T2D risk haplotypes of the *TCF7L2* gene in the Czech population sample: the association with FFAs composition.

Josef Vcelak[1], Daniela Vejrazkova[1], Marketa Vankova[1], Petra Lukasova[1], Olga Bradnova[1], Tereza Halkova[1], Jiri Bestak[1], Katerina Andelova[2], Hana Kvasnickova[1], Petra Hoskovcova[1], Karel Vondra[1], Jana Vrbikova[1], and Bela Bendlova[1]

[1]*Institute of Endocrinology, Prague, Czech Republic*

[2]*Institute for the Care of Mother and Child, Prague, Czech Republic*

Full adress of corresponding author

Josef Vcelak

Department of Molecular Endocrinology

Institute of Endocrinology, Narodni 8, 11694 Prague 1, Czech Republic

E-mail: jvcelak@endo.cz

Tel.: +420 224 905 265 Fax: +420 224 905 325

Short title

***TCF7L2* - association with FFAs composition**

SUMMARY

The association of transcription factor 7-like 2 (*TCF7L2*) gene variants with the pathogenesis of T2D, gestational diabetes and polycystic ovary syndrome (PCOS) was examined.

The study involved 1460 individuals: 347 T2D patients (D); 261 gestational diabetics (G); 147 offspring of T2D (O); 329 women with PCOS, and 376 controls (C). The SNPs: rs7901695; rs7903146; rs12255372 in the *TCF7L2* gene were genotyped. Anthropometric and biochemical parameters, oGTT derived indices were assessed. In addition, free fatty acids (FFAs) were evaluated in 183 non-diabetic women.

The CTT haplotype showed the strongest association with T2D with OR 1.57, p=0.0003. The frequency of the CTT/CTT haplotype was decreasing in following order: D 10.6, O 9.5, G 6.1, C 5.3 and PCOS 4.9 [%]. Among CTT carriers, significantly decreased levels of oGTT-stimulated insulin and C-peptide as well as proportions of fasting PUFAs were observed. The carriership of CTG/TCG was associated with gestational diabetes, OR 2.59, p=0.036.

The association of *TCF7L2* haplotypes with T2D and gestational diabetes but not with PCOS was confirmed. Novel association of *TCF7L2* with FFAs composition was found.

Key words: TCF7L2, haplotypes, type 2 diabetes, gestational diabetes, genetics, polycystic ovary syndrome, diabetes risk, free fatty acids, polyunsaturated fatty acids

INTRODUCTION

Common variants in the transcription factor 7-like 2 (*TCF7L2*) gene have been identified as the strongest genetic risk factors for type 2 diabetes (T2D) in multiple ethnic groups (Cauchi *et al.* 2006, De Silva *et al.* 2007, Tong *et al.* 2009, Ereqat *et al.* 2010, Palmer *et al.* 2010, Lin *et al.* 2010, Cruz *et al.* 2010, Wen *et al.* 2010, Gambino *et al.* 2010, Chauhan *et al.* 2010). At the individual level, the *TCF7L2* risk allele carriership increases T2D risk for 50%, the population attributable risk varies between 10-25% depending on allele frequency (Zeggini and McCarthy 2007, Cauchi and Froguel 2008, Yan *et al.* 2009). Common variants in *TCF7L2* also predicted future T2D among persons with impaired glucose tolerance (Florez *et al.* 2006, Lyssenko *et al.* 2007). However, the mechanisms by which these non-coding variants increase risk of T2D have been unknown so far.

TCF7L2 is expressed in most human tissues, including pancreatic beta cells, liver, omental as well as subcutaneous adipose tissue, and brain. Low is the expression in human skeletal muscle. Its expression was significantly decreased in subcutaneous and omental fat from obese T2D patients in comparison with obese normoglycemic subjects (Cauchi *et al.* 2006). On the other hand, the mRNA level of *TCF7L2* was 5-fold higher in pancreatic islets from T2D patients than normoglycemic donors. In human islets, *TCF7L2* expression associated positively with insulin gene expression but negatively with glucose-stimulated insulin release suggesting posttranscriptional defect in insulin secretion (Lyssenko *et al.* 2007). Recently, the tissue-specific alternative splicing of *TCF7L2* was identified and the expression of splicing forms was influenced by intronic variants of the gene (Osmark *et al.* 2009).

TCF7L2 is a transcription factor involved in the Wnt signaling pathway which plays a crucial role in cell proliferation, differentiation and apoptosis, in the maintenance of tissue homeostasis and probably in metabolic processes (Smith *et al.* 2007). The impairment of Wnt signaling is associated with many disorders ranging from cancer to the degenerative disorders such as Alzheimer disease. First evidence about the importance of Wnt signaling for pancreatic organogenesis was given by Papandopoulou and Edlund (2007) but there are discrepant data on the role of Wnt signaling in mature islets. Wnt signaling was found to be activated in T2D patients in comparison to normoglycemic donors. In non-diabetic mice model, high fat diet induced Wnt activation (Lee *et al.* 2008). The authors suggest the exaggerated activation as an adaptive mechanism promoting beta-cell proliferation

in early T2D but, in contrast, chronic pathway activation as a process leading to cell death. In addition, Wnt signaling seems to be both an inducer and effector of glucagon-like peptide-1 (GLP-1) (Gustafson and Smith 2008).

Most recently, two publications (Pearson 2009, Villareal *et al.* 2010) reviewed the possible mechanisms explaining how altered TCF7L2 production or function may cause diabetes. Among them, reduced insulinotropic effect of incretin hormones (esp. GLP-1 signaling in beta cells), the impaired insulin processing or release, and decreased beta cell mass seem to be the most probable etiological mechanisms.

The aim of our study was to examine the possible imapct of the three *TCF7L2* single nucleotide polymorphisms (SNPs) on the pathogenesis of T2D, gestational diabetes, and polycystic ovary syndrom (PCOS) in the Czech population representatives and to ascertain the influence of T2D-associated variants on hormonal and other clinical characteristics.

METHODS

Study subjects

1460 adult subjects aged 18-70 years meeting the folowing criteria of the selection entered the study: 347 patients suffering from type 2 diabetes mellitus diagnosed by the criteria of the World Health Organization (World Health Organization Expert Committee 1999); 261 gestational diabetics meeting the 0.5-1 year interval after childbirth without other pathologies (i.e. hormonal disturbances, infections, organ disorders, psychical mental illness etc.); 329 patients with PCOS defined according to the European Society of Human Reproduction and Embryology consensus (ESHRE/ASRM PCOS Consensus Workshop Group 2004), none of them was taking hormonal contraception at least three months before the day of the examination;

147 offspring of T2D patients (T2D was present in one or both parents) without other patologies, and 376 healthy controls without family history of T2D, PCOS, and gestational

diabetes. The study cohorts are characterized in Tab. 1. All the T2D patients were well compensated either only by diet (41.6 %), or by diet and peroral antidiabetic drugs (39.8 %), and/or by insulin (18.6 %). Except of the diabetics, most subjects have normal glucose tolerance, only 8 gestational diabetics, 7 offspring, 21 PCOS, and 10 controls were additionally found to have impaired glucose tolerance (i.e. glucose in 120 min. of oGTT $\geq$ 7.8 and <11.0 mmol/l), none of them had T2D.

The study protocol was in accordance with the institutional ethic guidelines and the national rules and all the subjects gave their written informed consent to participate in the study.

Clinical and biochemical characterization

Body weight, height, waist and hip circumferences were measured in all participants in order to calculate body mass index (BMI) and to evaluate body fat distribution by means of waist circumference and waist to hip ratio (WHR). Furthermore, 14 skinfolds were measured and body composition (% of subcutaneous fat mass, % of muscle mass, and % of bone mass) was then calculated using the ANTROPO program (Blaha 1991).

Venous blood samples were obtained after an overnight fast. Glucose metabolism was characterized by blood glucose (Beckman Glucose Analyser 2), immunoreactive insulin (IRI; Immunotech IRMA, Czech Rep), C-peptide (Immunotech IRMA, Czech Rep), proinsulin (DRG Diagnostics, Germany), and also by glucagon (IBL-International, Germany).

The 3-hour oral glucose tolerance test (oGTT) with 75g of glucose load was performed in all subjects except of T2D patients. Areas under the oGTT glycemic, C-peptide and insulin curves (AUC) were calculated. Lipid profile was assesed by total cholesterol (CH), HDL-CH, LDL-CH and triglycerides (TG) concentrations (analyser Integra 400+, Roche Diagnostics GmbH, Germany). To assess insulin sensitivity and beta-cell function, the homeostasis models assessment $1/HOMAR=1/(I_0*G_0/22.5)$ and $HOMAF=20*I_0/(G_0-3.5)$, Matsuda index = $10^4/\sqrt{(meanI_0*meanG_0*G_0*I_0)}$ and insulinogenic index=$(I_{30}-I_0)/(G_{30}-G_0)$ were used. Furthermore, products of insulin sensitivity and beta cell function, i.e. disposition indices were calculated. Basal free

fatty acids (FFA0) concentrations and also oGTT-influenced FFAs levels in 60th (FFA60) and 180th min (FFA180) were evaluated by NEFA C ACS-ACOD (Wako Chemicals GmbH, Germany). FFAs composition was assessed by gas chromatography (GC-14A instrument, Shimadzu, Kyoto, Japan) after the extraction and subsequent derivatization by isooctane-methylchloroformiate, 5:1. Heptadecanoic acid (C17:0) was used as internal standard (Husek et al. 2002). Saturated fatty acids (SFAs) fraction included C12:0, C14:0, C16:0, C18:0. Monounsaturated fatty acids (MUFAs) fraction consisted of C16:1n-7, C18:1n-7, C18:1n-9, C20:1n-9 and polyunsaturated fatty acids (PUFAs) fraction of C18:2n-6, C18:3n-3, C20:3n-6, C20:4n-6, C22:6n-3. The percentages of fatty acids subgroups (%SFA, %MUFA, %PUFA) out of the total FFAs derived from GC method were evaluated.

TCF7L2 genotyping and haplotype construction

DNA extracted from peripheral leukocytes (QIAamp DNA Blood Kit, QIAGEN, Germany) was used to genotype for rs7901695 (T>C); rs7903146 (C>T); rs12255372 (G>T) intronic variants by ABI TaqMan SNP Genotyping Assays (LightCycler 480 System, Roche). The positive and negative controls were used in every run. The particular haplotypes and haplotype combinations were generated by PHASE version 2.1. software (Stephens and Donnelly 2003).

Statistical analysis

For the statistical evaluation, NCSS 2004 (Statistical Solutions, Saugus, USA) software was used. Data are given as means ± SDs or percentages. The Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium of the genotype frequencies. Analysis of linkage disequilibrium (LD) and correlation coefficient between the SNPs (r^2) was performed by Haploview 4.1. (Barrett *et al.* 2005). Allele/genotype/haplotype frequencies were compared by Chi-sqaure test or Fisher´s exact tests. Odds ratios (OR) and 95% Confidence Intervals (CI) according to Woolf formula were calculated. Differences in biochemical and anthropometric data between the compared groups were tested by non-parametric Mann-Whitney test. When appropriate, data were adjusted for age or for age

and BMI or HOMA R using the GLM Anova. The p-values <0.05 (two tailed) were considered to be significant.

RESULTS

The frequencies of rs7901695 (T>C); rs7903146 (C>T); rs12255372 (G>T) of *TCF7L2* gene in our cohorts are given in Tab.2. All the genotype distributions were in Hardy-Weinberg equilibrium. The frequency of the risk alleles of all variants decreased in the following order: T2D patients (D), offspring of T2D (O), gestational diabetics (G), with the lowest number in PCOS patients (PCOS) and in controls (C). The genotype as well as allele distributions differed significantly between D and C in all tested polymorphisms (Chi-square test for genotype distribution: rs7901695, p=0.0008; rs7903146, p=0.0012; rs12255372, p=0.0013; for allele distribution rs7901695, p<0.00001; rs7903146, p<0.00001; rs12255372, p<0.00001) and between G and C (Chi-sqare test for genotype distribution: rs7901695, p=0.019; rs7903146, p=0.02; for allele distribution rs7901695, p=0.005; rs7903146, p=0.003; rs12255372, p=0.018).

The carriership of these particular three risk variants was significantly associated with T2D. The ORs are given in Tab. 3. The C allele in rs7901695 and T allele in rs7903146 were also associated with an increased risk of gestational diabetes (Tab 3).

The haplotype analysis was performed. The two polymorphisms rs7901695 and rs7903146 are in a strong linkage disequilibrium with correlation coefficient r^2=0.98 and thus build one haplotype block. The r^2 between the variants rs7903146 and rs12255372 is 0.91. PHASE v.2.1.1. generated 8 haplotypes (TCG in 66%, CTT in 26%, CTG in 3%, CCG in 2%, TCT in 1,6% and TTT, CCT, TTG all in <1% of subjects in our cohorts) and 13 haplotype combinations. Four most frequent haplotype combinations (TCG/TCG; CTT/TCG; CTT/CTT and CTG/TCG) were examined methodically with the following results: the frequency of the risk homozygous CTT/CTT haplotype combination was decreasing in the studied groups as follows: D 10.7%, O 9.5%, G 6.1%, C 5.3% and PCOS 4.9% (Tab. 2). The distribution of CTT carriers did not differ within the particular groups with respect to the BMI categorization (BMI < 25; ≥ 25 and <30; ≥ 30 kg/m^2 - data not shown). The estimated OR of T2D for the CTT haplotype carriers was 1.57 [95% CI 1.23-2.01], p=0.0003; for CTT homozygotes the OR

was 2.25 [1.25-3.98], p=0.006 (Tab. 3). Interestingly, the frequency of the fourth most often haplotype combination CTG/TCG was almost twice higher in G compared with all the other studied groups (7.7% vs 2.9% in C, 3.8% in D, 4.1% in O, and 4.3% in PCOS) resulting in the association of the CTG/TCG carriership with gestational diabetes (OR 2.59 [1.12-6.01], p=0.036 - data not shown).

Relationship between the haplotype combinations and anthropometric as well as biochemical data was tested in our particular cohorts of O, G, PCOS, and C. Analysed separately, we did not observe the influence of the particular haplotypes on body weight, body composition, fasting and stimulated glucose, insulin, proinsulin, C-peptide and glucagon levels and on any other screened parameter. To increase the statistical power, we pooled together all the non-diabetic women (n=942) for further analyses. After that, significantly decreased levels of oGTT–stimulated C-peptide and insulin in 30^{th}, 60^{th}, and 90^{th} min. as well as lower AUCs of these two hormones were apparent in CTT risk haplotype carriers when compared with non-risk TCG/TCG configuration (Fig.1). However, proinsulin, glucagon, HOMA F, insulinogenic index, HOMA R, Matsuda index and disposition indices did not differ between the haplotypes (Fig. 2-3). Concerning our D group, majority of diabetics (66%) was treated by derivatives of sulfonylurea and 18.6% were insulin-treated. As it is well known that this treatment influences parameters of glucose as well as lipid metabolism and body composition (United Kingdom Prospective Diabetes Study Group 1998), we did not evaluate possible association of the risk genotype with these markers in diabetic patients.

As a pilot study, we tested the interaction between the *TCF7L2* gene polymorphisms and FFAs composition in 183 non-diabetic women. Plasma levels of total fasting as well as 3-hour oGTT-influenced FFAs did not differ with respect to the haplotype. Interestingly, highly significant association of the risk haplotype CTT with decreased % of fasting PUFAs was observed ($p<0.001$) when compared to the non-risk TCG/TCG configuration, esp. due to lower proportion of (n-6) PUFAs. These results remained significant even after adjustments for age and BMI ($p=<0.001$, power=0.98) or HOMA R ($p=<0.001$, power=0.97). On the contrary, percentage of fasting MUFAs were significantly

increased in women carrying the CTT haplotype in comparison with TCG/TCG homozygotes (p=0.02, power=0.66), see Fig. 4.

DISCUSSION

Our study performed on large cohorts of the Czech type 2 diabetics, offspring of diabetic parent(s), gestational diabetics, women with PCOS, and healthy controls without family history of these disorders confirmed the association of T2D with the *TCF7L2* risk alleles rs7901695 (C), rs7903146 (T) and rs12255372 (T). The allele and genotype distribution of the *TCF7L2* variants in the Czech T2D patients and controls as well as the ORs associated with the risk alleles conforms to the published data from other European cohorts reviewed by Tong (*et al.* 2009). In the Czech representatives, risk of T2D associated with the homozygous risk haplotype configuration was higher compared to the risk associated with the homozygous status of the particular risk alleles evaluated separately. The highest OR for T2D was found for the CTT haplotype, which was also associated with decreased oGTT-stimulated insulin and C-peptide levels in non-diabetic women.

There are three novel findings in this study: 1) According to allele frequencies, the association of the tested risk alleles was stronger with T2D than with gestational diabetes. Surprisingly, the CTG/TCG haplotype combination was present twice as frequent in gestational diabetics compared with other cohorts and was associated with 2.6 fold increased risk of gestational diabetes. There are several published studies describing the association of *TCF7L2* as well as other T2D susceptibility variants with increased risk of gestational diabetes mellitus (Shaat *et al.* 2007, Lauenborg *et al.* 2009, Pappa *et al.* 2010) which supports the hypothesis that gestational diabetes and T2D are two sides of the same entity. 2) In our study, PCOS was not associated with *TCF7L2* variants. Our findings are supported by Barber (*et al.* 2007) who did not find any association between rs7903146 and rs12255372 and PCOS in subjects of British/Irish and Finnish origin as well as no association with androgen levels. Similarly, large study performed on PCOS women of European ancestry did not find any association between 56 SNPs mapping to *TCF7L2* and PCOS (Biyasheva *et al.* 2009). 3) The most exciting finding of this study was the association of the risk CTT haplotype with the fasting polyunsaturated and monounsaturated fatty acids. The non-diabetic female carriers of the CTT

haplotype showed significantly decreased % of PUFAs and increased % of MUFAs in comparison with the non-risk TCG/TCG configuration in spite of the similar total fasting free fatty acids levels. The impact of the different diet of CTT carriers and noncarriers is not probable so it seems that the *TCF7L2* influence the fate of FFAs in the organism. As the various types of FFAs have different effects on glucose level control, insulin sensitivity, insulin secretion and other metabolic pathways (De Santa *et al.* 2009), they could reciprocally influence the metabolic effect of the *TCF7L2* genotype *per se*. The association of *TCF7L2* variants with lipids independently of BMI (increased cholesterol and LDL-cholesterol levels in risk alleles carriers) was described for the first time by Sanhhera (*et al.* 2008) in Indian population. Rs7903146 and rs12255372 were also associated with high triglyceride levels in Mexican and Finnish families with familial combined hyperlipidemia (Huertas-Vazquez *et al.* 2008). Warodomwichit (*et al.* 2009) investigated the effect of *TCF7L2* variants on postprandial lipemia and other metabolic syndrome-related traits and their modulation by dietary fat. The authors identified significant interactions between rs7903146 T allele variant and PUFAs intake modulating fasting VLDL particle concentrations and postprandial triglycerides, chylomicrons, total VLDL and large VLDL concentrations. These unfavourable atherogenic interactions of T allele carriership with PUFAs were exclusively due to high proportion of (n-6) PUFA intake. Also recent study (Perez-Martinez *et al.* 2010) links the *TCF7L2* variants to the modulation of the postprandial lipid metabolism. Nevertheless, the atherosclerotic risk in communities (ARIC) study, in spite of dietary influences, did not find any association of *TCF7L2* SNPs with incident coronary heart disease, ischemic stroke, cardiovascular disease, all-cause mortality, or prevalent peripheral artery disease (Bielinski *et al.* 2008). On the other hand, association of *TCF7L2* rs11196224 with coagulation/inflammation was described recently in the LIPGENE cohort recruited from eight European countries (Delgado-Lista *et al.* 2011). In this study, there are also delineated remarkable interactions of *TCF7L2* SNPs with fatty acids, suggesting extraordinary pleiotropic regulatory potential of the *TCF7L2* gene. Elevated SFAs modulated effects of rs11196224, rs7903146, rs176855538, rs290481 on insulin secretion (insulin level, AIRg), insulin resistance (HOMA-IR), coagulation (tPA), and inflammation (IL-6).

Our findings provide evidence of interaction between *TCF7L2* variants and FFAs spectra and thus indicate involvement of the gene in lipid metabolism. However, further study is needed to elucidate the mechanisms by which *TCF7L2* risk haplotype really influences the proportion of fasting PUFAs (esp. linoleic), MUFAs as well as SFAs and to investigate further consequences of the interactions between the genetic and the environmental factors.

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Fig. 1 The influence of risk haplotype CTT of the *TCF7L2* gene on oGTT stimulated C-peptide and insulin in women
(AUC – area under the oGTT curve)

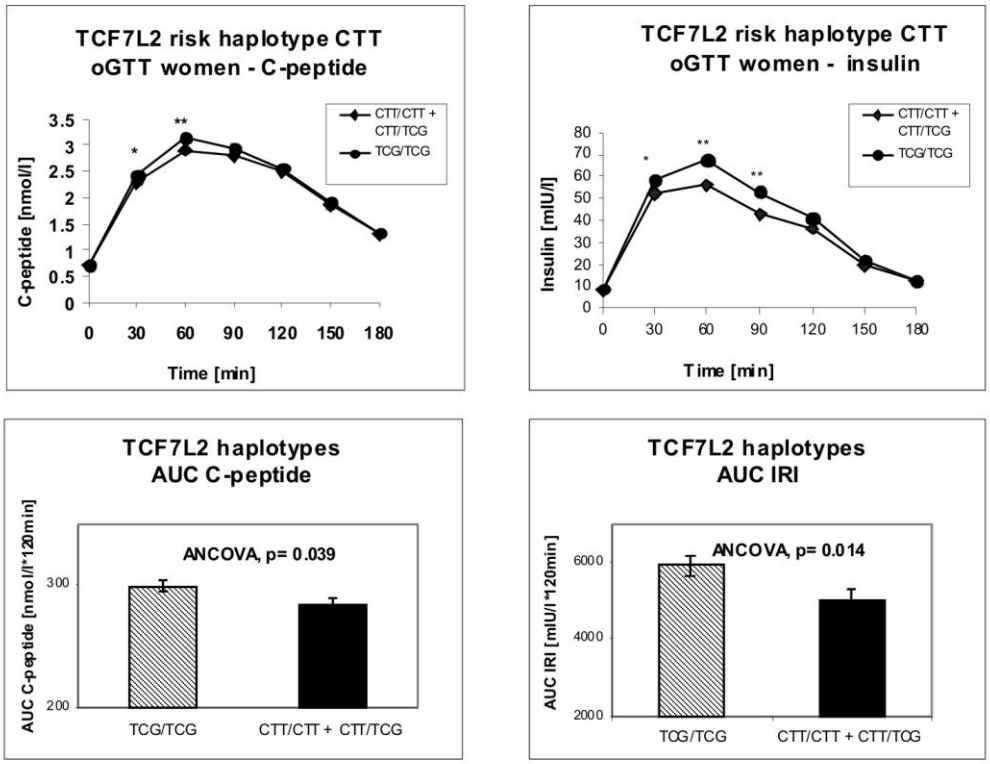


Fig. 2 The influence of risk haplotype CTT of the *TCF7L2* gene on fasting and oGTT stimulated proinsulin levels, proinsulin/insulin ratio, glucagon level and glucose/glucagon ratio in women
(ProIRI – proinsulin, IRI – immunoreactive insulin)

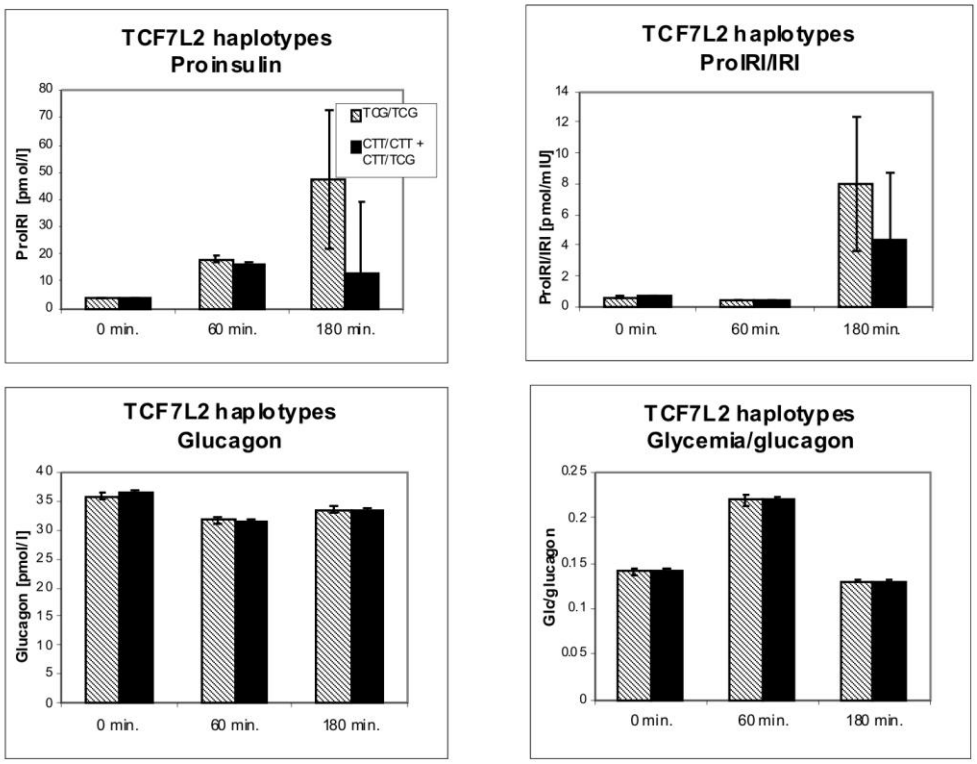


Fig. 3 The influence of risk haplotype CTT of the *TCF7L2* gene on insulin secretion and insulin sensitivity fasting and oGTT derived indices in women

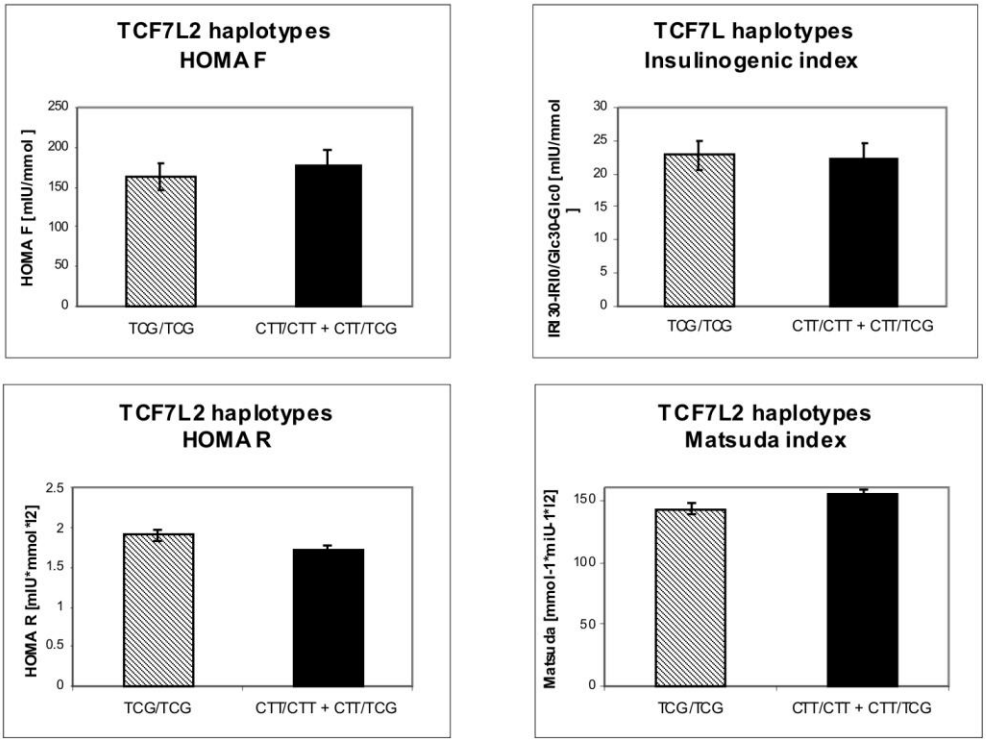
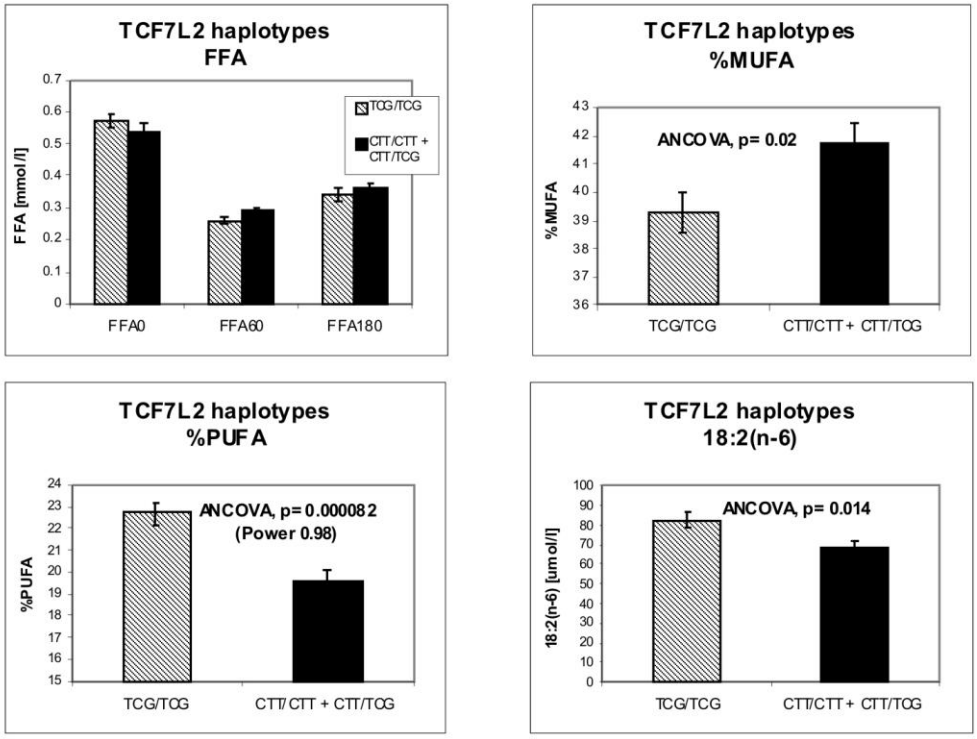


Fig. 4 The influence of risk haplotype CTT of the *TCF7L2* gene on fasting and oGTT stimulated free fatty acid levels, % of monounsaturated and polyunsaturated fatty acids and linoleic acid in women, data adjusted for age and BMI
FFA – free fatty acids, % MUFA - % monounsaturated FFA,
% PUFA - % polyunsaturated FFA, 18:2(n-6) – linoleic acid



Tab. 1 Study subjects

	n	Age (years)	BMI (kg/m^2)
T2D patients	**347**		
females	232	58.0 ± 9.9	31.8 ± 5.6
males	115	61.4 ± 7.4	29.7 ± 4.7
PCOS	**329**	27.5 ± 6.3	27.0 ± 6.6
Gestational diabetics	**261**	32.8 ± 4.9	23.8 ± 4.1
Offspring of T2D	**147**		
females	99	37.2 ± 12.8	25.1 ± 4.4
males	48	39.3 ± 10.1	26.7 ± 4.5
Controls	**376**		
females	253	29.9 ± 10.8	23.3 ± 4.4
males	123	29.4 ± 7.8	24.0 ± 2.9
Total	**1460**		

Tab. 2 *TCF7L2* gene polymorphisms rs7901695, rs7903146, rs12255372 - genotype, allele and haplotype frequencies in the Czech cohorts of T2D patients, gestational diabetics, offspring of T2D, patients with polycystic ovary syndrome and controls

rs7901695 (T>C)	**T2D patients**	**Offspring of T2D**	**Gestat. Diabetics**	**PCOS**	**Controls**
TT	40.5%	50.4%	40.7%	51.2%	53.0%
TC	46.4%	37.8%	49.8%	41.0%	40.1%
CC	**13.1%**	**11.8%**	**9.5%**	**7.7%**	**6.9%**
T carriership	63.7%	69.2%	65.6%	71.8%	73.1%
C carriership	**36.3%**	**30.8%**	**34.4%**	**28.2%**	**26.9%**
rs7903146 (C>T)					
CC	42.6%	50.0%	41.5%	54.0%	54.5%
CT	44.9%	39.2%	49.2%	38.6%	39.0%
TT	**12.5%**	**10.7%**	**9.3%**	**7.3%**	**6.5%**
C carriership	65.0%	69.6%	66.1%	73.3%	73.9%
T carriership	**35.0%**	**30.4%**	**33.8%**	**26.7%**	**26.1%**
rs12255372 (G>T)					
GG	43.5%	54.3%	47.3%	57.5%	54.8%
GT	43.8%	35.0%	44.1%	36.4%	39.0%
TT	**12.6%**	**10.7%**	**8.6%**	**6.1%**	**6.2%**
G carriership	65.4%	71.8%	70.0%	75.7%	74.3%
T carriership	**34.6%**	**28.2%**	**30.0%**	**24.3%**	**25.7%**
Haplotype combination					
TCG/TCG	36.4%	47.6%	38.3%	48.3%	48.9%
CTT/TCG	37.9%	32.0%	38.3%	32.8%	34.3%
CTT/CTT	**10.7%**	**9.5%**	**6.1%**	**4.9%**	**5.3%**
TCG carriership	65.1%	71.4%	69.4%	75.3%	74.6%
CTT carriership	**34.9%**	**28.6%**	**30.6%**	**24.7%**	**25.4%**

Tab. 3 Odds ratios for rs7901695 (T>C), rs7903146 (C>T), rs12255372 (G>T) and haplotype combination in *TCF7L2* gene in the Czech cohorts

rs7901695 (T>C)	risk homozygotes CC vs. others			C allele carriers vs. T allele carriers		
	OR*	[95%CI]	*p (Yates)*	OR	[95%CI]	*p (Yates)*
T2D patients vs. Controls	**2.04**	[1.22-3.40]	***0.0085***	**1.55**	[1.23-1.94]	***0.0002***
Offspring of T2D vs. Controls	1.82	[0.95-3.48]	*0.099*	1.21	[0.89-1.63]	*0.252*
Gestational diabetics vs. Controls (females)	1.60	[0.82-3.13]	*0.224*	**1.44**	[1.10-1.89]	***0.0098***
PCOS vs. Controls (females)	0.73	[0.34-1.55]	*0.501*	1.08	[0.83-1.41]	*0.595*
rs7903146 (C>T)	risk homozygotes TT vs. others			T allele carriers vs. C allele carriers		
	OR*	[95%CI]	*p (Yates)*	OR	[95%CI]	*p (Yates)*
T2D patients vs. Controls	**2.05**	[1.21-3.46]	***0.0092***	**1.52**	[1.22-1.92]	***0.0003***
Offspring of T2D vs. Controls	1.72	[0.87-3.37]	*0.164*	1.24	[0.90-1.68]	*0.192*
Gestational diabetics vs. Controls (females)	1.33	[0.70-2.51]	*0.476*	**1.41**	[1.08-1.84]	***0.0148***
PCOS vs. Controls (females)	1.02	[0.54-1.96]	*0.920*	1.01	[0.76-1.31]	*0.951*
rs 12255372 (G>T)	risk homozygotes TT vs. others			T allele carriers vs. C allele carriers		
	OR*	[95%CI]	*p (Yates)*	OR	[95%CI]	*p (Yates)*
T2D patients vs. Controls	**2.17**	[1.28-3.68]	***0.0054***	**1.52**	[1.21-1.91]	***0.0004***
Offspring of T2D vs. Controls	1.79	[0.91-3.55]	*0.131*	1.13	[0.83-1.54]	*0.474*
Gestational diabetics vs. Controls (females)	1.26	[0.65-2.44]	*0.621*	1.25	[0.95-1.65]	*0.129*
PCOS vs. Controls (females)	0.88	[0.45-1.74]	*0.848*	0.91	[0.69-1.20]	*0.545*
Haplotype combination	risk homozygotes CTT/CTT vs. TCG haplotype carriers			CTT haplotype carriers vs. TCG haplotype carriers		
	OR*	[95%CI]	*p (Yates)*	OR	[95%CI]	*p (Yates)*
T2D patients vs. Controls	**2.25**	[1.25-3.98]	***0.0065***	**1.57**	[1.23-2.01]	***0.0003***
Offspring of T2D vs. Controls	1.87	[0.92-3.83]	*1.224*	1.18	[0.86-1.62]	*0.352*
Gestational diabetics vs. Controls (females)	1.11	[0.54-2.31]	*0.921*	1.26	[0.94-1.69]	*0.142*
PCOS vs. Controls (females)	0.73	[0.34-1.55]	*0.523*	0.91	[0.69-1.22]	*0.604*

** ORs for recessive model*