

Variants in *PPARG*, *KCNJ11*, *TCF7L2*, *FTO*, and *HHEX* genes in South African subjects of Zulu descent with type 2 diabetes

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Abstract

Polymorphisms in a number of genes have consistently been associated with type 2 diabetes in various Caucasian populations. Little, however, is known of the association between these genetic risk markers and type 2 diabetes in sub-Saharan African subjects. The aim of the current study was to determine the association between common variants in the *PPARG*, *KCNJ11*, *TCF7L2*, *FTO* and *HHEX* genes in African (black) subjects of Zulu descent in KwaZulu-Natal, South Africa. The association between type 2 diabetes and rs1801282 (*PPARG*), rs5215 (*KCNJ11*), rs12255372 (*TCF7L2*), rs7903146 (*TCF7L2*) rs9939609 (*FTO*) and rs1111875 (*HHEX*) was determined in 178 South African Zulu subjects and 200 healthy ethnically matched control subjects.

rs1801282 (*PPARG*) and rs5215 (*KCNJ11*) were not found to be present in either the subjects with type 2 diabetes or the control subjects. No association between rs12255372 (*TCF7L2*), rs9939609 (*FTO*) and type 2 diabetes was found. Heterozygosity at rs7903146 (*TCF7L2*) was associated with type 2 diabetes (odds ratio 1.84, 95% confidence interval: 1.19–2.83, $p=0.0035$). Decreased frequency of homozygosity for the common allele at rs7903146 (*TCF7L2*) was observed in subjects with type 2 diabetes (odds ratio 0.54, 95% confidence interval: 0.34–0.84; $p=0.0043$). There was an increased frequency of C allele homozygosity in subjects with type 2 diabetes at rs1111875 (*HHEX*), of borderline significance (odds ratio 1.54, 95% confidence interval 0.97–2.44, $p=0.052$). Subjects with type 2 diabetes harbouring one or more of the risk alleles did not differ from those without genetic variation at the loci studied, with respect to age at diagnosis, blood pressure, body mass index or serum lipid levels.

We conclude that risk polymorphisms identified in Caucasian populations are not associated with type 2 diabetes in this group of South African subjects of Zulu descent, with the exception of rs7903146 (*TCF7L2*). The genetic risk for type 2 diabetes in sub-Saharan African subjects may reside in other, as yet unidentified, genes.

Introduction

The World Health Organization (WHO) Global Burden of Disease Study estimates that the number of people with diabetes in sub-Saharan Africa will rise from 7.15 million to 18.65 million by the year 2030 – an increase of 161%.¹ This massive increase in diabetes will progressively burden African health systems. Thus efforts to understand specific features of type 2 diabetes in the populations of sub-Saharan Africa, may assist in containing the burgeoning epidemic. It is widely recognised that type 2 diabetes has a complex genetic aetiology, in which a number of common genetic variants act in a coordinated fashion in conjunction with environmental factors, principally the accrual of excess body mass, to lead to clinical disease.² The clinical phenotype of type 2 diabetes in African populations has some unique characteristics, exemplified by the occurrence of ketosis-prone type 2 diabetes, predominantly in subjects of African origin.³ It is possible that these and other distinctive phenotypic features of type 2 diabetes in African populations have, at least partly, genetic origins that are different to those of Caucasian populations.

In Caucasian populations in Europe, the UK, Scandinavia and North America, a number of genetic loci have been shown to have consistent and reproducible association with type 2 diabetes.⁴ These include *PPARG*, *KCNJ11*, *PKN2*, *IGF2BP2*, *CDKAL1*, *SLC30A8*, *CDKN2A/CDKN2B*, *EXT/ALX4*, *FTO*, *TCF7L2* and *HHEX*.

There have been few studies on African populations and most of the data on genetic variants and type 2 diabetes are derived from studies in African American populations.⁵ A recent report describes the association between common variants at 12 genetic loci (*PKN2*,

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IGF2BP2, FLJ39370, CDKAL1, SLC30A8, CDKN2A/CDKN2B, EXT/ALX4, FTO, TCF7L2) and type 2 diabetes in a large group of African American subjects.⁶ Only rs7903146 (*TCF7L2*) was strongly associated with type 2 diabetes in that study. While African Americans predominantly originate from West Africa, the population in this study exhibited varying degrees of genetic admixture and thus differs from African subjects in sub-Saharan Africa. This variable admixture effect limits comparison of genetic risk for type 2 diabetes between African American and indigenous African subjects.

The current study was carried out in South African subjects of Zulu descent, living in the province of KwaZulu-Natal, to assess the role of selected common genetic variants, at loci shown to confer risk for type 2 diabetes in other populations.

Patients and methods

Subjects with type 2 diabetes (n=178) were consecutively enrolled from a tertiary Diabetes Clinic at Inkosi Albert Luthuli Central Hospital, Durban, South Africa. The diagnosis of type 2 diabetes was made according to standard clinical criteria. All subjects were treated with oral anti-diabetes medication or insulin or combination therapy. Basic anthropometric (weight, height), clinical (blood pressure) and laboratory information [serum urea, creatinine and lipids (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides), glycated haemoglobin (HbA_{1c}) and urine microalbumin/creatinine ratio] was collected from study subjects. The mean \pm SD age at diagnosis of type 2 diabetes was 45 \pm 10 years. The mean age of the type 2 diabetes subjects at enrolment into the study was 59 \pm 12 years. The mean body mass index (BMI) of study subjects was 35.8 \pm 6.5 kg/m² and the mean HbA_{1c} was 9.3 \pm 2.1%.

Control subjects (n=200) all had normal glucose tolerance during a 75 g oral glucose tolerance test. The mean age of the control subjects was 60 \pm 10 years and mean BMI 25.2 \pm 13.5 kg/m². The majority of subjects with type 2 diabetes (80%) and control subjects (78%) were female. All participants (study and control) were of Zulu descent.

Genomic DNA was extracted from whole blood by an in-house method. Variation at rs1801282 (*PPARG*), rs5215 (*KCNJ11*), rs12255372 (*TCF7L2*), rs7903146 (*TCF7L2*), rs9939609 (*FTO*) and rs1111875 (*HHEX*) was determined by allelic discrimination analysis using the Applied Biosystems Taqman chemistry on an Applied Biosystems 7500 Real-Time PCR instrument. The primer/probe set sequences for the Taqman assays were pre- or custom-designed by Applied Biosystems. Control samples representing all polymorphic genotypes were included in each 96-well plate. The Taqman Real-Time PCR assay conditions, as recommended by the manufacturer, were the same for all assays and consisted of an initial denaturation cycle (95°C for 10 min), followed by 40 amplification cycles (92°C for 15 sec, 60°C for 1 min and 60°C for 1 min). Data were analysed by the Applied Biosystems 7500 allelic discrimination software.

Single nucleotide polymorphism (SNP) determination was 100% successful in the subjects with type 2 diabetes and the control population. There was complete concordance with repeat testing.

All subjects gave written informed consent for participation in the study and approval was granted by the University of KwaZulu-Natal Bioethics Research Committee.

Differences in allele and genotype frequencies between cases and controls were calculated with Chi-squared and 2x2 tables were used to determine the odds ratios and 95% confidence intervals (Epi Info 2002). Student's t test was used to compare means of clinical and laboratory variables. A p value <0.05 was regarded as significant.

Results

The distribution of allele frequencies in subjects with type 2 diabetes and normoglycaemic control subjects is shown in Table 1. Single allele frequencies at each locus did not differ between subjects with type 2 diabetes and normoglycaemic control subjects. In particular, no variant allele was found at rs1801282 (*PPARG*) and rs5215 (*KCNJ11*) in either the study or control subjects.

Table 2 shows genotype frequencies at the loci studied, for subjects with type 2 diabetes and controls. Genotype frequencies were significantly different between subjects with type 2 diabetes and controls at SNP rs7903146 (*TCF7L2*). At this locus, homozygosity for the C allele (CC) was less frequent in the subjects with type 2 diabetes (odds ratio 0.54, 95% confidence interval 0.34–0.84, p=0.0043). Heterozygosity (CT) at rs7903146 (*TCF7L2*) occurred more frequently in the subjects with type 2 diabetes (odds ratio 1.84, 95% confidence interval 1.19–2.83, p=0.0035). No difference was found between subjects with type 2 diabetes and controls for the TT genotype at rs7903146 (*TCF7L2*). In addition, there was an increased frequency of CC homozygosity at rs1111875 (*HHEX*), but this was of borderline significance (odds ratio 1.54, 95% confidence interval 0.97–2.44, p=0.052). No significant association with type 2 diabetes was found at rs9939609 (*FTO*).

Amongst the subjects with type 2 diabetes, there was no difference in the frequency of individuals with and without a risk allele at each locus in relation to clinical and laboratory variables, including BMI, age at diagnosis, systolic or diastolic blood pressure, HbA_{1c}, prevalence of microalbuminuria, and serum lipid levels (data not shown).

This study has clearly shown that genetic risk for type 2 diabetes in a group of South African Zulu subjects differs from that described in Caucasian populations in Europe and North America. In particular, no variation at either of the European-derived susceptibility loci rs1801282 (*PPARG*) and rs5215 (*KCNJ11*) was found. An association between rs7903146 (*TCF7L2*) and type 2 diabetes was however observed, as has been reported from West African populations. A borderline association was found at rs1111875 (*HHEX*), but no association was found at rs9939609 (*FTO*).

Gene	SNP	Allele	Type 2 diabetes n=178	Control n=200	Odds ratio	95% CI	p value
<i>PPARG</i>	rs1801282	C T	1.0 0.0	1.0 0.0	–	–	–
<i>KCNJ11</i>	rs5215	G A	1.0 0.0	1.0 0.0	–	–	–
<i>TCF7L2</i>	rs12255372	G T	0.71 0.29	0.68 0.32	1.16 0.86	0.84–1.60 0.62–1.19	0.35 0.35
<i>TCF7L2</i>	rs7903146	C T	0.57 0.43	0.64 0.36	0.76 1.32	0.56–1.02 0.98–1.80	0.061 0.061
<i>FTO</i>	rs9939609	A T	0.57 0.43	0.55 0.45	0.90 1.11	0.67–1.21 0.82–1.50	0.470 0.470
<i>HHEX</i>	rs1111875	C T	0.85 0.15	0.79 0.21	1.42 0.71	0.96–2.10 0.48–1.05	0.0699 0.0699

Table 1 Allele frequencies at the studied loci in subjects with type 2 diabetes and controls (CI, confidence interval)

Gene	SNP	Genotype	Type 2 diabetes n=178	Control n=200	Odds ratio	95% CI	p value
<i>PPARG</i>	rs1801282	CC CT TT	1.0 0.0 0.0	1.0 0.0 0.0	–	–	–
<i>KCNJ11</i>	rs5215	GG GA AA	1.0 0.0 0.0	1.0 0.0 0.0	–	–	–
<i>TCF7L2</i>	rs12255372	GG GT TT	0.50 0.41 0.09	0.47 0.41 0.12	1.14 0.98 0.72	0.75–1.75 0.64–1.52 0.34–1.50	0.520 0.937 0.350
<i>TCF7L2</i>	rs7903146	CC CT TT	0.29 0.57 0.14	0.43 0.42 0.15	0.54 1.84 0.93	0.34–0.84 1.19–2.83 0.51–1.72	0.0043 0.0035 0.81
<i>FTO</i>	rs9939609	AA AT TT	0.30 0.55 0.15	0.30 0.49 0.21	0.67 1.29 0.97	0.38–1.18 0.84–1.99 0.61–1.55	0.142 0.215 0.902
<i>HHEX</i>	rs1111875	CC CT TT	0.72 0.25 0.03	0.62 0.34 0.04	1.54 0.66 0.87	0.97–2.44 0.41–1.05 0.44–1.72	0.052 0.065 0.683

Table 2 Genotype frequencies at the studied loci in subjects with type 2 diabetes and controls (CI, confidence interval)

The absence of the protective Ala12 *PPARG* allele in this African population is in keeping with an estimate that this SNP appeared in human evolution between 32 000 and 58 000 years ago after the split of populations into African and non-African.⁷ In a population-based study of 1441 African Americans, the Ala12 *PPARG* variant was identified in only 1.9% of subjects, including those with and without diabetes.⁸ Comparison of genetic markers for type 2 diabetes in African Americans and South African Zulus is, however, hampered by the variable influence of genetic admixture in African Americans, in whom European-derived genes occur at frequencies up to 22.5%.⁹ Since the Pro12 *PPARG* variant is associated with insulin resistance and increased BMI, it is possible that evolutionary pressure, based on environmental privation, facilitated propagation of the wild-type as it conferred survival advantage in previous generations. Furthermore, the risk of type 2 diabetes in South African Zulu subjects may be associated with other polymorphisms in the *PPARG* gene that have not been examined in this study.

The K variant in the E23K *KCNJ11* polymorphism has been associated with type 2 diabetes in many populations, including those in the UK, Japan and Europe.¹⁰ In the UK, the effect is modest, with an odds ratio of 1.18 (95% confidence interval 1.04–1.34, $p=0.01$). In vitro studies show that both the heterozygous and homozygous variant forms of E23K *KCNJ11* are associated with reduced ATP sensitivity thereby leading to lowered insulin secretion through over-activity of the pancreatic β -cell K_{ATP} channel.¹¹ In the present study, all 378 South African Zulu subjects were homozygous for the wild-type E allele at this locus. Thus, the K variant, found in European and Asian populations, appears to be rare or non-existent in South African Zulu subjects and is not involved in the genetic pathogenesis of type 2 diabetes in this population. Although very few other SNPs have been reported to occur in this gene, no data from South African or other African populations exist to exclude the possibility of involvement of other sites in the *KCNJ11* gene.

The *TCF7L2* gene was identified in 2006 by fine mapping of an area on chromosome 10q that demonstrated strong linkage to type 2 diabetes.¹² Since then, there has been widespread replication of the association between a number of SNPs in the *TCF7L2* gene and type 2 diabetes in many populations, mostly in Europe and North America.¹³ There are very few studies addressing the risk of *TCF7L2* and type 2 diabetes in African populations. In the recent study of Lewis and colleagues, rs7903146 (*TCF7L2*) showed strong association with type 2 diabetes in African Americans ($P_a=1.59 \times 10^{-6}$, after adjustment for admixture).⁶ Of four studies in West African populations, two (with study numbers similar to the present study), showed a significant association between the T (risk) allele at rs7903146 (*TCF7L2*) and type 2 diabetes.¹³ Combination of the data from all four West African studies (621 cases and 448 controls) showed an overall significant as-

sociation between the T allele at rs7903146 (*TCF7L2*) and type 2 diabetes, of relative risk (RR) 1.45, 95% confidence interval 1.19–1.77, $p=0.00021$, and a weaker association with the T allele at rs12255372 (*TCF7L2*), (RR 1.31, 95% confidence interval 1.01–1.69, $p=0.044$).¹³ In the present study of South African Zulu subjects, a significant association between the CT genotype at rs7903146 (*TCF7L2*) and type 2 diabetes was found. Significantly fewer subjects with type 2 diabetes harboured the CC genotype as compared to healthy controls, but the T (risk) allele was not found more frequently in subjects with type 2 diabetes. This is possibly a reflection of the relatively small numbers of subjects studied when compared to the larger numbers included in studies from Europe and North America. Notwithstanding this limitation, the present study shows that, as in other populations, rs7903146 (*TCF7L2*) conveys risk for type 2 diabetes in the South African Zulu population.

The *FTO* gene variant most widely studied (rs9939609) is located in intron 1 and is in linkage disequilibrium (LD) with a number of other SNPs within the first two introns and exon 2.^{14–16} In the present study, rs9939609 (*FTO*) failed to show any association with type 2 diabetes. Polymorphisms in the *FTO* gene have been reported to be associated with obesity.¹⁶ Despite the mean BMI of the study subjects in the present study being in the obese range, no association with BMI or waist circumference and the risk allele at this locus was found. In a study of obese African American children an association was observed with rs3751812 (*FTO*), but not with rs8050136 (*FTO*) (which is in strong LD with rs9939609), or with 11 other SNPs in the *FTO* gene.¹⁴ In contrast, in Caucasian American children, a strong association with obesity was shown for seven SNPs in the *FTO* gene, including rs8050136 and rs3751812, both of which exhibited complete LD with rs9939609 ($r^2=1$).¹⁴ In another study, none of seven SNPs at the *FTO* locus was associated with weight, hip circumference, or BMI in African Americans, but multiple SNPs were associated with each characteristic in European Americans and Hispanic Americans.¹⁵ Therefore, although the current study may have missed an association between obesity and *FTO* gene variants by not examining additional SNPs, the findings of other studies suggest the possibility that subjects of African ancestry may have different obesity-associated genetic markers.

HHEX belongs to a family of genes encoding transcription factors and appears to play a role in embryonic development of the pancreas.^{17–19} Several SNPs in the *HHEX* gene were identified in whole genome association studies and the associations confirmed in subsequent studies. Both rs1111875 (*HHEX*) and rs7923837 (*HHEX*) have been associated with type 2 diabetes in European and Japanese populations,^{20,21} but no studies have been reported in African populations. In the present study, borderline significance for an association with type 2 diabetes was observed for the CC genotype at rs1111875 (*HHEX*). The risk allele in previous studies was shown

to be C, with allele-associated odds ratio for type 2 diabetes in the region of 1.10–1.13.²² It is possible that the inclusion of bigger numbers of South Africans of Zulu descent would show a significant association with type 2 diabetes at this locus.

In conclusion, it appears, from this and similar studies, that the genetic architecture of type 2 diabetes in indigenous African populations differs from that described in Caucasian populations and raises the possibility that other, unidentified loci, may play a role in the development of the disease in African populations.

Declaration of competing interests

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References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27: 1047–53.
2. Gluiliherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; 9: 367–77.
3. Umpierrez GE, Smiley D, Kitabchi AE. Narrative review: ketosis-prone type 2 diabetes mellitus. *Ann Intern Med* 2006; 144: 350–7.
4. Barroso I. Genetics of type 2 diabetes. *Diabet Med* 2005; 22: 517–35.
5. Elbein SC. Evaluation of polymorphisms known to contribute to risk for diabetes in African and African-American populations. *Curr Opin Clin Nutr Metab Care* 2007; 10: 415–19.
6. Lewis JP, Palmer ND, Hicks PJ, et al. Association analysis of European-derived type 2 diabetes SNPs from whole genome association studies in African Americans. *Diabetes* 2008; 57: 2220–5.
7. Ruiz-Narváez E. Is the Ala12 variant of the PPARG gene an “unthrifty” allele? *J Med Genet* 2005; 42: 547–50.
8. Kao WHL, Coresh J, Shuldiner AR, et al. Pro12Ala of the peroxisome proliferator-activated receptor- γ 2 gene is associated with lower serum insulin levels in nonobese African Americans. *Diabetes* 2003; 52: 1568–72.
9. Parra EJ, Marcini A, Akey J, et al. Estimating African American admixture proportions by use of population-specific alleles. *Am J Hum Genet* 1998; 63: 1839–51.
10. Gloyn AL, Weedon MN, Owen KR, et al. Large-scale association studies of variants in genes encoding the pancreatic beta-cell K_{ATP} Channel Subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 2003; 52: 568–72.
11. Schwanstecher C, Meyer U, and Schwanstecher M. $K_{IR}6.2$ polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-Cell ATP-Sensitive K^+ Channels. *Diabetes* 2002; 51: 875–9.
12. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; 38: 320–3.
13. Helgason A, Pálsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 2007; 39: 218–25.
14. Grant SF, Li M, Bradfield JP, et al. Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. *PLoS ONE* 2008; 3: e1746.
15. Scuteri A, Sanna S, Chen WM, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007; 3: e115.
16. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007; 316: 889–94.
17. D’Elia A V, Tell G, Russo D, et al. Expression and localization of the homeodomain-containing protein HEX in human thyroid tumors. *J Clin Endocr Metab* 2002; 87: 1376–83.
18. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007; 445: 881–5.
19. Bort R, Martinez-Barbera JP, Beddington RS, Zaret KS: Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. *Development* 2004; 131: 797–806.
20. Staiger H, Stancáková, Zilinskaite J. A candidate type 2 diabetes polymorphism near the HHEX locus affects acute glucose-stimulated insulin release in European populations: results from the EUGENE2 study. *Diabetes* 2008; 57: 514–17.
21. Horikawa Y, Miyake K, Yasuda K. Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. *J Clin Endocrinol Metab* 2008; 93: 3136–41.
22. Saxena R, Voight BF, Lyssenko V, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007; 316: 1331–6.