Triglyceride Level Affecting Shared Susceptibility Genetic Variants in Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus represents a significant global health problem contributing to a considerably increased atherosclerotic burden and cardio- and cerebrovascular risk. Type 2 diabetes mellitus has high incidence and prevalence affecting both genders at any time of life worldwide. Presence of diabetes related phenotypes may represent risk for other metabolic diseases, cardio-, and cerebrovascular disorders, as well. The etiology of type 2 diabetes mellitus is complex; several environmental-, and genetic factors have been identified, which can contribute to the pathogenesis of the disease. Nowadays, numerous genetic loci have been shown to associate with type 2 diabetes mellitus and other diabetic traits, and the number of the identified susceptibility variants is expected to still grow due to rapidly developing field of candidate gene -, linkage-, and genome-wide association studies. The identified genetic variants can affect various metabolic pathways like glucose-, and lipid metabolism, signal transduction and can have effects on transcriptional- and translational levels. Among these, lipid alterations especially increased levels of triglycerides have been found to correlate to various disease phenotypes. It is also well known, that genetic variants identified in triglyceride metabolism are often shared as presence of these SNPs can confer risk for various disorders equally representing a susceptibility link between disorders. The scope of the current review is to summarize shared susceptibility variants, which have effect on the circulating lipid levels and represent risk for type 2 diabetes mellitus.

Keywords: Type 2 diabetes mellitus; T2DM; Gene; Susceptibility; SNP; Triglyceride; Genetic variants

Introduction

Type 2 (non-insulin dependent) diabetes mellitus (T2DM [MIM #125853]) is the most common form of diabetes accounting for approximately 90% of all diabetic cases in the world. It is one of the fastest growing diseases; its rate has intensively increased over the past two decades affecting more than 150 million people worldwide nowadays. This number is projected to rise to 366 million by the year 2030 as a consequence of an aging population and changes in lifestyle. The estimates of Wild et al. suggest that the major increase is expected in Asia, especially in India in the next 20 years [1-5].

The prevalence of T2DM shows strong ethnic differences and is very wide-ranging. Currently, T2DM is observed to be the most prevalent in India [6]. Besides, high-risk populations are Pima Indians and South Sea Islanders, where half of which suffer from T2DM, which could be explained by the thrifty hypothesis [7-9]. It is currently estimated that about 25.6 million adults over the age of 20 years have T2DM in the United States, which is 11.3% of all people in this age group; furthermore, nearly one-third of these cases are undiagnosed [10,11]. The European and Asian nations belong to the low-risk populations as the prevalence of T2DM is approximately 5% or less [12]. The differences in prevalence of T2DM among ethnic groups could be explained by the diverse population structure, some feature of the composition of diet, different lifestyle- and socio-economic factors. The incidence of T2DM increases with age, obesity, physical inactivity and unhealthy diet.

Until recently, T2DM was rarely observed in individuals under the age of 50, but nowadays, it is increasingly occurring at earlier ages affecting males and females equally [13]. T2DM has also been reported in children and adolescents worldwide [14].

T2DM is a complex, multifactorial disorder of carbohydrate metabolism, which is characterized by resistance to insulin-mediated glucose uptake, impaired insulin secretion, and increased hepatic glucose production as early phenomena leading to hyperglycemia, hyperlipidemia and pancreatic beta-cell dysfunction. The T2DM-related phenotypes create large pathophysiological spectrum from moderate to severe form [15,16]. The underlying pathophysiological mechanisms of T2DM have not yet been clearly elucidated. There is a growing number of evidence indicating that the dysfunction of the mitochondrial energy production machinery (OXPHOS), and abnormalities of the innate immune pathways could also play crucial role in development of insulin secretion [17-19]. T2DM is recognized as one of the major causes of morbidity and mortality representing serious health burden of the world [2]. The presence of uncontrolled T2DM can contribute to the development of late complications such as hyperglycemia, lethargy, atherosclerosis, coronary artery disease, stroke, Alzheimer disease, blindness, renal insufficiency, etc [2,17,20-25]. The etiology of T2DM is multifactorial. Complex interplay has been identified between environmental factors, like obesity, dyslipoproteinemia, oxidative stress, lifestyle (smoking, lacking of exercises, alcohol intake), family history of diabetes, and genetic alterations, which contribute to numerous biochemical pathways in the background of T2DM. Additionally, the heritability of T2DM is estimated to be 70%–80% [26]. T2DM has monogenic forms like maturity onset diabetes of the young (MODY) and maternally inherited...
diabetes and deafness (MIDD) [27,28]; however most of the cases are polygenic.

**Shared Alleles of Lipid Metabolism**

The identification of susceptibility genes is difficult due to the considerable heterogeneity of T2DM, and most associations have not been confirmed [29]. Approximately 40 susceptibility loci for the disease have been identified in Caucasian and Asian populations so far [30-35]. In order to recognize genetic loci associated with T2DM, linkage-, candidate gene studies, meta-analyses, and whole genome association studies (GWAS) have been widely performed for various populations [36]. Recently, the great technological development enables research groups to increase the number of identified T2DM-susceptible genetic loci using GWAS. As a result, genes such as CDKL1, SLC30A8, HHEX, EXT2, IGF2BP2, CDKN2B, LOC387861, FTO, TCF7L2, and BCL11A have been reported as susceptible genes for T2DM affecting various metabolic pathways like glucose metabolism, signal transduction, transcription-translation regulation, etc [35,37]. However, one of the most extensively studied elements in T2DM genetic research is the association among naturally occurring genetic variants, plasma lipid levels, particularly triglyceride levels and development of T2DM. Changes in the concentration of plasma lipids, triglyceride and cholesterol levels were found to confer independent risk factors for several metabolic traits, cardio- and cerebrovascular disorders. Plasma triglyceride levels are determined by numerous single gene variants, which may affect independently and/or in combination with other genetic- or environmental factors leading to the characteristic pathophysiological manifestations. The search for genetic variations affecting plasma triglyceride concentrations is still ongoing; several loci have been identified so far. Hereafter, we will summarize the most important genetic alterations affecting the lipid metabolism that can influence the susceptibility for T2DM.

**Apolipoprotein E (APOE)**

The apolipoprotein E is a 34 kDa molecule consisting of 299 amino acids. This plasma glycoprotein is responsible for stabilizing and solubilizing lipoproteins, and serves as a ligand facilitator for the clearance of lipoproteins like chylomicron reside and VLDL remnants [38-40]. The APOE gene on chromosome 19q13.2 has three codominant alleles e2, e3 and e4 and is determined by two SNPs (rs429358: g.112C>T) and rs7412: g.158T>C). The isoforms differ in single amino acids. This plasma glycoprotein is responsible for stabilizing and solubilizing lipoproteins, and serves as a ligand facilitator for the clearance of lipoproteins like chylomicron reside and VLDL remnants [38-40]. The APOE gene on chromosome 19q13.2 has three codominant alleles e2, e3 and e4 and is determined by two SNPs (rs429358: g.112C>T) and rs7412: g.158T>C). The isoforms differ in single amino acid substitutions ApoE2: 112Cys-158Cys; ApoE3: 112Cys-158Arg; ApoE4: 112Arg-158Arg); their relative frequencies in the Caucasian population are 0.08, 0.77, and 0.15 for e2, e3 and e4, respectively [41]. e2 allele is proved to have either pro- or anti-atherogenic nature, depending on its interactions with environmental and other genetic factors. Carriers of the e2 allele have lower total and LDL-cholesterol, higher triglyceride and apoE levels, while carriers of the e4 allele have lower levels of apoE, increased plasma levels of total-, LDL-, and VLDL-cholesterol compared to e3/e3 homozygotes. It has been concluded that e4 allele is a significant risk factor for atherosclerosis, CAD, CHD and MS [42,43]. In a case-control study on Thai population, APOE e4 allele was found to influence lipid profiles. Additionally, a significantly higher prevalence of e4 allele was found in T2DM patients group suggesting that e4 allele can be associated with elevated risk of T2DM [44]. However, other studies have shown contradictory results which follow: APOE gene polymorphism was not linked to the progression of islet dysfunction in T2DM [45]. No significant difference was detected in APOE genotype frequencies between hypertriglyceridemic and normotriglyceridemic among T2DM patients [46]; no significant effect of the APOE polymorphism was found on cholesterol levels among diabetics [47]. APOE polymorphism was not associated with lipids in men or women [48]. No association was found of APOE polymorphism with either T2DM or lipid variation [49].

**Apolipoprotein C3 (APOC3)**

The ApoCIII enzyme is an essential constituent of triglyceride-rich circulating particles, such as VLDL and chylomicrons. The protein synthesized by the liver and intestines serves as a marker of triglyceride-rich lipoprotein levels in the plasma. ApoCIII protein inhibits the hydrolysis of these particles catalyzed by lipoprotein lipase and their hepatic uptake mediated by apolipoprotein E. Therefore, the overexpression of the APOCIII gene leads to hypertriglyceridemia [50]. The APOCIII gene is located on chromosome 11q23. Variants in its sequence (rs1333049: g.-455T>C minor allele frequency (MAF) in Europeans (E): 0.492; and MAF in Asians (A): 0.511; g.-482C>T) have been shown to contribute to increase of APOCIII enzyme levels and hereby to hypertriglyceridemia, metabolic syndrome and CAD [51-53]. However, the examination of Pollin et al. revealed that carriers of p.19R-X APOCIII variant have lower serum triglyceride-, and LDL-cholesterol levels, and higher HDL-cholesterol levels. In addition, presence of this alteration confers decreased risk for atherosclerosis [54]. Dorfmeister et al. investigated g.-482C>T and g.1100C>T in patients with T2DM of European White, Indian Asian and Afro-Caribbean origin. Surprisingly, no significant association was found between lipid levels and APOCIII genetic variants in any ethnic group. Besides, only g.1100C>T variant was shown to be significantly frequent in European White patients with T2DM suggesting that this variant may have risk nature for T2DM but through mechanism other than effects on triglyceride levels [55].

**Apolipoprotein A5 (APOA5)**

This plasma protein, which has been identified in VLDL and HDL particles, is a complex regulator of triglyceride metabolism. APOA5 can facilitate the catabolism of chylomicron and VLDL particles. It contributes to the elimination of triglyceride-rich lipoprotein particles by hydrolysis of plasma triglycerides. APOA5 influences the triglyceride metabolism by two possible mechanisms: (1) enhancing intravascular triglyceride hydrolysis by activating the lipoprotein lipase, and/or (2) decreasing the serum concentration of triglycerides through inhibition of hepatic very-low-density lipoprotein-triglyceride production [56-58]. The protein encoding gene, which is the newly identified member of APOAI-APOCIII-APOAIV gene cluster, is located on chromosome 11q23 [59]. The minor alleles of the most frequently occurring variants, rs662799: -1131T>C (MAF(E): 0.017; MAF(A): 0.291), rs2266788: 12597>C (MAF(E): 0.084; MAF(A): 0.326), rs3135506: 56C>G (with Ser to Trp amino acid change, MAF(E): 0.326), rs2072560: 476G>A (MAF(E): 0.067; MAF(A): 0.256), have been reported to associate with elevated fasting or postprandial circulating triglyceride levels. Some of them were also found to be risk factors for hypertriglyceridemia, metabolic syndrome, cardio- and cerebrovascular diseases like ischemic stroke, CAD [57,58,60-71]. In a cohort of Indian patients, rs662799 C allele was shown to associate significantly with CAD-T2DM phenotype compared with CAD without T2DM [72]. On the basis of the results of a study conducted on T2DM patients with cerebral infarction Li et al. concluded that rs662799 C allele confers independent genetic risk for T2DM with cerebral infarction [73]. Dorfmeister et al. found in a study performed on T2DM patients with different ethnic origins that carriers of rs662799 C and rs3135506 G allele has elevated triglyceride levels in European Whites and Indian Asians, respectively. Besides, haplotypes
carrying rs662799 C allele showed significantly increased levels of triglyceride in the European Whites only; while, the haplotype defined by rs3135056 G allele showed significant triglyceride-raising effect in both Indian Asians and European Whites. In addition, no association was found between the two APOA5 variants and T2DM [55].

**Lipoprotein lipase (LPL)**

LPL catalyzes the hydrolysis of triglyceride-rich chylomicrons and VLDL particles; besides it functions as a ligand for receptor-mediated lipoprotein uptake, APOCII serves as a cofactor for LPL enzyme activity. LPL is synthesized by variety tissues, including skeletal muscle, heart, lung, brain, adipocytes, macrophages and smooth muscle cells [74,75]. Approximately 100 polymorphisms have been described in the LPL gene located on chromosome 8p22. Presence of these variants can lead to loss of function of the mature LPL protein or can modify the LPL enzyme activity. Therefore, these variants can alter plasma lipid levels and can confer risk for several diseases [76,77]. A number of studies have reported an association of p.291N>S and rs1801177: p.9D>N variants with increased triglyceride- and decreased HDL-cholesterol levels [78]. Carriers of these variants have been shown to have an increased risk of insulin resistance and metabolic syndrome [79]. The meta-analysis of Hu et al. indicates that the p.291N>S variant predisposes to CHD and T2DM and severe dyslipidemia, characterized by hypertriglyceridemia and low HDL-cholesterol levels [80]. Besides, rs328: p.447S-X (MAF(E): 0.125; MAF(A): 0.144) variant, which results in the formation of a premature stop codon, has been associated with reduced triglyceride- and increased HDL-cholesterol levels [81].

In Mexican families, this polymorphism can confer susceptibility for the development of hypertension and T2DM [82]. The LPL promoter -93T>G variant and p.9D>N polymorphism have been found to be in strong linkage disequilibrium. It has been demonstrated that co-occurrence of -93G and p.9D alleles has a triglyceride-lowering effect compared to non-carriers with TT/DD genotype [83,84]. In addition, p.312Lys>Arg, p.361Thr>InsA, and p.312Lys>InsC + p.291N>S alterations was found to contribute to hypertriglyceridemia observed in members of type 2 diabetic pedigrees [85]. Other alteration, rs343: g.13836C>A (MAF(E): 0.08; MAF(A): 0.169) was observed in association with T2DM and marginally with T2DM-related phenotypes like total- and HDL-cholesterol levels in Korean population [86].

**Adipose triglyceride lipase (ATGL)**

ATGL plays a crucial role in the turnover of fatty acids in adipose tissue and liver. It catalyzes the first step in the hydrolysis of triglycerides [87,88]. The gene encoding adipose triglyceride lipase is located on chromosome 11p15.5. In an Italian study, four selected variants within ATGL gene (rs7925131: MAF(E): 0.258; MAF(A): 0.283; rs7942159: MAF(E): 0.415; MAF(A): 0.613; rs66460720, rs1138693: p.481P-L; MAF(E): 0.225; MAF(A): 0.283) have been examined in familial type 2 diabetic pedigrees [85]. Other alteration, rs3135056 G allele has a triglyceride-lowering effect compared to non-carriers with TT/DD genotype [83,84]. In addition, p.312Lys>Arg, p.361Thr>InsA, and p.312Lys>InsC + p.291N>S alterations was found to contribute to hypertriglyceridemia observed in members of type 2 diabetic pedigrees [85]. Other alteration, rs343: g.13836C>A (MAF(E): 0.08; MAF(A): 0.169) was observed in association with T2DM and marginally with T2DM-related phenotypes like total- and HDL-cholesterol levels in Korean population [86].

**Potassium voltage-gated channel ( KCNQ1)**

This gene is located on chromosome 11p15.5 encoding a voltage-gated potassium channel, which is required for the repolarization phase of the cardiac action potential and plays a key role in water and salt transport in epithelial cells [101]. Several SNPs have been identified in the KCNQ1 gene. Two of these (rs2237892: C>T and rs2237895: A>C being in linkage disequilibrium; MAF(E): 0.075; MAF(A): 0.372 for rs2237892; MAF(E): 0.358; MAF(A): 0.030 for rs2237895) were found to be associated with T2DM in Asians [102]. Simultaneously, in another GWA study, Unoki et al. detected consistent association of SNPs in KCNQ1 region (rs2283228: C>T MAF(E): 0.076; MAF(A): 0.39; rs2237895: A>C; rs2237897: C>T MAF(E): 0.051; MAF(A): 0.458) with T2DM in independent case-control studies in Singaporean and Danish populations [103]. Since then, this correlation has also been confirmed [104-106]. Data of a meta-analysis, which involved more than 150 000 participants from a total of 25 articles, suggest that the rs2237892 and rs2237895 polymorphisms in KCNQ1 are associated with elevated T2DM susceptibility [107]. Potential mechanism of action has also been hypothesized; however, the exact mechanism has not yet been elucidated [104-106]. Additionally, Chen and coworkers described a strong association between CC genotype of rs2283228 and TT genotype in rs2237892 and increased levels of TG in a middle-aged Chinese population. Besides, lower levels of HDL-C and apoA1 were found in patients with CC genotype of rs2283228 [108]. These results provide new evidence that these variants in KCNQ1 gene have an effect on development of T2DM through lipid metabolism.
Glucokinase regulatory protein (GCKR)

This regulatory protein inhibits glucokinase in an allosteric manner by forming an inactive heterodimer. Hereby, it stabilizes and protects glucokinase from degradation [109-111]. GCKR is present in pancreatic islets and in the liver. The GCKR gene is located on chromosome 2p23 containing 19 exons [112,113]. Recently, genome-wide association studies identified naturally occurring variants in the GCKR gene [31,113-117]. The most widely studied PMs are the intronic rs780094 (MAF(E): 0.394; MAF(A): 0.599) and rs1260326: p.446Leu-Pro (MAF(E): 0.420; MAF(A): 0.593) variants, which affect the splicing site of the gene. Otherwise, these two variants are in strong linkage disequilibrium. It has been demonstrated in different ethnic groups that these variants reduce fasting plasma glucose concentration and insulin levels and improve insulin resistance, while inversely increase fasting and postprandial serum triglycerides through elevated glucokinase activity [31,115-124]. However, Jaromi et al. could not detect any association between rs1260326 variant and lipid levels in a stratified Hungarian stroke population [125]. Both functional GCKR variants were widely investigated as candidate susceptibility SNPs for several diseases like coronary artery disease, cardiovascular disease, and T2DM [31,116,126]. The results suggest that rs780094 variant does not contribute to CVD and CAD; however, both genetic variants have protective nature against T2DM as people carrying rs1260326 446Leu or rs780094: T allele variants have decreased risk for T2DM [31,115,118,123,127,128]. Results of a Japanese meta-analysis propose that GCKR rs780094 is a common variant for T2DM susceptibility in various ethnic groups [123].

Thrombospondin receptor (CD36)

CD36, a transmembrane protein on cell surface, was found to be an important regulator of lipid metabolism promoting endocytosis of oxidized LDL among others. Deficiencies in CD36 activity were found to elevate total cholesterol and triglyceride levels in diabetic CD36 deficient patients [129,130]. Besides, decreased activity of CD36 has been linked to several diseases like hypertension, hyperlipidemia, insulin resistance, metabolic syndrome, atherosclerosis and T2DM in Caucasian and African-American populations, as well [130-133]. Many sequence variations have been described in the CD36 gene on chromosome 7q11.2, which could be responsible for the protein deficiencies. The identified rs1761667: G>A (MAF(A): 0.667) has been identified to be associated with T2DM and increased risk of T2DM. Besides, in a study, which involved young Greek nurses, only the rs2230808: p.1587R>K (MAF(E): 0.839; MAF(A): 0.711) variant was shown to decrease LDL-cholesterol levels among three ABCA1 alterations (rs2230806: p.219R>K MAF(E): 0.792; MAF(A): 0.576; rs2230808: p.1587R>K and rs149313: p.883I>M MAF(E): 0.133; MAF(A): 0.576) [142].

Peroxisome proliferator-activated receptors (PPARs)

The members of this protein family are nuclear transcription factors which form heterodimers with retinoic acid X receptor and hereby can activate or repress transcription of various genes involved in lipid metabolism [143]. PPARs bind to specific DNA elements (peroxisome proliferator response elements, PPRE) on target genes through which they modulate DNA transcription [144]. PPARs consist of a DNA-binding domain stabilized by zinc cations bound to cysteine residues, a ligand-binding domain regulated by ligand binding and dimerization. One of the three common subtypes of PPARs is PPARγ, which is expressed in adipose tissue and gut. The PPARγ controls glucose and lipid metabolism, free fatty acid transport, cell proliferation, adipocyte differentiation and mitochondrial biogenesis [145]. Additionally, PPARγ has been implicated in the pathology of various diseases. The gene, which encodes PPARγ is located on chromosome 3p25. Polymorphisms in PPARγ gene may contribute to the risk of hypertriglyceridemia independently and/or in an interactive manner [146]. The rs1801282: Pro12Ala variant (MAF(E): 0.076; MAF(A): 0.056) is the most widely studied, common alteration of the PPARγ gene, which is highly prevalent in Caucasians. The 12Ala allele has been associated with a reduction of T2DM risk. This result was declared in 1998 and since then it has been confirmed in several studies [147]. Besides, other alterations in the PPARG gene, g.161C>T was shown to reduce the risk of severe atherogenesis by modulation of triglycerides and apolipoproteins in Chinese patients with CAD and T2DM [148].

Conclusion

Beside the above mentioned variants, there are other possible genetic markers that can contribute to the development of T2DM. Certain polymorphisms, such as those found in the MLX interacting protein-like (MLXIPL), angiopoietin-like 3 (ANGPLT3), human tribbles-1 (TRIB1), FADS gene cluster, have been found to confer risk for several diseases like dyslipidemia, hypertriglyceridemia, CHD, CVD. The role of these genes and genetic variants has not yet been investigated in association with T2DM. It is well known that the disorders above are associated with many metabolic abnormalities, in this particular case in lipid metabolism, which suggest that they might have also shared susceptibility genes and alleles. In numerous GWAS studies, variants in the GALNT, MLXIPL, TRIB1 and FADS genes have been found to have triglyceride-rising effect, which can serve as a link between increased triglyceride-related phenotypes. To discover these relations, further studies are needed on genetic variants in these genes.

Acknowledgements

This work was supported by grants from the Hungarian Scientific Research Foundation, OTKA K 103983 and by TAMOP SROP-4.2.2/B-10/1-2010-0029 Supporting Scientific Training of Talented Youth at the University of Pécs.
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