The E23K variant in the Kir6·2 subunit of the ATP-sensitive K^+ channel does not augment impaired glucose tolerance in Caribbean subjects with a family history of type 2 diabetes

Chidum Ezenwaka, Risha Kalloo, Mathias Uhlig[1], Robert Schwenk[1] and Juergen Eckel[1]

Unit of Pathology and Microbiology, Faculty of Medical Sciences, The University of the West Indies, St Augustine, Trinidad

[1]Institute of Clinical Biochemistry and Pathobiochemistry, German Diabetes Centre, Düsseldorf, Germany

(Requests for offprints should be addressed to C E Ezenwaka; Email: ezenwaka@tstt.net.tt)

Abstract

The E23K variant of the Kir6·2 gene has been shown to be associated with type 2 diabetes mellitus in Caucasian subjects. Because offspring of type 2 diabetic patients have a genetically increased risk of developing diabetes, we sought to identify the E23K variant of the Kir6·2 gene in offspring of Caribbean patients with type 2 diabetes and assess the contribution of this variant to impaired glucose tolerance in these subjects. Forty-six offspring of patients with type 2 diabetes and 39 apparently healthy subjects whose immediate parents were not diabetic ('control') were studied after an overnight fast. Anthropometric indices were measured and blood samples were collected. Fasting and 2 h plasma glucose, insulin and lipids were subsequently determined. Insulin resistance was calculated using the homeostatic model assessment technique. The offspring and control subjects had similar frequencies of the E23K polymorphism (52·6 vs 45·5%, $P>0·05$) and the frequency of the E23K variant did not differ significantly between gender and ethnic distributions, irrespectively of a family history of diabetes ($P>0·05$). There were no significant differences in biochemical risk factors for developing diabetes in offspring carriers of the E23K variant compared with offspring non-carriers of the mutation. Offspring with the E23K mutation had even significantly higher 2 h insulin concentrations when compared with control subjects. It is concluded that the presence of the Kir6·2 E23K genotype in Caribbean subjects with an immediate positive family history of diabetes does not confer significantly higher levels of biochemical risk factors for the development of type 2 diabetes.

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Introduction

Several research studies in different populations have confirmed that offspring and first-degree relatives of patients with type 2 diabetes mellitus are at increased risk of developing the disorder in later life (Haffner *et al.* 1988, Eriksson *et al.* 1989, Osei 1990, Warram *et al.* 1990, Ezenwaka *et al.* 1993, 2001*a*,*b*, Stewart *et al.* 1995, Gaillard *et al.* 1997, Snethalatha *et al.* 1998). Although the genetic component of type 2 diabetes is well established (Barnett *et al.* 1981, Newman *et al.* 1987), research on the genes associated with diabetes is still ongoing and inconclusive. Indeed, certain genetic variants that could affect insulin secretion and glucose utilisation have been identified. For example, it has been shown that a mutation in the gene encoding the sulphonylurea receptor subunit is associated with reduced insulin secretion and type 2 diabetes ('t Hart *et al.* 2000, Reis & Velho 2002). Furthermore, recent studies in Caucasian subjects showed that the E23K variant of the Kir6·2 gene is associated with type 2 diabetes (Gloyn *et al.* 2003, Nielsen *et al.* 2003, Riedel *et al.* 2003). Mutations in the genes encoding sulphonylurea receptor and Kir6·2 have significant implications in developing diabetes, given that the two proteins play important roles in beta-cell insulin secretion (Aguilar-Bryan *et al.* 1995, Inagaki *et al.* 1995). However, it is important to note that the impact of these mutations has not been confirmed in other populations. For instance, other studies of independent populations from The Netherlands, Germany and the USA have shown that mutations in the Kir6·2 gene are not associated with beta-cell dysfunction or insulin resistance (IR) or even detectable alterations in glucose-stimulated insulin secretion (Inoue *et al.* 1997, 't Hart *et al.* 2002, Tschritter *et al.* 2002). This implies that the role of the Kir6·2 variant in the development of type 2 diabetes is still controversial and warrants further investigation in other population groups. Therefore, it appears important to conduct research on the

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Kir6·2 variant (or any other mutation) in all ethnic groups, especially in genetically predisposed individuals such as offspring of type 2 diabetic patients. Thus, we sought to identify the E23K variant of the Kir6·2 gene in Caribbean subjects and compare the levels of biochemical risk factors for developing diabetes in subjects with and without the Kir6·2 E23K mutation.

Subjects and Methods

Offspring of diabetic patients

Forty-six offspring (24 on follow-up and 22 new recruits) of patients with type 2 diabetes participated in the study. The recruitment strategy for the subjects was as previously published (Ezenwaka *et al.* 2001*a*,*b*, 2004). Briefly, posters and flyers were distributed to type 2 diabetic patients visiting two major lifestyle disease clinics in Trinidad for onward distribution to their children. Offspring of diabetic patients who expressed interest in participating were invited to our research laboratory at the Eric Williams Medical Sciences Complex (EWMSC), Trinidad, to sign consent forms and to register for a study date. All offspring were advised to continue with their routine lifestyle until the day of the test.

Control subjects

Thirty-nine (14 on follow-up and 25 new recruits) apparently healthy individuals whose immediate parents were not diabetic served as 'control' subjects and were recruited through oral or poster/flyer advertisements from within the EWMSC. Students and workers from within the EWMSC have demonstrated their understanding of the requirements of medical research and are more aware of their family medical history (Ezenwaka *et al.* 2001*a*,*b*, 2004). Similarly to the offspring of diabetic patients, controls who expressed interest in participating in the study were required to visit our research laboratory for a thorough explanation of the study protocol, and to register and sign voluntary consent forms. They were also instructed to refrain from smoking on the morning of the test and to continue with their routine lifestyle and diet before the study.

Study protocol

The study protocol was reviewed and approved by the Faculty of Medical Sciences Ethics Committee. The protocol was essentially the same as has been recently published (Ezenwaka *et al.* 2004). All subjects were studied at our laboratory after an overnight fast (10–14 h). Using a research questionnaire, information on background characteristics of the subjects was obtained and anthropometric indices were measured. Weight (kg) and height (m) (in light clothing and without shoes) was measured using a standard scale and metre rule. Using a measuring tape, waist circumference (cm) was taken at the level of the umbilicus and hip circumference (cm) at the largest projection of the buttocks. Then, a 10 ml fasting blood sample was drawn from each subject and preserved in fluoride-oxalate (for glucose estimation), EDTA (for DNA extraction) and plain tubes (for serum lipid and insulin). Subsequently, subjects orally consumed 75 g anhydrous glucose dissolved in 250 ml water over 5 min. At 2 h of the study, a 5 ml blood sample was collected for plasma glucose and serum insulin estimation. Plasma and serum specimens were separated after centrifugation within 30 min of collection and stored at −20 °C.

DNA extraction and genotyping

Genomic DNA was isolated from whole blood samples using the QIAamp DNA blood Midi kit (Qiagen, Hilden, Germany). The DNA was amplified by the PCR technique using a forward primer (5′-GAATACGTCCTGA CACGCCT-3′) and a reverse primer (5′-GCCAGC TGCACAGGAAGGACAT-3′), which flanked the region containing the Kir6·2 gene (product size, 218 bp) (Nielsen *et al.* 2003). PCR was performed in a 50 µl volume containing 500 ng genomic DNA, 10 pmol of each primer, 10 mM dNTPs, 2·5 U PfuTurbo DNA polymerase (Stratagene, Heidelberg, Germany), 10 × polymerase buffer and water. The PCR conditions were an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 71 °C for 1 min and extension at 72 °C for 1 min, with a final extension of 5 min at 72 °C. The PCR product was purified using a QIAquick PCR purification kit (Qiagen) and subsequently digested with BanII enzyme (Roche Diagnostics, Mannheim, Germany) at 37 °C for 2 h and heat inactivated for 10 min at 65 °C. The digested probes were subjected to electrophoresis on a 3% agarose gel (Biozyme, Hess Oldendorf, Germany), and stained with ethidium bromide (Roth, Karlsruhe, Germany) for visualisation (Hani *et al.* 1998, Nielsen *et al.* 2003).

In the E23K point mutation of the Kir6·2 gene, substitution of G for A in the codon for glutamic acid resulted in the codon for lysine, that is GAG→AAG. Because of this substitution, BanII restriction enzyme was unable to recognise the sequence for cutting and hence no digestion products were obtained. Restriction fragment length polymorphism (RFLP) analysis of amplified wild type (+/+) probes with BanII generated two products (178 and 40 bp). However, since the E23K mutation of the Kir6·2 gene product leads to the loss of the recognition site for BanII, the digestion of heterozygous (+/−) mutated probes generated three products (218, 170 and 40 bp) and homozygous (−/−) mutated probes generated one product (218 bp) only.

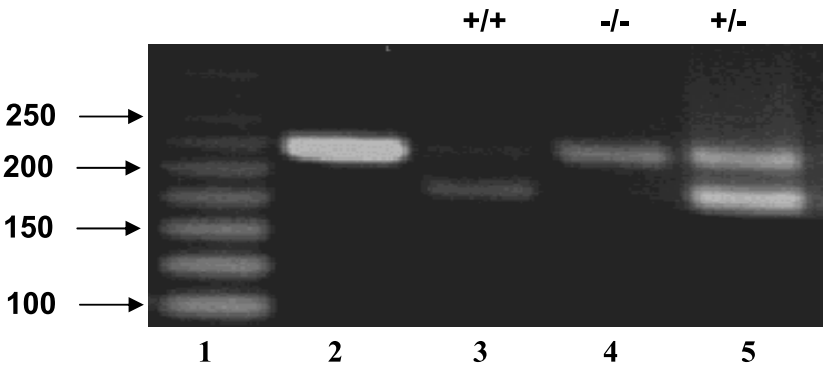

Figure 1 RFLP detection of the E23K variant of the Kir6·2 gene. (1) 25 bp ladder, (2) undigested sample – 218 bp, (3) wild type – 178 and 40 bp, (4) homozygous mutated – 218 bp, (5) heterozygous mutated – 218, 178 and 40 bp. The 40 bp fragments are not shown.

Biochemical analysis

Plasma glucose, serum triglyceride, total cholesterol and high density lipoprotein (HDL)-cholesterol concentrations were measured using enzymatic methods with commercial dry slide kits in a multi-channel auto-analyser (Johnson & Johnson Vitros 250; Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA). Low density lipoprotein (LDL)-cholesterol was calculated using the Friedwald equation (Friedwald *et al.* 1972). The serum insulin level was determined by ELISA using a Mercodia insulin ELISA kit (Mercodia AB, Uppsala, Sweden).

Statistics and calculations

The results are expressed as means ± S.E. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software was used in all analyses. IR, defined as the product of fasting serum insulin and plasma glucose divided by 22·5, was assessed using fasting serum insulin and plasma glucose concentrations in a homeostasis model assessment (Matthews *et al.* 1985). Comparisons of the mean differences in biochemical parameters in different sub-groups of subjects were performed using Student's *t*-tests while chi-square was used for non-parametric tests. A P value $<0{\cdot}05$ was considered statistically significant on two-tailed testing for all analyses.

Results

Figure 1 shows the RFLP detection of the E23K variant of the Kir6·2 gene. Of the 46 offspring of diabetic patients and 39 control subjects who participated in the study, PCR products could not be isolated in eight (17·4%) offspring and six (15·4%) control subjects. The anthropometric and biochemical parameters of these subjects (eight offspring and six controls) were excluded in the data analysis. Table 1 shows the distribution of Kir6·2 E23K variants in the 38 offspring of diabetic patients and 33 control subjects of which PCR products were isolated. The offspring and control subjects had similar frequencies of the E23K polymorphism (52·6 vs 45·5%, $P>0{\cdot}05$) and

Table 1 Ethnicity- and gender-related distribution of Kir6·2 E23K variants in offspring and control subjects

	Offspring genotype			Controls genotype		
	E23E (+/+)	E23K (+/−)	K23K (−/−)	E23E (+/+)	E23K (+/−)	K23K (−/−)
All (%)	18 (47·4)	20 (52·6)	0	17 (51·5)	15 (45·5)	1 (3·0)
Ethnicity						
African (%)	4 (22·2)	5 (25·0)	0	7 (41·2)	5 (33·3)	0
East Indian (%)	12 (66·7)	9 (45·0)	0	6 (35·3)	5 (33·3)	1 (100)
Mixed (%)	2 (11·1)	6 (30·0)	0	4 (23·5)	5 (33·3)	0
Gender						
Male (%)	8 (44·4)	7 (35·0)	0	6 (35·3)	9 (60·0)	1 (100)
Female (%)	10 (55·6)	13 (65·0)	0	11 (64·7)	6 (40·0)	0

Table 2 Selected biochemical parameters (mean ± S.E.) of all subjects with E23E and E23K genotypes

	Kir6·2 genotype	
	E23E (+/+) (*n*=35)	E23K (+/−) (*n*=35)
Parameter		
Sex (m/f)	14/21	16/19
Age (years)	28·0 ± 1·3	29·0 ± 1·4
BMI (kg/m^2)	25·6 ± 0·9	25·7 ± 0·9
Waist circumference (cm)	86·1 ± 2·2	85·8 ± 2·0
Fasting serum insulin (mIU/l)	9·2 ± 0·7	9·9 ± 0·8
2 h serum insulin (mIU/l)	84·7 ± 11·5	74·2 ± 10·3
Fasting plasma glucose (mmol/l)	4·7 ± 0·9	5·0 ± 0·1
2 h plasma glucose (mmol/l)	6·2 ± 0·4	6·3 ± 0·4
Fasting triglyceride (mmol/l)	1·4 ± 0·1	1·3 ± 0·1
Fasting total cholesterol (mmol/l)	4·5 ± 0·1	4·7 ± 0·2
Fasting HDL-cholesterol (mmol/l)	1·0 ± 0·1*	1·2 ± 0·1
Fasting LDL-cholesterol (mmol/l)	3·2 ± 0·1	3·2 ± 0·2
IR (pmol/mol/l)	14·3 ± 1·2	15·8 ± 1·3

*P<0·05 for comparison between E23E and E23K subjects.

the frequency of this variant did not differ significantly between gender and ethnic distributions, irrespectively of the family history of diabetes (Table 1, P>0·05). With the exception of plasma HDL-cholesterol concentration, there were no significant differences in biochemical risk factors for developing diabetes in all subjects with the heterozygous gene (E23K) mutation compared with carriers of the wild type gene (E23E) (Table 2, all P>0·05). After an oral glucose tolerance test (OGTT), control subjects with the E23K variant showed a tendency towards lower 2 h insulin concentrations than control carriers of the wild type (E23E), which did not reach statistical significance. However, offspring with the E23K mutation had significantly higher 2 h insulin and 2 h glucose concentrations compared with corresponding control subjects (Table 3, P<0·05). Importantly, the fasting and 2 h plasma glucose levels in offspring and control carriers of the E23K variant were not significantly higher compared with their respective wild type subjects. Furthermore, sub-analysis of the data showed that offspring carriers and non-carriers of the E23K variant had similar levels of fasting insulin and glucose, and 2 h insulin and glucose after 5 years of follow-up (Table 4, P>0·05).

Discussion

The present study identified the Kir6·2 E23K mutation in Caribbean subjects with and without an immediate family history of diabetes, and subsequently demonstrated that carriers and non-carriers of the E23K mutation had similar levels of glucose and insulin concentrations before and after an OGTT.

The identification of the Kir6·2 E23K mutation in Caribbean subjects with and without an immediate positive family history of diabetes is interesting. Trinidad and Tobago is a small country (population 1·3 million) with diabetes prevalence rate between 16 and 20% in its two major (people of African and East Indian descents) ethnic groups (Miller *et al.* 1996, Central Statistical Office 1998). It has been projected that by the year 2010, about 89 000 people in Trinidad and Tobago will be diagnosed with type 2 diabetes (Amos *et al.* 1997). Thus, research on susceptible genes that may increase the risk of developing

Table 3 Comparison of anthropometric and biochemical parameters (mean ± S.E.) of offspring and control subjects according to their Kir6·2 genotype

	Offspring (with immediate FH of diabetes)		Controls (without immediate FH of diabetes)	
	E23E (+/+) (*n*=18)	E23K (+/−) (*n*=20)	E23E (+/+) (*n*=17)	E23K (+/−) (*n*=15)
Parameter				
Sex (m/f)	8/10	7/13	6/11	9/6
Age (years)	32·1 ± 1·8	33·0 ± 1·9¶¶	25·3 ± 1·4#	23·7 ± 1·2
BMI (kg/m^2)	26·5 ± 1·4	26·9 ± 1·3	24·5 ± 1·2	24·2 ± 1·0
Waist circumference (cm)	89·4 ± 3·3	88·6 ± 2·8	82·6 ± 2·8	82·1 ± 2·9
Fasting serum insulin (mIU/l)	8·8 ± 1·0	9·9 ± 1·0	9·7 ± 0·9	9·9 ± 1·4
2 hour serum insulin (mIU/l)	91·4 ± 16·6	92·6 ± 16·4¶	77·1 ± 16·2	49·8 ± 6·3
Fasting plasma glucose (mmol/l)	4·9 ± 0·1	5·1 ± 0·2	4·5 ± 0·1#	4·8 ± 0·1+
2 hour plasma glucose (mmol/l)	7·0 ± 0·6	7·2 ± 0·5¶	5·4 ± 0·3#	5·1 ± 0·3
Fasting triglyceride (mmol/l)	1·6 ± 0·2	1·3 ± 0·1	1·2 ± 0·2	1·2 ± 0·2
Fasting total cholesterol (mmol/l)	4·6 ± 0·2	4·8 ± 0·2	4·5 ± 0·2	4·5 ± 0·3
Fasting HDL-cholesterol (mmol/l)	1·0 ± 0·04*	1·2 ± 0·1	1·0 ± 0·1	1·3 ± 0·1
Fasting LDL-cholesterol (mmol/l)	3·2 ± 0·2	3·4 ± 0·2	3·2 ± 0·2	3·0 ± 0·3
IR (pmol/mol/l)	14·5 ± 2·1	16·1 ± 1·7	14·2 ± 1·4	15·5 ± 2·2

FH, family history of diabetes; *P<0·05 for differences between +/+ offspring and +/− offspring; +P<0·05 for differences between +/+ 'controls' and +/− 'controls'; #P<0·05 for differences between offspring and 'control' subjects with +/+ genotype; ¶P<0·05, ¶¶P<0·01 for differences between offspring and 'control' subjects with +/− genotype.

Table 4 Similarities in insulin and glucose levels (mean ± S.E.) of offspring carriers and non-carriers of the E23K variant after 5 years of follow-up

	Offspring after 5 years of follow-up	
	E23E (+/+) (n=5)	E23K (+/−) (n=18)
Parameter		
Fasting serum insulin (mIU/l)	10·7 ± 2·4	10·0 ± 1·1
2 hour serum insulin (mIU/l)	111·8 ± 49·5	90·9 ± 18·2
Fasting plasma glucose (mmol/l)	4·9 ± 0·2	5·0 ± 0·2
2 hour plasma glucose (mmol/l)	7·3 ± 0·9	6·9 ± 0·5
Fasting triglyceride (mmol/l)	2·2 ± 0·3	1·3 ± 0·1**
Fasting total cholesterol (mmol/l)	5·1 ± 0·4	4·8 ± 0·2
Fasting HDL-cholesterol (mmol/l)	0·9 ± 0·1	1·1 ± 0·1
Fasting LDL-cholesterol (mmol/l)	3·7 ± 0·4	3·4 ± 0·2
IR (pmol/mol/l)	17·6 ± 4·7	16·0 ± 1·8

**P<0·01 for differences between +/+ offspring and +/− offspring.

diabetes is warranted. Although the E23K variant is widespread and has been identified in many populations (Inoue *et al.* 1997, 't Hart *et al.* 2002, Tschritter *et al.* 2002, Gloyn *et al.* 2003, Nielsen *et al.* 2003, Riedel *et al.* 2003), the finding of this mutation in 46–53% of subjects studied suggests that further investigation in larger cohorts is necessary in this population. Previous studies in other populations showed that the E23K polymorphism is associated with impaired glucose-induced insulin release (Gloyn *et al.* 2003, Nielsen *et al.* 2003, Riedel *et al.* 2003) and a trend towards this functional effect of the E23K mutation was only seen in our control subjects who have the E23K mutation, but not in offspring of diabetic patients (Table 3). However, recent evidence suggests that the impact of the E23K variant in beta-cell dysfunction and glucose metabolism is not similar in all populations (Inoue *et al.* 1997, 't Hart *et al.* 2002, Tschritter *et al.* 2002). Thus, the role of E23K polymorphism in developing diabetes remains controversial, especially as environmental factors, such as lifestyle and obesity, are relevant in the interpretation of the effect of gene mutation in different populations. For instance, sub-analysis of our data did not show any significant differences in the biochemical risk factors for developing diabetes in obese and non-obese subjects with the E23K variants (data not shown), while a previous study showed that the E23K polymorphism is associated with a significant increase in body mass index (BMI) (Nielsen *et al.* 2003).

Furthermore, our finding that the E23K polymorphism does not increase impaired glucose tolerance in carriers of the E23K genotype is consistent with previous reports (Inoue *et al.* 1997, 't Hart *et al.* 2002, Tschritter *et al.* 2002), and appears to suggest that the E23K variant does not essentially confer increased biochemical risk in persons with the mutation. This is in apparent contrast with the reports of Nielsen *et al.* (2003) and Reidel *et al.* (2003) in Caucasian subjects. Although the E23K gene was not identified in all the offspring of diabetic patients, there is no evidence in our study suggesting that carriers of this mutation have an increased biochemical risk for developing diabetes compared with non-carriers. However, the observed trend (P>0·05) towards a relative fasting and postprandial hyperglycaemia and hyperinsulinaemia in carriers of the E23K gene mutation could be explained by the apparently non-significantly higher IR levels (Table 3), which is consistent with our previous reports on the offspring of diabetic patients (Ezenwaka *et al.* 2001*a*,*b*). This is further supported by the results of the sub-analysis of offspring of diabetic patients on follow-up, which showed that carriers and non-carriers of the E23K variant had similar levels of glucose and insulin (Table 4). Therefore, there is no clear evidence in the current study to suggest that the presence of the E23K gene increases the biochemical risk of developing diabetes. It should, however, be noted that the number of subjects in the present study is relatively small and this might have reduced the statistical power of the tests. We intend to expand the present study to include subjects with overt type 2 diabetes with the aim of identifying the frequency of the E23K gene polymorphism in persons already living with diabetes. It is possible that such an enlarged study, with stronger statistical power, might be able to provide clearer evidence of the impact of the E23K variant in the antecedent biochemical markers for developing diabetes. In the meantime, the present results have demonstrated that the presence of the Kir6·2 E23K genotype in Caribbean subjects with an immediate or remote positive family history of diabetes did not increase impaired glucose tolerance in the subjects studied.

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References

Aguilar-Bryan L, Nicholas CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, Gonzalez G, Herera-Sosa H, Nguy K, Bryan J & Nelson DA 1995 Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* **268** 423–426.

Amos AF, McCarty DJ & Zimmet P 1997 The rising global burden of diabetes and its complications, estimates and projections to the year 2010. *Diabetic Medicine* **14** S7–S85.

Barnett AH, Effo C, Leslie RD & Pyke DA 1981 Diabetes in identical twins: a study of 200 pairs. *Diabetologia* **20** 87–93.

Central Statistical Office Republic of Trinidad and Tobago 1998 *Office of the Prime Minister. Population and Housing Census vol II: Age Structure, Religion, Ethnic Group, Education,* Port of Spain: Central Statistical Office.

Eriksson JE, Franssila KA, Ekstrand CS, Widen W & Schalin C 1989 Early metabolic defects in persons at increased risk for non-insulin dependent diabetes mellitus. *New England Journal of Medicine* **320** 337–343.

Ezenwaka CE, Akanji AO, Osei K, Adejuwon CA, O'Dorisio TM, Cotrell DA & Akinlade KS 1993 Glucose and insulin responses to intravenous glucose challenge in relatives of Nigerian patients with non-insulin dependent diabetes mellitus. *Diabetes Research and Clinical Practice* **20** 175–181.

Ezenwaka CE, Davis G & Offiah NV 2001*a* Evaluation of features of syndrome X in offspring of Caribbean patients with type 2 diabetes. *Scandinavian Journal of Clinical and Laboratory Investigation* **61** 19–26.

Ezenwaka CE, Davis G & Offiah NV 2001*b* Insulin secretion in glucose-tolerant offspring of type 2 diabetes patients in Trinidad, West Indies. *Southern Medical Journal* **94** 223–228.

Ezenwaka CE, Kalloo R, Uhlig M & Eckel J 2004 Relationship between adiponectin and metabolic variables in Caribbean offspring of patients with type 2 diabetes mellitus. *Hormone and Metabolic Research* **36** 238–242.

Friedwald WT, Levy RI & Fredrickson DS 1972 Estimation of the concentration of low-density lipoprotein in plasma without use of preparative ultracentrifuge. *Clinical Chemistry* **18** 499–502.

Gaillard TR, Schuster DP, Bossetti BM, Green PA & Osei K 1997 The impact of socioeconomic status on cardiovascular risk factors in African-Americans at high risk for type II diabetes: implications for syndrome X. *Diabetes Care* **20** 745–752.

Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S *et al.* 2003 Large-scale association studies of variants in genes encoding the pancreatic B-cell K_{ATP} channel subunits Kir6·2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* **52** 568–572.

Haffner SM, Stern MP, Hazuda HP, Mitchel BD & Patterson JK 1988 Increased insulin concentration in non-diabetic offspring of diabetic parents. *New England Journal of Medicine* **319** 1297–1301.

Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G & Froguel P 1998 Missense mutations in the pancreatic β-cell inwardly rectifying K^+ channel gene (Kir6·2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. *Diabetologia* **41** 1511–1515.

't Hart LM, Dekker JM, Van Haeften TW, Ruige JB, Stehouwer CD, Erkelens DW, Heine RJ & Maassen JA 2000 Reduced second phase insulin secretion in carriers of sulphonylurea receptor gene variant associating with type II diabetes mellitus. *Diabetologia* **43** 515–519.

't Hart LM, van Haeften TW, Dekker JM, Bot M, Heine RJ & Maassen JA 2002 Variations in insulin secretion in carriers of the E23K variant in the Kir6·2 subunit of the ATP-sensitive K^+ channel in the B-cell. *Diabetes* **51** 3135–3138.

Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S & Bryan J 1995 Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270** 1166–1170.

Inoue H, Ferrer J, Warren-Perry M, Zhang Y, Millns H, Turner RC, Elbein SC, Hampe CL, Suarez BK, Inagaki N *et al.* 1997 Sequence variants in the pancreatic islet B-cell inwardly rectifying K^+ channel Kir6·2 (Bir) gene: identification and lack of role in Caucasian patients with NIDDM. *Diabetes* **46** 502–507.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC 1985 Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28** 412–419.

Miller GJ, Maude GH & Beckles GLA 1996 Incidence of hypertension and non-insulin dependent diabetes mellitus and associated risk factors in a rapidly developing Caribbean community: the St James survey, Trinidad. *Journal of Epidemiology and Community Health* **50** 497–504.

Newman B, Sleby JV, King MC, Slemenda C, Fabsitz P & Friedman CD 1987 Concordance for type 2 (NIDDM) diabetes in male twins. *Diabetologia* **30** 763–768.

Nielsen EMD, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer C, Thorsteinsson B, Borch-Johnsen K, Hansen T & Pedersen O 2003 The E23K variant of Kir6·2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* **52** 573–577.

Osei K 1990 Increased basal glucose production and utilisation in non-diabetic first-degree relatives of patients with NIDDM. *Diabetes* **39** 597–601.

Reis AF & Velho G 2002 Sulfonylurea receptor-1 (SUR1): genetic and metabolic evidences for a role in the susceptibility to type 2 diabetes mellitus. *Diabetes and Metabolism* **28** 14–19.

Riedel MJ, Boora P, Steckley D, deVries G & Light PE 2003 Kir6·2 polymorphisms sensitize B-cell ATP-sensitive potassium channels to activation by acyl CoAs: a possible cellular mechanism for increased susceptibility to type 2 diabetes. *Diabetes* **52** 2630–2635.

Snethalatha C, Ramachandran A, Satyavani K, Latha E & Viswanathan V 1998 Study of genetic prediabetic South Indian subjects: importance of hyperinsulinemia and B-cell dysfunction. *Diabetes Care* **21** 76–79.

Stewart MW, Humphriss DB, Berrish TS, Barriocanal LA, Trajano LR, Alberti KG & Walker M 1995 Features of syndrome X in first-degree relatives of NIDDM patients. *Diabetes Care* **18** 1020–1022.

Tschritter O, Stumvoll M, Machicao F, Holzwarth M, Weisser M, Maerker E, Teigeler A, Haring H & Fritsche A 2002 The prevalent Glu23 Lys polymorphism in the potassium inward rectifier 6·2 (Kir6·2) gene is associated with impaired glucagon suppression in response to hyperglycemia. *Diabetes* **51** 2854–2860.

Warram JH, Blaise CM, Andrez S, Soeldner JS & Kahn CR 1990 Slow glucose removal rate and hyperinsulinaemia precede the development of type II diabetes in the offspring of diabetic parents. *Annals of Internal Medicine* **113** 909–915.

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