SHORT COMMUNICATION

Missense variants in the human peroxisome proliferator-activated receptor- γ 2 gene in lean and obese subjects

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Abstract

The peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2) is almost uniquely expressed in adipose tissue and is of major importance for fat cell differentiation and lipid metabolism. This study was undertaken to assess whether two missense variants in the PPAR γ 2 gene are associated with early-onset obesity. A previously described polymorphism encoding for an amino acid exchange in codon 12 (Pro12Ala) was detected with allele frequencies of 0.13 in 296 markedly obese children and adolescents and 0.14 in 130 lean individuals. A Pro115Gln variant, which had been linked to obesity in Germans in a previous association study, was not detected in any of our obese or lean subjects, who are also of German origin. We conclude from our data that these two variants in the PPAR γ 2 gene are unlikely to contribute to the high prevalence of early-onset obesity.

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Introduction

Overweight and obesity affect almost half of the population in many industrialized countries. The association with type 2 diabetes, dyslipidemia and hypertension creates increased morbidity and mortality risks for obese individuals. Obesity is viewed as a multifactorial disorder, with genetic factors playing an important pathogenic role.

Products of relevant genes may be involved in the regulation of food intake, energy expenditure or nutrient partitioning. The sum of presumably several genetic effects and their interaction with environmental and behavioral conditions, such as increased caloric and fat intake or decreased physical activity, can result in energy imbalance and increased fat mass.

In recent years, the discovery of leptin and other molecules secreted by fat cells has markedly changed our view of adipose tissue being a rather passive triglyceride store (1). Furthermore, the peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2) was cloned and identified as a central regulator of fat cell differentiation (2, reviewed in 3, 4). PPAR γ is a transcription factor that belongs to the family of nuclear receptors. Two isoforms, PPAR γ 1 and PPAR γ 2, are formed by alternative promoters and splicing. In contrast to the widespread

expression of PPAR γ 1, PPAR γ 2 is almost selectively expressed in adipose tissue. Overexpression of PPAR γ 2 in fibroblast cell lines causes lipid accumulation and differentiation into mature adipocytes (5). PPAR γ 2 mRNA is regulated by several nutritional and hormonal factors in both rodents and humans (6, 7). Endogenous ligands for PPAR γ include fatty acids and some prostaglandins, while even more attention has been attracted by the identification of the thiazolidinedione family of insulin sensitizers as acting via PPAR γ .

Because it is an attractive candidate gene for the dysregulation of energy balance, single strand conformational polymorphism-based screening was applied and two missense variants in the PPAR γ 2 coding sequence were identified (8–10). In the unique first exon, a CG base exchange results in a proline to alanine amino acid substitution in codon 12, while a GT base exchange in the second exon shared by both PPAR γ isoforms introduces a substitution of proline to glutamine in codon 115. Somewhat conflicting data have recently been published as to whether Pro12Ala is associated with obesity (11) or with a lower body mass index (BMI) (12). In the latter study, a decreased binding affinity of the mutated variant to its promoter response element and a decreased activation of transcription have been demonstrated in various cell lines (12). Pro115Gln has

recently been found in 4 of 121 severely obese individuals (10). Interestingly, the altered protein causes accelerated adipocyte differentiation and may be directly involved in increased triglyceride accumulation.

Given the above data and the importance of PPAR γ 2 for the regulation of energy storage, this study was undertaken to determine whether missense variants in the PPAR γ 2 gene are associated with early-onset human obesity in a German population.

Subjects and methods

A group of 296 extremely obese children and adolescents was recruited at the Children's Hospital Hochried and the Obesity Treatment Centre Insula, which both specialize in the inpatient treatment of extremely obese young individuals. Ninety-seven percent of the obese subjects had a BMI above the 95th percentile, 61% exceeded the 100th BMI percentile. The second study group of 130 underweight students was selected at the University of Marburg. They were characterized by a BMI below the 13th percentile, absence of somatic disorders and consumption of less than ten cigarettes per day. Probands with anorexia nervosa were excluded from the present sample of underweight individuals that represents an extension of a study group described previously (13), and both groups have been characterized in detail before (14).

Genetic studies were approved by the ethics committees of the Universities of Marburg and Hamburg. Written informed consent was obtained from all participants or, in case of minors, their parents.

EDTA anticoagulated venous blood samples were collected from the aforementioned individuals and leukocyte DNA was isolated as described (13). For restriction fragment length polymorphism (RFLP)-based genotyping, a 306 bp fragment including exon 1 of the human PPAR γ 2 gene and containing the Pro12Ala variant was amplified by PCR using oligonucleotides 5'-GCCAATTCAAGCCCAGTC-3' and 5'-CGTCCCCAATAGCCGTATC-3'. A 118 bp fragment containing the Pro115Gln variant was amplified using primers 5'-AATCAAAGTGGAGCCTGCATGT-3' and 5'-ATCTCCACAGACACGACATT-3'. Seventy-five nanograms genomic DNA were added to 20 μ l of reaction mixture (14) and amplification over 35 cycles was carried out in an automated thermal cycler at 94°C for 1 min, 55°C (Pro12Ala) or 50°C (Pro115Gln) for 1 min and 72°C for 2 min. Ten microliters PCR product were digested with HgaI (Pro12Ala) or HincII (Pro115Gln) and subjected to 3% agarose electrophoresis or 10% PAGE with ethidium bromide.

Results

The base exchange predicting the Pro12Ala variant introduces an HgaI site in the amplified sequence,

Figure 1 PCR-RFLP detection of PPAR γ 2 missense variants by PAGE, ethidium bromide staining and UV transillumination. Lanes 1, 5 and 9: pBR322 DNA-MspI marker. Lane 2: wildtype for codon 12 variant. Lane 3: heterozygous for codon 12 variant. Lane 4: homozygous for codon 12 missense mutation. Lane 6: wildtype for codon 115 variant (amplified from human genomic DNA). Lane 7: wildtype for codon 115 variant (amplified from a plasmid containing codon 115 wildtype DNA). Lane 8: codon 115 mutated variant (amplified from a plasmid containing codon 115 missense mutation; 22 bp fragment not visualized).

resulting in fragments of 220 and 86 bp for the mutated allele (Fig. 1).

RFLP analysis revealed an allele frequency of 0.13 for the Pro12Ala variant in 296 obese children and adolescents. Two hundred and twenty (74.3%) were homozygous for the wildtype allele, while seventy three (24.7%) were heterozygous and three (1.0%) were homozygous for the mutated allele.

In the lean group, 95 of 130 individuals (73.1%) were homozygous for the wildtype allele, while 35 (26.9%) were heterozygous for the variant with no homozygotes. Therefore, the frequency of the mutated allele in the lean group (0.14) was not different from the obese ($P = 0.98$ by χ^2 -test). The Pro115Gln variant that should have created an additional HincII site with fragments of 22 and 96 bp, in contrast to the 118 bp wildtype band, was found neither in 296 obese nor in 130 lean individuals.

A DNA fragment including the mutant variant provided by Dr M Ristow (Joslin Diabetes Centre, Boston, MA, USA) was used as a positive control to ensure the correct cut by the restriction enzyme HincII.

Discussion

The importance of PPAR γ 2 in lipid, glucose and energy metabolism is well established. Since PPAR γ 2 promotes adipocyte differentiation, it is an attractive candidate gene for states of altered triglyceride storage, such as obesity or conditions associated with underweight. Variants in the PPAR γ 2 gene could possibly lead to both a decreased or accelerated adipocyte differentiation and lipid accumulation. In a recent study, a variant in codon 115 was demonstrated to be an example of the latter (10). When a mutated PPAR γ 2 allele previously found in 4 of 121 obese German individuals was overexpressed in murine fibroblasts, it led to a markedly more pronounced adipocyte differentiation than wildtype

PPAR γ 2. This is presumably due to the fact that the mutation is next to a serine phosphorylation site in codon 114, which has been shown to be of major importance for the negative regulation of PPAR γ 2 gene expression (15).

It appeared tempting to speculate that this variant could be associated with early-onset human obesity. However, our RFLP analysis revealed that not a single individual within a large group of morbidly obese children and adolescents or lean individuals carried an allele with the mutation in codon 115. Because both of our study groups are of German origin, it appears that the reported variant (10) is rare in the German population. Thus, it seems unlikely that the Pro115Gln variant explains a relevant proportion of obesity in this population. Furthermore, a previously identified variant in codon 12 was associated neither with obesity nor with leanness.

Although we cannot fully exclude the possibility that an association may be found in other populations, our data make it rather unlikely that variants in the PPAR γ 2 gene contribute to the high prevalence of human obesity.

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References

- 1 Spiegelman BM & Flier JS. Adipogenesis and obesity: rounding out the big picture. *Cell* 1996 **87** 377–389.
- 2 Tontonoz P, Hu E, Graves RA, Budavari AI & Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes and Development* 1994 **8** 1224–1234.
- 3 Auwerx J, Martin G, Guerre-Millo M & Staels B. Transcription, adipocyte differentiation, and obesity. *Journal of Molecular Medicine* 1996 **74** 347–352.
- 4 Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998 **47** 507–514.
- 5 Tontonoz P, Hu E & Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 1994 **79** 1147–1156.
- 6 Vidal-Puig A, Jimenez-Linan M, Lowell BB, Hamann A, Hu E, Spiegelman B *et al.* Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *Journal of Clinical Investigation* 1996 **97** 2553–2561.
- 7 Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF *et al.* Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *Journal of Clinical Investigation* 1997 **99** 2416–2422.
- 8 Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP *et al.* Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochemical and Biophysical Research Communications* 1997 **241** 270–274.
- 9 Vigouroux C, Fajas L, Khallouf E, Meier M, Gyapay G, Lascos O *et al.* Human peroxisome proliferator-activated receptor-gamma 2: genetic mapping, identification of a variant in the coding sequence, and exclusion as the gene responsible for lipotrophic diabetes. *Diabetes* 1998 **47** 490–492.
- 10 Ristow M, Müller-Wieland D, Pfeiffer A, Krone W & Kahn CR. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *New England Journal of Medicine* 1998 **339** 953–959.
- 11 Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ *et al.* Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-gamma2 gene with obesity in two Caucasian populations. *Diabetes* 1998 **47** 1806–1808.
- 12 Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J *et al.* A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nature Genetics* 1998 **20** 284–287.
- 13 Hinney A, Barth N, Ziegler A, von Prittwitz S, Hamann A, Hennighausen K *et al.* Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. *Life Sciences* 1997 **61** L295–L303.
- 14 Hamann A, Tafel J, Büsing B, Münzberg H, Hinney A, Mayer H *et al.* Analysis of the uncoupling protein-1 (UCP1) gene in obese and lean subjects: identification of four amino acid variants. *International Journal of Obesity and Related Metabolic Disorders* 1998 **22** 939–941.
- 15 Hu E, Kim JB, Sarraf P & Spiegelman BM. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science* 1996 **274** 2100–2103.

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