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SHORT COMMUNICATION

Lack of association between polymorphism of the human cyclic GMP-dependent protein kinase gene and obesity

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OBJECTIVE: To investigate whether genetic variation in the cyclic GMP-dependent protein kinase gene (PRKG1) is associated with obesity.
METHODS: The study included 143 individuals from New York City area, NY, USA. The subjects were sampled on the basis of body mass index (BMI): obese (BMI ranging from 33.8 to 89.5 kg/m^2), and nonobese (BMI ranging from 16.0 to 29.4 kg/m^2). The association between C2276T polymorphism in PRKG1 gene and obesity was tested using linear regression analysis.
RESULTS: BMI levels were predicted by linear regression models adjusted for demographic factors. An analysis was performed twice: in individuals of all ethnical backgrounds and in European-Americans only. In both cases, genotype did not have a significant effect.
CONCLUSION: We found no evidence that the C2276T polymorphism in the PKRG1 gene is associated with obesity.
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Introduction

Obesity is a major health problem worldwide[1] influenced by both genetic and environmental factors. Association studies represent an important paradigm for investigation of complex traits, such as obesity. Using this approach, many candidate genes including β_2- and β_3-adrenergic receptors, leptin receptor, glucocorticoid receptor, and tumor necrosis factor have been assessed for association with obesity.[2] The cyclic GMP-dependent protein kinase (PKG) gene could be another candidate. PKG is a transduction pathway enzyme and operates in a variety of cell responses.[3] Allelic variation in the homologs of this gene has been linked to differences in food-related behaviors in fruitflies,[4] honey bees[5] and nematodes.[6] In *Drosophila melanogaster*, the product of the *for* gene, encoding PKG, might be a component of the Neuropeptide F (a homolog of human Neuropeptide Y (NPY)) signaling pathway, which is involved in regulation of food-related behaviors.[7] Moreover, PKG proteins are highly phylogenetically conserved.[8] Here, we tested relationships between a polymorphism of the PRKG1 gene, encoding cyclic GMP-dependent protein kinase, and obesity.

Methods

The genetic polymorphism in PRKG1 gene was assayed in 143 individuals from the New York City area. The study was approved by the IRB at the St Luke's-Roosevelt Institute for Health Sciences. Written informed consent was obtained from all subjects. Genomic DNA was extracted as previously described.[9] The C2276T polymorphism is located in the 3′UTR of the human PRKG1 gene (GenBank accession number NM_006258). A 236 bp fragment (positions 2129–2364) encompassing polymorphic position 2276 was amplified using PCR as previously described[9] with the following

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oligonucleotide primers: hPRKG1-F 5′-TTACCTGCTTCT GCCTTGCT-3′ and hPRKG1-R 5′-GTGAAAGGCTTTGCTT CAGG-3′ and annealing temperature of 64°C. In all, 10 μl of the PCR product were digested at 37°C for 16 h with 10 u of the restriction enzyme *Aci*I, specific for the sequence CCGC. The polymorphisms were detected after separation by electrophoresis on a 2% agarose gel in TEA at 100 V for 1.5 h. Gels were stained with ethidium bromide followed by visualization of separated DNA fragments under UV light. The T allele is not cut with *Aci*I and is seen as 236 bp band, whereas allele C is cut into two fragments of 148 and 88 bp. An association between the genetic polymorphism and body mass index (BMI) was tested using linear regression. Models were adjusted for age and gender. All analyses were performed using SAS version 9.0 (SAS Institute, Cary, NC, USA). A *P*-value <0.05 was considered significant.

Results

The C2276T polymorphism in the PRKG1 gene was examined in 143 individuals from the New York City area. Among them, 51 obese individuals were 15–63 y old (mean age is 36.0 y; s.d. = 10.9), with BMI ranging from 33.79 to 89.53 kg/m^2. The 92 nonobese individuals were 18–54 y old (mean age is 29.4 y; s.d. = 7.8), with BMI ranging from 16.02 to 29.46 kg/m^2. The ethnic makeup was 81% European-American, 14% African-American and 5% Hispanic-American. The summary of phenotype and genotype information is presented in Table 1.

BMI levels were predicted by linear regression models containing demographic factors (age and gender) and genotype categories recoded as dummy variables. Race was not included in the model due to the small number of African-Americans and Hispanic-Americans in the study. However, to assess if population stratification could affect results, analysis was performed twice: for individuals of all ethnical backgrounds (Table 2, model 1) and for European-Americans only (Table 2, model 2). In Model 1, only age had a significant effect (*P*-value = 0.0012). For model 2, the age effect was not significant (*P*-value = 0.0933). In both cases, neither gender nor genotype had significant effect. In summary, we found no compelling evidence that the genetic polymorphism C2276T in PRKG1 gene is associated with obesity.

Discussion

Cyclic GMP is the intracellular molecule common to the nitric oxide (NO) signaling pathway.[3] The cGMP-dependent protein kinase is a major receptor for cGMP in a variety of cells and has an evolutionary conserved structure.[10] Mammals have two PKG genes, PRKG1 and PRKG2 (also known as cGK I and cGK II). Two isoforms of the first type, α and β, are produced via alternative splicing and are expressed in cardiac and smooth muscle cells, platelets, cerebellum and other tissues.[10] PRKG1 knockout mice have a decreased lifespan, defects in relaxation of vascular, visceral, and penile smooth muscle, disturbed platelet adhesion and activation, and impaired guidance of sensory axons during embryogenesis.[11,12] The NO/cGMP pathway, where PRKG1 is one of the downstream players, mediates regulatory effects on key enzymes of fatty acid synthesis and oxidation and may be involved in physiological control of fatty acid metabolism in liver.[13] Leptin-deficient obese mice show significant changes in NO/cGMP signaling pathway.[14]

The functions of PKG in neurons have been much less clearly defined in mammals, but interesting observations have been made in evolutionarily distant organisms.[15,16] Allelic variation in the *for* gene encoding PRKG1 homolog in *Drosophila* results in differences in food-related behaviors.[4,16] The conserved role of PKG in food-related behavior has been demonstrated in the honey bee *Apis mellifera*[5] and in the nematode *Caenorhabditis elegans*.[6] Wu *et al* (2003)[17] speculated that the product of the *for* gene might be a component of the *Drosophila* Neuropeptide F (dNPF) signaling pathway,

Table 1 A summary of the genotype and phenotype information

	Ethnic background			
	European-American	*African American*	*Hispanic-American*	*Total*
Genotype				
CC	58	5	4	67
CT	13	0	0	13
TT	45	15	3	63
Phenotype				
Obese	31	16	4	51
Nonobese	85	4	3	92
Total	116	20	7	143

Table 2 Parameter estimates and significance levels for linear regression analyses

	Model		*Parameters*	
#	R^2	*Terms*	*Estimate (±s.e.)*	P-*value*
1	0.1279	Intercept	19.23±7.10	0.1955
		Sex	6.46±4.03	0.1109
		Age	0.57±0.17	0.0012
		CC	0.03±3.36	0.9940
		CT	−9.71±5.84	0.0987
2	0.0682	Intercept	15.32±7.82	0.0527
		Sex	3.13±4.72	0.5095
		Age	0.34±0.20	0.0933
		CC	3.91±3.75	0.2994
		CT	−5.33±5.86	0.3644

Model 1 was conducted using individuals of all ethnic backgrounds, and model 2 using European-Americans only.

which regulates food-related behaviors.[18] The dNPF, a homolog of human NPY, is a conserved hypothalamic neuromodulator that strongly increases food intake.[7]

Thus, we speculated that polymorphism in PRKG1 gene could be associated with obesity via a number of different mechanisms. The polymorphism found in the 3′UTR of the mature mRNA of PRKG1 at the position 2276 could be important for the regulation of translation of the mature mRNA or its stability.[9] We tested the relation between this polymorphism and obesity but did not detect significant association.

This study has several limitations. We observe departure from Hardy–Weinberg equilibrium in our sample, which is not uncommon.[19] The PRKG1 gene may be a good candidate from an evolutionary perspective, but its effect on obesity in humans may be minimal. At the time of the study, there was only one identified SNP in the coding region of the PRKG1 gene. Recently, more SNPs have been identified in this locus (over 200 in dbSNP database at NCBI of which 80 were confirmed by the International HapMap project[20]). Our results are limited in making a powerful conclusion due to the fact that we have only used a single polymorphism. In future studies, it would be interesting to test if any of the recently identified SNPs show association with obesity.

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