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Polymorphism in the 3′ Untranslated Region of *MTG8* Is Associated with Obesity in Pima Indian Males

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Obesity has a genetic component and predisposes for the development of type 2 diabetes mellitus. One approach to identifying new candidate genes for obesity is to explore potential regulatory factors expressed in fat tissue that may play a role in adipocyte development or metabolic control. Because we found relatively abundant mRNA levels of the putative transcription factor *MTG8* in human adipose tissue, a polymorphic microsatellite marker in the 3′ untranslated region of this gene was genotyped in 281 Pima Indians, a population with one of the highest reported rates of obesity. We detected a male-specific association with age-adjusted percentage body fat ($p = 0.0002$), body mass index ($p = 0.01$), waist circumference ($p = 0.008$), and thigh circumference ($p = 0.02$). Comparative analysis of all 13 *MTG8* exons in 30 Pimas did not reveal any genetic variants which could explain the association with obesity in males.

Obesity is a major risk factor for the development of type 2 diabetes mellitus (1, 2). Degree of adiposity has been found to have a strong genetic basis (3, 4) and is most likely polygenic (5, 6). However, no major genes contributing to the common forms of obesity in humans have yet been described.

One approach to identifying candidate genes that contribute to obesity is the investigation of potential regulatory factors that are highly expressed in adipose tissue. Presumably, these proteins may be more likely to affect adipogenesis or adipocyte metabolism. Based upon such selection criteria, we have identified a novel candidate gene, *MTG8,* which is abundantly expressed in human adipose tissue and skeletal muscle (J.K. Wolford and M. Prochazka, in press). The human *MTG8* gene (*M*yeloid *T*ranslocation *G*ene on *8q22*) was first identified by molecular characterization of the t(8; 21) (q22; q22.3) translocation breakpoint commonly found in acute myeloid leukemias (7, 8). The normal product of the *MTG8* gene is considered a transcription factor that is localized predominantly in the nucleus and contains several sequence motifs characteristic of DNA-binding proteins (9). Although the physiological role of MTG8 is still unknown, high conservation between the human and mouse homologues (99% identity at the amino acid sequence level) suggests an important biological function (10, 11).

Previously, we determined that *MTG8* mRNA is expressed at relatively high levels in several human organs and tissues, including adipose tissue (J.K. Wolford and M. Prochazka, in press). Based on this finding we hypothesized that *MTG8* might be a suitable candidate gene for obesity. To address this question, we have investigated the relationship between a highly polymorphic marker in the 3′ untranslated region (3′UTR) of the *MTG8* gene and obesity in the Pima Indians of Arizona. The Pimas represent an ideal population in which to study the genetic basis of obesity, primarily because they have one of the highest reported rates of obesity (12). More importantly, because the Pimas are a geographically isolated and relatively homogeneous population, mutations in genes that contribute to obesity are more likely to be shared among obese members of this tribe, in contrast with other, more heterogeneous populations.

Here we report that a highly polymorphic microsatellite marker in the last exon of *MTG8* is associated with obesity in Pima Indian males, as determined by a distinct allele distribution relative to percent body fat (PFAT) in this sample. To elucidate potential mechanisms by which *MTG8* could affect adiposity, we have also performed comparative sequencing of all 13 exons among selected subjects characterized by low or high PFAT associated with specific 3′UTR genotypes.

SUBJECTS AND METHODS

Subjects. Our study was comprised of genomic DNA samples prepared as previously described (13) from 281 non-diabetic, full-blooded

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Pima Indians (148 males, 133 females) who were not first-degree relatives and 54 unrelated Centre d'Etude Polymorphisme Humain (CEPH) Caucasian individuals obtained from the Human Genetic Mutant Cell Repository (Coriell Institute; Camden, NJ). Pima subjects selected for analysis of the *MTG8* 3′UTR microsatellite marker are participants in an ongoing longitudinal study of diabetes and obesity conducted among members of the Gila River Indian Community since 1965 (2). Parameters of obesity (including PFAT, body mass index, and waist/thigh circumference), insulin action, and insulin secretion were determined as described (2, 14).

Typing of the MTG8 3′UTR microsatellite marker. Amplification of the *MTG8* 3′UTR dinucleotide repeat (nucleotide positions +2872 to +2970) was performed using 1 pmol ^{32}P-labeled primer MTG8-CA3 (5′-TTGCAATGGGATGTATGAATAC) and 1 pmol unlabeled primer MTG8-CA4 (5′-TGTCAAAACTGCCAGAATGCT) in a 5 μl final reaction volume. Reaction components consisted of 50 ng genomic DNA, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM of each dNTP, and 0.025 U AmpliTaq DNA polymerase (Perkin Elmer; Norwalk, CT) and 5.5 ng TaqStart antibody (Clontech Laboratories; Palo Alto, CA). Samples were initially denatured at 96°C for 3 min, followed by 25 cycles of 20 sec at 96°C, 30 sec at 57°C, and 1 min at 72°C. After a final 5 min extension at 72°C, the labeled products were separated on a 6% sequencing gel and visualized by autoradiography. All PCR amplifications were performed using GeneAmp PCR System 9600 (Perkin Elmer).

DNA sequencing. Genomic DNA samples from 30 Pimas, selected to represent at least two homozygotes of each *MTG8* microsatellite allele, and 1 control CEPH were amplified using *MTG8* sequence-specific primers and sequenced directly using the Dye-Deoxy Terminator Cycle Sequencing FS kit and a 373A automated DNA Sequencer (Applied Biosystems Division of Perkin Elmer; Foster City, CA).

Statistical analyses. Statistical comparisons of the obesity-related quantitative traits were performed using the general linear modeling program of the SAS Institute (Cary, NC). In these analyses, parameters of obesity were the dependent variables; age and genotype, treated as a class variable, were the independent variables.

RESULTS AND DISCUSSION

To investigate the potential role of MTG8 in obesity in the Pimas, we first searched for an informative marker at this locus. A compound imperfect $(TA)_n$/$(CA)_n$ polymorphic repeat in the 3′UTR of the mouse *MTG8* homologue has been previously described (11). We therefore analyzed this repeat located at the corresponding position in the human gene for allelic variations. By PCR analysis of genomic DNA, initially from 112 Pimas and 54 CEPH Caucasians, we detected a total of 18 allelic variants of this segment (Figure 1). Fourteen alleles were found in the Pimas, including 2 (alleles 8 and 12) not observed in the Caucasians. In comparison, 16 alleles were present in Caucasians, of which four rare ones (alleles 15-18) were not detected in Pimas. The heterozygosity was 0.84 in the Pimas and 0.89 in Caucasians, consistent with high informativeness of this marker in both populations.

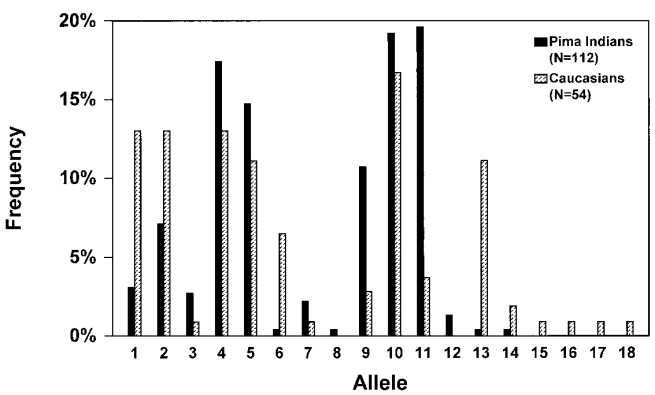

FIG. 1. Polymorphism of the *MTG8* 3′UTR microsatellite and distribution of alleles in 112 unrelated Pima Indians and 54 CEPH Caucasians.

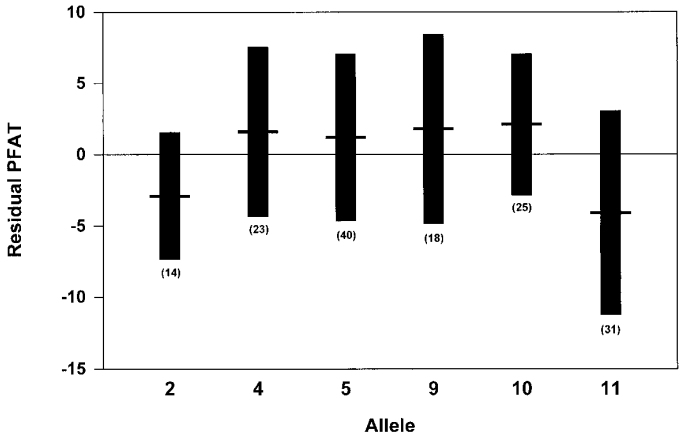

FIG. 2. Association of residual PFAT with six common alleles in non-diabetic Pima males. Residual PFAT for each individual was determined by linear regression analysis of the relationship between PFAT and age. Each bar represents mean ± SD and the number of alleles analyzed is shown parenthetically below each bar.

We subsequently genotyped the marker in 281 full-blooded, non-diabetic Pimas, who were not first-degree relatives, and found a male-specific association with several parameters of adiposity, including PFAT (N = 148 males; p = 0.0002; Figure 2), body mass index (p = 0.01), and waist, as well as thigh circumference (p = 0.008 and 0.02, respectively). Specifically, of the 6 most common alleles (Figure 1), alleles 2 and 11 were associated with a significantly lower PFAT, whereas alleles 4, 5, 9, and 10 were associated with higher PFAT (Figure 2). Interestingly, no statistically significant association with obesity was observed in females (N = 133). At present, we cannot explain why this locus is associated with obesity only in males. However, it is possible that gender-specific differences affecting the physiology of adipose tissue may obscure a relationship between this marker and obesity phenotype in women. Furthermore, the marker was not associated with age- and PFAT-adjusted phenotypic parameters of insulin action (fasting plasma insulin, low- and high-dose insulin-mediated whole body glucose uptake) or insulin secretion (acute and 2-hour insulin response to glucose) in either group (not shown).

Based on the results of our association study, we next determined the genomic structure of *MTG8* (J.K. Wolford and M. Prochazka, in press) and sequenced all 13 exons, plus adjacent intronic or untranslated regions in 30 Pimas selected for specific genotypes at the 3′UTR microsatellite marker. We did not find any sequence variants in either the Pima or CEPH DNAs. Based on these results, it is unlikely that *MTG8* directly impacts upon the development of obesity via disruptions in sequence and consequently, function. Although functional alterations of MTG8 are not expected, quantitative differences in either mRNA or protein levels cannot yet be ruled out. Quantification of *MTG8* transcript and protein will be necessary to address this possibility.

We have recently completed a genome-wide scan for genes contributing to diabetes and its subphenotypes, including obesity, in the Pimas. While the *MTG8* microsatellite was not included in this scan, those markers on 8q that were genotyped did not indicate any linkage of this region with parameters of obesity (14). However, it is well documented that a chromosomal region showing a factual association with a phenotype may not necessarily show a linkage with the same trait (15, 16). In light of this and the lack of genetic variance in the *MTG8* coding region, it is also possible that the 3′UTR microsatellite may be a marker for yet another gene in the 8q22 region which contributes to the association with obesity observed in Pima males.

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