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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Linlin Tang
Ningbo University

Changyi Wang
Ningbo University

Liyuan Han
Ningbo University

Jianping Ma
Ningbo University

Jia Cheng
Zhongshan Hospital Xiamen University
drchengjia@163.com Corresponding Author
ORCiD: https://orcid.org/0000-0003-3116-4035

Shiwei Duan
Ningbo University

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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_{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_{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_{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_{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 reveals 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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| Gene    | Chr | SNP     | Primer   | Sequence (5'-3')          |
|---------|-----|---------|----------|---------------------------|
| PIK3CD  | 1   | rs7518602 | 1st-primer | ACGTTGGATGATATTTTGGGTTGGGCACACAG |
|         |     |          | 2nd-primer | ACGTTGGATGTGGGGCTGACAGTGGTTTATGG |
| MKNK1   | 1   | rs3753359 | 1st-primer | ACGTTGGATGCAGGGCTGACAGTGGTTTATGG |
|         |     |          | 2nd-primer | ACGTTGGATGACAGGGCTGACAGTGGTTTATGG |
| PPP1CB  | 2   | rs7598876 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKCI   | 3   | rs481781  | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| MAPK9   | 5   | rs1363513 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKAA1  | 5   | rs461404  | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKAG2  | 7   | rs4078431 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKAR1B | 7   | rs4724904 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| GCK     | 7   | rs12702070| 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKACG  | 9   | rs4745515 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| FBP1    | 9   | rs7031924 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| HRAS    | 11  | rs7939028 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKAB1  | 12  | rs6490265 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PRKAG1  | 12  | rs2293446 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| KRAS    | 12  | rs7311692 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| PCK2    | 14  | rs4982856 | 1st-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
|         |     |          | 2nd-primer | ACGTTGGATGCTCTCCTCCAGTCCACGCAAC |
| Gene   | Position | SNV     | 1st Primer Sequence                        | 2nd Primer Sequence                        |
|--------|----------|---------|--------------------------------------------|--------------------------------------------|
| SOS2   | 14       | rs1955926 | ACGTTGGATGCCCAGACTCCAGCTATAAAC             | ACGTTGGATGCTCTCCTATGAGTAAGAAGAAC           |
| SHC4   | 15       | rs12900666| ACGTTGGATGACCTACAGATCATGAGCCCGGG           | ACGTTGGATGTGGCTTTAGCTCTTCTGCTTGGC         |
| PDPK1  | 16       | rs76318740| ACGTTGGATGCCCAAGGCTCAAGGCTACAA            | ACGTTGGATGGCTTTAGCTCTTCTGCTTGGC         |
| FASN   | 17       | rs7222326 | ACGTTGGATGCCTCCCTTTTCCTGACGCT             | ACGTTGGATGAGAAGGCTGGTTCAGCT              |
| ACACA  | 17       | rs11868124| ACGTTGGATGAGACCGCGCTGGTTCAGCT             | ACGTTGGATGGATGCTCTTACTGGAGGAC            |
| FLOT2  | 17       | rs4795473 | ACGTTGGATGATCAGACACTCGTCCCAGA             | ACGTTGGATGAGACCTGGTTCAGCTACG             |
| INSR   | 19       | rs1864009 | ACGTTGGATGACCGACACTTGCTCCCAGA             | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| PIK3R2 | 19       | rs3736328 | ACGTTGGATGATTTCCAGAAACGCTGCC              | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| CBLC   | 19       | rs2965143 | ACGTTGGATGTCAGCTTATGCTACTAAGGG            | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| MKNK2  | 19       | rs3810412 | ACGTTGGATGGAGAGAAAGATGGTGGCG              | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| SHC2   | 19       | rs73916989| ACGTTGGATGAGAGAGAGAGAGAGACAGCCT           | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| TRP10  | 19       | rs340141  | ACGTTGGATGCAAGGCTTCCTTCTTCAACC            | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |
| PRKX   | chrX     | rs1003351 | ACGTTGGATGAGAGGCTGGAGAGAAGAGAGAGACAGCCT   | ACGTTGGATGAGACCGCGCTGGTTCAGCT           |

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

| Genotype (counts) | $X^2$ | P  | HWE | Allele (count) |
|------------------|-------|----|-----|----------------|
| PIK3CD (rs7518602) | CC    | CT | TT  | C              |

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| Gene          | rs | T2D   | Prediabetes | Control | Odds Ratio | p-Value | 95% CI Low | 95% CI High | n1 | n2 | n3 | p   |
|--------------|----|-------|-------------|---------|------------|---------|------------|-------------|----|----|----|-----|
| MKNK1        |    | 1.832 | 1.139       | 0.329   | 1.000      | 0.4     | 0.244      | 0.848       |    |    |    | 0.4 |
| PPP1CB       |    | 4.558 | 1.174       | 0.893   | 1.000      | 0.1     | 0.556      | 0.64        |    |    |    | 0.05|
| PRKCI        |    | 3.575 | 0.921       | 0.521   | 1.000      | 0.089   | 0.556      | 0.64        |    |    |    | 0.01|
| PRKAA1       |    | 0.893 | 0.521       | 0.819   | 1.000      | 0.089   | 0.556      | 0.64        |    |    |    | 0.01|
| PRKAG2       |    | 0.302 | 1.136       | 1.136   | 0.000      | 0.302   | 0.567      | 0.788       |    |    |    | 0.01|
| PRKAR1B      |    | 0.866 | 1.000       | 1.000   | 0.000      | 0.302   | 0.567      | 0.788       |    |    |    | 0.01|
| GCK          |    | 1.000 | 1.000       | 1.000   | 0.000      | 0.302   | 0.567      | 0.788       |    |    |    | 0.01|
| Gene   | T2D   | Prediabetes | Control | p-value | OR   | 95% CI Low | 95% CI High |
|--------|-------|-------------|---------|---------|------|------------|-------------|
| PRKACG(rs4745515) | AA    | AG          | GG      |         |      |            |             |
| T2D    | 543   | 126         | 6       | 3.233   | 0.186| 0.834      | 1212        |
| Prediabetes | 197   | 44          | 4       | 2.611   | 0.261| 0.328      | 438         |
| Control | 477   | 86          | 4       |         |      |            |             |
| FBP1(rs7031924) | GG    | AG          | AA      | A       |      |            |             |
| T2D    | 527   | 166         | 10      | 0.173   | 0.917| 0.514      | 1220        |
| Prediabetes | 178   | 74          | 1       | 4.931   | 0.085| 0.023      | 430         |
| Control | 419   | 126         | 7       | 0.568   |      |            |             |
| HRAS(rs7939028) | CC    | CG          | GG      | G       |      |            |             |
| T2D    | 457   | 220         | 26      | 2.776   | 0.25 | 1.000      | 1134        |
| Prediabetes | 178   | 68          | 7       | 3.413   | 0.182| 0.818      | 424         |
| Control | 357   | 184         | 12      | 0.037   |      |            |             |
| PRKAB1(rs6490265) | CC    | CT          | TT      | C       |      |            |             |
| T2D    | 189   | 350         | 163     | 1.676   | 0.433| 1.000      | 728         |
| Prediabetes | 91    | 118         | 44      | 3.377   | 0.185| 0.606      | 300         |
| Control | 165   | 271         | 116     | 0.795   |      |            |             |
| PRKAG1(rs2293446) | GG    | GA          | AA      | G       |      |            |             |
| T2D    | 230   | 320         | 126     | 0.232   | 0.891| 0.434      | 780         |
| Prediabetes | 82    | 113         | 50      | 0.424   | 0.809| 0.362      | 277         |
| Control | 184   | 272         | 106     | 0.797   |      |            |             |
| KRAS(rs7311692) | CC    | CG          | GG      | C       |      |            |             |
| T2D    | 576   | 121         | 6       | 2.971   | 0.226| 1.000      | 1273        |
| Prediabetes | 220   | 32          | 1       | 0.923   | 0.63 | 1.000      | 472         |
| Control | 450   | 93          | 9       | 0.152   |      |            |             |
| PCK2(rs4982856) | CC    | CT          | TT      | C       |      |            |             |
| T2D    | 174   | 368         | 160     | 2.835   | 0.242| 0.223      | 716         |
| Prediabetes | 66    | 137         | 50      | 2.553   | 0.279| 0.207      | 269         |
| Control | 158   | 266         | 128     | 0.446   |      |            |             |
| SOS2(rs1955926) | CC    | CA          | AA      | C       |      |            |             |
|
| SNP          | Control | Prediabetes | T2D   |
|--------------|---------|-------------|-------|
| SHC4 (rs12900666) | CC: 233 CA: 262 AA: 71 | | |
| T2D          | 356     | 267         | 52    |
| Prediabetes  | 130     | 98          | 17    |
| Control      | 311     | 222         | 30    |
| 
| PDGK1 (rs76318740) | CC: 486 CT: 822 TT: 8 | | |
| T2D          | 512     | 159         | 5     |
| Prediabetes  | 180     | 60          | 6     |
| Control      | 428     | 128         | 10    |
| 
| FASN (rs7222326) | TT: 263 CT: 126 CC: 0 | | |
| T2D          | 521     | 142         | 12    |
| Prediabetes  | 197     | 47          | 1     |
| Control      | 447     | 113         | 4     |
| 
| ACACA (rs11868124) | CC: 262 CT: 118 TT: 0 | | |
| T2D          | 466     | 185         | 25    |
| Prediabetes  | 166     | 71          | 9     |
| Control      | 388     | 158         | 21    |
| 
| FLOT2 (rs4795473) | TT: 263 CT: 126 CC: 0 | | |
| T2D          | 352     | 301         | 50    |
| Prediabetes  | 121     | 116         | 16    |
| Control      | 281     | 215         | 57    |
| 
| INSR (rs1864009) | GG: 349 GT: 150 TT: 77 | | |
| T2D          | 166     | 351         | 159   |
| Prediabetes  | 45      | 125         | 76    |
| Control      | 127     | 286         | 152   |
| 
| PIK3R2 (rs3736328) | CC: 263 CG: 120 GG: 0 | | |
| T2D          | 376     | 252         | 48    |
| Prediabetes  | 144     | 87          | 15    |
| Control      | 316     | 224         | 27    |
| 
| CBLC (rs2965143) | AA: 263 AG: 120 GG: 0 | | |
| T2D          | 142     | 116         | 159   |
|                | T2D   | Prediabetes | Control | MKNK2(rs3810412) | T2D   | Prediabetes | Control | SHC2(rs73916989) | T2D   | Prediabetes | Control | TRIP10(rs340141) | T2D   | Prediabetes | Control | PRKX(rs1003351) | T2D   | Prediabetes | Control |
|----------------|-------|-------------|---------|------------------|-------|-------------|---------|------------------|-------|-------------|---------|------------------|-------|-------------|---------|---------------|-------|-------------|---------|
|                | 216   | 110         | 208     | CC               | 557   | 213         | 454     | CC               | 502   | 175         | 379     | CC               | 430   | 171         | 332     | AA            | 649   | 237         | 550     |
|                | 349   | 99          | 282     | CT               | 138   | 38          | 94      | CG               | 183   | 71          | 163     | CT               | 245   | 76          | 189     | AG            | 26    | 8           | 16     |
|                | 111   | 37          | 77      | TT               | 8     | 2           | 5       | GG               | 18    | 6           | 10      | TT               | 28    | 6           | 32      | GG            | 0     | 0           | 0       |
|                | 3.886 | 6.388       | 208     | C               | 1.64  | _           | _       | C                | 2.482 | 0.406       | 0.468   | C                | 6.785 | 0.816       | 0.822   | A             | 8.095 | _           | _       |
|                | 0.143 | 0.041       | 0.247   |                 | 0.44  | _           | _       |                 | 0.289 | 0.04       | 0.468   |                 | 0.331 | 0.816       | 0.822   |               | 0.371 | _           | _       |
|                | 0.154 | 0.069       | 0.468   |                 | 1.000 | _           | _       |                 | 0.034 | 0.036      | 0.036   |                 | 1.000 | 0.660       | 0.660   |               | 0.660 | _           | _       |
|                | 781   | 319         | 698     |                 | 1252  | 464         | 1002    |                 | 1187  | 421         | 921     |                 | 1105  | 418         | 853     |               | 1324  | 482         | 1116    |

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

**Figures**

![Figure 1](https://via.placeholder.com/150)

**Figure 1**

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

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplemental Table1.docx
- Supplemental Table 2.docx