Brief Genetics Report

A Ser311Cys Mutation in the Human Dopamine Receptor D2 Gene Is Associated With Reduced Energy Expenditure

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Brain dopaminergic pathways play a major role in the control of movement. Absence of the murine dopamine D2 receptor gene (drd2) produces bradykinesia and hypothermia. A Ser311Cys mutation of the human DRD2 produces a marked functional impairment of the receptor and is associated with higher BMI in some populations. We hypothesized that the Ser311Cys mutation of DRD2 may inhibit energy expenditure. Here we report that total energy expenditure (doubly labeled water) measured in 89 nondiabetic Pima Indians was 244 kcal/day lower in homozygotes for the Cys311-encoding allele when compared with those heterozygous and homozygous for the Ser311-encoding allele (P = 0.056). The 24-h resting energy expenditure (respiratory chamber) measured in 320 nondiabetic Pimas was also 87 kcal/day lower in homozygotes for the Cys311-encoding allele when compared with those heterozygous and homozygous for the Ser311-encoding allele (P = 0.026). These findings are the first evidence that a genetic mutation is associated with reduced energy expenditure in humans. Because the impact of this mutation on human obesity is small, we suggest that either the energy deficit induced is not large enough to significantly influence body weight in this population and/or that the Cys311-encoding allele is also associated with reduced energy intake. *Diabetes* 50:901–904, 2001

The human DRD2 gene has been mapped to a locus on human chromosome 11q23 (1). Three missense substitutions (Val96Ala, Pro310Ser, and Ser311Cys) have previously been identified in this receptor protein (2). The prevalence of the Ser311Cys receptor variant differs greatly across human populations. The prevalence of the Cys^{311} allele is 16% in Pima Indians, 3% in Caucasians, and 2.3% in the Japanese population (3–5). The Cys^{311} variant of the human DRD2 has been shown to markedly impair the ability of the receptor to modulate adenylyl cyclase activity (6). Studies in genetically heterogeneous human populations have shown weak associations between DRD2 variants and obesity (7–11).We have previously observed that the location of DRD2 on chromosome 11q23–24 is near the peak of linkage of a locus known to influence type 2 diabetes, obesity, and energy expenditure in Pima Indians (12,13) and that individuals with a Cys311-encoding allele have a higher BMI than those homozygous for the Ser311-encoding allele (14). Therefore, the aim of the present study was to assess whether the Cys^{311} variant of DRD2, a plausible candidate gene for obesity, promotes body weight gain by inhibiting energy expenditure in humans.

We measured total energy expenditure (TEE) using doubly labeled water in 89 nondiabetic Pima Indians (Table 1). Energy expenditure due to physical activity (EEACT) was measured in 53 of the 89 individuals. After adjusting for age, sex, and body composition, there was an overall tendency for the genotype to contribute to the interindividual variability of TEE ($P = 0.11$, df = 2), but not EEACT ($P = 0.49$, df = 2). In post hoc analyses, individuals who were homozygous for the Cys311-encoding allele had significantly lower mean TEE (−244 kcal/day; $P = 0.056$) than the other two genotypes combined (Fig. 1, upper panel). After adjusting for age, sex, and body composition, differences in mean EEACT were in the same direction; the homozygotes for the Cys311-encoding allele had a 184 kcal/day deficit compared with the other two genotypes, but the difference did not reach statistical significance ($P = 0.293$).

We also measured 24-h energy expenditure (24-h EE), sleeping metabolic rate (SMR), and spontaneous physical activity (SPA) using a respiratory chamber in 320 nondiabetic Pima Indians (Table 2). After adjusting for age, sex, and body composition, there was an overall tendency for the genotype to contribute to the interindividual variability of 24-h EE ($P = 0.07$, df = 2) and SMR ($P = 0.11$, df = 2), but not SPA ($P = 0.88$, df = 2). In post hoc analyses, individuals who were homozygous for the Cys311-encoding allele had a lower mean 24-EE (−87 kcal/day, $P = 0.026$) and SMR (−77 kcal/day, $P = 0.040$) than the other two genotypes combined (Figs. 1*B* and *C*). After adjusting for age, sex, and body composition, SPA was not different

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24-h EE, 24-h energy expenditure; EEACT, energy expenditure due to physical activity; SMR, sleeping metabolic rate; SNP, single nucleotide polymorphism; SPA, spontaneous physical activity; TEE, total energy expenditure.

TABLE 1
Physical characteristics of 89 nondiabetic Pima Indians with Ser311- or Cys311-encoding DRD2 alleles in whom we measured TEE

	Ser/Ser	Ser/Cys	Cys/Cys
n	58	25	6
Sex (M/F)	43/15	15/10	5/1
Age (years)	35 ± 12	34 ± 11	34 ± 10
Height (cm)	167 ± 6	167 ± 7	173 ± 8 *
Weight (kg)	89 ± 19	91 ± 16	97 ± 19
Body fat (%)	34 ± 10	37 ± 9	35 ± 6

Data are *n* or means ± SD. Sex distribution across genotypes (χ^2 = 2.18, P = 0.33, df = 2). *After adjustment for age and sex, Cys311 homozygotes were taller than the two other genotypes combined (P = 0.012). Body weight and percent body fat did not differ between genotypes, after adjusting for sex and age.

in individuals who were homozygous for the Cys311-encoding allele compared with the other two genotypes combined (7.1 ± 1.6 vs. 7.3 ± 2.1%, P = 0.72). Results were unaffected when adjusted for family membership.

These data indicate that, in humans, the Ser311Cys mutation, which is known to functionally impair DRD2, affects resting energy expenditure but not energy expenditure related to physical activity. This lack of effect on physical activity is somewhat surprising, considering that a reduction of spontaneous movement represents the main feature of both pharmacological and molecular inhibition of DRD2 (15,16). Because some homozygous drd2-deficient mice are hypothermic compared with their littermates (16), it is possible that the effect of Ser311Cys on resting energy metabolism is related to the role of DRD2 in the regulation of body temperature (17). However, we did not find an association between the Ser311Cys mutation and oral temperature in this group of Pima Indians (data not shown).

We have recently reported that among Pima Indians, individuals with a Cys311-encoding allele have a higher BMI than those homozygous for the Ser311-encoding allele (14). However, the Ser311Cys mutation did not account for the linkage to BMI on chromosome 11 (14). Furthermore, in the smaller group of Pima Indians in the present study, we did not find differences in weight and percentage of body fat between genotypes (Tables 1 and 2). The limited effect of the Ser311Cys mutation on body weight and composition indicates that the energy deficit induced by this mutation may not be large enough to significantly affect the development of obesity in adult Pima Indians. In a previous prospective study, however, we found that a difference in resting energy expenditure of 70 kcal/day was associated with differences in subsequent weight gain in Pima Indians (18). Thus, it is possible that the Ser311Cys mutation has concomitant inhibitory effects on energy intake in this population. Dopamine release in the brain is associated with pleasure and reward. Dopamine is required for hyperphagia in *ob/ob* mice (19) and dopamine-deficient mice gradually become aphagic and die of starvation (20). A reduction of 10–15% in water and food intake was reported in drd2-deficient mice (16). Studies to carefully characterize the eating habits of individuals who carry the Ser311Cys mutation are warranted. Neuroimaging techniques, such as positron emission tomography and functional magnetic resonance imaging have recently been employed to describe the neuroanatomical correlates of

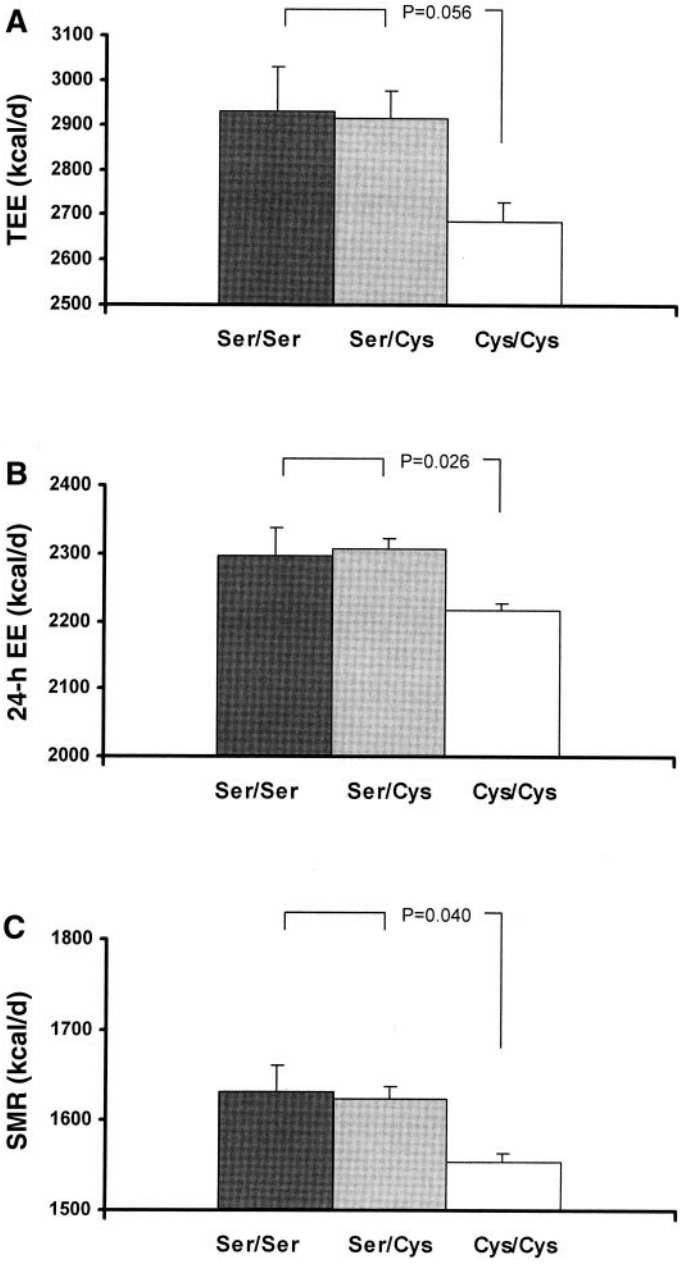

FIG. 1. Energy expenditure and SPA in nondiabetic Pima Indians with Ser311- or Cys311-encoding DRD2 alleles. TEE (*A*) was measured by doubly labeled water in 89 individuals (58 Ser/Ser, 25 Ser/Cys, and 6 Cys/Cys) and was adjusted for age, sex, and body composition. The 24-h EE (*B*) and SMR (*C*) were measured using a respiratory chamber in 320 individuals (217 Ser/Ser, 87 Ser/Cys, and 16 Cys/Cys) and were adjusted for age, sex, and body composition. The means ± SE are given for subjects homozygous for the Ser311 allele (■), heterozygous for the Ser311Cys alleles (▨), and homozygous for the Cys^{311} allele (□). Levels of significance refer to comparisons between Cys^{311} homozygous subjects and subjects homozygous or heterozygous for the Ser^{311} allele.

eating-related stimuli in humans (21,22). These techniques might be used in the future to further clarify the role, if any, of DRD2 in the neurophysiology of eating behavior in humans.

The function of dopamine in the central control of movement is paralleled by its neuroendocrine regulation of the pituitary gland. DRD2 is abundantly expressed in the anterior pituitary gland, where it mediates the inhibitory control of dopamine over synthesis and the release of

TABLE 2
Physical characteristics of 320 nondiabetic Pima Indians with Ser311- or Cys311-encoding DRD2 alleles in whom we measured resting energy expenditure

	Ser/Ser	Ser/Cys	Cys/Cys
n	217	87	16
Sex (M/F)	145/72	58/29	5/11
Age (years)	29 ± 9	28 ± 8	25 ± 4
Height (cm)	167 ± 8	167 ± 7	161 ± 10
Weight (kg)	91 ± 21	89 ± 19	87 ± 18
Body fat (%)	35 ± 10	36 ± 10	40 ± 6

Data are *n* or means ± SD. Sex distribution across genotypes (χ^2 = 8.37, P = 0.01, df = 2). Height, body weight, and percent body fat did not differ between genotypes, after adjusting for sex and age.

prolactin and POMC in the adenohypophysis (23). Mice lacking drd2 are hyperprolactinemic and show a time-dependent proliferation of lactotrophs, ultimately leading to pituitary tumors, hypoestrogenism, and infertility, especially in older animals (23). We measured the fasting plasma prolactin concentration in a group of 126 nondiabetic Pima Indians (83 male and 43 female subjects, 107 Ser/Ser and 19 Cys/Cys) who were homozygous for either the Ser311- or Cys311-encoding DRD2 allele. Prolactin was not related to age or body size. No sex differences were observed. The mean fasting plasma concentration was not different between genotypes (11.1 ± 8.9 and 12.7 ± 15.9 ng/ml in Ser/Ser and Cys/Cys, respectively; P = 0.43). In addition, a review of the available medical records did not reveal any information suggestive of pituitary-gonadal axis impairment in individuals with the Ser311Cys mutation (i.e., they had unremarkable reproductive histories and no evidence of pituitary adenomas). Our study could not test whether a minimal DRD2 function is sufficient to maintain normal prolactinemia or whether the effect of the Ser311Cys mutation on the pituitary-gonadal axis becomes manifest at a much older age in humans.

In conclusion, the Ser311Cys mutation of the human DRD2 has a significant inhibitory effect on energy expenditure, but it does not induce hyperprolactinemia. The limited impact of Ser311Cys on body weight suggests an inhibitory effect of this mutation on energy intake. Further studies are warranted to test this hypothesis.

RESEARCH DESIGN AND METHODS

Subjects for the present analysis were selected from among the participants in a genome-wide linkage study for loci influencing obesity and type 2 diabetes (12) who had been admitted to the metabolic ward for measurement of total energy expenditure (n = 89, Table 1) and resting energy expenditure (n = 320, Table 2). Concomitant measurements of TEE and 24-h EE during the same admission were available in 53 individuals. Body composition was determined by underwater weighing or dual energy X-ray absorptiometry (24). The institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community approved these studies, and subjects gave informed consent.

Energy expenditure. TEE was measured using $^2H_2{}^{18}O$, as previously described (25). Briefly, after an overnight fast, subjects were given a water dose of 0.126 g ^2H_2O and 2.39 g $H_2{}^{18}O$/kg total body water. Timed urine collections were obtained immediately after dosing and 7 days later, when the subjects had resumed their normal free-living activities. Deuterium (^2H) and ^{18}O enrichments were determined on a Finnigan MAT-251 and Delta-S isotope mass spectrometers (Thermoquest, San Jose, CA). Because the composition of the diet was not known, TEE was calculated using CO_2 production derived from the isotopic decay rates and energy equivalent of CO_2 at an arbitrary respiratory quotient of 0.866. EEACT was calculated from the difference between TEE and SMR plus 10% of TEE to take into account the estimate of the thermic effect of food. Resting energy expenditure was measured using the respiratory chamber as previously described (26). Briefly, volunteers entered the chamber at 7:45 A.M. after an overnight fast and remained in it until 7:00 A.M. the following morning. Subjects were fed a standardized diet (three meals and an evening snack) with the amount of calories calculated to achieve energy balance according to previously determined equations. The rate of energy expenditure was measured continuously, calculated for each 15-min interval of the 23 h in the chamber, and then extrapolated to 24 h (24-h EE). SPA was detected by radar sensors and expressed as the percentage of time over the 24-h period in which activity was detected. The SMR was calculated as the metabolic rate measured between 11:00 P.M. and 5:00 A.M., when the 15-min activity by radar was <1.5%.

Genotyping. Genotyping was done by Taqman allelic discrimination polymerase chain reaction using a model 7700 ABI Prism instrument (PE Applied Biosystems, Foster City, CA). The Ser311Cys polymorphism was typed as previously described (14). Two double-probe sets were used, at equal concentrations, to suppress a rare second His313His silent single nucleotide polymorphism (SNP) present just seven nucleotides downstream. In some cases the SNP was independently confirmed by direct DNA sequencing. We have previously sequenced the entire coding region, the intron/exon boundaries, the 3′ untranslated region, and 200 bp of the promoter for DRD2 in DNA samples from 20 Pima Indians. Eight unique SNPs and one STR marker were identified and genotyped in a group of 200 Pima Indians. One SNP in the promoter, which is positioned 250 kb 5′ to the coding region, showed no association with any phenotype. One SNP in the 3′ untranslated region and the Ser311Cys SNP were significantly associated with BMI. Therefore, these two SNPs were selected for further analysis (14). For the current study of energy expenditure, we focused on the Ser311Cys polymorphism because it has known functional consequences.

Analytical measurements. Fasting plasma prolactin concentration was measured using a commercially available radioimmunoassay in 126 nondiabetic Pima Indians who were homozygous for either the Ser311- or Cys311-encoding DRD2 allele. For each individual, measurements were obtained from the most recently stored (frozen at −20°C) plasma sample.

Statistical analysis. All analyses were performed using the procedures of the SAS software (SAS Institute, Cary, NC). The overall effect of genotype on energy expenditure was assessed by general linear regression analyses. In these analyses, energy expenditure was the dependent variable, whereas age, sex, body composition, and genotype (Ser/Ser, Ser/Cys, and Cys/Cys) were the independent variables. Because heterozygotes appeared to be phenotypically similar to subjects homozygous for the DRD2 Ser^{311} allele, the data from these subjects were pooled for statistical comparison with the DRD2 Cys^{311} homozygotes. The comparison between these groups was performed using the same linear regression analyses as described above, but this time a df of 1 was imposed for the genotype. Adjustment for family membership was performed using the GENMOD procedure of SAS. Unless indicated otherwise, results are presented as means ± SD.

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