

# PPAR $\gamma$ 2 and KCNJ11 – Two Promising Candidate Genes in the Aethiopathogenesis of Type 2 Diabetes Mellitus

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## SUMMARY

Type 2 diabetes mellitus (DM2) is a multifactor disease – both genetic and environmental factors are implicated in its aetiology. In spite of the enormous effort that has been applied to the task, unravelling the genetics of type 2 DM has been problematic. Recently increasing attention has been directed to two candidate genes that have been repeatedly described in association with DM2. They are the PPAR $\gamma$ 2 gene (peroxisome proliferator-activated receptor) and KCNJ11 (potassium channel inwardly rectifying). The family of nuclear receptors referred to as peroxisome proliferators-activated receptors play an important role in the regulation of the energy metabolism and adipogenesis. The KCNJ11 gene codes for a pore-forming subunit of the inwardly rectifying ATP sensitive K<sup>+</sup> channel, which is involved in the direct regulation of insulin secretion. Recent knowledge regarding the involvement of these two genes in complex metabolic pathways is summarized.

**Key words:** type 2 diabetes mellitus, insulin resistance, PPAR $\gamma$ 2 gene, KCNJ11 gene, genetic polymorphism, glucose homeostasis.

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**T**ype 2 diabetes mellitus (DM2) is one of the most common civilisation diseases; in western countries about 3–5% of the population is affected. Incidence increases with age and with the growing number of obese people. It is a multifactorial disease, involving both genetic and environmental factors. DM2 genetic studies are currently focused on the identification of the “candidate genes” affecting individual predisposition to DM2. Once a candidate gene is selected, the search starts for its genetic variants - usually single nucleotide polymorphisms. Although more than 250 candidate genes have been studied in relation to DM2, the results have not been very encouraging so far. Nonetheless, the association between DM2 and the polymorphisms of two genes has been confirmed. They are gene PPAR $\gamma$ 2, which is a transcription factor directly affecting adipocyte differentiation, and gene KCNJ11, which codes for one of the cellular structures indispensable for the correct regulation of insulin secretion, called Kir6.2, a K<sup>+</sup> channel subunit of pancreatic beta cells.

## THE FAMILY OF NUCLEAR RECEPTORS CALLED PPARs

PPARs are general transcription factors indispensable in the regulation of the cellular cycle; they participate in the inflammatory process and in immune response control (1), in carcinogenesis (2) and in atherogenesis (3). The interest of diabetologists and obesityologists has been aroused especially by the finding that PPAR $\gamma$  plays a key role in adipogenesis and also that its synthetic ligands include thiazolidindiones (4), substances that are increasingly used to improve insulin sensitivity in type 2 diabetic patients.

## CLASSIFICATION OF THE PPAR GENE FAMILY

Two types of PPARs are known:  $\alpha$ ,  $\beta$  (also called  $\delta$ ) and  $\gamma$ ; each of them is coded on a different chromosome of the human genome.

The gene for PPAR $\alpha$  is located on the chromosome 22 at locus 22q13.31. In man, it is expressed mainly in brown adipose tissue and the liver, also in the kidneys, heart and skeletal muscles. The target genes of PPAR $\alpha$  are genes participating in the lipid metabolism, i. e. in the transport of fatty acids to cells, their oxidation in microsomes, peroxisomes and mitochondria.

The gene for PPAR $\beta$  is located on the chromosome 6 at locus 6p21.2–p21.1. In man, it is expressed most abundantly in the colon and placenta, and also in the kidneys and heart. It affects the expression of acetyl-CoA synthase 2 in the brain and takes part in the process of embryonal implantation and decidualisation; there is a proven relation to colon cancer.

The PPAR $\gamma$  gene is located on the chromosome 3 at locus 3p25 (5, 6). Four forms of PPAR $\gamma$  are known: PPAR $\gamma$ 1, PPAR $\gamma$ 2, PPAR $\gamma$ 3 and PPAR $\gamma$ 4. All are originated by alternative splicing and are controlled by different promoters. The PPAR $\gamma$ 1 gene is expressed in many different tissues (adipose tissue, also slightly in bone marrow, the spleen, kidneys, liver, testes, brain, heart, skeletal muscles). The PPAR $\gamma$ 2 gene is expressed especially in adipose tissue. The PPAR $\gamma$ 3 gene is, as far as we know, expressed in the colon and in adipose tissue. Not much is known so far about the tissue distribution of the expression of the PPAR $\gamma$ 4 gene (7).

PPAR $\gamma$  is an activator of adipocyte differentiation. Combined with transcription factors it induces differentiation of pre-adipocytes in the mature adipose cells (8–11). The effect is mediated by genes active in adipose tissue. These include genes encoding

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proteins that participate in the regulation of the amount of fat deposits and in the transmission of signals affecting insulin sensitivity, such as the tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), lipoprotein lipase (LPL), adipocyte fatty acid binding protein 2 (aP2), acetyl-CoA synthase, fatty acid transport protein (FATP), carnitin palmitoyltransferase (CPT) or phosphoenol pyruvate carboxykinase (PEPCK).

### ROLE OF PPARs AND THE EFFECT OF THEIR LIGANDS AND CO-ACTIVATORS

PPARs whose function has been activated by any of the ligands (see below) bind to a specific area in the promotor of the target genes ("hormone response element"), thus modulating their transcription. PPARs can bind to the DNA promoter site only as heterodimers with retinoic acid receptor X (RXR) (12).

Natural PPAR ligands and functional activators include fatty acids and their derivatives (leucotriens, certain prostaglandins). Polyunsaturated fatty acids with long-chain (e. g. linolenic acid) binding primarily to PPAR $\alpha$ . Prostaglandin 15-dPGJ2 was found to be an effective functional activator of PPAR $\gamma$ . Other very potent PPAR $\gamma$  functional activators are derivatives of LDL oxidation products: 9-hydroxy and 13-hydroxyoctadecadienoic acid (HODE) and 15-hydroxyeicosatetraen-1-oic acid (15-HETE). Insulin, too, potentiates expression and adipogenic activity of both PPAR $\gamma$ 1 and PPAR $\gamma$ 2 in man. The indirect negative regulation of PPAR $\gamma$  expression by the effect of prostaglandin F $_2$  (PGF $_2$ ) through the mediation of MAP kinases is a known phenomenon.

Synthetic PPARs' ligands, especially ligands of PPAR $\alpha$ , belong to the group of fibrate hypolipidaemic drugs, like bezafibrate, fenofibrate, gemfibrozil and clofibrate. PPAR $\gamma$  ligands with a rather weaker affinity include NSAIDs (non-steroid anti-inflammatory drugs), e. g. indomethacin, phenoprofen, ibuprofen, flufenamic acid). Tyrosine-based agonists have a selective affinity to PPAR $\gamma$ . Antidiabetic agents of the thiazolidindion series (troglitazone, pioglitazone, rosiglitazone, ciglitazone, englitazone) have a strong affinity. The thiazolidindions' effect on PPAR $\alpha$  and  $\beta$  is markedly weaker. An interesting finding is that thiazolidindions stimulate preadipocyte differentiation; this fact was published before the connection with PPAR $\gamma$  was known (13). It is currently known that PPAR $\gamma$  activation through the mediation of thiazolidindions causes an increase in the number of small adipocytes, which, compared to older adipose cells, are more sensitive to insulin, particularly when glucose transport ability is concerned. This finding has contributed to the understanding of the thiazolidindion mode of action as a pharmacological agent that improves insulin sensitivity. Stimulation of new adipocytes formation, together with the potentiation of fatty acid distribution into adipocytes and triglyceride (TG) synthesis, explains the unfortunate consequences described in long-term treatment with some of the thiazolidindion drugs, such as weight increase (14). An improvement of sensitivity to endogenous insulin may also be achieved by the effect of RXR agonists, which form heterodimers with PPAR $\gamma$ .

### DESCRIBED POLYMORPHISMS IN THE PPAR $\gamma$ 2 GENE AND THEIR ASSOCIATION WITH OBESITY AND DM2

Since PPAR $\gamma$ 2 is a transcription factor with a direct effect on adipocyte differentiation, the connection between mutations and polymorphisms in the gene encoding this factor and the amount of reserve fat, or possibly DM2, has been investigated.

Two studies (15, 16) found the rare mutation of the PPAR $\gamma$ 2 gene at codon 113 (Pro113Gln substitution, also referred to in literature as Pro115Gln). The consequence of the mutation is increased gene activity and the subsequent acceleration of adipocyte differentiation, since phosphorylation of serine in the 112 position has been hindered. This phosphorylation under normal circumstances leads to inhibition of gene expression. Mutation has been described in the heterozygous state in 4 out of 358 examined unrelated Germans. All carriers were severely obese with a body mass index (BMI) of more than 37.9 kg/m $^2$  but with a preserved sensitivity to insulin (15). One carrier of this mutation was found in the group of individuals with significant insulin resistance (16); his BMI was 28.5 kg/m $^2$ . A wider influence on obesity prevalence in the European population is, however, excluded by virtue of the extreme rarity of the mutation.

On the other hand, polymorphism in the codon 12 (Pro12Ala) of exon B of gene PPAR $\gamma$ 2 is common. It is found with considerably varying frequency, depending on the ethnicity of the tested individuals. In the Hispanic population the minor allele 12Ala is present in up to 23%; in the European population and in Americans of European descent it is 12%; in Mexican Americans 10%; in Afro-Americans and the Japanese only 2–3%. Polymorphism reduces the transactivation capacity of the gene (17, 18); which results, according to some studies, in lower BMI and higher insulin sensitivity. The lower prevalence of minor allele 12Ala amongst type 2 diabetics is in accordance with the latter finding (19, 20). These observations are the outcome of "association studies", i. e. studies which follow generally the relation between a certain polymorphism and the phenotype characteristics of a certain group of individuals and require, for the purposes of worthwhile statistical processing and sufficiently strong tests, groups containing large numbers of individuals. Even when these prerequisites are met, the conclusions of association studies are not always unequivocal. They differ, for example, depending on the ethnicity of the tested population, but even within the same ethnic group there may be considerable discrepancies between particular studies. This is where meta-analyses come in. The snag to meta-analyses is the considerable publication attractiveness of positive observations ("publication bias") pointing to an association of the studied genetic polymorphism with a concrete disease, and the consequence is the greater representation of such positive observations in the final evaluation of the meta-analyses. Necessary caution notwithstanding, the results of the meta-analysis evaluating the association of polymorphism Pro12Ala with DM2 can be regarded as highly interesting (21). It has confirmed with high statistical probability the protective effect of allele 12Ala. It ensues from the study that if the whole population consisted of only 12Ala carriers, DM2 prevalence would decrease by 25%. Many partial association studies do not, however, confirm the protective effect of the minor allele in relation to DM2. The conclusions of Ek et al. (22), concerning the association of Pro12Ala polymorphism with obesity, are interesting in this respect. He describes higher BMI in homozygous 12Ala carriers among 752 obese Caucasian men; in the control, non-obese group of 869 men, however, homozygous alanine carriers had a significantly lower BMI. Some studies observed no association with BMI.

Several other polymorphisms of the PPAR $\gamma$ 2 gene (Pro495Leu, Val318Met, Phe388Leu, Arg425Cys) are associated with partial lipodystrophy, severe insulin resistance, DM2 and also hypertension.

At our centre we tested the Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene in a group of 350 type 2 diabetics and 880 non-diabetic individuals. We compared glucose metabolism parameters in 12Ala carriers and non-carriers. Higher insulin sensitivity in 12Ala carriers was confirmed only in the group of patients with DM2.

## POTASSIUM CHANNELS

ATP-sensitive potassium channels are present in the membranes of many types of cells of different tissues, e.g. skeletal muscle and heart muscle cells, smooth muscle cells, neurons and pancreatic beta cells. They belong to a broad group of membrane ion channels that coordinate cellular functions, such as neurotransmission, contraction or secretion. A characteristic feature of all ion channels is the intermittent fluctuation between open and closed state. The current state depends on the intracellular and extracellular concentration of substances that stimulate or inhibit opening of the channels; the channels can also react to changes of membrane potential. ATP-sensitive  $K^+$  channels of the pancreatic beta cells enable the transmission of intracellular metabolic changes into changes of electric activity of the plasma membranes of the cells and are a key factor in the regulation of insulin secretion. Closure of the channels is a key prerequisite for the release of insulin secretion, their opening leads to inhibition of insulin secretion (23).

### BETA CELLS $K^+$ CHANNEL: STRUCTURE AND FUNCTION

Potassium channels in pancreatic cells are complexes formed by an extensive regulatory component and the actual pore of the channel. The regulatory component consists of sulphonylurea receptors (SUR1); the channel pore consists of subunits called Kir6.2 (**Potassium inward rectifier 6.2**).

Both SUR1 subunits, and Kir6.2 subunits are necessary for the correct regulation of the metabolic function of the channel, for which ATP concentration inside the cell (ATP: ADP ratio) is of key importance. Where ATP concentration is low,  $K^+$  channels are usually open and allow potassium flow along the electrochemical gradient out of the cell; the membrane potential is thus maintained in a hyperpolarised state. Glucose intake and the subsequent rise in intracellular ATP concentration leads to closure of the channel by the binding of ATP on Kir6.2 subunits, triggering a cascade of reactions enabling insulin secretion. Potassium cannot escape from the cells through closed channels, which leads to depolarisation of the cellular membrane; this opens voltage dependent calcium channels, and the subsequent increase of intracellular calcium streaming along the concentration gradient into cells leads to the exocytosis of insulin granules from the pancreatic beta cells. Sulphonylurea and its derivatives stimulate insulin secretion by binding to their receptor, i. e. to the SUR1 subunit, leading to the closure of the  $K^+$  channel and secretion of insulin independently of ATP concentration in the beta cell (23). This effect of sulphonylurea and its derivatives is applied in DM2 therapy. On the other hand, ADP in bond with magnesium ions (Mg-ADP) also interacts with SUR1 subunits of the potassium channel; in this event, however, it causes their opening and thus acts as a significant inhibitor of insulin secretion.

### CHROMOSOMAL LOCALISATION OF POTASSIUM CHANNEL GENES

The gene coding for SUR1 is called ABCC8 (it belongs to the ATP-binding cassette family, subfamily C, member 8); the gene coding for Kir6.2 has been given the name KCNJ11 (it belongs to the potassium channel inwardly rectifying family, subfamily J, member 11). Both are located on the short arm of chromosome 11 (locus 11p15.1).

## POLYMORPHISMS AND MUTATIONS LINKED TO CHANGED FUNCTION OF POTASSIUM CHANNELS

As mentioned earlier, the correct regulation of the function of the potassium channel, and thus also balanced insulin secretion, require error-free function of both SUR1, and Kir6.2 subunits. This claim is confirmed by the congenital glycoregulation defect called persistent hyperinsulinemic hypoglycaemia of infancy (PHHI). This is a rare metabolic disorder that manifests as early as the neonate stage by severe hypoglycaemic states with inability to suppress insulin secretion. The affected molecule may be the channel regulatory SUR1 subunit, but also the pore forming subunit Kir6.2. The disease can be caused, moreover, by mutations of other molecules involved in the glycoregulating mechanisms, e. g. glucokinase (24), glutaminedehydrogenase (25) and others. It is not always possible to detect the mutation responsible and find effective therapy; in severe cases, maintenance of an acceptable range of glycaemia values and the protection of the child against mental retardation requires the performance of a partial or subtotal pancreatectomy. Manifestations of PHHI were first linked to mutations in the SUR1 gene (26–28), and currently more than 20 different substitutions, insertions and deletions are known in the introns and exons of this gene associated with a varying degree of severity of the disease, from mild, pharmacologically manageable manifestations to total absence of beta cell potassium channel activity. In the Kir6.2 subunit, too, several mutations leading to PHHI symptoms have been described (29, 30). On the other hand, mutations and polymorphisms are known that lead to higher potassium channel activity in beta cells and thus to the reduction of the insulin secretion capacity. Recent studies have shown that heterozygous mutations in KCNJ11 cause permanent neonatal diabetes (PND) either alone (R201C, R201H) or in association with developmental delay, muscle weakness and epilepsy (V59G, V59M). Most of patients with PND who were insulin-dependent were able to discontinue insulin injections and achieve excellent control with sulphonylurea tablets (31) \*.

These activating mutations can be an important factor predisposing or leading directly to the development of common forms of DM2. Such polymorphisms have recently aroused much interest among scientists and doctors.

### E23K POLYMORPHISM

The most frequently discussed genetic variants of this type include in particular the substitution of lysine for the common glutamic acid at codon 23 of the gene encoding subunit Kir6.2 of the potassium channel; the substitution is marked as polymorphism E23K accordingly to the single-letter acronyms for amino acids. The substitution elevates the threshold of ATP concentration necessary for the closure of potassium channels, thus slightly reducing insulin secretion (32). Since the minor allele K encoding lysine is represented quite profusely (approximately in 30–40%, depending on the ethnic origin of the studied population), its even relatively small influence on insulin secretion capacity may represent a large predisposition load for the population.

An association study including 2,036 individuals (33) studied the relation of polymorphism E23K to DM2. The frequency of the minor allele and genotype distribution among a group of type 2 diabetics was compared with healthy controls. The risk allele K was present more frequently in diabetics (40% compared to 36% in the control group) and the genotype comparison revealed a higher percentage of risk homozygous combination of KK alleles amongst diabetics (16% compared to 13% in the controls). On the other hand, compared to diabetics the controls had more frequent protec-

tive EE combinations (42% against 36%). The study concluded that the E23K polymorphism is associated with a slightly increased risk of DM2. The study performed at our centre, on a group of 400 non-diabetic individuals, showed that the KK genotype is associated with the function of pancreatic beta cells, specifically with a lower insulinogenic index. The metaanalytical processing of published studies with this theme leads to the conclusion that the estimate of the population load to which the E23K polymorphism contributes is 15% in the Caucasian population (34). This means that if during evolution lysine were not substituted for glutamic acid in the gene encoding Kir6.2 at codon 23, and all people were carriers of the EE genotype, there would be 15% less of type 2 diabetics in the Caucasian population. In spite of the mentioned pitfalls of a meta-analytical approach to the assessment of risk represented by a certain polymorphism for a given disease, the E23K polymorphism, due to the strength of its association with DM2, ranks currently among the most important ones.

The importance of mapping the genetic background of polygenic diseases, like DM2 or obesity, is based especially on the fact that the detection of concrete polymorphisms, determination of their frequency in populations of different ethnic origin, the estimation of their risk rate in a given population and, especially, thorough knowledge of their function in a complex aetiopathogenesis, gives affected individuals, in whom the development of the disease has been affected by a concrete genotype, a chance of very precisely targeted treatment. Pharmacological agents could affect the function of the modified gene directly, thus reducing significantly the severity of the disease and presenting minimum side effects for the treated person.

#### Abbreviations

15-HETE	- 15-hydroxyicosatetraen-1-oic acid
ABC	- ATP-binding cassette
aP2	- adipocyte fatty acid binding protein 2
BMI	- body mass index
CPT	- carnitin palmitoyltransferase
DM2	- type 2 diabetes mellitus
FATP	- fatty acid transport protein
HODE	- 9-hydroxy and 13-hydroxy octadecadienoic acid
KCNJ	- potassium channel inwardly rectifying
Kir	- potassium inward rectifier
LPL	- lipoprotein lipase
NSAID	- nonsteroidal anti-inflammatory drugs
PEPCK	- phosphoenol pyruvate carboxykinase
PHHI	- persistent hyperinsulinaemic hypoglycemia of infancy
PND	- permanent neonatal diabetes
PPAR	- peroxisome proliferator-activated receptor
RXR	- retinoic acid receptor X
SUR	- sulphonylurea receptor
TG	- triglyceride
TNF	- tumour necrosis factor

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## COMMENTARY

# Comment on J. Vejražková and B. Bendlová “PPAR $\gamma$ 2 and KCNJ11 - Two Promising Candidate Genes in the Aethiopathogenesis of DM2”

Diabetes mellitus type 2 is a disease characterised by a very interesting interaction between genetics and the environment. This disease cannot actually occur without a genetic load. A person whose parents are both diabetics has an almost 100% chance of being diabetic too. Also the second monozygotic twin is almost certain to be diabetic. Many specialists travel to study the American Pima Indians, who have an almost 100% prevalence of diabetes mellitus. A hundred years ago, when Czech anthropologist Aleš Hrdlička studied this people, they probably had no diabetes at all, and when they were observed in the 1940s by Joslin, their prevalence of diabetes was only slightly higher than other populations. Our own ancestors, too, did not suffer so much from diabetes, and as late as the 1970s there were only about 2% of diabetics. Today the figure is actually higher than that stated by the authors – it is 7%, and this is in a situation when there is no nationwide population diabetes screening. A screening study executed in Bavaria (a population whose diet and genetics may well differ only slightly from our own) gave a 10% prevalence of diabetes. The latter figure points to the fact that diabetes mellitus is evoked by the environment, but on a genetic basis. The possibility of molecularly genetic detection of individuals at high risk of diabetes would be very significant. The whole world is searching for diabetes and metabolic syndrome genes.

Following sentences from our book “Prevention of Diabetes” (1) are reflecting the state of knowledge two years ago and have been logically followed up by the authors of the reviewed article:

“At a molecular-genetic level 2 regions associated with diabetes mellitus type 2 have been established – the NIDDM1 locus on chromosome 2q, identical with the pro calpain-10 gene, and NIDDM 2 locus on chromosome 12q, where the gene at issue is unknown. We are leaving out the whole area of MODY diabetes, which has no direct reference to this article and diabetes mellitus type 2.

Other candidate genes have been established in association with the metabolic syndrome and insulin resistance. Insulin resistance is hereditary in about 60% of cases, insulinaemia in only 30%. There are known candidate genes for obesity, hypertriglyceridaemia, hypertension, insulin resistance, appetite and energy output. They include IRS proteins, amyline, insulin, insulin receptor, GLUT 1, 2, 4 transporters, glycogen synthase, fatty acid binding globulin, CD36-FAT – parts of LDL receptors, PPA receptors and FOXC2 gene-transcription factor genes. As regards a defect of the last factor, it is easy to induce insulin resistance in mice by diet. The disease then has similar features to the human metabolic syndrome. New mutations in the region of gamma receptors associated with typical early development of the metabolic syndrome in man have recently been described.”

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In December 2004, together with my co-author M. Haluzík, we sent off a book on PPAR receptors to the printers (2). The relation of gamma subtypes to diabetes and the metabolic syndrome is discussed there on less than two full pages. The work of the authors of the article is thus a good supplement, moreover containing the authors' own data from the Czech population.

It has long been suspected that the structure and function of the beta cell potassium channel plays a role in the genetics of diabetes mellitus type 2. Sulphonylure antidiabetics and related substances have a very diverse effect on individuals. It was finally admitted several years ago that diabetes mellitus type 2 is really a disease of the pancreas and not only a disease caused by insulin resistance. The mechanisms of insulin secretion must therefore be affected by genes affecting the function of potassium channels. In this regard, too, the article on the KCNJ11 gene, which has an essential role in insulin secretion, is very interesting and coherent.

The authors have prepared a good review of the possible influence of certain genes in the genesis of diabetes mellitus type 2. We may have made a step forward from NIDDM 1 and 2 genes in the detection of persons threatened by obesity and diabetes mellitus type 2.

I think this is not the final step and that many more DM2 candidate genes will be discovered. This does not detract from the essence of the interesting and convincing article on two small chapters in the pathophysiology of diabetes mellitus type 2.

#### REFERENCES

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