

Short Communication

Tagging rs10811661 Variant at *CDKN2A/2B* Locus Is Not Associated with Type 2 Diabetes Mellitus in Czech Population

(polymorphism / diabetes / Czech population / *CDKN2A/2B*)

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Abstract. Genome-wide association studies have resulted in the identification of the *CDKN2A/2B* locus as an important genetic determinant of type 2 diabetes mellitus development. The aim of this study was to investigate the role of this locus in the development of type 2 diabetes mellitus in Czech Slavonic population. Groups of 1,149 type 2 diabetic patients and a group of 2,312 healthy controls, both of Czech origin, were successfully genotyped for the rs10811661 *CDKN2A/2B* tagging polymorphism. The “risky” TT genotype frequencies were almost identical in both examined groups (69.3 % in patients and 68.9 % in controls, $P = 0.52$; OR [95% CI] = 1.02 [0.87 – 1.19] for TT versus C allele carriers). Similar negative results were obtained when males ($P = 0.93$) and females ($P = 0.23$) were analysed separately. We have not confirmed the association between rs10811661 SNP and susceptibility to the type 2 diabetes mellitus in Czech Slavonic population.

Introduction

It is estimated that there could be about 300 millions of individuals with type 2 diabetes around the world. Diabetes is a complex chronic disease with polygenic background. Both environmental and genetic factors in-

teract together and influence the onset and further development of this disease.

So far performed genome-wide association studies (GWAs) have detected several genetic variants (for example within the *TCF7L2*, *FTO*, *KCNJ11*, *SLC30A8*, *ADAMTS9*, *JAZF1* and *CDC123* genes) with different reproducibility associated with type 2 diabetes (Sanghera and Blackett, 2012). Despite the fact that GWAs identified almost 50 candidate loci involved in diabetes onset and development, these variants account together for only about 10 % of the entire diabetes heritability (Imamura and Maeda, 2011).

One of the first detected and widely analysed DNA markers is the common variant rs10811661 on the 9p21.3 locus. The marker is located in an intergenic region between the genes coding for the cyclin-dependent kinase inhibitors *CDKN2A* and *CDKN2B* (OMIM acc. Nos. 600160 and 600431), where also the *ANRIL* gene (antisense noncoding RNA in the *INK4* locus) coding for regulatory RNA is present (Pasmant et al., 2011). Interestingly, another independent set of SNPs located in the same region is associated with myocardial infarction (Palomaki et al., 2010; Hubáček et al., 2012a).

CDKN2A/2B encode two kinase inhibitors, which play an important role in β -cell regeneration (for review see Bao et al., 2012), and thus the functional connection to the type 2 diabetes is obvious.

The results published so far have focused mainly on West-European and Asian individuals. Therefore, our study investigated whether rs10811661 SNP within the *CDKN2A/2B* region is associated with enhanced risk of type 2 diabetes in Czech Slavonic population.

Material and Methods

Altogether, we have collected 1,153 and successfully genotyped 1,149 patients (717 males and 432 females aged 18–86 years) with type 2 diabetes mellitus (according to the World Health Organization (WHO) criteria) at a single centre.

As controls, 2,339 (1,072 males and 1,267 females, aged 29–68 years) diabetes mellitus-free individuals at

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Abbreviations: *CDKN2A/2B* – cyclin-dependent kinase inhibitors 2A and 2B, CI – confidence interval, GWASs – genome-wide association studies, OR – odds ratio, PCR – polymerase chain reaction, SNP – single-nucleotide polymorphism, WHO – World Health Organization.

the time of examination were selected. This group represents a 3-year cohort of a selected 1% Czech Caucasian population sample. The individuals were recruited in nine districts in 1997/1998 and re-invited in 2000/2001 according to the WHO protocol ("MONICA Project". Manual WHO/MNC 82.2, Nov. 1983).

The ethics committee of the Institute approved the study. Body height, weight, waist and hip circumference were measured according to standardized WHO protocols. Body mass index (BMI) was calculated as weight in kg over squared height in meters. Plasma lipid parameters were analysed by the WHO Regional Lipid Reference Centre, IKEM, Prague in a Roche COBAS-MIRA autoanalyser (Roche Diagnostic System, Basel, Switzerland), using reagents from Boehringer Mannheim Diagnostics (Indianapolis, IN) and Hoffmann-La Roche (Basel, Switzerland).

The rs10811661 variant was analysed by polymerase chain reaction and restriction analysis. Oligonucleotides 5' tga aga cat tag aac acc ata acc ttt cc and 5' tag gag gag cca gaa gac aga tca gg with restriction enzyme *Bsp*HI were used for the genotyping. The uncut PCR product (143 bp) represents allele C, and restriction fragments of 94 bp and 49 bp allele T.

The Hardy-Weinberg test (<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>) was applied to confirm the independent segregation of the alleles. The χ^2 tests and odds ratio (95% CI) were calculated according to http://www.physics.csbsju.edu/cgi-bin/stats/contingency_form.sh?nrow=2&ncolumn=3, and <http://www.hutchon.net/ConfidOR.htm>. P values lower than 0.05 were considered to be significant.

Results and Discussion

The genotyping call rate was 98.8 % for controls and 99.7 % for patients. Within the groups under study, the genotype frequencies were within the Hardy-Weinberg equilibrium (P = 0.69 for controls and P = 0.53 for type

2 diabetes mellitus patients) and the results confirmed the independent segregation of the alleles. The frequency of the protective minor C allele (0.17) in non-diabetic controls was similar to the frequencies found in other Caucasian populations (summarized by Cugino et al., 2012).

In our study, we have not confirmed the previously mentioned association between the rs110811661 marker and type 2 diabetes mellitus (TT vs. CC homozygotes, P = 0.261; OR 1.29; 95% CI 0.82 – 2.04) (Table 1).

Similarly negative results were obtained when dominant (CC homozygotes vs. carriers of the T allele; P = 0.255), codominant (TT vs. TC vs. CC; P = 0.522) or recessive (TT homozygotes vs. carriers of the C allele; P = 0.822) models were used. Further, the numbers of alleles did not differ between the groups (P = 0.635).

Finally, no significant differences were obtained when males (P = 0.88 for codominant model) and females (P = 0.70 for codominant model) were analysed separately in any statistical model used (Table 1).

Our results are in contrast with so far described results. Even three simultaneously published meta-analyses (Bao et al., 2012; Cugino et al., 2012; Li et al., 2012) detected the common TT genotype to be associated with type 2 diabetes mellitus with OR about 1.25. Still, the effect is significantly more expressed in Asian population, where the frequency of the protective C allele is almost three times higher than in Caucasians. Further and interestingly, some differences are detectable even within the White European population. Most importantly, the geographically closest study was performed in the Bavarian region (where the lifestyle is very similar to the lifestyle in the region of Bohemia) in Germany and also found no association between the rs10811661 SNP and type 2 diabetes (Herder et al., 2008).

It is of extraordinary interest that the SNP rs10811661 is located on chromosome 9 at the 9p21 region, which has been widely recognized as a disease-associated region in different GWAs. The region has been associated not only with type 2 diabetes (Sanghera and Blasket,

Table 1. Frequencies of the rs10811661 genotypes between healthy controls and type 2 diabetes mellitus patients. Further, P* values for dominant[#], codominant[†] and recessive[‡] model of analysis are given.

Population	Controls		DMII patients		OR (95% CI) Crude	P	P*
	N	%	N	%			
TT	1593	68.9	796	69.3	1.19 (0.82–2.04)	0.261	0.822 [‡]
TC	649	28.1	326	28.4	1.30 (0.81–2.07)	0.263	0.522 [†]
CC	70	3.0	27	2.3	1.0		0.255 [#]
Males	Controls		DMII patients		OR (95% CI) Crude	P	P*
	N	%	N	%			
TT	730	69.0	493	68.8	1.09 (0.63–1.91)	0.753	0.915 [‡]
TC	294	27.8	203	28.3	1.12 (0.63–1.98)	0.703	0.924 [†]
CC	34	3.2	21	2.9	1.0		0.734 [#]
Females	Controls		DMII patients		OR (95% CI) Crude	P	P*
	N	%	N	%			
TT	863	68.8	303	70.1	2.11 (0.88–5.05)	0.088	0.609 [‡]
TC	355	28.3	123	28.5	2.07 (0.86–5.06)	0.100	0.233 [†]
CC	36	2.9	6	1.4	1.0		0.088 [#]

2012), but also with cardiovascular disease (Palomaki et al., 2010), various forms of cancer (Bishop et al., 2009; Wrensch et al., 2009) and last but not least also with neurodegenerative disease (Koppers et al. 2013).

Genetic variants within this region are associated with diseases of differing pathogenesis. Even in the case that there are some unifying processes such as between type 2 diabetes mellitus and myocardial infarction, the relevant polymorphisms are independent of each other. In the case of rs10811661, SNP is associated with type 2 diabetes mellitus in most but not all studies, but is not associated with cardiovascular disease. Vice versa, we have shown that the rs10757254 SNP at 9p21 is highly associated with the risk of acute coronary syndrome in Czechs (Hubáček et al., 2012a), but in the same population we have not detected any association with type 2 or type 1 diabetes mellitus (Hubáček, unpublished data).

Many results underline the importance of this region in determination of the predisposition to various diseases, but mostly without clear causality. The studies suggest some so far unclear and non-described multifunctional and likely regulatory mechanism(s) of this region. The importance of such region(s) could be of the highest level, despite the fact that, in some cases, the effect on the disease is not general. The outputs of the genome-wide screenings underline the importance of such regions associated with different diseases, but without clear mechanisms leading to the disease onset and development. As another example we could mention the *FTO* gene, which is associated not only with obesity (Jacobsson et al., 2012) and diabetes (Herder et al., 2008), but also with cardiovascular disease (Doney et al., 2009; Hubacek et al., 2010), end-stage renal disease (Hubacek et al., 2012b) and some types of cancer (Dlouhá et al., 2012).

Despite the studies published so far (Čejková et al., 2007; Cejkova et al., 2008; Lukášová et al., 2008, Kincl et al., 2009; Hubáček et al., 2011; Gu et al., 2012; Horová et al., 2012; Včelák et al., 2012), the knowledge about the genetic determination of type 2 diabetes mellitus in Czech Central European population are sparse. In our study, we have extended the information about the genetic variants that are putatively associated with type 2 diabetes mellitus in this region by another piece of puzzle.

Based on our and some other results we conclude that the rs10811661 SNP within the *CDK2A/2B* region on chromosome 9 is likely not associated with type 2 diabetes mellitus in the Central European region.

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