants without diabetes (D'=0.952) and in those with diabetes (D'>0.999), in normal weight (D'=0.937), overweight (D'=0.943) and obese participants (D'=0.999). *TCF7L2*-**rs12255372** is in linkage disequilibrium (LD) with *TCF7L2*-**rs7903146** overall (D'=0.635), in participants without diabetes (D'=0.648) and in those with diabetes (D'=0.602), in normal weight (D'=0.614), overweight (D'=0.648) and obese participants (D'=0.641).

In linear analyses adjusted for age, sex, BMI and HOMA IR (Table 3), minor allele (T) of ENPP1-rs997509 was associated with a higher risk of prevalent type 2 diabetes under a recessive model [odds ratio (95% confidence interval), 4.60 (1.07 to 19.86); p = 0.040], but with no evidence of significant statistical interaction with BMI categories. The TCF7L2-rs7903146 minor allele showed a borderline significant association with a high risk of T2DM [1.43 (1.00 to 2.04); p= 0.053] in log-additive models (Table 3).

Table 3 –logistic regression models showing the effects of genes on prevalent diabetes risk.

| SNP | Allele | Overall | | BMI categories*SNP interaction |
		Effects size (95%CI)	p	
Recessive model				
ENPP1-rs997509	T/T	4.60 (1.07 to 19.86)	0.040	0.173
ENPP1-rs1044498	A/A	1.48 (0.89 to 2.47)	0.134	0.059
FTO-9941349	T/T	1.10 (0.49 to 2.43)	0.822	0.155
FTO-rs3751812	T/T	0.85 (0.26 to 2.81)	0.937	0.374
TCF7L2-rs12255372	T/T	1.56 (0.61 to 4.00)	0.361	0.160
TCF7L2-rs7903146	T/T	1.57 (0.64 to 3.86)	0.329	0.972
Log additive model				
ENPP1-rs997509	T	0.98 (0.62 to 1.54)	0.915	
ENPP1-rs1044498	A	0.99 (0.72 to 1.36)	0.954	
FTO-9941349	T	1.40 (1.00 to 1.96)	0.052	
FTO-rs3751812	T	1.22 (0.83 to 1.80)	0.307	
TCF7L2-rs12255372	T	1.28 (0.89 to 1.83)	0.191	
TCF7L2-rs7903146	T	1.43 (1.00 to 2.04)	0.053	

Models are adjusted for age, sex, BMI and HOMA IR. Effect estimates are odd ratio and 95% confidence intervals. *Abbreviation:* **ENPP1,** ectonucleotide pyrophosphatase/phosphodiesterase gene; **FTO,** fat mass and obesity-associated gene; **TCF7L2,** transcription factor 7-like 2 gene.

Although the distribution of alleles and genotypes of the FTO-rs9941349 were not significant between the subgroups (Table 2), the minor allele was marginally significantly associated with a high risk of T2DM [1.40 (1.00 to 1.96); p = 0.052] in log-additive models (Table 3). The inferred haplotype association analysis showed a negative effect of the rs997509-rs1044498 (TA) and rs9941349- rs3751812 (CT) on the risk of T2DM, however, the haplotypes occurred at very low frequencies (Table 4).

Table 4 – Logistic regression models showing the haplotype effects of genes on prevalent diabetes risk

SNP 1	SNP 2	Haplotype	Estimated frequency (%)	Overall	
				Effects size (95%CI)	p
ENPP1-rs997509	*ENPP1*-rs1044498	CA	48.4	1 (reference)	
		CC	39.6	0.98 (0.70 to 1.38)	0.904
		TA	0.2	$7.213^{(-10)}$ [$7.213^{(-10)}$ to $7.213^{(-10)}$]	<0.0001
		TC	11.8	1.01 (0.62 to 1.65)	0.969
	Global effects				0.753
FTO-rs9941349	*FTO*-rs3751812	CG	73.1	1 (reference)	
		CT	0.5	$4.407^{(-9)}$ [$4.407^{(-9)}$ to $4.407^{(-9)}$]	<0.0001
		TG	7.6	1.52 (0.88 to 2.64)	0.135
		TT	18.8	1.34 (0.90 to 2.00)	0.150
TCF7L2-rs12255372	*TCF7L2*-rs7903146	GC	67.8	1 (reference)	
		GT	12.2	1.49 (0.91 to 2.45)	0.110
		TC	5.3	1.31 (0.64 to 2.73)	0.455
		TT	14.5	1.41 (0.90 to 2.20)	0.129

Models are adjusted for age, sex, body mass index and HOMA-IR. Effect estimates are odd ratio and 95% confidence intervals for qualitative traits. *Abbreviation: **ENPP1***, ectonucleotide pyrophosphatase/phosphodiesterase gene; ***FTO***, fat mass and obesity-associated gene; ***TCF7L2***, transcription factor 7-like 2 gene.

Discussion

In the present study we demonstrated that, in a recessive model, the minor allele of the ENPP1 rs997509 is associated with a high risk of T2DM. The presence of both ENPP1-rs997509 T alleles conferred a 7% higher risk of prevalent T2DM. However, when analysed as a haplotype with rs1044498 (TA), the direction of association changed and this effect was abolished by the presence of the rs1044498 C allele. The haplotype association should be analysed with caution due to the low frequency of the TA haplotype (0.2%). The rs1044498 minor allele failed to show any association with T2DM risk, similar to studies conducted elsewhere in other ethnic population groups[13,16]. This is in contrast to findings of other studies conducted in Europeans and Chinese[12,14] which demonstrated increased risk of T2DM among carriers of the 121QQ genotype.

The ENPP1-encoded protein inhibits insulin-signalling through interaction with and autophosphorylation of the insulin receptor beta-subunit, thus causing insulin resistance[15]. However, in the present study we did not find any significant association between ENPP1 SNPs and indicators of insulin resistance/sensitivity. Instead, in a previous study we demonstrated that the PPARG-Pro12 allele was associated with insulin resistance in this mixed ancestry cohort[21]. Like ENPP1, PPARG plays a role in insulin sensitivity. Although the role of ENPP1 SNPs in insulin resistance was not evident in the present study, combined the findings of our two studies and based on the functions of the two proteins suggest that insulin resistance rather than a defect in insulin secretion is a primary defect that may lead to the development of T2DM in this population group. Further supporting this hypothesis is the marginal association between TCF7L2 and T2DM without any effect on the measure of beta-cell function (HOMA-B%).

The two TCF7L2 polymorphisms have been reported to influence the development of T2DM across sever-

al population groups worldwide[30-32]. While this is the case in other population groups, some studies reported a weak[33,34] or no association[35-38]. Other studies highlighted the effect of the TCF7L2 polymorphisms on the progression of T2DM[35,39], in some ethnic population groups these variants were associated with responses to medication[40,41], and in others their effect on the disease was modulated by the type of diet[42-44]. Our results [1.43 (1.00 to 2.04), p= 0.053] are comparable to those reported for other African ethnic population groups[17, 18,20]. It is possible that TCF7L2 polymorphisms have different effects in various ethnic population groups depending on the diet.

Insulin resistance is often related to obesity, and several studies have demonstrated that the association of ENPP1 and FTO polymorphisms with T2DM is mediated by obesity[8,9,45,46]. However, our study failed to show any association between ENPP1, FTO and obesity. The FTO-rs9941349 was marginally associated with an increased risk of T2DM independent of obesity in our study population. The finding that the rs9941349 polymorphism is marginally associated with the risk of T2DM raises the possibility that this SNP is in linkage disequilibrium with a causal locus that is yet to be identified in mixed ancestry population. In African-Americans, this polymorphism was positively associated with BMI[47]. It is possible that the different effects of the rs9941349 are attributable to different linkage disequilibrium blocks harbouring FTO polymorphisms in these two ethnic population groups. A comprehensive analysis of FTO polymorphisms in a large population of South African mixed-ancestry is warranted to clarify their effect on T2DM and obesity.

Limitations
Our study had limitations. We investigated for the first time the role of ENPP1, FTO and TCF7L2 polymorphisms in a South African mixed ancestry ethnic population with a unique genetic architecture. However, only two SNPs in each gene were studied, and therefore we cannot rule out the possible role of other variants within these genes. Due to financial constraints, ancestry informative markers were not used to account for population admixture, and there is a possibility that population admixture interfered with the association analysis. However, when tested none of the SNPs deviated from HWE. Association of ENPP1, FTO and TCF7L2 polymorphisms with high risk of T2DM may

have also been confounded by lifestyle effects as reported elsewhere. For example, Moore and co-workers[48] showed that the significant association between ENPP1 and increased incidence of T2DM was mediated by lifestyle or metformin intervention. Ortega-Azorin and co-workers[49] also found an interaction between FTO and mediterranean diet in determining T2DM, with carriers of the FTO-rs9939609 minor allele on a low Meddiet having a higher risk of prevalent T2DM than individuals homozygous for the major allele. In a European-American population group, Mattei and co-workers[50] observed a significant interaction between the TCF7L2-rs12255372-TT risk genotype and fat intake for changes in BMI, total fat mass, and trunk fat mass at 6 month of lifestyle intervention. The study suggested that this effect may help in inducing better glycemic control for such individuals predisposed to T2DM. It is therefore possible that the marginal association between FTO and TCF7L2 polymorphisms may be strengthened by inclusion of environmental factors in the analysis, which was not possible in our study due to lack of information on these variables. Due to a small sample size, we did not match cases of T2DM and controls by BMI to investigate whether or not obesity modulates the effect of polymorphisms on T2DM.

Conclusion
Our study has conducted a genetic association of the FTO, ENPP1, and TCF7L2 polymorphisms with T2DM and related traits. We found significant association between the rs997509 SNP while the rs7903146 and rs9941349 demonstrated borderline correlation with T2DM in the South African mixed ancestry ethnic population. A genome-wide association analysis in a large sample size is required to identify polymorphisms that may predispose mixed ancestry individuals to T2DM, accounting for ancestry genetic background.

Disclosure statement
None for all co-authors.

Contribution statement: YYY: conception and design, acquisition and interpretation of data, preparation of the first draft and approval of final draft. JHM acquisition and interpretation of data, and approval of final draft. APK: analysis and interpretation of data, revision for important intellectual content and approval of final draft. RTE: acquisition of data, revision for important intellectual content and approval of final draft. TSP: revision for important intellectual content and approval of final draft. TEM: conception and design, acquisition and interpretation of data, revision for important intellectual content and approval of final draft.

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