CpG single nucleotide polymorphisms of the insulin signaling pathway associated with the risk of prediabetic status/type 2 diabetes in Chinese population

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Abstract

Aims: Cytosine-phosphate-guanine single nucleotide polymorphisms (CpG SNPs) have been identified as new markers in human diseases. We aimed to evaluate the contribution of 29 CpG SNPs in the insulin signaling pathways to the risk of prediabetes and type 2 diabetes (T2D) in Han Chinese.

Materials and methods: We recruited 708 T2D subjects, 253 prediabetes subjects and 575 healthy controls from 16 community health service centers (CHSC) in Shenzhen Nanshan district. Detailed clinical information and DNA samples were collected. A total of 29 CpG SNPs of the insulin signaling pathways were sequenced using the MassARRAY platform. Binary logistic regression was applied to evaluate the association between these 29 CpG SNPs with prediabetes and T2D after adjusted the relevant confounding factors. The potential interactions among the 29 CpG SNPs were conducted by the generalized multifactor dimensionality reduction (GMDR).

Results: Significant associations are found between KRAS rs7311692 [Padjusted = 0.009, OR = 0.562(0.363-0.868) by allele] and CBLC rs2965143 [Padjusted = 0.008, OR = 1.591(1.128-2.242) under the dominant model] with prediabetes. A further breakdown analysis by gender identify a significant association between MAPK9 rs1363513 and T2D risk in males [Padjusted = 0.004, OR = 0.57(0.399-0.836) by allele], while FLOT2 rs4795473 is associated with prediabetes in females (P = 0.007 by genotype). However, no significant effect of potential interaction is observed among the 29 CpG SNPs.

Conclusion: These results indicated that KRAS rs7311692, CBLC rs2965143 and FLOT2 rs4795473 are related with prediabetes, while MAPK9 rs1363513 was associated with male T2D.

1. Introduction

Type 2 diabetes (T2D) is a complex human disease contributed by genetic, environmental factors, and their interactions[1]. The incidence of T2D is increasing rapidly in both developed and developing countries[1]. As an intermediate process between normal glucose tolerance (NGT) and T2D, prediabetes is the major risk factor of T2D and its incidence is also soaring in recent years[1].

Single nucleotide polymorphisms (SNPs) have been identified as genetic markers of T2D. A large number of susceptibility loci have been validated in different ethnic populations according to the Genome Wide Association Studies (GWAS)[2]. However, all of the known genetic polymorphisms together can only account for about 5% of the phenotypic variance, therefore there is considerable missing heritability of T2D.

Recently, epigenetic mechanisms including DNA methylation and histone modification et al are found to be involved in the regulation of β -cell function in pancreatic islets[3]. DNA methylation is regarded as a bridge that connected the environmental influence and genetic background, that plays an important role in the pathogenesis of T2D. Interestingly, DNA methylation usually occurs on the CpG sites, which may influence the gene expression by regulating the transcription process [4]. The DNA methylation levels are commonly analyzed at clusters of CpG sites in the gene promoter and are used for indication of epigenetic effects[5]. It has been assumed that the gene functions may be influenced by disease related SNPs that correlating with DNA methylation status, and the introduction or removal of the CpG sites may contribute to the molecular mechanism of T2D development[3]. Moreover, CpG-SNPs have been found to be associated with many human diseases, such as coronary heart disease, schizophrenia, obesity et al[6, 7]. Human CpG SNPs might act as new potential molecular markers in many diseases.

In this study, we hypothesize that CpG SNPs (SNPs involved in the CpG sites) of the insulin signal pathway may be new markers for the development of prediabetes and T2D, and there may be potential interactions among the 29 CpG SNPs in insulin signal pathway. Many SNPs of the candidate genes in insulin signal pathway have been identified as significant loci for T2D. Furthermore, it has been reported that CpG SNPs contributed to T2D onset by changing the DNA methylation levels in human pancreatic islets. Thereby, the purpose of our study is to explore the association between CpG SNPs in insulin signal pathway and the susceptibility of prediabetes and T2D, which is helpful to elucidated their clinical value in T2D development.

2. Materials And Methods

2.1. Samples collection

A total of 1,536 subjects consisted of 708 T2D subjects, 253 prediabetes subjects and 575 healthy controls were included in our study. The subjects were collected from 16 community health service centers (CHSC) in Shenzhen Nanshan district of China. The subjects of T2D and prediabetes were diagnosed according to the American Diabetes Association guidelines. Subjects with hypertension, coronary heart disease or other serious diseases were excluded in this study. Detailed information about age, body mass index (BMI), waist: hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), uric acid (UA), creatinine (Cre) were also documented. The study protocol was approved by the Ethical Committee of Shenzhen Nanshan Center for Chronic Disease Control, and the informed written consents were obtained from all the subjects.

2.2. CpG-SNP selection

The selection criteria of CpG-SNPs were as follows: (1) the selected CpG-SNPs were on the promoter of genes in insulin signal pathway; (2) the minor frequencies of the selected CpG-SNPs were over 10% in HapMap HCB population; (3) CpG-SNPs with design problems or failed assays were excluded. Finally, 29 CpG-SNPs in insulin signal pathway were included in our study (Figure 1). All of the CpG-SNPs were able to introduce or remove a CpG site.

SNP genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using the nucleic acid extraction analyzer (Lab-Aid 820, Xiamen, China). DNA concentration was determined using the NanoDrop 1000 spectrophotometer (Thermal Scientific Co. Ltd., Wilmington, USA). The PCR cycles included an initial denaturation

stage 94 °C for 15 sec, 45 cycles of denaturation for 20 sec at 94 °C, an annealing phase conducted at 56 °C for 30 sec, a primer extension at 72 °C for 1 min and a final extension for 3 minutes at 72 °C. Genotyping of the 29 CpG-SNPs was performed on the Sequenom MassARRAY platform. The primer sequences of the 29 CpG-SNPs were shown in Table 1.

2.3. Statistical analysis

Continuous variables among the three groups were analyzed by one-way analysis of variance. The genotype and allele frequencies of CpG-SNPs between cases and controls were analyzed by Chi-square test under dominant and recessive models. Binary logistic regression analysis was used to analyze the association between CpG-SNPs and prediabetes/T2D risk after adjusted the corresponding confounders. Besides, we also conducted the sub-group analysis stratified by genders.

To reduce type I error induced by multiple tests, $P < 0.01$ was adopted as the significant threshold. The above statistical tests were performed by SPSS software (SPSS Inc., version 16.0, Chicago, IL, USA). The consistency of genotype frequencies (Hardy–Weinberg equilibrium, HWE) were performed by Arlequin program (version 3.5). The potential interactions among the 29 CpG-SNPs were performed by generalized multifactor dimensionality reduction (GMDR). A two-sided $P < 0.05$ were considered to be significant.

3. Results

The characteristics of the included 1536 subjects were presented in Supplemental Table 1. There were significant differences of clinical features among the three groups in different comparisons. The comparison results between insulin signal pathway genotype distributions and allele frequencies among T2D subjects, prediabetes subjects and healthy controls were summarized in Table 2.

Significant association between *KRAS*rs7311692 and prediabetes was revealed in the multivariate analysis after adjusted for age, BMI, SBP, DBP, TC, TG, UA and Cre [prediabetes/controls: $P_{\text{adjusted}} = 0.009$, OR = 0.562 (0.363 - 0.868) by allele]. In addition, we found a significant association between *CBLC*rs2965143 and prediabetes following the adjustment of age, BMI, SBP, DBP, TC, TG, UA, Cre [prediabetes/controls: $P_{\text{adjusted}} = 0.008$, OR = 1.591(1.128 - 2.242) under the dominant model].

A further breakdown analysis by gender revealed that *MAPK9*rs1363513 was significantly associated with T2D risk following the adjustment of age, BMI, WHR, SBP, DBP, TG, IDL,UA and Cre in male subjects [T2D/controls: $P_{\text{adjusted}} = 0.004$, OR = 0.57 (0.399-0.836) by allele] as shown in Supplemental Table 2. We also observed a significant association between *FLOT2*rs4795473 and prediabetic status in females (prediabetes/controls: $P_{\text{adjusted}} = 0.007$ by genotype). However, we didn't observe any potential interaction among these 29 CpG-SNPs by GMDR analysis (data not shown).

4. Discussion

In this study, we investigated the association between T2D risk and 29 CpG SNPs of gene promoters that selected from insulin signal pathway in 1536 subjects. Our study revealed that the allele frequencies of *KRAS* rs7311692 and *CBLC* rs2965143 were associated with prediabetic status. As the influence of gender can contribute to the development of T2D, which is mainly due to the mediation of sex hormones, we also performed a subgroup analysis stratified by gender. Significant associations of *MAPK9* rs1363513 with male T2D and *FLOT2* rs4795473 with female prediabetes were observed.

To the best of our knowledge, this is the first report about CpG SNPs with susceptibility to status of prediabetes and T2D. Prediabetes is an intermediate stage of hyperglycemia, which is a high risk factor of T2D onset [8]. This study reveal that *KRAS* rs7311692, *CBLC* rs2965143 and *FLOT2* rs4795473 were associated with prediabetes. It has been reported that there was an interaction between glucose levels and diabetes risk SNPs, which perform an effect on the insulin secretion[9–11]. Therefore, the T2D/prediabetes related CpG SNPs might have similar biological effect on insulin secretion that resulting in blood glucose variability. Additionally, prediabetes related SNPs, such as at *PERK* rs6750998 and *XBP1* rs2239815, were also found associated with homeostasis model assessments of insulin resistance[12]. Interestingly, we observe significant associations for *KRAS* rs7311692, *CBLC* rs2965143 and *FLOT2* rs4795473 with prediabetic status. Our study provide new genetic evidences indentified contributions of CpG SNPs of insulin signal pathway to the molecular pathologic mechanism of insulin resistance in human prediabetes.

Insulin resistance and β -cell dysfunction are main crucial metabolic disorder in T2D[13], a large number of SNPs of T2D candidate genes exerted an influence on the onset of T2D by regulating the insulin secretion and sensitivity. Taqi et al reported that T2D-associated SNPs could affect gene function by altering its DNA methylation level[14]. Similar mechanism existed between the CpG SNPs and DNA methylation status, that can influence the binding ability of DNA-binding proteins resulted in gene regulation. It is important to understand the molecular mechanisms of CpG SNPs of insulin signal pathway for further clarifying the pathogenesis of T2D.

DNA methylation is critical for gene transcription regulation and chromosome conformation[15]. In general case, increased DNA methylation level of the promoter results in decreased gene expression. In order to understand the mechanism of T2D, it is necessary to validate the link between CpG SNPs and DNA methylation. Hence, the analyses of DNA methylation status that surrounding the CpG SNP sites of the insulin signal pathway are needed for further epigenetic study.

The merits of our study include: firstly, we recruited a homogeneous population with strict inclusion and exclusion criteria; secondly, our study may provide a novel hint in clarifying the molecular mechanism of prediabetes; thirdly, we explored the potential interactions among the 29 CpG SNPs with GMDR method. However, there are some limitations in our study: the sample size is not very large and we didn't test the relationship between the prediabetes/T2D related CpG SNPs and DNA methylation levels.

5. Conclusions

In conclusion, we found four CpG SNPs are significantly associated with prediabetes/T2D in the insulin signal pathways. These genetic locus may be potential biomarkers with clinical value in the assessment of the T2D risk.

Declarations

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Author's contributions

Changyi Wang and Shiwei Duan contributed to the design of the study. Liyuan Han performed statistical analysis; Jia Cheng and Linlin Tang contributed to the drafting of the manuscript; all authors participated in generation, collection, assembly, analysis, and interpretation of data. All authors read and approved the final manuscript.

Declaration of Competing Interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Primer sequences and the information of 29 CpG-SNPs

Gene	Chr	SNP	Primer	Sequence (5'-3')	Extension primer sequence (5'-3')
<i>PIK3CD</i>	1	rs7518602	1st-primer	ACGTTGGATGATATTTTGGGGTGGCACACG	gcatGACACACACCCAAAATGTGACT
			2nd-primer	ACGTTGGATGTTTGCAGAGGTGACCAGTGTG	
<i>MKNK1</i>	1	rs3753359	1st-primer	ACGTTGGATGCAGAGGCTGCAGTGTATG	GATGCCGCATCACCT
			2nd-primer	ACGTTGGATGCAGAGCATCCACCTATTGAG	
<i>PPP1CB</i>	2	rs7598876	1st-primer	ACGTTGGATGCTCTCTCCACGTCCACGGC	ggggaGGCGCCTGCGCCACCG
			2nd-primer	ACGTTGGATGTCTGCGTAACCGGGACTTC	
<i>PRKCI</i>	3	rs481781	1st-primer	ACGTTGGATGGCTGATCATGGCATCAAAC	ctcgCGCGTGAAAGCACCCCTC
			2nd-primer	ACGTTGGATGCTAGAGCTGGTCATTGTCTC	
<i>MAPK9</i>	5	rs1363513	1st-primer	ACGTTGGATGATGGGAAAACCGTGGAGAG	CCACTCTTCTAGTCCCCAA
			2nd-primer	ACGTTGGATGGTCAACCATTGTTCCCACTC	
<i>PRKAA1</i>	5	rs461404	1st-primer	ACGTTGGATGGTAACATGCATTAGGCACTG	tttgGCTTCTCTGCAATATCTTCCT
			2nd-primer	ACGTTGGATGCTAAGCTCAAAGGCAGAAAC	
<i>PRKAG2</i>	7	rs4078431	1st-primer	ACGTTGGATGACACCATAGGCCTTTACTC	agggGTCCTGCGAAAGCAC
			2nd-primer	ACGTTGGATGAGAGATGATCCTTTGGGTGG	
<i>PRKAR1B</i>	7	rs4724904	1st-primer	ACGTTGGATGTGGTTTCCAGGTATCTGGAC	tccaaCCCACCCGGGCCACGAGC
			2nd-primer	ACGTTGGATGCACCCTCCTCCTGCCTGA	
<i>GCK</i>	7	rs12702070	1st-primer	ACGTTGGATGATCTGAACAGGTGGCAAAGG	CACTACAGTGACCGC
			2nd-primer	ACGTTGGATGACCATGGTGACCACTCAATC	
<i>PRKACG</i>	9	rs4745515	1st-primer	ACGTTGGATGTTCTAAGCTCCCTTTTCATC	ATCCCCAAGTATTTGGAAA
			2nd-primer	ACGTTGGATGCTTGAAAAATCCCCAAGTAT	
<i>FBP1</i>	9	rs7031924	1st-primer	ACGTTGGATGTTTTGAAGGTAGAGCTGATG	TCTCACATATCTCATCTGTCAA
			2nd-primer	ACGTTGGATGAGTTTCTCAGGCCTCAAACC	
<i>HRAS</i>	11	rs7939028	1st-primer	ACGTTGGATGAGCCGAGCTCTGACTGCGA	ggGGTCGCTGCTGACCCG
			2nd-primer	ACGTTGGATGTCCTGCGCCGGTCGCTGCT	
<i>PRKAB1</i>	12	rs6490265	1st-primer	ACGTTGGATGTTCCGGCGACTCCGTAAGT	AGCCTCGCGCGTTTAGC
			2nd-primer	ACGTTGGATGTGGAATCGAGATAGCCTCGC	
<i>PRKAG1</i>	12	rs2293446	1st-primer	ACGTTGGATGCATGACCCTCGCCGTCAGC	tcAACCGCCCTCCCCCGGGTCCTC
			2nd-primer	ACGTTGGATGAGGCAAGGAACCCACCCTTC	
<i>KRAS</i>	12	rs7311692	1st-primer	ACGTTGGATGATAGTAAATGGATGCGCGCC	cctccGACCGGTCTCCACAGAGAAG
			2nd-primer	ACGTTGGATGCCGCTGACCGTCTCCACA	
<i>PCK2</i>	14	rs4982856	1st-primer	ACGTTGGATGCAGCCGCACATGATGTAAT	AGCTTGTGTTGCCACC
			2nd-primer	ACGTTGGATGCCCTCGTTTCTAGCTTGTTT	
<i>SOS2</i>	14	rs1955926	1st-primer	ACGTTGGATGCCAGACTCCAGCTATAAAC	tgCATGGTCTAGTAGTTTGACA
			2nd-primer	ACGTTGGATGCTCCTATGAGTAAAGAGAAC	
<i>SHC4</i>	15	rs12900666	1st-primer	ACGTTGGATGACTACAGATCATTAGCCGGG	cccaTTCTGCTCTGCCCTCCC
			2nd-primer	ACGTTGGATGTTTTAGCTCTTCTGCTCTGC	
<i>PDPK1</i>	16	rs76318740	1st-primer	ACGTTGGATGCCAAGGGTCAAGGGTCACAA	CTGCCCCCTCTCTCCC
			2nd-primer	ACGTTGGATGTTTGACCTTCAGCTGCCCCCT	
<i>FASN</i>	17	rs7222326	1st-primer	ACGTTGGATGCCTCCCTTTTCTGACCGCT	GTGCCTGGCCCCGCA
			2nd-primer	ACGTTGGATGAGACACCTGTGGCCTCACAA	
<i>ACACA</i>	17	rs11868124	1st-primer	ACGTTGGATGAGAACGCGCTGTTGCACTC	tGGAGTTTCACACTTGTGCT
			2nd-primer	ACGTTGGATGTGTGCATCCTTTACTGGACG	
<i>FLOT2</i>	17	rs4795473	1st-primer	ACGTTGGATGGTAAATACACCACTGTGC	CAGGAAGAAAGAAAGGAGT
			2nd-primer	ACGTTGGATGAGGAACAGCTCCGAGTTCAC	
<i>INSR</i>	19	rs1864009	1st-primer	ACGTTGGATGCAGCGACACTCGTCCAGA	CTGGGCCGGGGCGCAC
			2nd-primer	ACGTTGGATGTGGCTTGGGTCTCGCCCCT	
<i>PIK3R2</i>	19	rs3736328	1st-primer	ACGTTGGATGATTTCCAGAAACGCTCCGC	GGCCGAACACTCTCTAC
			2nd-primer	ACGTTGGATGTTTCGAAATGGCCGAACAC	
<i>CBLC</i>	19	rs2965143	1st-primer	ACGTTGGATGTGTAGTCTAGTTACTAGGG	ttccTTGCAGCCTTGACCTC
			2nd-primer	ACGTTGGATGTGTTGTTGTTGGATCACCCTC	
<i>MKNK2</i>	19	rs3810412	1st-primer	ACGTTGGATGGAAGAAGAAAGTAGGTGGCG	GCACGCTTGGAGTCCCA
			2nd-primer	ACGTTGGATGTGCACCACCAGCAGCTTG	
<i>SHC2</i>	19	rs73916989	1st-primer	ACGTTGGATGTGAGGAGAGAGCAGCCCCA	aaGCAGGGCGCAGAGAG
			2nd-primer	ACGTTGGATGTTGGGAGGGTGCGCACTG	
<i>TRIP10</i>	19	rs340141	1st-primer	ACGTTGGATGCAAGAAGTCTTCTCAACC	ttgcCCTAGACGCCTGGAGC
			2nd-primer	ACGTTGGATGTTAGTGGGACGAACTAGAC	
<i>PRKX</i>	chrX	rs1003351	1st-primer	ACGTTGGATGTGCACCCAAGCCCGAAGAT	ccctACTTCTGCTGAGCTAC
			2nd-primer	ACGTTGGATGTTTTGAACTTCACCTCTGC	

Table 2. Association of 29 variants with type 2 diabetes and prediabetes in the whole sample set

	Genotype			χ ²	P	HWE	Allele		P	OR(95%CI)	Dominant model			Ref		
	(counts)						(counts)				(Adjusted)	(Adjusted)	P		OR(95%CI)	P
													(Adjusted)		(Adjusted)	(Adjusted)
<i>PIK3CD</i> (rs7518602)	CC	CT	TT				C	T								
T2D	557	109	5	1.832	0.4	1.000	1223	119	0.229 ^a	0.826(0.605-1.128) ^a	0.302 ^a	1.195(0.852-1.677) ^a	0.29			
Prediabetes	197	47	2	0.329	0.848	1.000	441	51	0.584 ^b	1.114(0.757-1.639) ^b	0.48 ^b	0.858(0.561-1.312) ^b	0.70			
Control	455	104	7			0.653	1014	118								
<i>MKNK1</i> (rs3753359)	GG	CG	CC				G	C								
T2D	409	260	34	2.824	0.244	0.401	1078	328	0.864 ^a	1.019(0.820-1.267) ^a	0.826 ^a	0.971(0.744-1.266) ^a	0.35			
Prediabetes	153	87	12	1.139	0.566	1.000	393	111	0.84 ^b	1.029(0.778-1.362) ^b	0.985 ^b	0.997(0.706-1.408) ^b	0.57			
Control	328	187	37			0.157	843	261								
<i>PPP1CB</i> (rs7598876)	CC	CT	TT				C	T								
T2D	379	269	55	4.558	0.102	0.448	1027	379	0.402 ^a	0.914(0.740-1.128) ^a	0.142 ^a	1.218(0.936-1.586) ^a	0.40			
Prediabetes	143	95	15	1.37	0.504	1.000	381	125	0.232 ^b	0.843(0.636-1.116) ^b	0.21 ^b	1.242(0.885-1.743) ^b	0.66			
Control	294	231	28			0.046	819	287								
<i>PRKCI</i> (rs481781)	CC	CT	TT				C	T								
T2D	454	213	36	1.174	0.556	0.101	1121	285	0.116 ^a	0.837(0.670-1.045) ^a	0.132 ^a	1.236(0.938-1.629) ^a	0.35			
Prediabetes	159	83	11	0.893	0.64	1.000	401	105	0.515 ^b	0.911(0.687-1.207) ^b	0.738 ^b	1.061(0.750-1.500) ^b	0.32			
Control	341	178	33			0.138	860	244								
<i>MAPK9</i> (rs1363513)	TT	TG	GG				T	G								
T2D	459	193	24	3.575	0.167	0.515	1111	241	0.05 ^a	0.780(0.608-1.000) ^a	0.087 ^a	0.782(0.90-1.036) ^a	0.14			
Prediabetes	169	70	7	0.921	0.631	1.000	408	84	0.251 ^b	0.827(0.598-1.144) ^b	0.318 ^b	0.831(0.577-1.196) ^b	0.37			
Control	402	152	11			0.521	956	174								
<i>PRKAA1</i> (rs461404)	TT	CT	CC				T	C								
T2D	393	245	38	0.089	0.957	1.000	1031	321	0.616 ^a	0.946(0.763-1.173) ^a	0.432 ^a	1.249(0.717-2.176) ^a	0.80			
Prediabetes	145	83	17	0.52	0.771	0.292	373	117	0.326 ^b	0.872(0.663-1.146) ^b	0.31 ^b	1.414(0.724-2.762) ^b	0.47			
Control	327	204	34			0.819	858	272								
<i>PRKAG2</i> (rs4078431)	GG	AG	AA				G	A								
T2D	168	362	173	0.94	0.625	0.449	698	708	0.238 ^a	0.894(0.743-1.077) ^a	0.414 ^a	0.882(0.653-1.191) ^a	0.68			
Prediabetes	58	122	73	2.375	0.305	0.615	238	268	0.033 ^b	0.770(0.606-0.979) ^b	0.185 ^b	0.766(0.516-1.136) ^b	0.03			
Control	145	274	133			0.866	564	540								
<i>PRKAR1B</i> (rs4724904)	CC	CG	GG				C	G								
T2D	288	320	95	0.302	0.86	0.680	896	510	0.785 ^a	1.027(0.8437-1.246) ^a	0.79 ^a	0.964(0.739-1.258) ^a	0.87			
Prediabetes	96	122	35	1.136	0.567	0.788	314	192	0.364 ^b	1.121(0.876-1.433) ^b	0.237 ^b	0.812(0.575-1.147) ^b	0.91			
Control	231	252	69			1.000	714	390								
<i>GCK</i> (rs12702070)	TT	CT	CC				T	C								
T2D	543	126	6	3.233	0.186	0.834	1212	138	0.729 ^a	0.941(0.669-1.325) ^a	0.926 ^a	1.018(0.701-1.478) ^a	0.28			
Prediabetes	197	44	4	2.611	0.261	0.328	438	52	0.039 ^b	1.526(1.021-2.279) ^b	0.057 ^b	0.654(0.423-1.013) ^b	0.19			
Control	477	86	4													
<i>PRKACG</i> (rs4745515)	AA	AG	GG				A	G								
T2D	322	287	67	1.901	0.387	0.788	931	421	0.559 ^a	1.059(0.873-1.286) ^a	0.324 ^a	0.877(0.675-1.139) ^a	0.75			
Prediabetes	115	106	24	1.125	0.57	1.000	336	154	0.38 ^b	1.118(0.872-1.434) ^b	0.127 ^b	0.766(0.544-1.079) ^b	0.60			
Control	261	235	70			0.128	757	375								
<i>FBP1</i> (rs7031924)	GG	AG	AA				G	A								
T2D	527	166	10	0.173	0.917	0.514	1220	186	0.511 ^a	1.097(0.832-1.446) ^a	0.608 ^a	0.923(0.681-1.252) ^a	0.47			
Prediabetes	178	74	1	4.931	0.085	0.023	430	76	0.57 ^b	1.109(0.776-1.587) ^b	0.306 ^b	0.821(0.563-1.197) ^b	0.99			
Control	419	126	7			0.568	964	140								
<i>HRAS</i> (rs7939028)	CC	CG	GG				C	G								
T2D	457	220	26	2.776	0.25	1.000	1134	272	0.784 ^a	1.034(0.812-1.317) ^a	0.843 ^a	1.028(0.781-1.353) ^a	0.15			
Prediabetes	178	68	7	3.413	0.182	0.818	424	82	0.429 ^b	0.878(0.653-1.213) ^b	0.213 ^b	1.245(0.870-1.782) ^b	0.31			
Control	357	184	12			0.037	898	208								
<i>PRKAB1</i> (rs6490265)	CC	CT	TT				C	T								

T2D	189	350	163	1.676	0.433	1.000	728	676	0.011 ^a	1.270(1.055-1.528) ^a	0.034 ^a	0.731(0.546-0.977) ^a	0.04
Prediabetes	91	118	44	3.377	0.185	0.606	300	206	0.292 ^b	0.881(0.696-1.115) ^b	0.37 ^b	1.176(0.825-1.677) ^b	0.40
Control	165	271	116			0.795	601	503					
<i>PRKAG1</i> (rs2293446)	GG	GA	AA				G	A					
T2D	230	320	126	0.232	0.891	0.434	780	572	0.792 ^a	1.025(0.852-1.233) ^a	0.804 ^a	0.965(0.732-1.273) ^a	0.43
Prediabetes	82	113	50	0.424	0.809	0.362	277	213	0.716 ^b	0.958(0.758-1.210) ^b	0.715 ^b	0.936(0.655-1.336) ^b	0.82
Control	184	272	106			0.797	640	484					
<i>KRAS</i> (rs7311692)	CC	CG	GG				C	G					
T2D	576	121	6	2.971	0.226	1.000	1273	133	0.552 ^a	0.911(0.671-1.238) ^a	0.805 ^a	1.043(0.746-1.458) ^a	0.14
Prediabetes	220	32	1	0.923	0.63	1.000	472	34	0.009 ^b	0.562(0.363-0.868) ^b	0.023 ^b	1.736(1.078-2.797) ^b	0.05
Control	450	93	9			0.152	993	111					
<i>PCK2</i> (rs4982856)	CC	CT	TT				C	T					
T2D	174	368	160	2.835	0.242	0.223	716	688	0.758 ^a	0.971(0.807-1.169) ^a	0.668 ^a	0.937(0.698-1.260) ^a	0.33
Prediabetes	66	137	50	2.553	0.279	0.207	269	237	0.6 ^b	0.938(0.740-1.190) ^b	0.632 ^b	0.911(0.623-1.333) ^b	0.16
Control	158	266	128			0.446	582	522					
<i>SOS2</i> (rs1955926)	CC	CA	AA				C	A					
T2D	294	295	87	0.901	0.637	0.357	883	469	0.464 ^a	1.074(0.887-1.300) ^a	0.379 ^a	1.125(0.865-1.464) ^a	0.84
Prediabetes	106	111	29	0.282	0.868	1.000	323	169	0.558 ^b	1.078(0.839-1.383) ^b	0.559 ^b	1.107(0.787-1.556) ^b	0.74
Control	233	262	71			0.927	728	404					
<i>SHC4</i> (rs12900666)	CC	CA	AA				C	A					
T2D	356	267	52	2.971	0.226	0.848	979	371	0.604 ^a	0.945(0.763-1.170) ^a	0.824 ^a	0.971(0.747-1.261) ^a	0.39
Prediabetes	130	98	17	0.923	0.63	0.872	358	132	0.283 ^b	0.859(0.650-1.134) ^b	0.426 ^b	0.871(0.620-1.223) ^b	0.28
Control	311	222	30			0.267	844	282					
<i>PDPK1</i> (rs76318740)	CC	CT	TT				C	T					
T2D	512	159	5	0.2801	0.246	0.052	1183	169	0.902 ^a	0.983(0.744-1.298) ^a	0.666 ^a	0.935(0.690-1.267) ^a	0.66
Prediabetes	180	60	6	0.764	0.683	0.618	420	72	0.354 ^b	1.175(0.836-1.651) ^b	0.581 ^b	0.881(0.599-1.295) ^b	0.45
Control	428	128	10			0.854							
<i>FASN</i> (rs7222326)	TT	CT	CC				T	C					
T2D	521	142	12	3.035	0.219	0.478	1184	166	0.087 ^a	1.286(0.964-1.717) ^a	0.157 ^a	0.797(0.581-1.091) ^a	0.11
Prediabetes	197	47	1	0.342	0.945	0.483	441	49	0.261 ^b	0.791(0.526-1.191) ^b	0.329 ^b	1.237(0.807-1.896) ^b	0.32
Control	447	113	4			0.379	1007	121					
<i>ACACA</i> (rs11868124)	CC	CT	TT				C	T					
T2D	466	185	25	0.354	0.838	0.230	1117	235	0.95 ^a	1.008(0.796-1.276) ^a	0.811 ^a	0.966(0.731-1.278) ^a	0.68
Prediabetes	166	71	9	0.084	0.959	0.666	403	89	0.29 ^b	1.174(0.872-1.580) ^b	0.286 ^b	0.824(0.577-1.176) ^b	0.64
Control	388	158	21			0.315	934	200					
<i>FLOT2</i> (rs4795473)	TT	CT	CC				T	C					
T2D	352	301	50	4.911	0.086	0.193	1005	401	0.272 ^a	1.121(0.914-1.374) ^a	0.931 ^a	1.012(0.779-1.313) ^a	0.01
Prediabetes	121	116	16	0.749	0.687	0.099	358	148	0.36 ^b	1.129(0.871-1.464) ^b	0.998 ^b	1.000(0.716-1.398) ^b	0.04
Control	281	215	57			0.103	777	329					
<i>INSR</i> (rs1864009)	GG	GT	TT				G	T					
T2D	166	351	159	2.070	0.355	0.354	683	669	0.305 ^a	0.907(0.753-1.093) ^a	0.523 ^a	1.105(0.813-1.504) ^a	0.30
Prediabetes	45	125	76	2.388	0.303	0.695	215	277	0.192 ^b	1.173(0.923-1.491) ^b	0.231 ^b	0.772(0.505-1.179) ^b	0.34
Control	127	286	152			0.799	540	590					
<i>PIK3R2</i> (rs3736328)	CC	CG	GG				C	G					
T2D	376	252	48	3.196	0.202	0.547	1004	348	0.631 ^a	1.053(0.853-1.300) ^a	0.799 ^a	1.034(0.799-1.338) ^a	0.07
Prediabetes	144	87	15	1.6	0.449	0.723	375	117	0.577 ^b	0.922(0.695-1.255) ^b	0.285 ^b	1.204(0.857-1.690) ^b	0.37
Control	316	224	27			0.138	856	278					
<i>CBLC</i> (rs2965143)	AA	AG	GG				A	G					
T2D	216	349	111	3.886	0.143	0.154	781	571	0.239 ^a	1.122(0.926-1.360) ^a	0.342 ^a	0.875(0.663-1.153) ^a	0.33
Prediabetes	110	99	37	6.388	0.041	0.069	319	173	0.04 ^b	0.772(0.604-0.988) ^b	0.008 ^b	1.591(1.128-2.242) ^b	0.71
Control	208	282	77			0.247	698	436					
<i>MKMK2</i> (rs3810412)	CC	CT	TT				C	T					

T2D	557	138	8	1.64	0.44	1.000	1252	154	0.204 ^a	1.215(0.899-1.642) ^a	0.231 ^a	0.818(0.589-1.136) ^a	0.48
Prediabetes	213	38	2	–	–	0.683	464	42	0.727 ^b	0.929(0.612-1.408) ^b	0.683 ^b	1.098(0.700-1.725) ^b	0.91
Control	454	94	5	3.565	0.468	1.000	1002	104					
<i>SHC2</i> (rs73916989)	CC	CG	GG				C	G					
T2D	502	183	18	2.482	0.289	0.773	1187	219	0.465 ^a	0.910(0.706-1.172) ^a	0.4 ^a	0.131(0.849-1.509) ^a	0.96
Prediabetes	175	71	6	0.406	0.816	0.822	421	83	0.859 ^b	1.030(0.743-1.428) ^b	0.904 ^b	0.978(0.678-1.410) ^b	0.80
Control	379	163	10			0.127	921	183					
<i>TRIP10</i> (rs340141)	CC	CT	TT				C	T					
T2D	430	245	28	2.214	0.331	0.371	1105	301	0.369 ^a	0.903(0.722-1.129) ^a	0.572 ^a	1.080(0.826-1.413) ^a	0.24
Prediabetes	171	76	6	6.785	0.034	0.660	418	88	0.061 ^b	0.748(0.552-1.013) ^b	0.13 ^b	1.314(0.922-1.872) ^b	0.09
Control	332	189	32	8.095		0.469	853	253					
<i>PRKX</i> (rs1003351)	AA	AG	GG				A	G					
T2D	649	26	0	–	–	1.000	1324	26	0.187 ^a	1.655(0.783-3.493) ^a	0.187 ^a	0.604(0.286-1.276) ^a	–
Prediabetes	237	8	0	–	–	1.000	482	8	0.54 ^b	1.343(0.523-3.452) ^b	0.54 ^b	0.745(0.290-1.914) ^b	–
Control	550	16	0	–	–	1.000	1116	16					

a: adjusted for age, BMI,SBP, DBP,TG, IDL, Cre; b: adjusted for age, BMI,SBP, DBP,TC, TG, UA, Cre.

Figures

Figure 1

29 CpG-SNPs of genes collected from insulin signaling pathway.

Supplementary Files

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