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Genetic variants in potassium channels are associated with type 2 diabetes in Mongolian population

Zagaa Odgerel[1], **Hee S Lee**[1], **Narnygerel Erdenebileg**[2], **Suren Gandbold**[3], **Munkhjargal Luvsanjamba**[4], **Nyamkhishig Sambuughin**[5], **Sainbileg Sonomtseren**[6], **Purevdulam Sharavdorj**[3], **Erdenezul Jodov**[6], **Khasag Altaisaikhan**[6], and **Lev G Goldfarb**[1]

[1]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

[2]Infectious Diseases and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

[3]National Institute of Forensic Science, Ulaanbaatar, Mongolia

[4]Ministry of Health, Ulaanbaatar, Mongolia

[5]Uniformed Services University of the Health Sciences, Bethesda, MD, USA

[6]Health Sciences University of Mongolia, Ulaanbaatar, Mongolia

Abstract

Objectives—Recent genome-wide association studies (GWAS) have identified more than 40 common sequence variants associated with type 2 diabetes (T2D). However, the results are not always the same in populations with differing genetic backgrounds. We evaluated a hypothesis that a North Asian population living in a geographic area with unusually harsh environmental conditions developed unique genetic risks.

Methods—We performed a population-based association study with 21 single-nucleotide polymorphisms (SNPs) in 9 genes selected according to the results of GWAS conducted in other populations. The study participants included 393 full-heritage Mongolian individuals, 177 diagnosed with T2D and 216 matched controls. Genotyping was performed by TaqMan methodology.

Results—The strongest association was detected with SNPs located within the potassium-channel coding *KCNQ1* (highest OR=1.92; P=3.4×10^{-5}) and *ABCC8* (OR=1.79; P=5×10^{-4}) genes. Genetic variants identified as strongly influencing the risk of T2D in other populations such as those in *KCNJ11* or *TCF7L2* genes did not show statistically significant association in Mongolia.

Conclusions—The strongest T2D risk-associated SNPs in Mongolians are located within 2 of 3 tested potassium-channel coding genes; accumulated variations in these genes may be related to environmental exposure to extreme cold.

Keywords

type 2 diabetes; genetic association; potassium channels; Mongolia; *KCNQ1*; *ABCC8*

Correspondence, Lev G Goldfarb, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Room 4S06, 5625 Fishers Lane, Bethesda, Maryland 20892-9404, USA. Tel: 1-301-4021480; Fax: 1-301-4966341; GoldfarbL@ninds.nih.gov.

Disclosure
None declared.

Introduction

Genome-wide association studies have identified more than 40 genomic loci associated with the risk for type 2 diabetes mellitus.[1] The results of GWAS conducted in European or American mixed populations[2] were partly inconsistent with subsequent studies in Asians,[3,4] suggesting that specific gene variants may independently influence risk of T2D in populations having unique demographic origins and history. The differences were most striking between the signals reported for the *KCNQ1* locus that were strong in Asians (OR 1.42; $p=2.5\times10^{-40}$ [3]) but have not reached significance in several studies of Europeans,[2] before a weaker association (OR 1.08; $p=2.8\times10^{-13}$) was detected with another SNP.[5] Conversely, *TCF7L2* considered the strongest candidate in the Europeans[2] was not as prominent in at least some Asians populations.[6] The new challenge in the post-GWAS era is to validate the genetic associations through replication of the candidate genes in various populations because the efficiency of individual gene-directed medications may depend on genetic background.[7]

Northern populations are exposed to unique ecological conditions including severe chronic cold stress, marked seasonality, sparse vegetation, and low overall energy availability.[8] It has been hypothesized that survival in the harsh environment may be associated with the accumulation of risk alleles that predispose to T2D.[9] The Mongolian population selected for this study has generally low but rapidly increasing prevalence of T2D (currently 8.9% of the population at age >25). [10]

The purpose of this study was to replicate genetic associations previously reported in GWAS and map more densely the candidate regions in order to detect risk alleles predisposing Mongolians to T2D.

Methods

Study population

Mongolia is one of the most sparsely populated areas in the world with 1.6 inhabitants per square kilometer. The average life expectancy is 61.6 years for males and 67.8 for females. T2D prevalence in Mongolia grew among adult urban population from 3.2% in 1999 to 8.9% in 2009.[10] Patients were diagnosed using the World Health Organization diagnostic criteria, officially registered, and received treatment for this condition. Each patient underwent annual follow-up that included an exam, biochemical tests, and adjustment of treatment regiments. The non-diabetic control group included age, gender and place of residence matched individuals in which diabetes has been excluded. Control individuals were not related to the T2D patients or each other. The study population included 393 full-heritage Mongolian individuals, of which 177 were patients affected with T2D at various stages of the disease and 216 were control subjects. The T2D-affected individuals in our study were on average in their mid-50s at the time of sample collection and did not differ significantly from the current age of control individuals ($P=0.52$). The patients had an increased body mass index (30.5±9.0 *vs.* 27±4.7 in controls; $P=0.0008$) and fasting plasma glucose level (12.45±5.6 *vs.* 6.5±2.1 in controls; $P=0.0001$) and HbA1c (9.1±2.4 *vs.* 5.5±0.9 in controls; $P=0.0001$). The study was in compliance with the Helsinki Declaration. Research protocols were approved by a local Ethics Committee and Institutional Review Boards of participating institutions. Informed consent was obtained from each participant.

Genotyping

For the purposes of this analysis, we selected 21 SNPs in 9 genes, *ABCC8, CDKN2A/B, CDKAL1, KCNJ11, KCNQ1, HHEX, PPARg, SCL30A8,* and *TCF7L2,* shown to be associated with T2D in GWAS or association studies with individual genetic markers

performed in other populations. SNPs were genotyped using Taqman® SNP Genotyping assays with TaqMan Universal PCR Master Mix (No AmpErase® UNG) or TaqMan® Genotyping Master Mix (both from Applied Biosystems, Foster City, CA). All reactions were run on an ABI7300 system and analyzed using SDS 1.2 software (Applied Biosystems, Foster City, CA). The results were consistent with direct sequencing of selected samples and/or testing with dHPLC on automated WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE).

Statistical analysis

We tested association between candidate gene SNPs and T2D using logistic regression. Estimated odd ratios (ORs) are equivalent to those obtainable from Cochran-Armitage trend test. Under the widely accepted additive genetic model for this disorder, trend test is more robust to deviations from Hardy-Weinberg equilibrium, hence preferred to other tests such as the ones calculated by contrasting allele frequencies or homozygosity frequencies.[11] Study-wide threshold *P* value for association was set at 0.0024 by applying Bonferroni correction for testing 21 SNPs. Formal statistical tests and parameter estimations including 95% confidence intervals were carried out using SAS/STAT 9.2 (SAS Institute Inc, Cary, NC). Pair-wise estimates for linkage disequilibrium (r^2) between neighboring markers located in the same locus were calculated using Haploveiw (Broad Institute of MIT and Harvard, USA, version 4.1).

Results

The prevalence of type 2 diabetes in Mongolia has increased three-fold in the last 10 years, with an alarming tendency of further growth. A total of 21 SNPs in 9 genes were genotyped in 177 T2D patients and genotype profiles compared to 216 controls. Initially, variations in four genes, *PPARg, CDKAL1*, *KCNQ1*, and *ABCC8*, have shown weak association with T2D. Denser mapping with additional follow-up SNPs was carried out within the *KCNQ1* and *ABCC8* candidate loci. *P*-values for 3 SNPs in *KCNQ1* (rs2237892, rs2237895, and rs2237897) and 2 SNPs in *ABCC8* (rs1799858 and rs2074308) overcame the $P=0.0024$ threshold adjusted for multiple testing (Table). The three positively associated *KCNQ1* SNPs are located within intron 15, and the *ABCC8* signals are in a relatively short fragment between intron 11 and exon 14 (Figure). The strongest signals were detected with marker rs2237897 in *KCNQ1* gene (OR=1.92; $p=3.4\times10^{-5}$) and rs2074308 in *ABCC8* (OR=1.79; $p=5\times10^{-4}$). Of the neighboring SNPs with positive association, the rs1799858 and rs2074308 were in low linkage disequilibrium ($r^2=0.134$), and the three SNPs located within the *KCNQ1* exon 15, rs2237892, rs2237895, and rs2237897, showed moderate correlation, $r^2=0.324$ and $r^2=0.444$, respectively. Marker rs7903146 in *TCF7L2* frequently replicated in large-scale T2D studies showed no evidence of association in the Mongolians (OR=1.17; $P=0.488$). The second most replicated marker rs5219 of *KCNJ11* also failed to show signs of association (OR=1.07; $P=0.645$). Signals in *PPARg* and *CDKAL1* genes did not overcome the *P*-value threshold adjusted for multiple testing. We were likewise unable to confirm the association with two SNPs within the retinol binding protein 4 gene reported in the only previous study of T2D in Mongolia.[12]

Discussion

We found variants rs2237892, rs2237895, and rs2237897 located in intron 15 of the *KCNQ1* gene to be associated with T2D in Mongolians. Similar results have previously been obtained in Japanese cohorts and replicated in Chinese, Korean, Swedish and Danish populations.[3,4,5] Our studies in the Mongolian population confirm these earlier reports. *KCNQ1* located on chromosome 11p15.5 encodes a pore-forming subunit of voltage-gated potassium channel that is expressed in β-cells. The channel has a role in insulin secretion.

Follow-up genetic studies have shown that the intron 15 *KCNQ1* risk alleles are associated with impaired insulin secretion presumably through altering the *KCNQ1* gene expression in β-cells.[3,13] Rare mutations in *KCNQ1* are known to cause potassium channel dysfunction leading to a form of cardiac long QT syndrome (LQT1) and serious arrhythmias, ventricular fibrillation and cardiac arrest.[14]

The second strong signal associated with risk of T2D in Mongolians was detected in the *KCNJ11/ABCC8* region located on chromosome 11p15.1. *KCNJ11* encodes the inwardly rectifying potassium channel Kir6.2, and *ABCC8* codes for sulfonylurea receptor 1 (SUR1); they together form a β-cell adenosine triphosphate–sensitive potassium channel (K_{ATP}). K_{ATP} channels regulate insulin release from β-cells. Rare heterozygous activating mutations in these genes are the cause of permanent neonatal diabetes.[15] The *KCNJ11* E23K variant was consistently found to be associated with T2D in multiple studies, but this SNP is in complete linkage disequilibrium with a non-synonymous S1369A variant in the neighboring *ABCC8* gene.[16] In comparative experiments it was the *ABCC8* A1369 and not the *KCNJ11* K23 allele that was responsible for the effect of decreased ATP inhibition.[17] These and other studies led to a hypothesis that the role of *ABCC8* in T2D development is underestimated and that the 11p15.1 chromosomal region needs to be analyzed for further contributing variants.[18] Indeed, rare mutations in exons 4,5, and 7 of *ABCC8* exerting a relatively minor or no effect on K_{ATP} channel activity still caused type 2 diabetes in adult patients.[19] Our data contribute a new observation indicating that two SNPs in *ABCC8* are in fact associated with the disease in Mongolians.

Populations living in cold environments require higher energy use and can significantly enhance fat utilization by increasing insulin sensitivity[20] through minute changes in genes that could have been critical for survival. The *KCNQ1* encoded potassium channel is known to be reacting to cold environment: swimming in cold water causes prolongation of the QT interval and results in syncope and frequent drawning in patients with long QT syndrome associated with mutations in *KCNQ1*.[21] Dramatic improvements within the past 10–20 years of living conditions and work requirements promoting sedentary lifestyle in combination with the traditional habits of meat and animal fat consumption resulted in an abrupt and extensive exposure to the risks of type 2 diabetes.[10]

The estimated effects of the associated variants seem larger than in other reports, which may reflect the fact that the Mongolian population is smaller and more homogeneous than the previously studied populations. At the same time, as the sample size in this study is small and statistical power limited, negative results on several loci such as *KCNJ11* or *TCF7L2* associated with T2D in other populations (including Asians[22,23]) are not strong enough for exclusion and need further evaluation.

Genetic variants predisposing to T2D are expected to influence the choice of therapeutic strategies. A recent study[24] evaluated the effects of widely used T2D treatment modalities in individuals possessing intron 15 *KCNQ1* risk alleles. After Repaglinide treatment, the TT homozygotes at rs2237892 showed lower 2-h glucose levels than the C allele carriers. Repaglinide is a fast-acting insulin secretagogue that initiates insulin secretion by closing the ATP-dependent potassium channels. Rosiglitazone showed a less pronounced effect. In infants with neonatal diabetes due to mutations in the ATP-sensitive potassium channel encoded by *ABCC8* and *KCNJ11* genes the disease can be successfully treated with oral sulfonylurea-based medications rather than lifelong insulin injections.[25]

In conclusion, this study replicates data regarding the role of *KCNQ1* variants in the development of type 2 diabetes in an independent ancient Asian population. The association with *ABCC8* genetic variants indicates their participation in the causation of type 2 diabetes

in Mongolians, most likely as a result of functional interaction with the effects of other potassium channel gene.

- The significant finding of the study is establishing of an association between genetic variants in potassium-channel genes *KCNQ1* and *ABCC8* and the development of type 2 diabetes in the Mongolian population.
- The study adds that potassium channels known to be affecting the adjustment to harsh environmental conditions may accumulate genetic defects that predispose to type 2 diabetes.

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References

1. Bonnefond A, Froguel P, Vaxillaire M. The emerging genetics of type 2 diabetes. Trends in Molecular Medicine. 2010; 16:407–416. [PubMed: 20728409]
2. Zeggini E, Scott LJ, Saxena R, Voight BF. Meta-analysis of genome-wide association data andlarge-scale replication identifies additional susceptibility loci for type 2 diabetes. Nature Genetics. 2008; 40:638–645. [PubMed: 18372903]
3. Yasuda K, Miyake K, Horikawa Y, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nature Genetics. 2008; 40:1092–1097. [PubMed: 18711367]
4. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nature Genetics. 2008; 40:1098–1102. [PubMed: 18711366]
5. Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nature Genetics. 2010; 42:579–589. [PubMed: 20581827]
6. Ng MC, Park KS, Oh B, et al. Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2 and FTO in type 2 diabetes and obesity in 6,719 Asians. Diabetes. 2008; 57:2226–2233. [PubMed: 18469204]
7. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461:747–753. [PubMed: 19812666]
8. Cox, CB.; Moore, PD. Biogeography: an ecological and evolutionary approach. 5th ed. Oxford: Blackwell Scientific Publications; 1993.
9. Chakravarthy MV, Booth FW. Eating, exercise, and "thrifty" genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases. J Applied Physiology. 2004; 96:3–10.
10. WHO Mongolian STEPS survey on the prevalence of noncommunicable disease risk factors in 2009. WHO, MOH, Public Health Institute; 2010.
11. Elston, RC.; Olson, JM.; Palmer, L. Biostatistical genetics and genetic epidemiology. John Wiley and Sons; 2002. p. 208
12. Munkhtulga L, Nakayama K, Utsumi N, et al. Identification of a regulatory SNP in the retinol binding protein 4 gene associated with type 2 diabetes in Mongolia. Human Genetics. 2007; 120:879–888. [PubMed: 17006670]
13. Jonsson A, Isomaa B, Tuomi T, et al. A variant in the KCNQ1 gene predicts future type 2 diabetes and mediates impaired insulin secretion. Diabetes. 2009; 58:2409–2413. [PubMed: 19584308]
14. Neyroud N, Tesson F, Denjoy I, et al. A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nature Genetics. 1997; 15:186–189. [PubMed: 9020846]

15. Flanagan SE, Clauin S, Bellanne-Chantelot C, 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. Human Mutation. 2009; 30:170–180. [PubMed: 18767144]

16. Florez JC, Burtt N, de Bakker PI, et al. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes. 2004; 53:1360–1368. [PubMed: 15111507]

17. Hamming KS, Soliman D, Matemisz LC, Niazi O, Gloyn AL, Light PE. Coexpression of the type 2 diabetes susceptibility gene variants KCNJ11 E23K and ABCC8 S1369A alter the ATP and sulfonylurea sensitivities of the ATP-sensitive K(+) channel. Diabetes. 2009; 58:2419–2424. [PubMed: 19587354]

18. van de Bunt M, Gloyn AL. Genetic association to molecular mechanism. Curr. Diabetes Reports. 2010; 10:452–466.

19. Tarasov AI, Nicolson TJ, Riveline JP, et al. A rare mutation in ABCC8/SUR1 leading to altered ATP-sensitive K+ channel activity and beta-cell glucose sensing is associated with type 2 diabetes in adults. Diabetes. 2008; 57:1595–1604. [PubMed: 18346985]

20. Vlasova NV, Gitel'zon II, Okladnikov IuN. Lipid metabolism in Far North aborigines. Vopr. Pitan. 1975; 5:53–56. [PubMed: 1210203]

21. Ackerman MJ, Tester DJ, Porter CJ. Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. Mayo Clin Proc. 1999; 74:1088–1094. [PubMed: 10560595]

22. Hu C, Zhang R, Wang C, et al. PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese population. PLoS One. 2009; 4:7643.

23. Xu M, Bi Y, Xu Y, et al. Combined effects of 19 common variations on type 2 diabetes in Chinese: results from two community-based studies. PLoS One. 2010; 5:e14022. [PubMed: 21103332]

24. Yu W, Hu C, Zhang R, et al. Effects of KCNQ1 polymorphisms on the therapeutic efficacy of oral antidiabetic drugs in Chinese patients with type 2 diabetes. Clin Pharmacol Ther. 2011; 89:437–442. [PubMed: 21289621]

25. Gloyn AL, Siddiqui J, Ellard S. Mutations in the genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) in diabetes mellitus and hyperinsulinism. Human Mutations. 2006; 27:220–231.

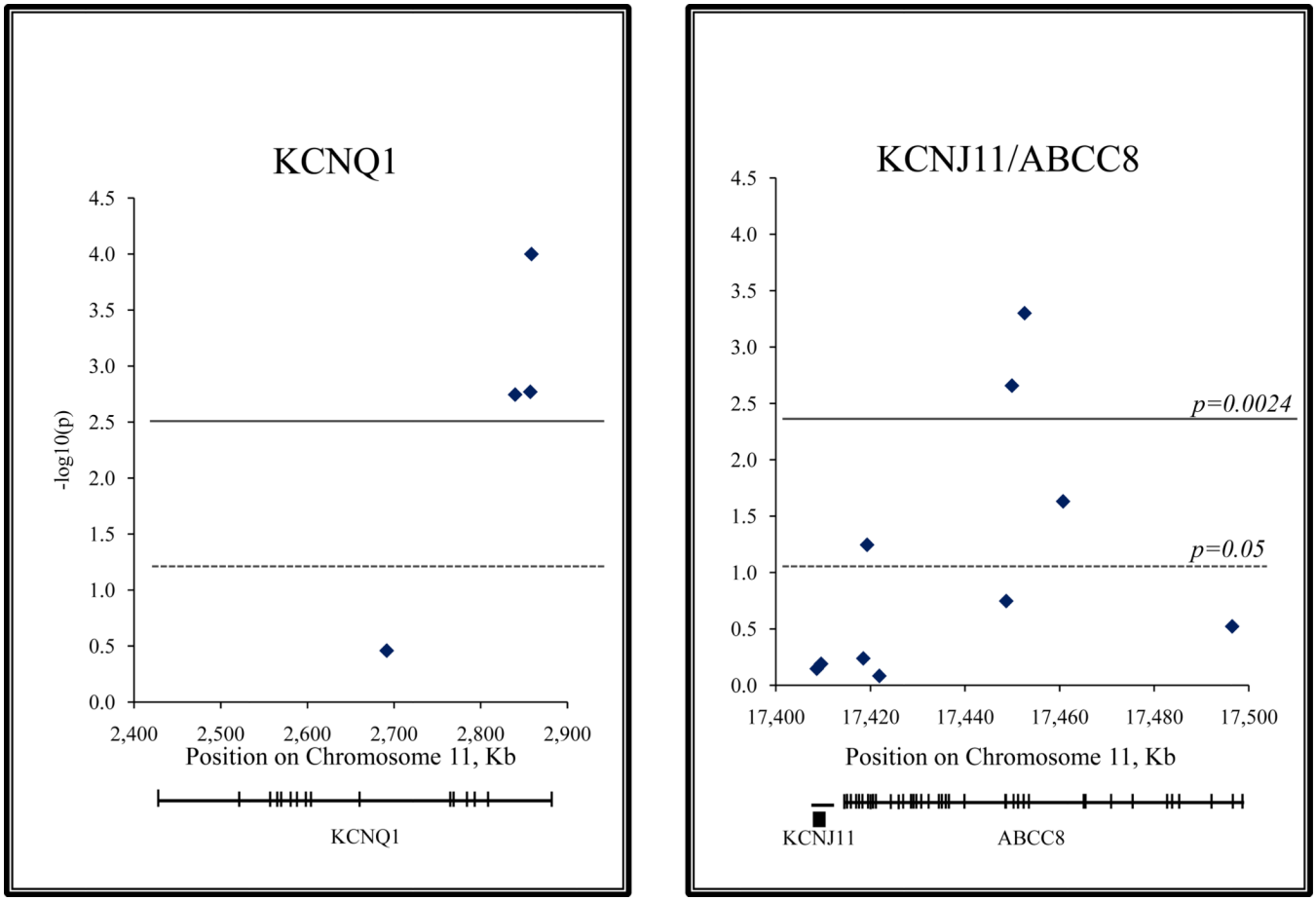

Figure.

Plots of T2D association signals in *KCNQ1* (Panel A) and *KCNJ11/ABCC8* (Panel B) genes detected in the Mongolian population. *P*-values for the association (in $-\log_{10}$ scale) are shown for each SNP as diamonds and plotted according to their location on chromosome 11. The strongest *P*-values for 3 SNPs in *KCNQ1* (rs2237892, rs2237895, and rs2237897) and 2 SNPs in *ABCC8* (rs1799858 and rs2074308) overcame the *P*-value threshold adjusted for multiple testing (*P*=0.0024).

Table

Results of genotyping with 21 SNPs in Mongolian patients with type 2 diabetes

Chromosome number	rs_number	Gene	SNP position within the gene	Risk allele	Risk allele frequency Cases/Controls		OR	95% CI	P-value
3	rs1801282	PPARg	P12A	C	0.89	0.94	1.93	1.16–3.34	0.015
3	rs3856806	PPARg	H477H	T	0.16	0.14	1.13	0.77–1.67	0.962
6	rs7754840	CDKAL1	Intron 5	C	0.32	0.39	1.30	1.02–1.88	0.038
8	rs13266634	SCL30A8	Exon R276W	C	0.57	0.64	1.31	0.96–1.72	0.091
9	rs10811661	CDKN2 A/B	3’-flanking region	T	0.67	0.68	1.04	0.74–1.38	0.940
10	rs1111875	HHEX	3’-flanking region	C	0.32	0.32	1.00	0.77–1.39	0.820
10	rs7903146	TCF7L2	Intron 2	C	0.94	0.93	1.17	0.68–2.08	0.488
11	rs231361	KCNQ1	Intron 11	G	0.31	0.34	1.15	0.86–1.54	0.348
11	rs2237892	KCNQ1	Intron 15	C	0.63	0.73	**1.69**	**1.21–2.32**	**0.0020**
11	rs2237895	KCNQ1	Intron 15	C	0.29	0.34	**1.70**	**1.22–2.38**	**0.0020**
11	rs2237897	KCNQ1	Intron 15	C	0.52	0.67	**1.92**	**1.41–2.59**	**3.4×10^{-5}**
11	rs5215	KCNJ11	Exon 1 V337I	T	0.66	0.68	1.06	0.79–1.42	0.714
11	rs5219	KCNJ11	Exon 1 K23E	C	0.67	0.68	1.07	0.80–1.44	0.645
11	rs757110	ABCC8	Exon 33 A1369S	A	0.66	0.68	1.09	0.81–1.46	0.577
11	rs1799859	ABCC8	Exon 31 R1273R	G	0.67	0.72	1.35	0.99–1.84	0.057

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Chromosome number	rs_number	Gene	SNP position within the gene	Risk allele	Risk allele frequency Cases/Controls		OR	95% CI	P-value
11	rs2074311	ABCC8	Intron 29	G	0.68	0.69	1.03	0.77–1.39	0.827
11	rs1799854	ABCC8	Exon 16	G	0.56	0.51	1.22	0.92–1.62	0.179
11	rs1799858	ABCC8	Exon 14 K649K	T	0.16	0.23	**1.78**	**1.23–2.58**	**0.0022**
11	rs2074308	ABCC8	Intron 11	T	0.22	0.33	**1.79**	**1.29–2.50**	**5×10^{-4}**
11	rs2237982	ABCC8	Intron 10	C	0.79	0.85	1.54	1.07–2.24	0.023
11	rs1048099	ABCC8	Exon 2 P69P	A	0.65	0.69	1.16	0.87–1.56	0.301