Association of the KCNJ11 Variant E23K with Type 2 Diabetes in Indo-Trinidadians

LG Boodram[1,2], K Miyake[2], MG Hayes[3], GI Bell[2], BN Cockburn[1,2]

ABSTRACT

Objective: *To examine the effect of genetic variation in* KCNJ11 *on the risk of Type 2 diabetes mellitus in Trinidadians.*

Methods: *The coding and bordering intron-exon regions of the* KCNJ11 *gene were sequenced in 168 diabetic and 61 non-diabetic subjects who historically were thought to be of South Asian Indian ancestry, as well as 66 diabetic and 59 non-diabetic subjects of African ancestry. Allele and haplotype frequency differences were calculated between cases and controls and linkage equilibrium was assessed across the* KCNJ11 *region.*

Results: *We identified novel missense mutations in both subject groups including A94P and R369C in a diabetic Indo-Trinidadian subject, S113G in a non-diabetic Indo-Trinidadian subject, and S118L in a diabetic Afro-Trinidadian subject. It is unknown if these mutations are pathogenic as other family members were not available for study. Additionally, the common variant E23K was associated with Type 2 diabetes in the Indo-Trinidadian group (OR = 1.797 [1.148–2.814], p = 0.0098).*

Conclusions: *Rare variants in* KCNJ11 *are segregating in the Indo- and Afro-Trinidadian populations and further studies are needed to determine their contribution, if any, to the overall prevalence of diabetes in these groups. Common variants such as E23K may increase the risk in the Indo-Trinidadian population.*

Keywords: KCNJ11, E23K, Type 2 diabetes, Indo-Trinidadian

La Asociación de la Variante E23K de KCNJ11 con la Diabetes de Tipo 2 en Indo-Trinitenses

LG Boodram[1,2], K Miyake[2], MG Hayes[3], GI Bell[2], BN Cockburn[1,2]

RESUMEN

Objetivo: *Examinar el efecto de la variación genética en* KCNJ11 *sobre el riesgo de la diabetes tipo 2 en trinitenses.*

Métodos: *Las regiones codificantes y las regiones de la frontera intrón-exón del gen KCNJ11 fueron secuenciadas en 168 sujetos diabéticos y 61 no diabéticos – históricamente de ascendencia del sur de la India – así como 66 sujetos diabéticos y 59 no diabéticos, de ascendencia africana. Se calcularon las diferencias de la frecuencia de los aleles y los haplotipos entre los casos y los controles, evaluándose asimismo el equilibrio de ligamiento de la región de* KCNJ11.

From: [1]Department of Life Sciences, Faculty of Science and Agriculture, The University of the West Indies, Trinidad and Tobago, [2]Department of Medicine, The University of Chicago, Illinois, USA, and [3]Division of Endocrinology, Metabolism and Molecular Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA.

Correspondence: Dr LG Boodram, Department of Life Sciences, Faculty of Science and Agriculture, The University of the West Indies, St Augustine, Trinidad and Tobago. E-mail: 1boodram@hotmail.com, boodrala@carec.paho.org

Resultados: *Se identificaron novedosas mutaciones de sentido erróneo en ambos grupos de sujetos, incluyendo A94P y R369C en un sujeto indo-trinitense diabético, y S118L en un sujeto afro-trinitense diabético. No se sabe si estas mutaciones son patogénicas ya que no se disponía de otros miembros de la familia para estudiar el caso. Además, la variante E23K estaba asociada con la diabetes tipo 2 en el grupo indo-trinitense (OR = 1.797 (1.148–2.814),* p = *0.0098).*

Conclusiones: *Variantes raras en el* KCNJ11 *se están segregando en las poblaciones indo – y afro-trinitenses, y se requieren estudios ulteriores para determinar si de algún modo contribuyen a la prevalencia general de la diabetes en estos grupos. Las variantes comunes como la E23K pueden aumentar el riesgo en la población de indo-trinitense.*

Palabras claves: KCNJ11, E23K, diabetes tipo 2, indo-trinitenses

West Indian Med J 2011; 60 (6): 605

INTRODUCTION

The ATP-sensitive potassium channel (K_{ATP}) plays a critical role in insulin secretion by coupling glucose metabolism to electrical activity of the cells (1). The β-cell K_{ATP} comprises two different types of subunits, an ATP-sensitive, inwardly rectifying K^+ channel (Kir 6.2) subunit (2), and a sulphonylurea receptor (SUR1) subunit (3). Rare mutations in the genes encoding these two proteins *(KCNJ11* and *ABCC8,* respectively) can result in hyper- as well as hypoglycaemia (4) and common variants have been associated with Type 2 diabetes (5). The significant linkage disequilibrium (LD) across the region of the adjacent *ABCC8* and *KCNJ11* genes has made it difficult to attribute clear diabetes susceptibility risk to common variants in either gene (4, 5).

In this study, we sequenced *KCNJ11* in unrelated Indo- and Afro-Trinidadian subjects with early-onset (age < 40 years) Type 2 diabetes mellitus as well as in non-diabetic controls. We report the replication of a positive association of the E23K variant with Type 2 diabetes in the Indo-Trinidadian population as well as the discovery of novel synonymous and non-synonymous variants in both groups.

SUBJECTS AND METHODS

Subjects in this study were recruited as previously described (6). Study approval and informed consent were obtained from the local hospital ethics committees and participating subjects respectively. Briefly, the study population consisted of 234 early-onset diabetic (age of disease onset < 40 years) and 120 non-diabetic subjects who were of African Ancestry and those historically considered to be of South Asian Indian ancestry. The clinical characteristics of this population are shown in Table 1.

Mutation screening

KCNJ11 in both cases and controls was screened for mutations and common variants by DNA sequencing. The coding region and the intron–exon boundaries of *KCNJ11* were amplified from genomic DNA by PCR with the use of previously described primers (5) and the sense strand of the PCR product sequenced using an ABI PRISM 3730 DNA

Table 1: Clinical characteristics of study population

	Indo-Trinidadians		**Afro-Trinidadians**	
Male/female ratio	**Diabetic (n = 168) 46/123**	**Control (n = 37) 15/22**	**Diabetic (n = 66) 13/53**	**Control (n = 35) 11/24**
Age (years)	45.11 ± 12.03	51.68 ± 17.22	49.11 ± 12.35	44.53 ±16.91
Age of diagnosis (years)	30.29 ± 8.43	–	32.5 ± 11.2	–
BMI (kg/m^2)	26.05 ± 4.47	*25.08 ± 5.03*	29.29 ± 6.10	23.99 ± 6.91
Initial treatment				
Diet/OHA/Insulin/Insulin + OHA	11/128/30/0		1/51/6/8	
Present treatment:				
Diet/OHA/Insulin/Insulin + OHA	0/54/108/7		0/11/44/11	
Retinopathy (%)	21		26	
Proteinuria (%)	13		12	
Neuropathy (%)	34		49	
Foot ulcers (%)	5		6	
Stroke (%)	4		0	
Peripheral vascular disease (%)	33		57	
Hypertension (%)	41		37	

Data for quantitative variables are mean ± SD. BMI: body mass index; OHA: oral hypoglycaemia agent. Note that complete clinical data were not available for all control subjects screened for mutations.

sequencer (Applied Biosystems, Foster City, CA). Novel variants were confirmed by sequencing the antisense strand.

Statistical analysis

Differences in the allele frequency between cases and controls, and departures from Hardy-Weinberg equilibrium (HWE), were assessed with standard asymptotic χ^2 tests or Fisher's exact tests when cell counts were less than five. Odds ratios for SNPs with rare alleles were calculated using Finetti (7). Given the multiple testing issues of conducting tests on 11–12 SNPs in each of two populations, the significance of the association tests was assessed through permutation in Haploview 4.0 (8). Linkage disequilibrium across the *KCNJ11* (measured in terms of D′ and r^2) and the estimation of haplotype frequencies, were also calculated using Haploview.

Table 2: Association of *KCNJ11* polymorphisms with Type 2 diabetes in Indo-Trinidadians

dbSNP	Postion[1]	nt[2]	Codon[3]	Amino acid change	Label	Maj/ Min	Genotype count						Allele frequency				p_a[4]	p_e[5]	OR[6]	95% CI[7]
							Cases (n = 168)			Controls (n = 61)										
							Maj/ Maj/	Maj/ Min	Min/ Min	Maj/ Maj/	Maj/ Min	Min/ Min	Maj	Min	Maj	Min				
rs5219	17366148	67	23	GAG (Glu > AAG (Lys)	E23K	G/A	55	85	28	28	31	2	0.580	0.420	0.713	0.287	0.010	0.050	1.797	(1.148 – 2.814)
	17366107	280	94	GCC (Ala) GGC (Pro)	A94P	C/G	167	0	1	61	0	0	0.994	0.006	1.000	0.000	0.393	1.000	1.831	(0.087 – 38.410)
	17365906	309	103	AGC (Ser) > AGT (Ser)	S103S	C/T	165	3	0	61	0	0	0.991	0.009	1.000	0.000	0.295	0.958	2.571	(0.132 – 50.139)
	17365878	337	113	AGC (Ser) > GGC (Gly)	S113G	A/G	168	0	0	60	1	0	1.000	1.000	0.992	0.008	0.097	0.800	0.120	(0.005 – 2.974)
rs5218	17365645	57	190	GCC (Ala) > GCT (Ala)	A190A	C/T	128	39	1	46	15	0	0.878	0.122	0.877	0.123	0.979	1.000	0.991	(0.527 – 1.864)
rs5216	17365414	801	267	CTC (Leu) > CTG (Leu)	L267L	C/G	160	7	1	59	2	0	0.973	0.027	0.984	0.016	0.521	1.000	1.651	(0.357 – 7.632)
rs1800467	17365407	808	270	CTG (Leu) > GTG (Val)	L270V	C/G	166	2	0	61	0	0	0.994	0.006	1.000	0.000	0.393	1.000	1.831	(0.087 – 38.410)
rs5215	17365206	1009	337	ATC (Ile) > GTC (Val)	1337V	A/G	54	86	28	28	31	2	0.577	0.423	0.713	0.287	0.008	0.045	1.819	(1.166 – 2.839)
	17365156	1059	353	CAC (His) > CAT (His)	H353H	C/T	167	1	0	61	0	0	0.997	0.003	1.000	0.000	0.546	1.000	1.095	(0.037 – 22.560)
	17365110	1105	369	CGC (Arg >) TGC (Cys)	R369C	C/T	167	1	0	61	0	0	0.997	0.003	1.000	0.000	0.546	1.000	1.095	(0.037 – 22.560)
	17364977	1238	c.* + 65[3]		c.* + 65A > G	A/G	54	86	28	28	31	2	0.577	0.423	0.713	0.287	0.008	0.045	1.819	(1.166 – 2.839)

[1] Position in db SNP Build 35; [2] Nucleotide position from start ATG at 17366783; [3] c.*+65A>G denotes an A to G substitution 65 nucleotides 3' of the translation termination codon;
[4] Asymptotic *p*-value from χ^2 test; [5] Empiric *p*-value from permutations; [6] Odds ratio for the minor allele; [7] Confidence interval of the odds ratio

RESULTS

We identified a total of 19 SNPs of varying frequencies in the study population. Common variants such as E23K, A190A, I337V and c.*+65A>G were detected in both Indo- and Afro-Trinidadians, however, in all instances, the minor alleles were found at higher frequencies in the Indo- than Afro-Trinidadian group (Tables 2 and 3). Rare variants were also identified including four not previously described: A94P, S113G, S118L and R369C. The missense mutations, A94P and R369C, were detected in diabetic Indo-Trinidadian subjects, and S113G was found in a non-diabetic Indo-Trinidadian subject with no family history of diabetes. The variant S118L was found in a diabetic Afro-Trinidadian subject. Other family members were not available for genotyping and it is unknown if A94P, S118L and R369C co-segregate with diabetes and thus could be pathogenic.

Two SNPs showed departure from HWE in the Indo-Trinidadian cases (L267L, $p = 0.009$; A94P, $p = 0.003$). The E23K polymorphism was associated with Type 2 diabetes in the Indo-Trinidadian (OR = 1.797 [1.148, 2.814], $p = 0.0098$). In the Indo-Trinidadian diabetic group, there is considerable LD across the three associated SNPs ($r^2 > 0.97$ for all three pairwise comparisons of E23K, I337V, and c.*+65A>G) such that these SNPs each generate significant associations ($p = 0.0098, 0.0084$, and 0.0084, respectively) which are not independent of one another. For all these variants, a greater risk appeared to be associated with the minor allele, and the associations appear robust to multiple testing issues as they remain significant at $p \leq 0.05$ when assessed empirically through permutations. No significant associations were found among the Afro-Trinidadian population, although power calculations suggest we do not have sufficient power (< 10%) to detect even a nominally significant association due to the small sample size and low minor allele frequency. The LD detected across these three primary SNPs was somewhat lower in the Afro-Trinidadian diabetic ($r^2 = 0.70$) and control groups ($r^2 = 0.72$). Given the robustness of the associations and the pattern of high LD across the gene, we investigated whether the combination of risk alleles at each of the SNPs combined into a risk haplotype performed better. We estimated the frequencies of haplotypes made of the four most common SNPs (those with frequency greater than 4%), three of which showed evidence of association. Table 4 indicates that the GCAA haplotype at E23K—A190A—I337V—nt65.A/G is protective, while the ACGG haplotype confers risk to Type 2 diabetes mellitus in this population. Given the near complete LD between the individual SNPs, and therefore the similar association results between these SNPs and their combined haplotype, there appears to be a single association signal in this gene.

DISCUSSION

Variants in *KCNJ11* have been associated with Type 2 diabetes mellitus in several populations (1–5). In the present study, the common variant E23K was associated with Type 2

Table 3: Association of *KCNJ11* polymorphisms with Type 2 diabetes in Afro-Trinidadians

dbSNP	Postion[1]	nt[2]	Codon[3]	Amino acid change	Label	Maj/ Min	Genotype count						Allele frequency				p_a[4]	OR[5]	95% CI[6]
							Cases (n = 66)			Controls (n = 59)									
							Maj/ Maj/	Maj/ Min	Min/ Min	Maj/ Maj/	Maj/ Min	Min/ Min	Maj	Min	Maj	Min			
rs5219§	17366148	67	23	GAG (Glu) > AAG (Lys)	E23K	G/A	62	3	1	55	4	0	0.962	0.038	0.966	0.034	0.866	1.122	(0.294 – 4.276)
	17366107	108	36	GTG (Val) > GTA (Val)	V36V	G/A	62	4	0	58	1	0	0.970	0.030	0.992	0.008	0.218	3.656	(0.464 – 28.809)
	17365862	353	118	TCG (Ser) > TTG (Leu)	S118L	C/T	65	1	0	59	0	0	0.992	0.008	1.000	0.000	0.343	2.703	(0.109 – 67.003)
rs5218	17365645	570	190	GCC (Ala) > GCT (Ala)	A190A	C/T	61	5	0	52	5	0	0.962	0.038	0.958	0.042	0.856	0.890	(0.251 – 3.151)
	17365557	678	226	CCC (Pro) > CCT (Pro)	P226P	C/T	66	0	0	58	1	0	1.000	0.000	0.992	0.008	0.289	0.296	(0.012 – 7.326)
rs5215§	17365206	1009	337	ATC (Ile) > GTC (Val)	I337V	A/G	60	5	1	53	6	0	0.947	0.053	0.949	0.051	0.938	1.045	(0.341 – 3.202)
rs5214	17365126	1089	363	TCA (Ser) > TCG (Ser)	S363S	A/G	62	4	0	58	1	0	0.970	0.030	0.992	0.008	0.218	3.656	(0.464 – 28.809)
	17365120	1095	365	CGC (Arg) > CGT (Arg)	R365R	C/T	64	2	0	59	0	0	0.985	0.015	1.000	0.000	0.179	4.540	(0.216 – 95.537)
rs41282930	17365061	1154	385	TCT (Arg) > TGT (Cys)	S385C	C/G	65	1	0	59	0	0	0.992	0.008	1.000	0.000	0.343	2.703	(0.109 – 67.003)
	17365016	1199	c.*+26	—	c.*+26G > A	G/A	66	0	0	58	1	0	1.000	0.000	0.992	0.008	0.289	0.296	(0.012 – 7.326)
	17364977	1238	c.*+65		c.*+65A > G/T	A/(G/T)	59	63	1	55	4	0	0.939	0.061	0.966	0.034	0.324	1.839	(0.548 – 6.168)
	17364963	1252	c.*+79		c.*+79G > A	G/A	65	1	0	59	0	0	0.992	0.008	1.000	0.000	0.343	2.703	(0.109 – 67.003)

[1] Position in db SNP Build 35; [2] Nucleotide position from start ATG at 17366783; [3] c.*+65A>G denotes an A to G substitution 65 nucleotides 3' of the translation termination codon;
[4]Asymptotic *p*-value from χ^2 test; [5] Odds ratio for the minor allele (minor G and T alleles combined for this analysis); [6] Confidence interval of the odds ratio
§ SNP not in Hardy Weinberg equilibrium (HWE) in the Afro-Trinidadians cases: E23K, $p = 0.002$ and I337V, $p = 0.046$.

Table 4: Association of Kir6.2 (*KCNJ11*) haplotypes with Type 2 diabetes in Indo-Trinidadians

SNP				Haplotype frequency			
E23K	A190A	I337V	nt65.A/G	Case	Control	p_a[1]	p_e[2]
G	C	A	A	0.452	0.59	0.0091	0.0152
A	C	G	G	0.417	0.287	0.0115	0.0176
G	T	A	A	0.122	0.123	0.9786	1

[1]Asymptotic *p*-value from χ^2 test
[2]Empiric *p*-value from permutations

diabetes mellitus in Indo-Trinidadians, whose ancestors are believed to have historically originated from the northern state of Uttar Pradesh on the Indian subcontinent. This association was not detected in a previous study involving a comparable group of North Indians (9). We also identified a number of rare missense mutations in both Indo- and Afro-Trinidadian subjects with diabetes. Further studies are needed to determine the contribution, if any, of these rare variants to the overall prevalence of diabetes in these groups.

ACKNOWLEDGEMENTS

This study was supported in part by US Public Health Service Grant DK-20595 and a gift from the Kovler Family Foundation. KM was supported in part by a fellowship from the Manpei Suzuki Diabetes Foundation. MGH was supported by a mentor-based fellowship from the American Diabetes Association to Nancy J Cox.

REFERENCES

1. Remedi MS, Nichols CG. Hyperinsulinism and diabetes: genetic dissection of beta cell metabolism-excitation coupling in mice. Cell Metab 2009; **10:** 442–53.
2. Inagaki N, Gonoi T, Clement IV JP, Namba N, Inazawa J, Gonzalez G et al. Reconstitution of IKATP an inward rectifier subunit plus the sulfonylurea receptor. Science 1995; **270:** 1166–70.
3. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement IV JP, Boyd III AE, Gonzalez G et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 1995; **268:** 423–6.
4. Flanagan SE, Clauin S, Bellanné-Chantelot C, de Lonlay P, Harries LW, Gloyn AL et al. Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. Hum Mutat 2000; **30:** 170–80.
5. Florez JC, Burtt N, de Bakker PIW, Almgren P, Tuomi T, Holmkvist J et al. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 2004; **53:** 1360–8.
6. Cockburn BN, Bermano G, Boodram LG, Teelucksingh S, Tsuchiya T, Mahabir D et al. Insulin promoter factor-1 mutations and diabetes in Trinidad: identification of a novel diabetes associated mutation (E224K) in an Indo-Trinidadian family. Clin Endocrinol Metab 2004; **89:** 971–8.
7. Strom TM, Wienker TF. De Finetti Program 2004. [Accessed Jan 15 2011]. Available from: http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl
8. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; **21:** 263–5.
9. Sanghera DK, Ortega L, Han S, Singh J, Ralhan SK, Wander GS et al. Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: *PPARG2 (Pro12Ala)*, *IGF2BP2*, *TCF7L2* and *FTO* variants confer a significant risk. BMC Medical Genetics 2008; **9:** 59.