Association of Common Variants of *CDKN2A/2B* rs10811661 (C/T) and *WFS1* rs6446482 (C/G) to Type 2 Diabetes Mellitus in the Indian Population of Eastern Uttar Pradesh

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Abstract

Aim: Recent genome-wide association studies (GWAS) have identified several unsuspected genes that significantly increase the risk of type 2 diabetes mellitus (T2DM). We aimed to replicate the association of a common variant each in *CDKN2A/2B* (rs10811661) and *WFS1* (rs6446482) in the population of Eastern Uttar Pradesh, India. These variants have been identified to be differentially associated with T2DM in different ethnic groups in previous GWAS.

Results: We found SNP rs10811661 of *CDKN2A/2B* (OR 1.50, 95% CI 1.109-2.032, $P=0.009$) and SNP rs6446482 of *WFS1* (OR 1.43, 95% CI 1.074-1.896, $P=0.014$) as significant risk factors for T2DM in Eastern Uttar Pradesh population. Normal Glucose-Tolerant (NGT) subjects carrying risk allele of rs10811661 (C/T) and rs6446482 (C/G) polymorphisms had significantly higher Fasting Plasma Glucose (FPG) and 2-hour Postprandial Plasma Glucose (2h-PPPG) levels compared to non-carriers.

Conclusion: Our study replicates the association of well established common variants of *CDKN2A/2B* rs10811661 (C/T) and *WFS1* rs6446482 (C/G) with type 2 diabetes in the population of Eastern Uttar Pradesh, India. Interestingly, our data show larger effect size for both of the SNPs than those reported in European populations.

Keywords: Association studies; Body mass index; *CDKN2A/2B*; *WFS1*; Ethnicity; Single Nucleotide Polymorphism (SNP); Type 2 diabetes mellitus

Introduction

Type 2 Diabetes Mellitus (T2DM) is a substantial health issue worldwide with increasing prevalence at alarming rate [1]. India leads the world with largest number of diabetic patients (around 40.9 million in 2006 which is expected to rise to ~70 million by 2025) and is therefore termed as “Diabetes capital of the world” [2]. T2DM is characterized by pancreatic beta cell dysfunction and insulin resistance as a result of the interaction of genetic and environmental factors. Association of a number of genes with T2DM has been shown in the recent past [3]. Recent GWAS have identified several unsuspected genes (with previously unknown functions in pathology of T2DM) associated with T2DM [4-8]. However, the underlying molecular mechanisms in the development of diabetes remain poorly understood. In the present study, we have examined the association of the most significant genetic variant of loci *CDKN2A/2B* (rs10811661) and *WFS1* (rs6446482) with diabetes in the population of Eastern Uttar Pradesh, India. These variants have been found to be convincingly associated with T2DM in Caucasian populations [6-14]. However, recently Nemr et al. [15] have found that *CDKN2A/2B* rs10811661 is not associated with T2DM in Lebanese.

CDKN2A and *CDKN2B* are adjacent Cyclin dependent kinase inhibitor genes on chromosome 9p. SNP rs10811661 located upstream of *CDKN2A/2B* showed genome-wide significant association to T2DM (OR 1.20, $P=7.8 \times 10^{-15}$) in all data meta-analysis. SNP rs10811661 may have a long-range effect on one of the genes, or may influence a gene not yet annotated [6]. The region of association is limited to a 9 kb region flanked by strong recombination hot-spots, in which there are multiple conserved non-coding sequences but no known genes or

microRNAs [9]. *CDKN2A* and *CDKN2B* encode p16^{INK4a} and p15^{INK4b} respectively. p16^{INK4a} inhibits *CDK4*, a powerful regulator of pancreatic beta cell replication. In mice, *Cdkn2a* over-expression leads to islet hypoplasia and diabetes [8].

WFS1 encodes wolframin, a transmembrane glycoprotein that maintains calcium homeostasis of the endoplasmic reticulum. Mutations in this gene causes Wolfram syndrome (OMIM 222300), characterized by diabetes insipidus, juvenile-onset non-autoimmune diabetes mellitus, optic atrophy and deafness. Disruption of *WFS1* gene in mice causes overt diabetes or impaired glucose tolerance depending on genetic background. Both humans and mice deficient in wolframin show pancreatic beta cell loss. Thus, *WFS1* is critical for survival and function of insulin-producing pancreatic beta cells. Four of the SNPs rs10010131, rs6446482, rs752854 and rs734312 (*H611R*) in the *WFS1* locus have been convincingly shown to be associated with T2DM in Caucasians, having odds ratios (ORs) 0.90~0.92 and P values $1.3 \times 10^{-4} \sim 1.4 \times 10^{-7}$ [10]. Replication of the association between variants in *WFS1* and risk of type 2 diabetes was also shown by Franks et al. [16] in European population. Statistically significant associations between

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the major alleles of three variants of *WFS1* rs10010131, rs1801213/rs7672995 and rs734312 and prevalent T2D in the DESIR cohort at baseline was observed by Cheurfa et al. [17]. Also, the most frequent haplotype at the haplotype block containing the *WFS1* gene modulated insulin secretion and was associated with an increased risk of type 2 diabetes.

Material and Methods

Sample collection

Samples were collected from Eastern Uttar Pradesh in this case-control study. Blood from diabetic patients and normal healthy controls (>35 years) was collected after informed consent according to the approved protocol by the Institutional Ethical Committee of Banaras Hindu University from the patients attending out-patient departments of Institute of Medical Sciences, Banaras Hindu University, Heritage Hospital and Prakash Pathology, Varanasi.

Screening of the study subjects

We have genotyped two single nucleotide polymorphisms, *CDKN2A* rs10811661 and *WFS1* rs6446482 in 517 unrelated individuals from Eastern Uttar Pradesh, India, including 271 type 2 diabetic patients and 246 ethnically matched control subjects. Subjects were diagnosed diabetic according to WHO criteria (1999). Subjects were included in the diabetes group if they had fasting glucose concentrations ≥ 126 mg/dl or 2-hour glucose concentrations ≥ 200 mg/dl after a 75 g Oral Glucose Tolerance Test (OGTT). Clinical history of diabetes and associated complications as well as the family history were recorded. Non-diabetic control subjects were chosen based on the absence of a history of diabetes in the subject and among first-degree relatives, as well as normal glucose tolerance, confirmed by a 75 g oral glucose tolerance test. After screening with standard OGTT, age, gender and Body Mass Index (BMI) matched 246 normal healthy controls were enrolled from the population undergoing routine health check-up.

Anthropometric and biochemical evaluation

Anthropometric measurements including weight, height, and waist were obtained using standard protocol. The BMI was calculated as the weight in kilograms divided by the square of height in meters. Clinical and biochemical data (fasting plasma glucose (FPG) and 2 hour postprandial plasma glucose (PPPG)) were obtained as part of our study protocol.

DNA analysis and genotyping

Blood sample (4-5 ml) was taken in 0.5 M EDTA (Sigma, USA) vials. Genomic DNA was extracted from peripheral blood leucocytes using the standard salting-out method.

CDKN2A/2B: We genotyped 271 diabetic subjects and 246 healthy

controls. Polymorphic region of *CDKN2A/2B* (rs10811661) was PCR amplified using a forward primer: 5'-ATAAGCGTTCTTGCCCTGTC-3' and reverse: 5'-GTCAAAAACCTTCCCCATCC-3'. The cycling conditions were 94°C for 5 minutes, followed by 32 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 7 minutes. The PCR products of 121 bp were digested with BspHI (Bangalore Genie, India) for the rs10811661 (C/T) polymorphism. The resulting products were electrophoresed on a 3 % agarose gel.

WFS1: We genotyped 234 diabetic subjects and 234 normal healthy controls (smaller sample size than *CDKN2A/B* is due to the genotyping failure in some of the samples). A 136 bp polymorphic region of rs6446482 was PCR-amplified using forward primer: 5'-TGGTCACCTCACAGCTCAGT-3' and reverse primer: 5'-TGCAAGAGGAGAGGAGTCG-3' followed by RsaI (Bangalore Genie, India) digestion for the rs6446482 (C/G) polymorphism. The cycling conditions were 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 7 minutes. The resulting products were electrophoresed on a 3% agarose gel.

Statistical analysis

Clinical information of the T2DM patients and healthy controls was recorded as shown in table 1. Data on quantitative characteristics are expressed as mean $\pm$ SD. Allele and genotype frequencies were compared between T2DM patients and controls using 2 $\times$ 2 and 2 $\times$ 3 contingency tables respectively with χ^2 test. Odds ratio (OR) with 95% Confidence Interval (CIs) was determined to describe the strength of association. *P* values of less than 0.05 were considered statistically significant.

Results

The study group cases had significantly higher values of BMI (*P*=0.024 for *CDKN2A/2B*; *P*=0.0027 for *WFS1*); waist circumference (*P*=0.0128 for *CDKN2A/2B*; *P* < 0.0001 for *WFS1*); FPG (*P*<0.0001 for both the SNPs) and 2h-PPPG (*P*<0.0001 for both the SNPs) compared to controls. The cases were also found to be of significantly higher age group than healthy controls (Table 1). *CDKN2A/2B* (rs10811661) and *WFS1* (rs6446482) polymorphisms show Hardy-Weinberg distributions in our study group. The genotype and allele distributions of the SNPs, rs10811661 and rs6446482 are significantly different between the T2DM and control groups. The major allele T of rs10811661 of *CDKN2A/2B* (OR 1.50, 95% CI 1.109-2.032; *P*=0.009) shows modest effect size and the major allele C of rs6446482 of *WFS1* also shows modest effect size (OR 1.43, 95% CI 1.074-1.896; *P*=0.014) (Tables 2 and 3) indicating that these variants are risk factors associated to type 2 diabetes in this population. Our data support the association of rs10811661 of *CDKN2A/2B* to T2DM under both dominant (*P*=0.0270;

Variables	CDKN2A/2B			WFS1		
	Cases	Control	P-value	Cases	Control	P-value
Number	271	246		234	234	
M:F	177:94	139:107		144:90	135:99	
^a Age (yrs)	52.80 $\pm$ 9.64	50.84 $\pm$ 11.48	<0.0001	51.95 $\pm$ 8.35	50.72 $\pm$ 11.74	<0.0001
^a Duration of diabetes (yrs)	3.95 $\pm$ 4.83	-		3.78 $\pm$ 4.60	-	
^a BMI (Kg/m ²)	25.85 $\pm$ 5.38	24.28 $\pm$ 4.81	0.0240	26.16 $\pm$ 5.44	25.05 $\pm$ 4.73	0.0027
^a Waist Circumference (cm)	87.84 $\pm$ 9.36	86.32 $\pm$ 10.12	0.0128	88.5 $\pm$ 8.46	85.49 $\pm$ 10.16	<0.0001
^a FPG (mg/dl)	154.54 $\pm$ 65.97	87.75 $\pm$ 20.30	<0.0001	148.59 $\pm$ 65.39	87.32 $\pm$ 19.30	<0.0001
^a 2hr PPPG (mg/dl)	229.46 $\pm$ 87.94	120.53 $\pm$ 18.73	<0.0001	221.55 $\pm$ 86.46	120.83 $\pm$ 18.64	<0.0001

^aMean $\pm$ S.D

Table 1: Clinical characteristics of the study populations for *CDKN2A/2B* and *WFS1*.

OR 1.50, 95% CI 1.05 - 2.14) and recessive ($P=0.0308$; OR 2.62, 95% CI 1.06-6.49) models, while rs6446482 of *WFS1* shows association under only dominant model ($P=0.0205$; OR 1.54, 95% CI 1.07-2.22) (Table 4).

Further, in Normal Glucose-Tolerant (NGT) subjects, FPG and 2h-PPPG levels were found to be significantly higher in carriers of at risk allele (genotypes TT+CT) of rs10811661 of *CDKN2A/2B* (C/T) polymorphism ($P=0.0031$ and $P=0.049$ for FPG and 2h-PPPG, respectively) compared to non-carriers (genotype CC) (Table 5). Similarly, carriers of at risk allele of rs6446482 of *WFS1*(C/G) polymorphism (genotypes CC+CG) also showed significantly higher FPG and 2h-PPPG levels ($P=0.007$ and $P=0.029$ for FPG and 2h-PPPG, respectively) compared to non-carriers (genotype GG) (Table 6). Compared to the carriers of the risk genotypes of both rs1081166 of *CDKN2A/2B* and rs6446482 of *WFS1* together in T2DM group,

the FPG and 2h-PPPG levels were not significantly different from the carriers of risk genotypes of either *CDKN2A/2B* (rs1081166) or *WFS1* (rs6446482) separately (Table 7).

Discussion

Asian Indians have an increased risk of developing T2DM. Characteristic “Asian Indian Phenotype” (higher body fat percent but a lower lean mass for a given BMI, central obesity leading to high insulin resistance) make them more susceptible to the disease [2]. Most of the studies related to search for T2DM susceptibility loci have been conducted in populations of European decent. Since different gene-environment interactions operate in different populations to increase risk of developing diabetes, the association studies of western populations are required to be replicated in different Asian Indian

Genotype/Allele	T2DM frequency (%)	Control Frequency (%)	χ^2	P-value	OR	95% CI
CDKN2A/2B rs10811661	n=271	n=246				
T/T	183 (0.675)	143 (0.581)	5.721	0.028	2.925	1.201 - 7.110
C/T	81 (0.30)	87 (0.354)	2.574	0.123	2.128	0.852 - 5.298
C/C	7 (0.025)	16 (0.065)				
C	95 (0.175)	119 (0.240)				
T	447 (0.825)	373 (0.760)	6.968	0.009	1.50	1.109-2.032

Table 2: Genotype /Allele frequency distribution of CDKN2A/2B rs10811661(C/T) variant among control subjects and type 2 diabetes patients and their Odds Ratio (OR).

Genotype/Allele	T2DM frequency (%)	Control Frequency (%)	χ^2	P-value	OR	95% CI
WFS1 rs6446482	n=234	n=234				
C/C	135 (0.58)	110 (0.47)	3.408	0.065	1.809	0.965-3.389
C/G	80 (0.34)	96 (0.41)	0.380	0.538	1.228	0.642-2.346
G/G	19 (0.08)	28 (0.12)				
C	350 (0.73)	316 (0.67)	6.017	0.014	1.43	1.074-1.896
G	118 (0.27)	152 (0.33)				

Table 3: Genotype /Allele frequency distribution of WFS1 rs6446482 (C/G) variant among control subjects and type 2 diabetes patients and their Odds Ratio (OR).

CDKN2A/2B rs10811661 (C/T)		WFS1 rs6446482 (C/G)	
Dominant model	Recessive model	Dominant model	Recessive model
TT vs. TC+CC	TT+TC vs. CC	CC vs. CG+GG	CC+CG vs. GG
OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
1.50 (1.05-2.14)	2.62 (1.06-6.49)	1.54 (1.07-2.22)	1.54 (0.83-2.84)
P-value=0.0270	P-value=0.0308	P-value=0.0205	P-value=0.1663

Table 4: Comparison of dominant and recessive models for CDKN2A/2B and WFS1.

Characteristics	Type 2 diabetics (n=271)			Control (n=246)		
	CC (n=7) (Non-risk group)	TT+CT (n=264) (Risk group)	P-value	CC (n=16) (Non-risk group)	TT+CT (n=230) (Risk group)	P-value
Fasting plasma glucose	122.50 ± 20.28	154.03 ± 53.09	0.045	80.38 ± 05.72	86.34 ± 10.36	0.003
2h Plasma glucose	217.50 ± 51.44	240.59 ± 74.21	0.439	117.22 ± 11.16	124.44 ± 21.94	0.049

Table 5: Correlation of the CDKN2A/2B rs10811661(C/T) genotype with FPG and 2h-PPPG.

Characteristics	Type 2 diabetics (n=234)			Control (n=234)		
	GG (n=19) (Non-risk group)	CC+CG (n=215) (Risk group)	P-value	GG (n=28) (Non-risk group)	CC+CG (n=206) (Risk group)	P-value
Fasting plasma glucose	126.72 ± 54.89	151.12 ± 66.17	0.047	83.53 ± 12.84	88.46 ± 21.21	0.007
2h Plasma glucose	202.44 ± 87.48	223.84 ± 86.26	0.256	117.37 ± 13.57	126.87 ± 19.83	0.029

Table 6: Correlation of the WFS1 rs6446482 (C/G) genotype with FPG and 2h-PPPG.

CDKN2A/2B rs10811661 (TT+CT) + WFS1 rs6446482 (CC+CG)		CDKN2A/2B rs10811661 (TT+CT)		WFS1 rs6446482 (CC+CG)	
FPG (mg/dl)	2h-PPG (mg/dl)	FPG (mg/dl)	2h-PPG (mg/dl)	FPG (mg/dl)	2h-PPG (mg/dl)
143.536 ± 62.087	216.764 ± 84.286	143.006 ± 59.355 (P=0.467)	216.699 ± 86.448 (P=0.497)	145.480 ± 64.206 (P=0.396)	219.996 ± 89.921 (P=0.378)

Table 7: Combined effect of risk genotypes of CDKN2A/2B and WFS1 in T2DM group on FPG and 2h PPG compared with the risk genotypes of CDKN2A/2B and WFS1 separately (Mean ± S.D.).

populations, especially populations with a high prevalence of diabetes such as India. Moreover, Indian populations are not genetically homogeneous and are unique in their genetic origin and the mutations underlying susceptibility to complex disease [18]. The result shows the evidence for modest effect of *CDKN2A/2B* (rs10811661) and *WFS1* (rs6446482) on susceptibility to T2DM in the population from Eastern Uttar Pradesh. The *CDKN2A/2B* (OR 1.5) shows modest effect size similar to other reports in some populations [6-9,11]. In our study *WFS1* (OR 1.43) shows larger effect size compared to European populations [10,16,17] and the susceptible variant attain statistical significance in the present study.

Recent GWAS and meta-analysis provide convincing evidence for *CDKN2A/2B* gene region to be involved in T2DM [6-9,11,13,14]. Meta-analysis of genotype data from GWAS in northern Europeans have confirmed that SNPs rs10811661 and rs564398 in the *CDKN2A/2B* region are T2DM susceptibility variants, although the combined evidence for rs10811661 is far stronger than that for rs564398 [6,8,9]. GWAS in French-Canadian obtained nominal association signals for proxies ($r^2 \geq 0.9$) of rs10811661 (rs2383208, $P=2 \times 10^{-4}$) and rs564398 (rs1063192, $P=2 \times 10^{-2}$) [7]. A strong association between the major allele of rs10811661 and T2DM was reported in French Europids [19], Chinese Hans population [20], and Korean population [21]. In a Danish population, variants of *CDKN2A/2B* was found to be highly associated with T2DM with an OR of 1.30 per risk allele and the SNPs within *CDKN2A/2B* loci impaired glucose induced insulin release in healthy Danes [22]. Association of variant of *CDKN2A/2B* was modestly replicated in Asians but not replicated in African Americans and Pima Indians [23]. Recent GWAS in Diabetes Prevention Program (DPP) have shown *CDKN2A/2B* (rs10811661) as a potential intervention-interaction site showing response to treatment with Troglitazone by improving insulin sensitivity [24]. A strong association between *CDKN2A/2B* (rs10811661) and T2DM has been reported in Chinese population with an Odds ratio 1.406 [25]. Similar Odds ratio has been reported for *CDKN2A/2B* (rs10811661) OR 1.3 by Chauhan et al. [26] in the populations of a region of North-Western India. We report a higher OR 1.5 for the same SNP rs10811661 of *CDKN2A/2B* in the Eastern region of India. India represents one of the largest human diversity, consisting of 4635 culturally and anthropologically well-defined populations with little or no gene flow between them [18]. The deviation in our results from those of Chauhan et al. [26] may probably be due to this genetic heterogeneity. Bao et al. [13] have also reported OR 1.28 for *CDKN2A/2B* rs10811661 in a recent meta-analysis.

Association of the *WFS1* with T2DM has been reported in several UK studies and one of the Ashkenazi Jewish populations where 1,536 SNPs in 84 candidate genes (including *WFS1*) that regulate pancreatic beta cell development, growth, function and survival, were genotyped in the association study of 9,533 cases and 11,389 controls. A haplotype with rs1801208 (R456H) and rs734312 (H611R) in *WFS1* showed a nominal association with T2DM in Japanese subjects [27]. A meta-analysis in Caucasians for the *WFS1* gene showed strong evidence of statistical association [16]. In an accompanying report from DPP showed that the carriers of the protective variants in *WFS1* exhibited a trend towards increased insulin secretion [28]. Recent study in Korean population does not show association between *WFS1* rs734312 and T2DM [21]. Another SNP for *WFS1* (rs10010131) did not show significant association to T2DM in Chinese populations [25]. A case-control study in Japanese population has shown a marginal association between *WFS1* rs12511742 and T2DM. However, association between rs6446482 and T2DM was not apparent in the same case-control study, although the OR was found to be in the same direction as that in

Caucasians, probably due to lower minor allele frequency of the SNP in Japanese (0.02 in Japanese and 0.33~0.42 in Caucasians) [29]. We show association with modest effect size (OR 1.43) for *WFS1* rs6446482 in the present study group.

We show modest effect size similar to other reports (loc. cit.) for *CDKN2A/2B* rs10811661 (OR 1.50, 95% CI 1.109-2.032; $P=0.009$) indicating that it can be a susceptible variant predisposing to T2DM in the population of Eastern Uttar Pradesh, India. For *WFS1* rs6446482 (OR 1.43, 95% CI 1.074-1.896; $P=0.014$) the effect size is larger compared to other studies, implicating it to be a predisposing risk factor for T2DM in our population and the gene variant showed statistical significant association. The significantly higher values for fasting plasma glucose and 2-hour plasma glucose levels in the TT+CT genotype group of rs10811661 of *CDKN2A/2B* and CC+CG genotype group of rs6446482 of *WFS1* polymorphisms among NGT subjects further support our finding that the 'T' allele of *CDKN2A/2B* and 'C' allele of *WFS1* are risk alleles for type 2 diabetes mellitus. However, our data does not show any significant difference when the FPG and 2 h PPG values of the risk genotypes of both the variants combined together were compared with the risk genotypes of both *CDKN2A/2B* and *WFS1* separately. Recently, Hribal et al. [30] also showed that TT genotype of rs10811661 polymorphism is associated with impaired insulin release and IGT suggesting that this variant may contribute to T2D by affecting beta cell function. Study at a larger scale with functional characterization is warranted to reliably confirm the association of the above variants and define their role(s) in future preventive measures for T2DM.

In conclusion, we replicate the association of well established common variants of *CDKN2A/2B* rs10811661(C/T) and *WFS1* rs6446482 (C/G) with type 2 diabetes in the population of Eastern Uttar Pradesh, India. Interestingly, our data for both of the SNPs show larger effect size than those reported in European populations. To our knowledge this is the first report in this population and provides valuable information for comparison with other ethnic groups as well as in determining disease susceptibility in this population. However, in view of the genetic diversity of Indians, both these results need to be replicated in other ethnic groups.

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References

1. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15: 539-553.
2. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C (2007) Epidemiology of type 2 diabetes: Indian scenario. *Indian J Med Res* 125: 217-230.
3. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, et al. (2008) Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N Engl J Med* 359: 2220-2232.
4. Frayling TM (2007) Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 8: 657-662.
5. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, et al. (2007) A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316: 889-894.
6. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316: 1341-1345.

7. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, et al. (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445: 881-885.
8. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, et al. (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316: 1336-1341.
9. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burt NP, et al. (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316: 1331-1336.
10. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, et al. (2007) Common variants in *WFS1* confer risk of type 2 diabetes. *Nat Genet* 39: 951-953.
11. Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661-678.
12. Zmyslowska A, Borowiec M, Antosik K, Szalecki M, Stefanski A, et al. (2011) Wolfram syndrome in the Polish population: novel mutations and genotype-phenotype correlation. *Clin Endocrinol (Oxf)* 75: 636-641.
13. Bao XY, Xie C, Yang MS (2012) Association between type 2 diabetes and *CDKN2A/B*: a meta-analysis study. *Mol Biol Rep* 39: 1609-1616.
14. Shea J, Agarwala V, Philippakis AA, Maguire J, Banks E, et al. (2011) Comparing strategies to fine-map the association of common SNPs at chromosome 9p21 with type 2 diabetes and myocardial infarction. *Nat Genet* 43: 801-805.
15. Nemr R, Almawi AW, Echtaay A, Sater MS, Daher HS, et al. (2012) Replication study of common variants in *CDKAL1* and *CDKN2A/2B* genes associated with type 2 diabetes in Lebanese Arab population. *Diabetes Res Clin Pract* 95: e37-e40.
16. Franks PW, Rolandsson O, Debenham SL, Fawcett KA, Payne F, et al. (2008) Replication of the association between variants in *WFS1* and risk of type 2 diabetes in European populations. *Diabetologia* 51: 458-463.
17. Cheurfa N, Brenner GM, Reis AF, Dubois-Laforgue D, Roussel R, et al. (2011) Decreased insulin secretion and increased risk of type 2 diabetes associated with allelic variations of the *WFS1* gene: the Data from Epidemiological Study on the Insulin Resistance Syndrome (DESIR) prospective study. *Diabetologia* 54: 554-562.
18. Indian Genome Variation Consortium (2008) Genetic landscape of the people of India: a canvas for disease gene exploration. *J Genet* 87: 3-20.
19. Duesing K, Fatemifar G, Charpentier G, Marre M, Tichet J, et al. (2008) Strong association of common variants in the *CDKN2A/CDKN2B* region with type 2 diabetes in French Europeans. *Diabetologia* 51: 821-826.
20. Wu Y, Li H, Loos RJ, Yu Z, Ye X, et al. (2008) Common variants in *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, *SLC30A8*, and *HHEX/IDE* genes are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. *Diabetes* 57: 2834-2842.
21. Lee YH, Kang ES, Kim SH, Han SJ, Kim CH, et al. (2008) Association between polymorphisms in *SLC30A8*, *HHEX*, *CDKN2A/B*, *IGF2BP2*, *FTO*, *WFS1*, *CDKAL1*, *KCNQ1* and type 2 diabetes in the Korean population. *J Hum Genet* 53: 991-998.
22. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, et al. (2007) Studies of association of variants near the *HHEX*, *CDKN2A/B*, and *IGF2BP2* genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes* 56: 3105-3111.
23. Rong R, Hanson RL, Ortiz D, Wiedrich C, Kobes S, et al. (2009) Association analysis of variation in/near *FTO*, *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *LOC387761*, and *CDKN2B* with type 2 diabetes and related quantitative traits in Pima Indians. *Diabetes* 58: 478-488.
24. Moore AF, Jablonski KA, McAteer JB, Saxena R, Pollin TI, et al. (2008) Extension of type 2 diabetes genome-wide association scan results in the diabetes prevention program. *Diabetes* 57: 2503-2510.
25. Hu C, Zhang R, Wang C, Wang J, Ma X, et al. (2009) *PPARG*, *KCNJ11*, *CDKAL1*, *CDKN2A-CDKN2B*, *IDE-KIF11-HHEX*, *IGF2BP2* and *SLC30A8* are associated with type 2 diabetes in a Chinese population. *PLoS One* 4: e7643.
26. Chauhan G, Spurgeon CJ, Tabassum R, Bhaskar S, Kulkarni SR, et al. (2010) Impact of common variants of *PPARG*, *KCNJ11*, *TCF7L2*, *SLC30A8*, *HHEX*, *CDKN2A*, *IGF2BP2*, and *CDKAL1* on the risk of type 2 diabetes in 5,164 Indians. *Diabetes* 59: 2068-2074.
27. Kawamoto T, Horikawa Y, Tanaka T, Kabe N, Takeda J, et al. (2004) Genetic variations in the *WFS1* gene in Japanese with type 2 diabetes and bipolar disorder. *Mol Genet Metab* 82: 238-245.
28. Florez JC, Jablonski KA, McAteer J, Sandhu MS, Wareham NJ, et al. (2008) Testing of diabetes-associated *WFS1* polymorphisms in the Diabetes Prevention Program. *Diabetologia* 51: 451-457.
29. Mita M, Miyake K, Zenibayashi M, Hirota Y, Teranishi T, et al. (2008) Association study of the effect of *WFS1* polymorphisms on risk of type 2 diabetes in Japanese population. *Kobe J Med Sci* 54: E192-199.
30. Hribal ML, Presta I, Procopio T, Marini MA, Stancakova A, et al. (2011) Glucose tolerance, insulin sensitivity and insulin release in European non-diabetic carriers of a polymorphism upstream of *CDKN2A* and *CDKN2B*. *Diabetologia* 54: 795-802.