Brief Genetics Report

Association Between Common Polymorphisms of the Proopiomelanocortin Gene and Body Fat Distribution

A Family Study

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Rare mutations in the proopiomelanocortin (*POMC*) gene cause severe early-onset childhood obesity. However, it is unknown whether common variants in *POMC* are responsible for variation in body weight or fat distribution within the commonly observed range in the population. We tested for association between three polymorphisms spanning the *POMC* gene and obesity phenotypes in 1,428 members of 248 families. There was significant association between genotypes at the C8246T ($P < 0.0001$) and C1032G ($P = 0.003$) polymorphisms and waist-to-hip ratio (WHR) corrected for age, sex, smoking, exercise, and alcohol consumption. Each T allele at C8246T (or G allele at C1032G) was associated with a 0.2-SD–higher WHR in a codominant fashion. When WHR was additionally corrected for BMI, thus providing a measure of body fat distribution throughout the range of BMI, there remained significant evidence for association with both markers that was of similar magnitude and statistical significance. There was no association between genotype at any polymorphism and BMI or plasma leptin level. These data show that genetic variants at the *POMC* locus influence body fat distribution within the normal range, suggesting a novel role for *POMC* in metabolic regulation. *Diabetes* 54: 2492–2496, 2005

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ACTH, adrenocorticotrophic hormone; CHD, coronary heart disease; POMC, proopiomelanocortin; WHR, waist-to-hip ratio.

Abdominal obesity is a major risk factor for type 2 diabetes, coronary heart disease (CHD), colon cancer, and several other common and serious conditions. Although the definition of obesity typically relies on BMI, several studies suggest that, with respect to risk stratification, measurement of the waist-to-hip ratio (WHR) may be a superior predictor both of type 2 diabetes and CHD, possibly because of specific metabolic abnormalities accompanying central, rather than generalized, obesity (1,2). Both WHR and BMI are heritable traits, and various studies suggest that genes account for 25–70% of the observed variability (3,4).

Melanocortin signaling in the hypothalamus plays a central role in the control of energy homeostasis. The proopiomelanocortin (*POMC*) gene is expressed in response to leptin signaling by neurons of the hypothalamic arcuate nucleus. Intracellular posttranslational processing of the POMC propeptide by prohormone convertase 2 leads to the production in these neurons of α-, β-, and γ-melanocyte–stimulating hormones. These peptides signal to downstream target neurons in the lateral hypothalamus that express the melanocortin receptors MC3R and MC4R with resultant decrease in food intake and increase in energy expenditure (5). Rare mutations in the *POMC* gene cause monogenic, severe, early-onset obesity in humans; however, the influence of common polymorphisms in *POMC* on obesity phenotypes in less extreme individuals is unclear (6). *POMC* is also a precursor of adrenocorticotrophic hormone (ACTH); pathological ACTH excess results in severe central obesity. We have investigated the role of common polymorphisms of the *POMC* gene on abdominal obesity in a large family study.

RESEARCH DESIGN AND METHODS

Subject collection and phenotyping. The collection strategy of this family study has been previously described (7). Briefly, families were ascertained through a proband with essential hypertension. To be suitable for the study, families were required to consist of at least three siblings clinically assessable for blood pressure if at least one parent of the sibship was available to give blood for DNA analysis and to consist of at least four assessable siblings if no parent was available. Where members of the sibship were found to be hypertensive, families were extended and the spouses and offspring of hypertensive sibs collected. Thus, the majority of the individuals in the family collection have blood pressure values within the normal range, and the family

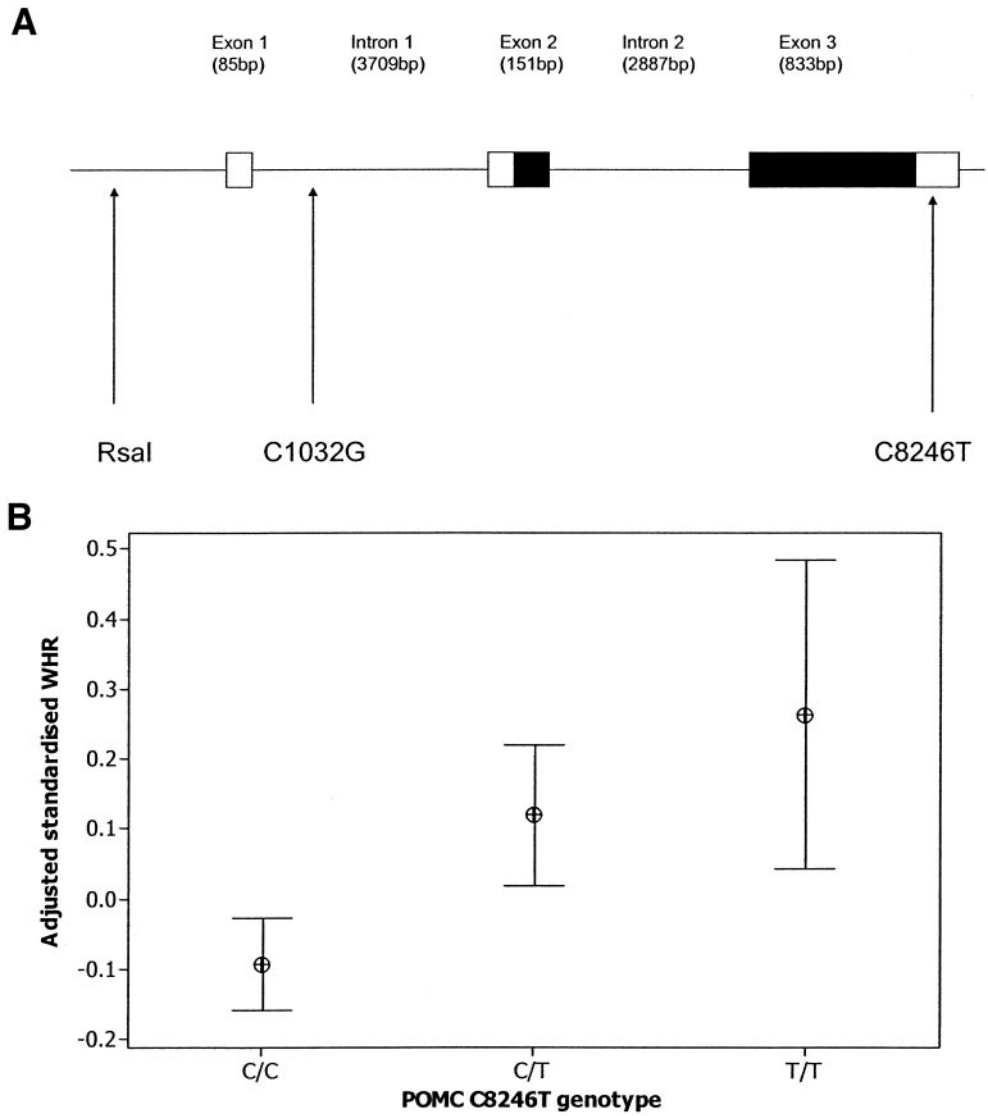

FIG. 1. *A*: Schematic representation of *POMC*. Exons are shown by boxes (□, untranslated regions; ■, coding regions). Vertical arrows indicate the positions of the typed polymorphisms. *B*: Association between genotypes at the *POMC* C8246T polymorphism and WHR. WHR was adjusted for age, sex, smoking, alcohol consumption, and physical exercise. Error bars represent 95% CIs for means.

collection includes some extended families, though most are nuclear families. A full medical and lifestyle history was taken. Anthropometric measurements including height, weight, and waist and hip circumferences were made (waist measured at the natural waist, and hip measured at the level of the greater trochanters). A total of 1,428 individuals from 248 families participated in the study.

Laboratory methods. Plasma leptin was measured using a commercially available enzyme-linked immunosorbent assay kit (Linco Research, St. Charles, MO) with sensitivity 0.5 ng/ml, intra-assay precision 2.6–4.6%, and interassay precision 2.6–6.2%. Plasma cortisol and 24-h urinary excretion rates of cortisol metabolites were measured as previously described (8). We used the sum of urinary tetrahydrocortisol, allo(tetrahydrodeoxycortisol), and tetrahydrodeoxycortisol as a measure of total cortisol production.

The *POMC* gene occupies a short genomic segment of ~7.5 kb on chromosome 2p24. No common polymorphisms exist within the coding sequence of the gene in Caucasians. We therefore studied three common noncoding polymorphisms, which spanned the gene (Fig. 1*A*), by PCR, restriction enzyme digestion, and agarose gel electrophoresis. The *Rsa*I polymorphism 1798 bp 5′ to the beginning of exon 1 (representing a C/T substitution, dbSNP ID rs3754860) was genotyped using primers 5′GTAACT TCAGGAGGCTGCTGG3′ and 5′CCAGTGATTCTAAATGCAGTTC3′, annealing temperature 52°C and 0.5 mmol/l $MgCl_2$, and restriction digestion with *Rsa*I. A C/G polymorphism within the first intron (referred to here as C1032G, dbSNP ID rs1009388) was typed using primers 5′CTCGACAAACTTTCTGCGC3′ and 5′CGGTGAGCAGAGATCACC3′, annealing temperature 69°C and 1.5 mmol/l $MgCl_2$, and restriction enzyme *Ava*I. The C8246T polymorphism in the 3′ untranslated region (referred to in some previous studies as C7566T, dbSNP ID rs1042571) was genotyped using primers 5′AGCCCCGCAGCGATGGT3′ and 5′CCTTTCACGCTCACTAAGTCCTGTG3′, annealing temperature 63°C and 1.5 mmol/l $MgCl_2$, and restriction enzyme *Mbo*II. Reference individuals of known genotype were included in each run.

Statistical analysis. Mendelian inheritance of all genotypes was checked using PedCheck (9). Possible genotyping errors not producing Mendelian inconsistencies were screened for by confirming the correspondence of genotype frequencies to Hardy-Weinberg proportions in the genotyped founders (n = 269–275 for the different polymorphisms) and by examining the families for recombination in this short genomic segment using MERLIN (10). The estimated genotyping error rate was <1%. Disequilibrium coefficients and haplotype frequencies were estimated in the founders using FUGUE (11). Phenotypes of interest were examined for normality and log transformed in the case of BMI and plasma leptin. Significant covariates of these phenotypes were then determined by linear regression using MINITAB software; the adjusted values from these regressions were standardized (so each phenotype has a mean of 0 and an SD of 1) and used in the genetic analyses. Association between genotypes and the adjusted phenotypes was assessed in the families by calculating identity-by-descent vectors for each individual using MERLIN followed by variance components analysis using the QTDT (quantitative transmission disequilibrium test) program (12). Empirical significance levels were determined by Monte Carlo permutation of 5,000 replicates (13). A three-locus measured genotype analysis was also carried out using the

TABLE 1
Characteristics of study population

Variable	*n*	Min	Lower quartile	Median	Upper quartile	Max	R^{2}*
Age (years)	1,425†	18.7	35.7	50.9	60.9	90.7	—
BMI (kg/m^2)	1,402	16.7	23.1	25.4	28.2	51.8	15.2
WHR	1,357	0.56	0.78	0.85	0.91	1.22	48.7
Plasma leptin (ng/μl)	1,319	1.1	4.6	8.6	15.3	116.6	46.4

*After correction for age, sex, alcohol consumption, smoking behavior, and exercise habit. †52.4% were female, and 36.1% were classified as hypertensive.

Pedigree Analysis Package (PAP version 5.0, available at http://hasstedt.genetics.utah.edu/pap5) in which multilocus additive effects (i.e., no gene interactions) were fitted. Linkage disequilibrium was allowed in the exact likelihood calculations as well as residual intrafamilial correlations in a class D regressive model. A hierarchical analysis was performed to evaluate the fit of nested models.

RESULTS

Characteristics of the study subjects are shown in Table 1. Sixty percent of families comprised between four and six genotyped members. Median values for BMI, WHR, and plasma leptin lie within the normal range for an unselected U.K. Caucasian population. Correction for age, sex, alcohol consumption, smoking, and habitual exercise accounted for ~15% of the total variability in log BMI and 46–49% of the total variability in WHR and log plasma leptin level. The heritability of the phenotypes after correction was 35% for log BMI, 28% for WHR, and 36% for log plasma leptin (all $P < 0.0001$).

Genotyping was successful for >95% of all subjects for all polymorphisms. Genotype frequencies at all polymorphisms satisfied Hardy-Weinberg equilibrium (Table 2). Linkage disequilibrium was, as expected, strong in this small genomic region; D′ values were 0.868 (between C8246T and C1032G), 0.964 (between C8246T and *Rsa*I), and 0.933 (between C1032G and *Rsa*I), respectively. There were four common haplotypes of the three polymorphisms (online appendix Table 1 [available at http://diabetes.diabetesjournals.org])

There was evidence for significant association between genotypes at the C8246T polymorphism and WHR (0.197 ± 0.047 [means ± SE] higher-adjusted standardized WHR per T allele, $P < 0.0001$) and between genotypes at the C1032G polymorphism and WHR (0.133 ± 0.042 higher-adjusted standardized WHR per G allele, $P = 0.002$) (Table 3). There was no significant association between genotypes at the *Rsa*I polymorphism and WHR ($P = 0.145$) (Table 3). There was also no significant association between any polymorphism and BMI, plasma leptin level, plasma cortisol, or urinary cortisol metabolite excretion (data available on request). To investigate more specifically the effect of *POMC* polymorphisms on fat distribution, we additionally adjusted the WHR phenotype for log BMI. Log BMI was responsible for an additional 12.5% of the variability in adjusted WHR ($P < 0.0001$). Following adjustment for log BMI, there remained significant association between genotypes at the C8246T polymorphism and WHR (0.184 ± 0.044 [means ± SE] higher-adjusted standardized WHR per T allele, $P < 0.0001$) and between genotypes at the C1032G polymorphism and WHR (0.119 ± 0.040 higher-adjusted standardized WHR per G allele, $P = 0.003$). There was no association between genotypes at the *Rsa*I polymorphism and WHR ($P = 0.292$) (Table 3). These associations remained significant at the $P < 0.01$ level after Monte Carlo simulation of 5,000 replicates, and significance (at $P < 0.05$) persisted after application of a Bonferroni correction for 12 analyses. There was no evidence of heterogeneity in the strength of the association between men and women ($F_{1,1,315} = 2.043$, $P = 0.153$). Measured genotype analysis using the Pedigree Analysis Package showed no support for additional genetic effects over the C8246T marker on the WHR phenotype ($P > 0.45$) and no evidence for dominance effects encoded by C8246T ($P = 0.44$). Models robust to stratification fitted in the QTDT program (that is, incorporating transmission from heterozygous parents only) confirmed support for the association at C8246T ($P = 0.026$) and C1032G ($P = 0.0127$). The proportion of the total variance in the WHR phenotype associated with the C8246T polymorphism (narrow-sense heritability) was estimated to be 1.1% (95% CI 0.2–2.7) (Fig. 1*B*). Post hoc power calculations indicated that the cohort had >90% power to detect a 0.2 SD difference between genotypes at either the C8246T or C1032G polymorphisms.

DISCUSSION

Patients with a complete deficiency of POMC due to homozygous or compound heterozygous loss-of-function mutations exhibit a characteristic syndrome marked by childhood-onset severe obesity, red hair, and hypocortisolism (14). With respect to nonsyndromic obesity, four mutation screening studies involving 601 nonsyndromic obese subjects have shown missense or nonsense mutations in just 7 individuals, implying that coding sequence variation in *POMC* is a very uncommon cause of nonsyndromic obesity (15–18). Two genome-screening studies, in French obese sibling pairs and in Mexican-American ex-

TABLE 2
Genotype and allele frequencies at polymorphisms typed

Polymorphism	Genotype	*n*	Allele frequency	Heterozygosity (%)
RsaI	+/+	705	$P_+ = 0.713$	39.4
	+/−	538	$P_- = 0.287$	
	−/−	123		
	All	1,366		
C1032G	C/C	639	$P_c = 0.684$	43.1
	C/G	588	$P_g = 0.316$	
	G/G	138		
	All	1,365		
C8246T	C/C	861	$P_c = 0.787$	33.5
	C/T	465	$P_t = 0.213$	
	T/T	64		
	All	1,390		

TABLE 3
Association between *POMC* polymorphisms and WHR

Polymorphism	Genotype	*n*	Adjusted for age, sex, smoking, alcohol, and exercise			Adjusted for age, sex, smoking, alcohol, exercise, and BMI		
			Mean	SE	*P* value for trend	Mean	SE	*P* value for trend
RsaI	+/+	670	0.026	0.039	0.145	0.012	0.035	0.262
	+/−	504	−0.021	0.046		−0.047	0.045	
	−/−	120	−0.117	0.078		−0.0114	0.077	
C1032G	C/C	608	−0.084	0.038	0.003	−0.093	0.036	0.003
	C/G	555	0.053	0.046		−0.001	0.043	
	G/G	132	0.175	0.085		0.175	0.081	
C8246T	C/C	817	−0.093	0.033	<0.0001	−0.110	0.032	<0.0001
	C/T	440	0.119	0.051		0.064	0.046	
	T/T	62	0.263	0.111		0.283	0.107	

tended families, suggested strong evidence for linkage of plasma leptin levels to the region of chromosome 2 harboring the *POMC* gene (19,20). Association between haplotypes of the *Rsa*I and C8246T polymorphisms and plasma leptin levels (though not BMI or WHR) was subsequently shown in the Mexican-American cohort, with higher plasma leptin observed in the haplotype comprising a present *Rsa*I restriction site (+ in this study) and a T allele at C8246T (21). Although we found no association between any genotype and plasma leptin, the same T allele at C8246T is associated with higher WHR in the present study. No association between *POMC* polymorphisms and either plasma leptin or BMI was found in the French cohort (22). No previous study has reported either positive or negative findings with respect to common *POMC* polymorphisms and WHR.

The finding in the present study that *POMC* polymorphisms affect WHR even after adjustment for BMI suggests that the principal effect of these common polymorphisms is on susceptibility to central rather than generalized obesity. Since dysregulation of appetite and energy expenditure (known to be mediated via hypothalamic actions of POMC) might be expected to predispose to generalized obesity, our observations may reflect actions of POMC-derived peptides whose effects on obesity are extrahypothalamic. Posttranslational processing of POMC differs in different tissues and yields a large number of such peptides. For example, β-endorphin is produced in lymphocytes, macrophages, and the small intestine in addition to the central nervous system (23). Plasma concentrations of β-endorphin are higher in obese individuals even after successful weight loss when compared with nonobese control subjects, and gene-targeted mice that have normal melanocortin signaling but produce no β-endorphin are obese and hyperphagic (24). β-Lipotropin, another POMC-derived peptide, is an important promoter of lipolysis in adipocytes and is present in higher concentrations in the plasma of centrally obese subjects (25). Further research will be necessary to investigate whether the polymorphisms we have typed are correlated with plasma and tissue concentrations of these and other POMC-derived peptides. Although differences in ACTH production caused by *POMC* polymorphisms would be a strong candidate mechanism for an effect on central obesity, we found no association between *POMC* variants and steroid phenotypes in this study.

Previous studies that have examined the *POMC* gene and obesity phenotypes have in general enrolled participants with significantly higher BMIs and plasma leptin levels than the present study. Thus, one limitation of our study is that, since the majority of our study population has BMIs in the high normal rather than the obese range, it is not possible to rule out absolutely effects of common *POMC* variants on BMIs >30 kg/m^2 or on the highest end of the distribution of plasma leptin level. However, it seems unlikely that strong effects on these phenotypes are present within the range of BMI and plasma leptin present in our sample (which would encompass ~80% of the U.K. population). Furthermore, no previously reported study has found association between *POMC* polymorphisms and BMI, even in highly selected samples. Strengths of our study include its large size, the genotyping of three informative polymorphisms spanning *POMC* (thus likely capturing all common variation in this small gene), its family-based design, which circumvents concerns about population stratification, and its detailed ascertainment, which permits statistical correction of obesity phenotypes for important lifestyle confounders.

Central obesity may be a more powerful predictor of both CHD and type 2 diabetes risk than generalized obesity. These *POMC* polymorphisms would be suitable candidates for testing in large-scale case-control studies of genetic susceptibility to these diseases. Although their effect on abdominal obesity is relatively modest, they are common and thus could potentially contribute significantly to the population attributable risk.

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