Absence of Association Between Genetic Variation of the β_3-Adrenergic Receptor and Metabolic Phenotypes in Oji-Cree

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OBJECTIVE — To assess the association between the common missense variant, Y64R, in the gene encoding the β_3-adrenergic receptor, *ADRB3*, and intermediate phenotypes related to obesity and NIDDM in Canadian Oji-Cree.

RESEARCH DESIGN AND METHODS — We determined genotypes of the *ADRB3* Y64R polymorphism in 508 clinically and biochemically well-characterized adult Oji-Cree, of whom 115 had NIDDM. We tested for associations with multivariate analysis of variance.

RESULTS — We found the *ADRB3* R64 allele frequency to be 0.40 in this population, which is the highest yet observed in a human population. Furthermore, 15% of subjects were R64/R64 homozygotes, compared with a virtual absence of homozygotes in European study samples. However, we found no statistically significant associations of the *ADRB3* Y64R genotype either with the presence of NIDDM, with indexes of obesity, or with intermediate quantitative biochemical traits related to NIDDM.

CONCLUSIONS — Despite the very high frequency of the *ADRB3* R64 allele in this sample of aboriginal people, it was not associated with any metabolic phenotype. This suggests that the *ADRB3* R64 allele is probably not a major determinant of obesity or NIDDM in these aboriginal Canadians.

A preponderance of recent scientific evidence indicates that there are strong heritable influences on obesity (1) and NIDDM (2). The strategies to define these genetic determinants include *1*) linkage analysis using genotypes derived from genomic scanning in order to find new quantitative trait loci; and 2) analysis of the association of phenotypes with defined genomic variants within candidate genes (1,2). For both obesity and NIDDM, genes whose products act within the adrenergic system are good candidates for association studies. For example, the β_3-adrenergic receptor *ADRB3* plays a role in the regulation of catecholamine-induced lipolysis and in the regulation of energy balance (1). A missense mutation in the *ADRB3* gene, Y64R, results in the substitution of a tryptophan by an arginine in the first intracellular loop of *ADRB3* (3–5). Numerous studies in several populations have evaluated the genetic association of the *ADRB3* R64 allele with traits such as the presence and/or severity of NIDDM, BMI, waist-to-hip ratio (WHR), weight gain, abdominal fat mass, fat cell volume, and age at onset of obesity (3–19). The results of these studies have been inconsistent at best; such inconsistencies may have been due to factors such as population admixture and differences in linkage disequilibrium between the studied variant and other putative functional variants at the locus. Alternatively, some study samples may have had too small a number of subjects, particularly homozygotes for the *ADRB3* R64 allele, to have had sufficient power to find associations with these traits.

The Oji-Cree of the Sandy Lake Reserve in Northern Ontario have one of the highest reported prevalences of NIDDM among human populations (20). We have previously reported associations of candidate gene variants with several metabolic phenotypes in the Sandy Lake Oji-Cree (21–26). We felt that this population would be suitable to study for associations between the genetic variation of *ADRB3* and both NIDDM and obesity-related phenotypes.

RESEARCH DESIGN AND METHODS

Study subjects

The community of Sandy Lake, Ontario, is located about 2,000 km northwest of Toronto, in the subarctic boreal forest of central Canada. The community is isolated and is accessible only by air during most of the year. Most members of the community speak both English and Oji-Cree, a member of the Algonkian family of languages (20). Historically, the ancestors of the contemporary residents of this region lived a nomadic hunting-gathering subsistence typical of other Algonkian-speaking peoples of the northeastern subarctic. Since the development of the reservation and residential school systems, the lifestyle has changed radically from physically active to sedentary. The primary source of food has changed from wildlife with supplementation by roots and berries to processed foods high in animal fats.

A total of 728 members of this community, aged ≥10 years (72% of the total population), participated in the Sandy Lake Health and Diabetes Project (20). Assessments included a questionnaire to assess medical history, including a previous diagnosis of NIDDM. Subjects were assessed

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Abbreviations: ANOVA, analysis of variance; OGTT, oral glucose tolerance test; PBF, percent body fat; p.c., postcibal; WHR, waist-to-hip ratio.

Table 1—*Allele and genotype frequencies of* ADBR3 *Y64R in Sandy Lake NIDDM and non-NIDDM subjects*

	NIDDM	Non-NIDDM	*P* value
n	115	393	—
R64 frequency	0.391	0.403	NS (0.90)
R64/R64 frequency	0.139	0.153	NS (0.80)

without shoes and wearing either cotton examination gowns and underclothes or light athletic clothing. Each measurement was performed twice, and their average was used in the analysis. Height was measured to the nearest 0.1 cm using an Accustat wall-mounted stadiometer (Genentech, South San Francisco, CA) with heels together and buttocks, back, shoulders, and head touching the wall. Weight was measured to the nearest 0.1 kg using a standard hospital balance beam scale (Health-O-Meter, Bridgeview, IL). BMI was defined as weight/height^2 (kg/m^2). WHR was determined as described previously (20). Percent body fat (PBF) was estimated by bioelectrical impedance analysis using the TBF-201 Body Fat Analyzer (Tanita, Tokyo, Japan) and an equation based on total body water (27). The method of estimation of PBF was independently validated in 98 subjects using dual-emission X-ray absorptiometry (28). The reproducibility of PBF estimates in a subsample had an intraclass correlation coefficient of 0.99 (data not shown). The project was approved by the University of Toronto Ethics Review Committee.

Biochemical and genetic analyses

Plasma samples were obtained with informed consent. Exclusion criteria included an inadequate blood sample available for all biochemical and/or genetic determinations. Volunteers gave plasma samples after fasting overnight for 8–12 h. Blood was centrifuged at 2,000 rpm for 30 min, and the plasma was stored at −70°C. Concentrations of fasting glucose were determined as described previously (20). Concentrations of fasting plasma insulin were determined by radioimmunoassay (Pharmacia, Uppsala, Sweden). A standard 75-g oral glucose tolerance test (OGTT) (Glucodex; Rougier, Chambly, Quebec, Canada) was then administered, and a second blood sample was collected after 120 min for plasma glucose determination. Volunteers were excluded from the OGTT if they had physician-diagnosed diabetes and were currently receiving treatment with insulin and/or oral hypoglycemic agents or if they had a fasting blood glucose >11.1 mmol/l. Volunteers who were pregnant at the time of recruitment had their OGTT deferred until 3 months postpartum. NIDDM and impaired glucose tolerance were diagnosed using established criteria (29,30). Genotypes for *ADRB3* codon 64 were determined as described elsewhere (3). All samples were run with known genotypic controls.

Statistical analysis

The significance of deviations of observed genotype frequencies from those predicted by the Hardy-Weinberg equation were evaluated with χ^2 tests. SAS (Version 6.1) was used for all statistical comparisons (31). The distributions of BMI, WHR, PBF, plasma concentrations of fasting and 2-h postcibal (p.c.) glucose, and insulin were significantly non-normal in this data set. Therefore, for parametric statistical analyses, each quantitative variable was transformed and subjected to analysis of normality as described elsewhere (21–26). Analysis of variance (ANOVA) was performed using the general linear models procedure to determine the sources of variation for transformed quantitative traits, with *F* tests computed from the type III sums of squares. This form of sums of squares is applicable to unbalanced study designs and reports the effect of an independent variable after adjusting for all other variables included in the model (21–26). Dependent variables were transformed BMI, WHR, PBF, plasma concentrations of fasting and 2-h p.c. glucose, and insulin. Independent variables were *ADRB3* codon 64 genotype, age, sex, and NIDDM status for obesity-related traits, with BMI included for fasting and 2-h p.c. glucose and insulin.

RESULTS

Baseline phenotypes in whole sample

Sufficient DNA and phenotypic information were obtained from 508 adult subjects (57% women). A total of 115 subjects had newly or previously diagnosed NIDDM. The mean ± SD age, BMI, PBF, plasma fasting and 2-h p.c. glucose, and insulin were, respectively, 35.0 ± 13.5 years, 28.1 ± 5.26 kg/m^2, 37.3 ± 12.0%, 6.80 ± 3.46 mmol/l, 7.29 ± 4.56 mmol/l, and 131.7 ± 110.3 U/l. Forty subjects took medication for hypertension; almost all of these took ACE inhibitors.

Allele and genotype frequencies

The observed frequency of the *ADRB3* R64 allele was 0.40, which was more than three times higher than that reported in Caucasians and slightly higher than that reported in Japanese study samples (1,3–5). The observed genotype frequencies did not deviate from those predicted by the Hardy-Weinberg equation. There was no difference in the allele frequency of R64 in NIDDM and non-NIDDM subjects (Table 1). There was no difference in the frequency of R64/R64 homozygotes in NIDDM and non-NIDDM subjects (Table 1).

Genetic determinants of variation in BMI, WHR, and PBF

The results of the ANOVA are shown in Table 2. Because ANOVA takes multiple comparisons into account, we did not adjust the levels of nominal significance. For BMI, WHR, and PBF, there were significant associations with age, sex, and NIDDM status but no significant associations with *ADRB3* genotype (Table 2). For plasma concentrations of fasting and 2-h p.c. glucose and fasting insulin, there were significant associations observed with age, sex, BMI, and NIDDM status but no significant associations with *ADRB3* genotype (Table 2).

CONCLUSIONS — In this study of aboriginal Canadians, we found that *1*) the *ADRB3* R64 allele frequency was 0.40 in this population, which is the highest yet observed in a human population; 2) 15% of subjects were R64/R64 homozygotes, compared with a very low prevalence of homozygotes in European study samples; and 3) there was no statistically significant association of the *ADRB3* Y64R genotype either with the presence of NIDDM, with indexes of obesity, or with intermediate quantitative biochemical traits related to NIDDM. Thus, despite the very high frequency of the *ADRB3* R64 allele in this sample of aboriginal people, it was not associated with any metabolic phenotype. This suggests that the *ADRB3* R64 allele is probably not a major determinant of obesity or NIDDM in these aboriginal Canadians.

Table 2—*Summary of association analyses of* ADBR3 *Y64R with quantitative metabolic phenotypes in Sandy Lake*

	BMI	WHR	PBF	Plasma glucose		Plasma insulin
				Fasting	2-h p.c.	
Age	<0.0001	<0.0001	<0.0001	0.013	NS (0.65)	<0.0001
Sex	<0.0001	0.0042	<0.0001	0.0005	0.0014	0.039
BMI	—	—	—	<0.0001	<0.0001	<0.0001
NIDDM status	<0.0001	0.0027	0.0002	<0.0001	<0.0001	<0.0001
ADBR3 genotype	NS (0.26)	NS (0.69)	NS (0.14)	NS (0.41)	NS (0.36)	NS (0.98)

The product of the *ADRB3* gene is one of three β-adrenergic receptor subtypes, which have been shown to affect adipocyte lipolysis and oxygen consumption (32). All three belong to the R7G family of receptors coupled to G-proteins, and all are characterized by an extracellular glycosylated NH_2-terminal and an intracellular COOH-terminal region and seven transmembrane domains, linked by three extracellular and three intracellular loops (33). In contrast to the β_1 and β_2 subtypes, the β_3 subtype is resistant to short-term desensitization mediated by phosphorylation (33). Also, dexamethasone and insulin upregulate expression of β_1 and β_2 subtypes, while they downregulate expression of β_3 (33). Such findings suggest a specific physiological role for the β_3 subtype in different tissues or at different stages of development (33).

During the past 2 years, the *ADRB3* R64 allele has been heavily investigated for its potential association with obesity, NIDDM, and related intermediate traits. These studies began in earnest after the positive associations reported in three extremely prominent early publications (3–5). Enthusiasm for a possible physiological role for this common variant has subsequently been dampened by the more recent publication of numerous negative associations (1,34–37). A thorough review of this topic concluded, perhaps somewhat hyperbolically, that, despite the early suggestions of significance, the evidence that this variant was associated with any of these traits was, at best, inconsistent (1). Our findings in the Oji-Cree would be consistent with this conclusion. Alternatively, it remains possible that the physiological impact of the *ADRB3* R64 allele varies in different populations, and, thus, it may not be surprising that the results observed in Caucasians may differ from those seen in aboriginal populations, such as the Oji-Cree. Finally, it remains possible that there may be associations with other intermediate phenotypes that we did not measure, such as resting metabolic rate, weight gain, insulin sensitivity, or age at diagnosis of NIDDM.

The initial positive reports might have represented publication bias of positive association results, a pattern that has been observed with reports of genetic associations with other phenotypes (38). This tendency to publish positive results sooner than negative ones may explain the ongoing appearance of newer studies reporting positive associations between *ADRB3* R64 and phenotypes that are even more remote from intermediary metabolism. For example, association of *ADRB3* R64 with the more remote phenotype of diabetic proliferative retinopathy has recently been reported (39), concurrent with the publication of a preponderance of negative associations between *ADRB3* R64 and more proximal phenotypes, such as the presence and severity of NIDDM and related intermediate traits (34–37).

The recent demonstration of the absence of a functional impact of the *ADRB3* R64 (40) adds ballast to the evolving impression that this common variation is not an important determinant of human phenotypes. Furthermore, the absence of point mutations or rearrangements in the *ADRB3* coding sequence from subjects with rare disorders, such as lipoatrophic diabetes (41), also supports the notion that structural or coding sequence variation in this gene may not be important in human pathophysiology. However, the results to date do not rule out a possible role for variation in regulation of expression of this gene as a potentially important determinant of human phenotypes. Thus, our results suggest the absence of an effect of the *ADRB3* R64 variant, or indeed other variants, on a chromosomal haplotype marked by this allele in the Oji-Cree on phenotypes such as NIDDM and obesity. Such an interpretation is consistent with the aggregate of experimental data to date.

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