CLINICAL STUDY

Identification of a deletion variant in the gene encoding the human α_{2A} -adrenergic receptor

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Abstract

Objective: The α_2 -adrenergic receptors are involved in the effects of catecholamines on energy metabolism. Of three known subtypes with differential expression, α_{2A} -adrenergic receptors are also localized in adipose tissue where they counteract the lipolytic activity of β -adrenergic receptors. This study was undertaken to assess whether variants in the α_{2A} -adrenergic receptor gene are associated with body weight.

Design and methods: Single strand conformation polymorphism (SSCP) screening and subsequent sequencing were applied to determine genetic variants in DNA samples from individuals with obesity, those of normal weight and those underweight.

Results: Analysis of the coding region resulted in the identification of an 18 bp deletion, with no other mutation found. Of 429 genotyped subjects, 7 carried the deletion, with no significant differences between lean and obese subjects. A previously identified polymorphism in the promoter of the α_{2A} -adrenergic receptor gene also did not show an association with any of the tested body weight categories.

Conclusion: Our data suggest that variants in the α_{2A} -adrenergic receptor gene are unlikely to contribute to the predisposition for the lean or obese state.

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Introduction

Catecholamines play an important role in the regulation of metabolism in white and brown adipose tissue (1–3). Epinephrine and norepinephrine exert their action via adrenergic receptors, which belong to the family of seven transmembrane-segment receptors. In adipocytes, activation of β_1 -, β_2 - and β_3 -adrenergic receptors by catecholamines leads to the stimulation of adenylate cyclase and generation of cAMP, mediated by the G protein G_s . Conversely, sympathetic activation of the α_2 -adrenergic receptor leads to inhibition of adenylate cyclase and cAMP production via the G protein G_i . Intracellular cAMP levels in adipocytes control activity of protein kinase A which, in turn, regulates activation of hormone-sensitive lipase and thereby the lipolytic process. In brown adipocytes, initiation of lipolysis is a prerequisite for activation of thermogenesis (4).

In rodents, the β_3 -adrenergic receptor is of major importance for lipolysis and thermogenesis, while the presence and functional importance of α_2 -adrenergic

receptors in adipocytes are questionable (5). Furthermore, in murine fat cell lines, α_{2A} -adrenergic receptors have not been found (2). The human fat cell, on the other hand, contains predominantly β_1 - and β_2 -adrenergic receptors responsible for the activation of lipolysis and α_{2A} -adrenergic receptors with the ability to counteract lipolytic activity (6). Three genes for the α_2 -adrenergic receptor located on different chromosomes have been identified, encoding for subtypes α_{2A} , α_{2B} and α_{2C} . As cellular distribution for the various subtypes is markedly different, only the α_{2A} -adrenergic receptor encoded on chromosome 10q23–q25 is responsible for the observed effects in adipose tissue (7).

Various pharmacological approaches using microdialysis and selective α_2 - and β -adrenergic agonists and antagonists have been used to gain insight into the adrenergic control of fat cell function and nutritive blood flow in the surrounding tissue. Blockade of human α_2 -adrenergic receptors *in vitro* promotes enhancement of catecholamine-induced lipolysis (2, 8). Interestingly, heterogeneous distribution of α_2 -adrenergic receptors in various fat deposits seems to

be involved in tissue and sex-specific differences of lipolytic activity (9, 10). Adipocytes from femoral subcutaneous fat deposits are less responsive to the lipolytic action of epinephrine than adipocytes from the omental or abdominal subcutaneous region. Furthermore, in gluteal fat depots of women low concentrations of epinephrine have a reduced lipolytic effect compared with fat of the same region obtained from men. This observation goes along with a higher number of α_2 -adrenergic receptors in female gluteal adipocytes, which could be responsible for the enhanced tendency of women to accumulate fat in this region.

Genetic factors play an important role in the regulation of energy metabolism and body weight. Products of relevant genes may be involved in the regulation of food intake, energy expenditure or nutrient partitioning (11). 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 contrast, genetic factors could also predispose to leanness. Variants in the α_{2A} -adrenergic receptor could alter lipolytic activity in adipose tissue, making the α_{2A} -adrenergic receptor an attractive candidate gene for the dysregulation of energy balance. A mutation in the coding region of the α_{2A} -adrenergic receptor could deplete its antilipolytic action, resulting in reduced lipid storage and resistance to diet-induced obesity. Possibly, such a mutation in the α_{2A} -adrenergic receptor could also change its modulation of brown fat thermogenesis.

Besides their local effect in adipose tissue, α_2 -adrenergic receptors play an important role in the regulation of the sympathetic tone and could thereby influence energy balance (12). Therefore, a defect in the α_{2A} -adrenergic receptor protein could via various mechanisms predispose to a low body weight. On the other hand, a polymorphism in the promoter of the α_{2A} -adrenergic receptor gene could, depending on its effect on gene transcription, predispose to either underweight or obesity. Earlier studies had identified two restriction fragment length polymorphisms (RFLPs) in the α_{2A} -adrenergic receptor gene (DraI and Bsu36I) (13, 14). Controversial findings regarding the possible association of the DraI RFLP with hypertension have been reported (15, 16). In women, the DraI RFLP was shown to be associated with body fat distribution independent of body weight (17).

This study was undertaken to identify possible variants in the coding region of the α_{2A} -adrenergic receptor gene and to assess their association with different categories of body weight. In addition, we assessed the frequency of another previously identified polymorphism in the promoter of the α_{2A} -adrenergic receptor gene (18).

Subjects and methods

A cohort of extremely obese children and adolescents was recruited at the Children's Hospital Hochried and the Obesity Treatment Center Insula, which both specialize in the in-patient treatment of extremely obese young individuals. Ninety-seven percent of the obese subjects had a body mass index (BMI) above the 95th percentile, and 61% exceeded the 100th BMI percentile, as determined in a large and representative epidemiological sample of the German population (19). For the purpose of this paper, we refrained from including information on age of onset of obesity because recall errors are most likely. The second study cohort of underweight students was recruited at the University of Marburg. They were characterized by the criteria of a BMI below the 13th percentile, absence of somatic disorders and consumption of less than 10 cigarettes per day. The present sample of underweight probands represents an extension of a study group described previously (20) and both cohorts have been characterized in detail elsewhere (21, 22). Mean $\pm$ s.d. age in the obese and the lean study groups was 13.7 ± 2.3 years and 25.4 ± 4.0 years respectively. The obese study group comprised 53% females, while 46% of the lean individuals were female. Two adult cohorts were recruited at the University of Hamburg, Germany: a group of adults with late onset obesity, BMI 38.5 ± 5.0 kg/m², age 53.4 ± 13.7 years, and a control group of normal weight individuals with a BMI of 22.6 ± 1.6 kg/m², age 50.2 ± 18.4 years.

The ethics committees of the participating universities approved all genetic studies. Written informed consent was obtained from all participants or, in the case of minors, their parents. According to the published sequence of the intronless α_{2A} -adrenergic receptor (23), the following primer pairs for the amplification of the α_{2A} -adrenergic receptor coding sequence were generated: pair 1, 5'-CCTTCATCTT CCGCCAGGAG-3' and 5'-GTAGCCCATGACCTCGTTGG-3' (fragment size 348 bp, annealing temperature (T_a) 61°C); pair 2, 5'-CGCTCGTCATCCCTTCTCG-3' and 5'-CCGCCGCCGCCCTTCTCTC-3' (286 bp, T_a 63°C); pair 3, 5'-TCCCGCCGCTCATCTCCATC-3' and 5'-CACGC GGGTGCGACGCTTGG-3' (191 bp, T_a 62°C); pair 4, 5'-CTTCGCTCCCTGCCTCATCA-3' and 5'-GGTCCAGCGCG TCGGTGTCG-3' (263 bp, T_a 63°C); pair 5, 5'-CGACACC GACGCGCTGGA-3' and 5'-GCGCTTCTCGAGTTCTGC-3' (256 bp, T_a 64°C); pair 6, 5'-CGACACCGACGCGCTG GAC-3' and 5'-GCCTGCAGTCAGCGTGAG-3' (538 bp, T_a 59°C). Seventy-five nanograms genomic DNA isolated from blood leukocytes were added to 20 μ l reaction mixture containing 0.4 μ mol/l of each primer, 0.625 U Taq DNA polymerase, 200 μ mol/l of each dNTP and 2 μ l PCR reaction buffer (Applied Biosystems, Weiterstadt, Germany) and 5–10% DMSO. Amplification was carried out in an automated thermal cycler (Biometra, Göttingen, Germany) over 35 cycles

Table 1 Frequency of the deletion variant in the human α_{2A} -adrenergic receptor gene in different categories of body weight.

	Allele frequency		Genotype frequency		
	Wildtype	Deletion	WT/WT n (%)	WT/D n (%)	D/D n (%)
Obese children and adolescents (n = 195)	0.995	0.005	193 (99.0)	2 (1.0)	0 (0)
Obese adults (n = 48)	1.0	0.0	48 (100)	0 (0)	0 (0)
Normal weight subjects (n = 58)	0.983	0.017	56 (96.5)	2 (3.5)	0 (0)
Underweight subjects (n = 128)	0.988	0.012	125 (97.6)	3 (2.4)	0 (0)

WT = Wildtype, D = deletion.

 χ^2 -test: obese children/adolescents vs underweight subjects $P = 0.350$; obese children/adolescents vs normal weight subjects $P = 0.196$; obese adults vs underweight subjects $P = 0.452$.

at 94°C for 1 min, at the indicated annealing temperature for 1 min and at 72°C for 2 min, with template-free controls included in each experiment.

For single strand conformational polymorphism (SSCP) analysis, aliquots of PCR products were separated on a Multiphor II Electrophoresis Unit using CleanGels 48S (Pharmacia Biotech, Freiburg, Germany) at 14°C and visualized by silver staining, all according to the manufacturer's instructions. Amplified fragments were cloned into the TA vector (Invitrogen, Groningen, The Netherlands) and isolation of plasmid DNA was performed with the JET Quick Plasmid Miniprep (Genomed, Bad Oeynhausen, Germany). Sequencing was carried out with the dRhodamine Terminator Ready Reaction Kit with Amplitaq DNA Polymerase FS on an ABI Prism 377 (Applied Biosystems).

Genotyping for the identified deletion was performed by agarose gel electrophoresis after amplification of genomic DNA with primer pair 4 (see above). Long and short alleles were identified after staining with ethidium bromide based on their different electrophoretic migration rates.

A 502 bp fragment of the α_{2A} -adrenergic receptor gene promoter was amplified using the primer pair 5'-TCACACCGGAGGTTTCATTCCCTCG-3' and 5'-TCCGAC

GACAGCGCGAGTT-3' (T_a 63°C). Genotyping for the C/G polymorphism at position -1291 of the α_{2A} -adrenergic receptor gene promoter was performed by digestion with the restriction enzyme MspI, followed by agarose gel electrophoresis.

Differences in allele frequencies between cohorts of different body weight were evaluated by χ^2 -test.

Results

With each primer set, PCR yielded amplicons of the expected sizes. SSCP screening of the intronless coding region of the α_{2A} -adrenergic receptor gene using 6 different primer pairs and sequencing of PCR fragments with altered migration patterns resulted in the identification of an insertion/deletion polymorphism. Compared with the published wildtype sequence, 18 base pairs were deleted in a region of the gene corresponding to amino acids 255–260 of the α_{2A} -adrenergic receptor. No further variants were detected.

Allele and carrier frequencies of the deletion variant were determined by subsequent genotyping in cohorts of individuals with different body weights, as shown in Table 1. Of the 429 genotyped probands, 7 were heterozygous for the deletion: 2 of 195 obese children and adolescents, 3 of 125 underweight subjects and 2

Table 2 Frequency of the promoter polymorphism in the human α_{2A} -adrenergic receptor gene in different categories of body weight.

	Allele frequency		Genotype frequency		
	C	G	C/C n (%)	C/G n (%)	G/G n (%)
Obese adults (n = 107)	0.776	0.224	65 (60.7)	36 (33.7)	6 (5.6)
Normal weight subjects (n = 60)	0.750	0.250	34 (56.7)	22 (36.7)	4 (6.7)
Underweight subjects (n = 118)	0.771	0.229	71 (60.2)	40 (33.9)	7 (5.9)

 χ^2 -test: obese vs underweight subjects $P = 0.909$ obese vs normal weight subjects $P = 0.594$; normal weight vs underweight subjects $P = 0.656$.

of 56 normal weight subjects, with 0 of 48 obese adults. Nobody homozygous for the deletion was detected. No significant differences in allele frequencies between cohorts of different body weights were assessed by χ^2 -test. Combining both obese cohorts for statistical analysis versus subjects with normal weight or underweight resulted in a *P* value of 0.123.

Genotyping for the C/G polymorphism at position -1291 of the α_{2A} -adrenergic receptor gene was carried out by RFLP analysis, since the polymorphism introduces an additional *MspI* site. This resulted in the predicted fragment sizes of 174, 165, 116, 62 and 5 bp for the wildtype allele compared with 165, 121, 116, 62, 53 and 5 bp for the mutant allele. As shown in Table 2, RFLP analysis revealed no significant differences in allele or genotype frequencies between the study groups.

Discussion

Given its role in the regulation of lipolysis in adipose tissue and systemic control of sympathetic tone, defects in the α_{2A} -adrenergic receptor could predispose an individual to alterations in body weight. Since the α_{2A} -adrenergic receptor has the potency to counteract the effects of the various β -adrenergic receptors on lipolysis and thermogenesis, it is an attractive candidate gene for states of altered triglyceride storage, such as underweight. Our results, however, indicate that variability in the α_{2A} -adrenergic receptor gene is not involved in different body weight regulation in humans. In the entire coding sequence, only one variant was found and was shown to be very rare among all genotyped individuals. The prevalence of a more frequent polymorphism in the promoter region of the α_{2A} -adrenergic receptor gene was not significantly different between individuals of different weight categories.

The deletion variant is located in the third cytoplasmic domain of the α_{2A} -adrenergic receptor, deleting amino acids 255–260 (Pro-Glu-Arg-Ser-Ala-Gly). It is not known whether this specific region is of critical relevance for the function of the α_{2A} -adrenergic receptor, e.g. G protein coupling.

Mice completely lacking functional α_{2A} -adrenergic receptors due to disruption of the gene in embryonic stem cells were reported to have an increased sympathetic activity (24). While this was associated with resting tachycardia, body weight was reported to be normal. However, this does not exclude the possibility that the α_{2A} -adrenergic receptor has a role to play in human body weight regulation. It is well documented that an increase in sympathetic tone can promote weight loss in humans, as observations with ephedrine and caffeine, other sympathomimetic compounds and sibutramine have shown (25, 26). Also, mild to moderate effects of yohimbine and other α_2 -adrenoceptor antagonists on body weight, lipid

mobilization and other features of energy metabolism have been demonstrated in humans, dogs and other mammalian species (2). According to the concept of susceptibility genes, a relevant defect in the α_{2A} -adrenergic receptor gene would only be expected to represent one of presumably multiple genetic factors predisposing an individual towards the development of underweight or obesity. The development of the phenotype would generally depend on interactions between genes and environmental conditions, such as diet and exercise. In this context, genetic defects in the α_{2A} -adrenergic receptor could possibly prevent excess lipid storage when humans or animals are exposed to a high fat diet.

An *MspI* RFLP in the promoter region of the α_{2A} -adrenergic receptor gene was shown to result from a C/G substitution 1291 bp upstream from the transcription start site (18, 27, 28). Previous association studies have focussed on human hypertension and psychiatric disorders, but did not detect statistically different prevalences between phenotypically different cohorts. No studies have been performed regarding the possible effect of this variant on α_{2A} -adrenergic receptor gene expression and receptor density. However, we did not detect any evidence that such a possible effect of the polymorphism could be associated with body weight regulation. Since all our study groups are of German origin, we cannot fully exclude the possibility that association may be found in other ethnic populations. In summary, our data make it rather unlikely that variants in the α_{2A} -adrenergic receptor gene contribute to interindividual differences in body weight.

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