THE PPARGC1A - GLY482SER POLYMORPHISM (RS8192678) AND THE METABOLIC SYNDROME IN A CENTRAL ROMANIAN POPULATION

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Abstract

Background. The peroxisome proliferator-activated receptor- γ co-activator 1- α (PPARGC1A), a key transcription factor involved in the control of metabolism and energy homeostasis, is an important biological and positional candidate of the metabolic syndrome. Association studies of its polymorphisms, however, yielded inconsistent sometimes conflicting results, pointing to important ethnic differences, which call for replication in various populations.

Objective. In order to study its most common - potentially functional - polymorphism Gly482Ser (rs8192678), we carried out a case-control study in a central Romanian population.

Material and methods. Two hundred and ninety six patients affected by the metabolic syndrome diagnosed according to the International Diabetes Federation proposed criteria and 166 middle-aged control subjects have been investigated. Genotyping was done by PCR-RFLP, using the restriction enzyme MspI.

Results. While the G(Gly)/A(Ser) allele frequencies (66.89/33.11 vs. 71.68/28.31 %) and GG/GA/AA genotype distribution (45.27-43.24-11.48 vs. 54.21-34.93-10.84 %) differed in the metabolic syndrome and control group, the risk of developing the metabolic syndrome did not reach the limit of statistical significance (OR=1.43; p=0.06, CI 95%: 0.97-2.09). Metabolic parameters in the two study groups did not show significant differences according to the genotype (p>0.05).

Conclusion. rs8192678 could be a functional polymorphism contributing to the development of the metabolic syndrome, but probably its effect is minor, and might depend on gene-gene and gene-environment interactions. Clarification of very small effects would require larger sample sizes.

Key words: metabolic syndrome, PPARGC1A polymorphism.

INTRODUCTION

Metabolic pathways involve a high number of transcription factors. A key role is attributed to the peroxisome proliferator-activated receptor- γ co-activator 1- α (PPARGC1A) that appears critical in the regulation of metabolism and energy homeostasis, from adaptive thermogenesis to metabolic responses during fasting and exercise. Being involved in numerous metabolic mechanisms, energy production and utilization (i.e. mitochondrial biogenesis, cellular respiration - oxidative phosphorylation, fatty acid β -oxidation, peroxisome remodeling, gluconeogenesis, glycogenolysis, glucose uptake, determination of muscle fiber type, adipogenesis, angiogenesis, induction of ROS-detoxifying enzymes, pancreatic beta cell insulin secretion, regeneration and apoptosis), it appears as an important biological but also positional candidate for the susceptibility to the much debated, nonetheless very prevalent metabolic syndrome pathology (1-3). Association studies of its polymorphisms, however, yielded controversial, sometimes conflicting results, pointing to important ethnic differences, which impose the reproduction of such investigations in various populations and by different recruitment approaches. rs8192678 appears as the most common and potentially functional polymorphism of the gene, most often reported as being related to individual disturbances comprised by the metabolic syndrome diagnosis (4).

Given the controversial findings and the potential direct practical implication in patient management of a genotype-based risk assessment, we aimed to explore the possible effect of the PPARGC1A Gly482Ser polymorphism on the metabolic syndrome, to our best knowledge yet uninvestigated in the local population.

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MATERIAL AND METHODS

We have carried out a case-control study in a central Romanian population on 166 middle-aged non-obese control subjects and 296 patients affected by the metabolic syndrome diagnosed according to the criteria proposed by the International Diabetes Federation as detailed previously (5), after obtaining informed written consent according to the protocol approved by the local Institutional Ethics Committee.

Genotyping was carried out by PCR-RFLP. Amplification took place using the primer pair 5'-CAAGTCCTCCAGTCCTCAC-3' and 5'-GGGGTCTTTGAGAAAATAAGG-3' (Eurogentec), by an initial denaturation of 5 min at 95°C, followed by 38 cycles of 95°C – 45 sec, 60°C – 45 sec, 72°C – 45 sec, and ending the reaction with a final elongation of 10 min at 72°C. Using the restriction enzyme MspI (FastDigest, ThermoScientific) - an isoschizomer of HpaII that cleaves both the methylated and unmethylated CCGG restriction site, digestion took place in 5 min at 37°C, which was followed by electrophoresis and the separation of the restriction fragments in a 2 % agarose gel.

For the statistical analysis, we used IBM SPSS Statistics 20 and GraphPad InStat 3.06, considering results statistically significant if $p < 0.05$.

RESULTS

In the metabolic syndrome and control group, the average age was 59.6 ± 13.43 vs. 56.17 ± 12.68 years, and the male/female sex ratio $41.5/ 58.5$ vs. $43.93/ 56.06\%$. The metabolic characterization of the two groups is summarized in Table 1.

Digestion with MspI allows genotyping the polymorphism, as the substitution G→A in the suspected risk variant results in the loss of the restriction site present in the wild type allele (611 bp and 366 bp+245 bp, respectively). The interpretation of the genotype based on the electrophoresis of the restriction fragments in a series of samples is shown in Figure 1.

In the metabolic syndrome and control group, allele frequencies were 66.89 and 33.11 vs. 71.68 and 28.31 % for G (Gly) and A(Ser) respectively, while de GG (Gly/Gly) - GA (Gly/Ser) - AA (Ser/Ser) genotype distribution was 45.27-43.24-11.48 vs. 54.21-34.93-10.84%, in agreement with the Hardy-Weinberg equilibrium. The rs8192678 polymorphism associated risk of the metabolic syndrome did not reach the limit of statistical significance (OR=1.43; $p=0.06$, CI 95%: 0.97-2.09, Fisher's Exact Test - two-sided p value).

Clinical parameters according to the genotype in the two groups are shown in Table 2.

DISCUSSION

Despite the continuous debate related to its diagnostic methodology or existence as a distinct clinical entity, the metabolic syndrome research demands a special attention, motivated by both epidemiological data and intriguing relationships with important morbidities, from cardiovascular disorders to Alzheimer disease or certain types of cancer. The complex clinical picture, however, can be considered a major impediment in the study of its etymology, including the genetic aspects. While obesity is heterogeneous, and not all overweight persons manifest the syndrome, there is a metabolically unhealthy, abdominal or visceral

Table 1. Characterization of the study groups*

	Metabolic syndrome patients	Control persons
Body Mass Index (Mean±SD, kg/m ²)		
• Males	30.62±4.62	24.32±3.12
• Females	31.1±5.78	23.55±3.14
Waist Circumference (Mean±SD, cm)		
• Males	108.06±13.61	81.73±6.44
• Females	93.56±13.81	82.61±7.37
Fasting Glucose (mean±SD, mg/dL)	120.71±42.49	93.93±17.89
Systolic Blood Pressure (Mean±SD, mmHg)	145.19±23.42	126.05±20.77
Diastolic Blood Pressure (Mean±SD, mmHg)	85.73±13.32	78.24±11.9
Triglyceride ((Mean±SD, mg/dL)	202.76±132.42	103.09±61.13
HDL-Cholesterol (Mean±SD, mg/dL)		
• Males	48.95±14.95	51.82±12.17
• Females	50.13±17.96	56.1±11.59
Total Cholesterol (Mean±SD, mg/dL)	209.39±53.08	192.03±36.25

* $P < 0.05$, Mann-Whitney U test

Table 2. Metabolic parameters according to the genotype*

	Metabolic syndrome patients			Control persons		
	GG	GS	SS	GG	GS	SS
Body Mass Index (kg/m ²)						
• Males	29.79±3.63	31.32±5.75	30.97±3.48	24.01±2.97	23.63±3.4	25.94±3.27
• Females	31.7±7.12	30.49±4.58	31.18±4.94	23.67±2.28	24.58±4.18	25.05±3.82
Waist Circumference (cm)						
• Males	106.04±10.36	108.75±15.97	112.91±14.23	91.15±4.5	84.83±6.17	92.22±8.62
• Females	96.13±14.7	91.35±13.7	95.19±9.78	82.85±7.27	81.5±7.69	89.16 ±13.7
Fasting Glucose (mg/dL)	124.01±42.49	123.75±45.69	113.35±27.86	93.19±10.94	99.22±13.55	94.56±16.19
Systolic Blood Pressure (mmHg)	147.03±23.6	143.02±22.72	151.85±23.37	122.58±18.91	136.35±24.06	116.00±4.18
Diastolic Blood Pressure (mmHg)	85.94±13.51	84.72±12.76	90.38±14.52	76.69±11.90	82.17±12.69	76±6.51
Triglyceride (mg/dL)	198.51±137.69	213.73±140.07	187.24±90.59	107.61±73.85	89.92±35.04	115.57±34.15
HDL-Cholesterol (mg/dL)						
• Males	49.16±15.68	49.8±15.46	46.23±12.94	52.46±8.13	50.5±12.76	52.2±5.09
• Females	49.89±17.12	50.85±19.77	53.91±17.32	53.9±9.28	55.9±13.3	50.09±9.52
Total Cholesterol (mg/dL)	210.78±52.43	214.74±50.37	201.71±54.81	190.71±38.97	194.45±34.1	192.42±30.13

* Data are represented as mean±SD; p>0.05 by one-way ANOVA

Figure 1. Genotyping by PCR-RFLP. (lane 2, 5, 10, 14, 15 - GG (Gly/Gly) homozygous samples; lane 4, 7, 12, 13 - AA (Ser/Ser) homozygous samples; lane 1, 3, 6, 9, 11 - GA (Gly/Ser) heterozygous samples; lane 8 – DNA ladder 50 bp).

obesity which regularly associates the more or less complete clinical picture, so the International Diabetes Federation proposed criteria with increased waist circumference as a mandatory sign appeared as the best approach to diagnose the disorder (5). According to the adipocentric view, visceral obesity with consecutive lipotoxicity and the common form of insulin resistance may be the central component of the pathogenic process that develops apparently on a multifactorial background. Clinical manifestations may emerge after an exposure to an unhealthy lifestyle in the presence of an inherited predisposition involving a polygenic system that comprises risk alleles of candidate genes, probably varying from patient to patient. Complex gene-gene and gene-environment interactions may lead ultimately to the disease and its complications. Among

the candidate genes involved, transcription factors could have an important role by producing an abnormal gene expression in the various metabolic pathways and energy homeostasis, constituting the rationale of the focus of our studies.

The peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α , PPARGC1A) appears as an important biological and positional candidate for the susceptibility of the metabolic syndrome related pathology, insulin resistance, obesity and type 2 diabetes. Together with PGC-1 β and PRC, it belongs to a small family of transcriptional co-activators exerting its function through the control of nuclear and mitochondrial gene expression by regulating other transcription factors. Originally identified as a PPAR- γ co-activator, it has been found to be a multifunctional regulatory factor for other key transcription factors including the nuclear receptors for various hormones (e.g. GC, ER, TR, PPAR- γ and α , TFAM, RAR, MEF2C, HNF-4a, FOXO, NRF-1). The gene (PPARGC1A, ID: 10891) has been mapped to 4p15.1 - a region found by linkage analysis to be associated with various metabolic syndrome related traits (e.g. body mass index, abdominal subcutaneous fat, plasma fatty acid, fasting insulin, blood pressure) (1, 2, 4, 6-9). Comprising 13 exons, it encodes a 92 kDa protein expressed primarily in tissues with the highest energy use and rich in mitochondria (e.g. brown adipose tissue, slow-twitch skeletal muscle, heart, kidney) and involved in the metabolic syndrome

development associated processes such as peripheral and central insulin sensitivity or insulin secretion (i.e. muscle, adipose tissue, liver, pancreas); gene expression varies in the different tissue types, where the protein may have specific, even opposite functions (1, 6). In insulin resistance, PPARGC1A as well as the genes responsive to its effect and involved in oxidative metabolism appear down-regulated in a coordinated manner (10).

The central role of the PGCs in the collaboration of the mitochondrial and nuclear genome is sustained also by the fact that if knocked-down, failure of mtDNA gene expression occurs. Not only it coordinates various sets of genes, but as a sensor of metabolic, hormonal and inflammatory signals, PPARGC1A functions as a molecular switch adapting gene activity to varying conditions from cold to starving or exercise, (2, 3, 11-13) which sustain the need of its genetic and nutrigenetic investigation in the metabolic syndrome. In PPARGC1A null mice - with age and more severely in females - body fat increases, mitochondrial number and function are reduced, exercise capacity as well as thermogenic response is impaired, and in starvation hepatic steatosis develops, although surprisingly the animals appear less susceptible to diet-induced insulin resistance (6).

Several polymorphisms of the gene situated both in exon and intron sequences have been described. Among the common single nucleotide polymorphisms, rs8192678 (in exon 8, G1444A/Gly482Ser) and rs3736265 (in exon 9, C1835T/Thr612Met) correspond to missense mutations situated in coding sequences. rs8192678, a G→A substitution leading to the change of glycine with serine in codon 482 (Gly482Ser), is the most important polymorphism of PPARGC1A (1, 4). It may be a functional point mutation, though findings have been contradictory: it has been reported to associate with a reduced gene expression, but transfection and bioinformatics analyses offered conflicting results (10-13). Binding sites for known transcription factors appear not to be altered by the mutation. Reduced activity, however, might be explained by altered efficacy of the interaction with the controlled transcription factors (i.e. exon 8 encodes for amino acids 293-598, a domain that appears to interact with PPAR-γ, so a conformational change induced might reduce the affinity of binding). It has been speculated that altered gene expression might lead to mitochondrial biogenesis and dysfunction by impaired mitochondrial transcription factor (Tfam) activation, and might contribute to insulin resistance by impaired

metabolic pathways (e.g. PPAR-mediated adipocyte differentiation, lipid oxidation, gluconeogenesis in the liver or glucose transport in the muscles), (4, 12-14) making it an important target for genetic studies of the metabolic syndrome.

According to Human Genome Diversity data and the metabolic syndrome related studies carried out in various regions, the frequency of the minor allele shows important differences (35-36% in Europe; about 39% in Asia, 25% in the Americas, and it is almost absent in Africa), being considered a candidate for the thrifty gene hypothesis, with the highest frequency reported in certain Pacific populations (14-17). According to Neel's hypothesis offering an evolutionary explanation for the diabetes epidemics witnessed, the polymorphism may have been positively selected by starvation and cold, assisting survival by promoting fat and energy storage, that today in the presence of food abundance and a sedentary lifestyle functions as a risk allele contributing to increased disease susceptibility.

Epidemiological studies revealed inconsistent results regarding the association of rs8192678 with the metabolic syndrome pathology, in general investigated separately by its components. Data show important ethnic and gender differences, and are sometimes contradicting. The negative effect of the Ser482 allele has been described on metabolic and cardiovascular traits, such as insulin sensitivity and secretion, measures of obesity, lipid and glucose concentrations, adiponectin level, aerobic fitness (1, 2, 4, 14); conversely, association of the Gly482 allele with hypertension has been reported in Austrians which could, however, reflect linkage disequilibrium with another mutation (18). Nonetheless, most often it has been investigated in association with the type 2 diabetes. While some studies confirmed an increased risk, others failed to demonstrate a significant effect, or on the contrary, observed a decreased risk in the presence of the minor allele. An increased risk has been reported in some British, Danish, Slovenian, Tunisian, Iranian, North Indian, Chinese or Japanese studies (19-24), while similar investigation carried out in the Hispanics or various Caucasian populations from both Northern and Southern European countries failed to confirm the relationship (4, 9, 26, 27).

In general, association studies often offer inconclusive and sometimes conflicting results. A possible explanation could be the population differences characterized by both genetic and environmental heterogeneity. Allele frequencies may vary by ethnicity (e.g. Ser482 frequency is higher

in the Chinese than in Caucasians). Such studies are usually carried out on single populations, case selection is often inadequate, sample sizes for the clarification of a modest effect are often too small, and population specific gene-gene or gene-environment interactions are not taken into account (28). Conflicting results require the reproduction of the analysis in various populations, and to our best knowledge and a Pubmed search, the association of PPARGC1A Gly482Ser with obesity, type 2 diabetes or the metabolic syndrome has not been addressed in Romania yet.

As compared to the majority of association studies undertaken in type 2 diabetes, our approach was slightly different by the selection of a related but more complex and more common, however less distinct and less severe pathology represented by the metabolic syndrome phenotype. While the allele frequencies and genotype distribution in the two study groups were comparable to those reported in other populations of Caucasian origin, and differed according to disease status, the risk for developing the metabolic syndrome associated with the presence of the polymorphism did not reach the limit of statistical significance. There are very few studies investigating the relationship of the SNP with the metabolic syndrome. In the population-based DanMONICA study, no difference in allele frequency in the affected and healthy persons was found, though it should be noted that the syndrome was diagnosed according to the National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATPIII) proposed system, in which visceral obesity is not a mandatory element. Ambyea *et al.* consider that their inability to replicate the association of the polymorphism that was found with type 2 diabetes in the same population could be explained by a specific diabetes susceptibility of the variant not reflected in the metabolic syndrome related phenotypes (17). In a Mexican population, conversely the Ser482 variant was found protective against disease development, even in the obese (29).

The failure to detect a significant increase in the risk associated with an individual SNP may be due to the fact that it has no sufficient impact on disease susceptibility. While genome wide association studies (GWAS) failed to detect an association, meta-analyses demonstrate a slightly increased risk for type 2 diabetes in the presence of the Ser allele (i.e. OR varying from 1.07 to 1.11) (30, 31). In the case of such a very small effect as reported in type 2 diabetes, the sample size to clarify a relationship in the metabolic syndrome is obviously not large enough. By sampling a middle-

aged population with approximately equal gender and ethnicity ratios, we tried to address factors that may further complicate the elucidation of a complex multi-factorial pathology. Statistically significant differences according to the genotype of metabolic and anthropometric parameters comprising the metabolic syndrome criteria could not be demonstrated in either group, similarly to the DanMONICA study investigating the metabolic syndrome, (17) though tendencies of body weight, lipid level or blood pressure changes and a suggested over-dominant effect inconsistently reported in the literature could perhaps exist (4, 6, 10, 14, 18).

Evidence from humans and animals have shown that PPARGC1A activity is responsive to lifestyle factors, which further complicates the correct interpretation of the obtained data. Special attention should be given to confounding factors, and it has been suggested that the effect could be dependent on obesity (14). In our cases abdominal obesity was a basic condition by definition; regression analysis of the polymorphism and BMI in the study group showed an independent effect ($r^2 < 0.75$). Gene-gene and gene-lifestyle interaction studies will be required to gain a more precise view of the PPARGC1A polymorphism role in the metabolic syndrome pathology. Haplotype analysis instead of the individual SNP studies could increase not only the efficacy of the identification of major polymorphisms involved in the etiology, but also of the synergistic effect of different variants (Thr394Thr, Asp475Asp and Thr528Thr appear in linkage disequilibrium with Gly482Ser - the only missense variant with a minor allele frequency above 10%) (19).

Genotyping could assist the identification of an increased disease susceptibility, prediction of impaired glucose tolerance conversion to overt diabetes, development of complications (e.g. diabetic nephropathy), or response to drugs and other preventive-therapeutic measures (i.e. acarbose, dietary interventions, administration of supplements, physical exercise) (6, 32-36), thus potentially contributing to a more efficient management as part of a truly state of the art personalized, predictive and preventive genomic medicine. Nutrigenetic studies could help in developing strategies of targeted intervention by identifying persons who would require most and would respond best to specific prophylactic interventions (e.g. rs8192678 has been shown to be related to an increased alcohol consumption, also associating with an increased breast cancer risk in this group)(37).

Moreover, the gene may contribute to the explanation of Barker's hypothesis about the intrauterine origins of common adult disorders comprised by the metabolic syndrome. Prenatal social stress by an altered glucose homeostasis, malnutrition inducing mitochondrial dysfunction mediated by PPARGC1A and leading to ROS induced pancreatic beta cell dysfunction, or the hypomethylation of the gene by methyl-donor deficiency may result in long term impaired lipid oxidation, emphasizing that disease prevention should start as early as the time of conception (38-40).

In conclusion, rs8192678 could be a functional polymorphism contributing to the development of the metabolic syndrome, though its effect is probably minor, acting in the context of a predisposing polygenic system dependent on gene-gene and gene-environment interactions which require further investigations. Despite the inconclusive results, the master transcriptional regulator of metabolism PPARGC1A remains an intriguing candidate gene for the metabolic syndrome associated pathology, and insights could have multiple direct practical implications.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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