# A Quantitative Trait Locus Influencing BMI Maps to the Region of the β-3 Adrenergic Receptor

**Braxton D. Mitchell, Shelley A. Cole, Anthony G. Comuzzie, Laura Almasy, John Blangero, Jean W. MacCluer, and James E. Hixson**

**The β-3 adrenergic receptor (*ADRB3*) has been implicated as a regulator of energy expenditure, and a polymorphism in codon 64 of this gene (Trp64Arg) has been associated in some studies with obesity and insulin resistance. However, many studies have failed to detect an effect of this variant, and the importance of the Trp64Arg variant in human obesity remains controversial. We performed a quantitative linkage analysis of the *ADRB3* and obesity, using 12 markers (including the intragenic Trp64Arg polymorphism) spanning a 57-cM region of chromosome 8. The study population consisted of 470 individuals from 10 large multigenerational families of Mexican-American ancestry residing in San Antonio, TX. In two-point analysis, logarithm of odds (LOD) scores >1.0 were observed for six markers surrounding *ADRB3* in a 33-cM region spanned by markers D8S1477 and D8S1136. The multipoint LOD score was 3.21, occurring between markers D8S1121 and *ADRB3*, ~2–3 cM from *ADRB3*. Adjusting for the presence of the Arg64 allele or excluding from the analysis the 11 individuals homozygous for the Arg64 allele did not reduce the evidence for linkage. A genome scan was conducted at 10 cM map density to detect other loci influencing variation in BMI. Multipoint LOD scores >1.0 were observed in four other regions, including two on chromosome 17, one on chromosome 6q, and one on chromosome 2p. These data suggest that the *ADRB3* should continue to be regarded as a strong candidate gene for obesity even though evidence for an effect of the Trp64Arg polymorphism could not be established. It is also possible that a gene closely linked to *ADRB3* may influence susceptibility to obesity. *Diabetes* 48:1863–1867, 1999**

Human obesity has a multifactorial etiology that includes a substantial heritable component (1). On the basis of its biological role in fat metabolism, it has been speculated that the β-3 adrenergic receptor (*ADRB3*) may be one of the genes that influences accumulation in body fat. This receptor, which is expressed in adipose tissue, mediates the rate of lipolysis in response to catecholamine, and chemical agents that selectively block this receptor are potent stimulators of metabolic rate and adipose tissue thermogenesis (2,3). Recently, a common polymorphism in this gene has been identified, occurring in the first intracellular loop of the receptor, which is characterized by an amino acid substitution of tryptophan by arginine at position 64 (Trp64Arg) (4).

Studies in a variety of populations have reported associations between the Trp64Arg variant of the *ADRB3* and features related to both obesity and insulin resistance (5,6). Despite these findings, the role of this variant, and of the *ADRB3* gene itself, in human disease remains controversial. Numerous studies have failed to detect effects of the Trp64Arg variant on these traits and even among those studies that have reported associations, there are differences in which phenotypes are associated with the variant. These misgivings have led some experts to conclude that the Trp64Arg mutation probably has little clinical relevance to obesity (7). The lack of consensus about the role of *ADRB3* in obesity is illustrated by the fact that two recent meta-analyses examining the effect of this mutation on BMI have been published, with one concluding that the Arg64 allele was significantly associated with increased BMI (5), and the other concluding that it was not (6).

To date, all evidence supporting a role of the *ADRB3* in human obesity has been based on findings from association studies of the Trp64Arg variant. To provide more general evidence for involvement of the *ADRB3* gene, we have performed a quantitative trait linkage analysis of BMI with genetic markers in the region of the *ADRB3*. To put these results in context, we have also conducted a genome scan of BMI. Our study is based in a Mexican-American population, one in which we have previously detected an association of obesity with the Arg64 variant (8).

## RESEARCH DESIGN AND METHODS

The San Antonio Family Heart Study (SAFHS) is a population-based family study designed to identify the genetic determinants of atherosclerosis and its risk factors (9). Using a house-to-house recruitment procedure, we identified 40- to 60-year-old residents with large families from low-income neighborhoods in San Antonio, TX, and invited them and their first-, second-, and third-degree relatives to participate. The invitation to participate was extended regardless of the individual's body size or any other preexisting medical condition. The current study is based on the initial set of 470 individuals from 10 large families recruited into the SAFHS, on whom we have completed extensive genotyping.

Participating subjects received a medical examination in our clinic in the morning following a 12-h fast. Blood samples were obtained for DNA processing and for characterization of glucose, insulin, and a broad panel of lipids and lipoproteins. Subjects were instructed to remove their shoes and to don examination gowns, and height and weight were recorded by trained research nurses. The BMI, defined as weight (in kilograms) divided by height (in meters) squared, was used as a measure of overall adiposity.

From the Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas.

Address correspondence and reprint requests to Braxton D. Mitchell, PhD, Department of Genetics, Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245–0549. E-mail: bmitchel@darwin.sfbr.org.

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*ADRB3*, β-3 adrenergic receptor; IBD, identity by descent; LOD, logarithm of odds; SAFHS, San Antonio Family Heart Study.

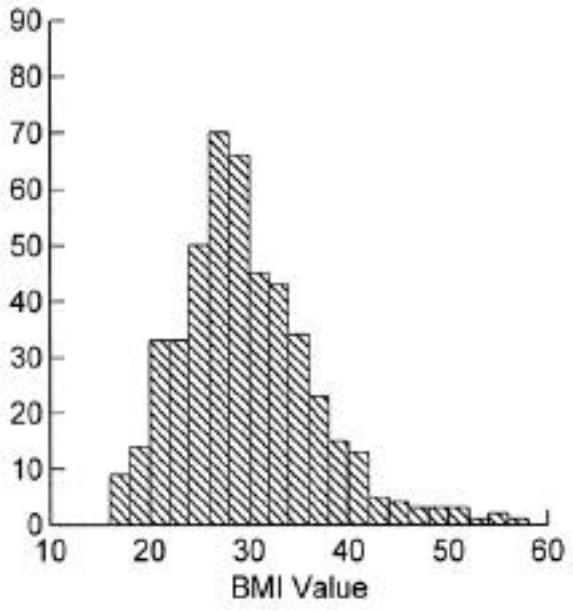


**FIG. 1. Distribution of BMI in the population.**

DNA was isolated from lymphocytes for genotyping. The DNA was amplified with fluorescently labeled primer pairs from MapPairs Human Screening Set (version 6; Research Genetics, Huntsville, AL) that detect highly polymorphic microsatellite markers. Genotypes were generated for a total of 380 microsatellite markers from 22 autosomes with an average spacing between markers of 10.0 cM. Marker genotypes were analyzed using Applied Biosystems (Perkin Elmer, Foster City, CA) Model 377 DNA Sequencers and Gene-scan and Genotyper DNA Fragment Analysis software. The Trp64Arg polymorphism of *ADRB3* was genotyped by polymerase chain reaction amplification of leukocyte DNA (forward primer 5′-CGCCCAATACCGCCAACAC-3′, reverse primer 5′-CCACCAGGAGTCCCATCACC-3′) and cleavage with *Bst*N1, as described by Walston et al. (4). Several additional markers were genotyped in the region of *ADRB3* to increase map density. In a 50-cM region spanning *ADRB3*, a total of 12 markers were typed, including: D8S136, D8S382, D8S1477, D8S1121, *ADRB3*, D8S255, D8S1110, D8S1113, D8S1136, D8S1119, GAAT1A4, and D8S1132. The distances between markers were computed from our data using the CRI-MAP software program (10). Allele frequencies were estimated using maximum likelihood methods.

We tested for linkage with the genetic markers using a variance component methodology as implemented in the SOLAR software package (11). This program models the expected genetic covariances between relatives as a function of the identity by descent (IBD) relationships at a marker locus. Briefly, the total phenotypic variance can be partitioned into components attributable to covariate effects, overall genetic similarity among individuals (i.e., residual heritability), and locus-specific heritability (i.e., linkage). Model parameters are obtained using maximum likelihood methods, and the hypothesis of linkage is evaluated by likelihood ratio test, by testing whether the variance attributable to allele sharing between pairs of individuals is significantly greater than zero (i.e., is $\sigma^2_{QTL} > 0$?). The model has been extended for multipoint analysis, using the multipoint approximation approach of Fulker et al. (12) to estimate IBD sharing at arbitrary chromosomal locations (e.g., at 1-cM intervals), generalized for use in extended families (11). Sex and sex-specific age terms (both age and age squared) were included as covariates in the linkage analysis.

We performed a simulation study to evaluate the probability of obtaining false evidence for linkage. Following the approach of Iturria et al. (13), we permuted phenotypes among study subjects in such a way as to maintain the heritability of BMI. We forced this constraint on the permutation by first simulating a polygenic trait having heritability equal to that which we observed for BMI (i.e., 54%). We then rank ordered all study subjects according to the value of the simulated trait, and then replaced the highest value of the simulated trait with the highest value of the observed trait, replaced the second-highest value of the simulated trait with the second-highest value of the observed trait, and so forth, until all simulated values had been replaced by observed values. This strategy enabled us to retain both the original phenotypic distribution and original genotypic information for the analyses. In addition, the order imposed on the permutation ensured that the original trait heritability was preserved. We then tested for linkage of the permuted (unlinked) trait to the *ADRB3* locus, performing 10,000 replications. The probability of obtaining false evidence for linkage was defined as the proportion of these replicates in which we observed a logarithm of odds (LOD) score higher than a specified value.

## RESULTS

The frequency of the Arg allele in this population was 18.8%. Genotype and allele frequencies were in Hardy-Weinberg equilibrium. BMI in this population was 29.7 ± 6.9 kg/m$^2$ (mean ± SD). The distribution of BMI in the total population is shown in Fig. 1. The mean age of study participants was 38.8 years, and diabetes was present in 15.8% of subjects. The characteristics of the population are shown in Table 1 according to sex and genotype at *ADRB3*. There was no evidence for an association of the Arg64 allele with obesity. In fact, the mean BMI in the four women homozygous for this allele was substantially lower than that in women having at least one Trp64 allele.

*ADRB3* has been localized to chromosome 8p12–p11.1, and the 11 additional markers used to assess linkage to this gene spanned chromosomal bands 8p12 to 8q23.3. The distances (in cM) between markers and the two-point LOD scores associated with the nine markers spanning the region of linkage are shown in Fig. 2. LOD scores >1.0 were observed for the two markers to the pter side of *ADRB3* (up to 7.5 cM away) and for the four markers to the qter side of *ADRB3* (up to 25.2 cM away). The LOD score corresponding to the *ADRB3* polymorphism was 0.97, and LOD scores for the two immediate flanking markers, D8S1121 and D8S255, were 3.50 and 1.25, respectively.

In multipoint analysis, the peak LOD score was 3.21, occurring between D8S1121 and *ADRB3*, ~2.5 cM to the pter side of *ADRB3* (Fig. 3). At this location, heritability at the quantitative trait locus (QTL) accounted for ~37% of the variability in BMI after adjusting for the effects of age and sex. Including diabetes as an additional covariate in the multipoint analysis did not substantially alter these results (LOD score = 3.32 at this same position).

In a previous analysis, using a less dense marker map, we reported a two-point LOD score of 2.2 between serum leptin concentrations and marker D8S1110, located ~10 cM pter to *ADRB3* (14). Using multipoint analysis and the present set of markers, we observed a LOD score of 1.28 at the position of

TABLE 1
Characteristics of study population according to sex and Trp64Arg genotype

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | Trp/Trp | Trp/Arg | Arg/Arg | Trp/Trp | Trp/Arg | Arg/Arg |
| *n* | 172 | 88 | 4 | 137 | 70 | 7 |
| % Diabetic | 14.6 | 19.3 | 0 | 15.6 | 14.3 | 14.3 |
| Age (years) | 38.7 ± 16.2 | 37.5 ± 16.3 | 28.8 ± 3.9 | 39.9 ± 17.3 | 38.2 ± 17.2 | 41.0 ± 23.4 |
| BMI (kg/m$^2$) | 30.6 ± 7.1 | 30.7 ± 8.2 | 24.5 ± 4.1 | 29.3 ± 6.3 | 27.4 ± 5.4 | 29.2 ± 3.9 |

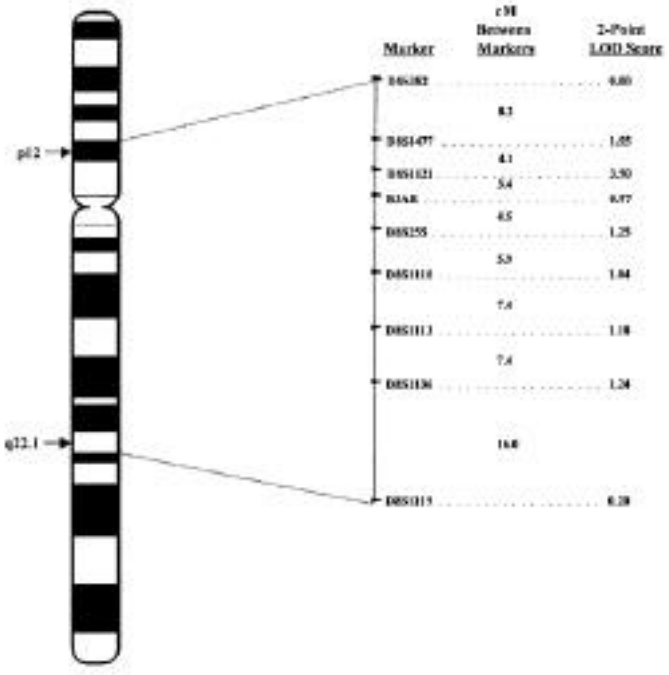


**FIG. 2. Chromosome 8 markers in the region of *ADRB3* and two-point LOD scores for linkage to BMI.**

*ADRB3*, although the peak multipoint LOD score (LOD = 1.59) occurred 6 cM to the qter side of *ADRB3*.

We conducted a simulation to determine the probability of obtaining a multipoint LOD score as high as 3.21 for an unlinked locus. For each replication, we simulated a polygenic trait and calculated the LOD score associated with the multipoint IBD matrix at *ADRB3*. The mean heritability computed from the 10,000 replications was 52%, indicating excellent recovery of the generating value for the heritability. In only one of the 10,000 replications did we observe a LOD score >3.21 (this being a value of 3.30), suggesting that the probability of obtaining false evidence for linkage at *ADBR3* was 0.0001. This value matches closely the *P* value of 0.000121 that corresponds to our observed LOD score of 3.21.

To determine if the observed linkage with BMI might be attributable to the Arg64 allele, we repeated the linkage analysis after including presence or absence of the Arg64 allele in the linkage model as an additional covariate (coded as 0 for Arg64 allele absent or 1 for allele present). The resulting LOD score was 3.19. We also tested the hypothesis that the observed linkage might be attributable to individuals homozygous for the Trp64Arg mutation by removing the 11 subjects homozygous for Arg64 from our analysis and reevaluating evidence for linkage. This analysis corresponds to a test of whether the effect of the Arg64 allele is inherited as a recessive trait. The resulting LOD score with these individuals removed also changed very little (LOD = 3.36), suggesting that the observed linkage was not attributable primarily to Arg64 homozygotes.

Results from the genome scan of BMI are shown in Table 2. Suggestive evidence for linkage was detected at a locus on chromosome 17q, where we observed an LOD score of 2.33 occurring ~65 cM from pter near marker D17S1293. With the exception of the *ADRB3* region on chromosome 8p and this locus on chromosome 17, LOD scores >1.0 were observed in only three other regions: one on chromosome 6q, ~177 cM from pter (LOD score = 1.53); one on chromosome 17p, ~21 cM from pter (LOD score = 1.34); and the third on chromosome 2p, ~60 cM from pter (LOD = 1.16). The chromosome 2 locus is one for which we have reported evidence for linkage with serum leptin levels in a previous study (14).

## DISCUSSION

Because there is controversy about the possible role of the Trp64Arg variant in human obesity, we evaluated evidence for

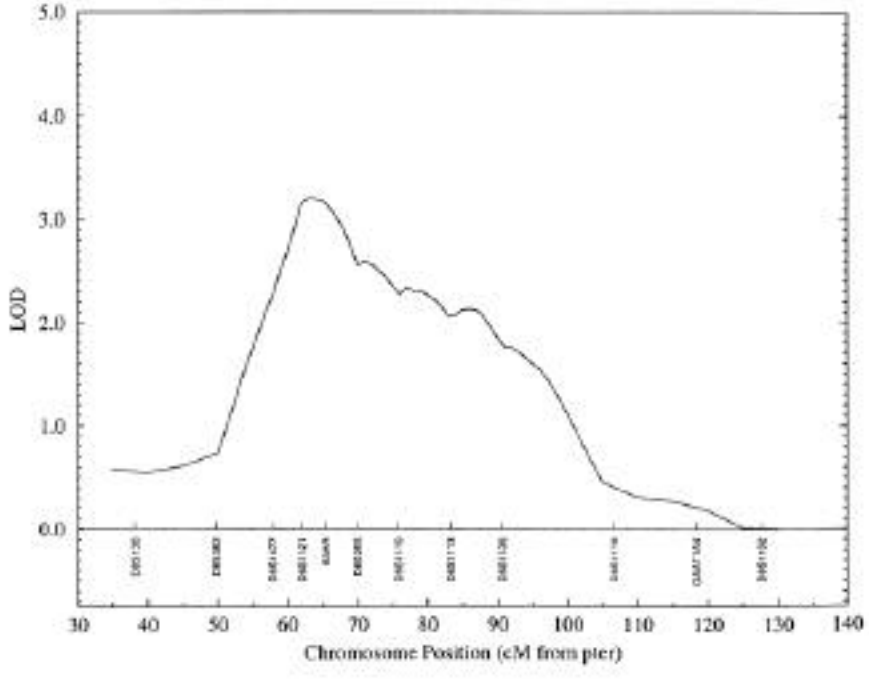


**FIG. 3. Multipoint linkage analysis of BMI with chromosome 8 markers.**

TABLE 2
Chromosomal regions showing evidence for linkage to BMI (LOD score >1.0) in genome-wide scan

| Chromosome | Position from pter (cM) | Closest linked marker(s) (map position) | Peak LOD score |
|---|---|---|---|
| 2 | 60 | D2S1788 (62) | 1.16 |
| 6 | 177 | D6S1008 (179) | 1.53 |
| 8 | 63 | D8S1121 (62), *ADRB3* (65) | 3.21 |
| 17 | 21 | D17S786 (23) | 1.34 |
| 17 | 64 | D17S1293 (64) | 2.33 |

linkage between obesity and the region of chromosome 8 that includes *ADRB3*. Our analysis provides strong evidence for the presence of a gene in this region that influences variation in BMI. In fact, peak evidence for linkage in the current analysis occurred at a location less than 3 cM away from *ADRB3*, although the 1 LOD unit confidence region surrounding this location (i.e., the region corresponding to peak LOD score – 1) spanned a 24-cM region. When we screened for linkage to BMI throughout the entire genome, we identified only four other loci for which the LOD scores were >1.0, one of which occurred on chromosome 2p (LOD = 1.16), in a region that we have previously shown to be linked to serum leptin levels (13). Through simulations, we estimated that the probability of obtaining an LOD score to the *ADRB3* region as high as 3.21 assuming no linkage was 0.0001.

Linkage studies of obesity have been carried out in several other populations (15–19), but to date, none has reported evidence for linkage between obesity measures and markers in the region of *ADRB3*. However, two of these studies (15,16) were based on families ascertained on two obese siblings (one with extreme obesity), and a third was based in Pima Indians, another population with a high prevalence of obesity (17,18). It is possible that the effect of *ADRB3* on obesity is relatively modest and is diluted out by the effects of other genes and/or lifestyle factors in these populations.

Two different studies of Mexican Americans from San Antonio have provided evidence for an association between the Trp64Arg polymorphism and obesity-related traits (8,20). In one study, the Trp64Arg polymorphism was associated with both 2-h insulin concentrations and BMI (20). In the second study, which was based on this same population, an association was observed between the Trp64Arg polymorphism and several different obesity-related traits, although the association could only be detected in siblings who were matched for a QTL on chromosome 2 that we had previously shown to be linked to variation in serum leptin concentrations (8). Interestingly, there is no evidence of an association between the Trp64Arg polymorphism and obesity in the overall population, as shown in this study. One reason for this apparent discrepancy might be related to the fact that by matching on sibs, our previous study ensured that the sib pairs discordant for the Trp64Arg polymorphism were also discordant for other markers across this chromosomal region.

There is an emerging body of evidence from functional studies suggesting that the Trp64Arg polymorphism may compromise activity of the receptor. Two recent studies have reported that the Arg64 allele is associated with lower lipolytic activity of β-3 adrenoreceptors isolated from human omental adipocytes (21,22), although not all researchers have confirmed this result (23). Hoffstedt et al. (22) observed decreased sensitivity to β-3 adrenoreceptor agonist in subjects with the Arg64 haplotype, but also identified two additional genetic variants in the *ADRB3* that were in almost complete association, leaving open the possibility that the Arg64 variant is nonfunctional but might be in linkage disequilibrium with functional mutations elsewhere in the gene. At least one study has reported an effect of the Arg64 mutation on ligand-mediated cAMP accumulation (24).

Despite the prior associations that have been reported between the Trp64Arg polymorphism and obesity, and the emerging evidence supporting a functional defect of this mutation, we were unable to attribute the observed linkage to the mutation in this population. Neither adjusting for the presence of the Arg64 allele nor removing subjects homozygous for this allele diminished evidence for linkage. In fact, it is perhaps not surprising that neither strategy reduced evidence for linkage, given the lack of an association between the Arg64 allele and BMI in the overall population. However, we cannot rule out the possibility that the Arg64 allele influences obesity in combination with mutations at other genes, such as that implicated on chromosome 2p (14). It is also possible that the Arg64 mutation may be only one of several functional mutations in this chromosomal region that contribute to variation in body fat.

Finally, even though peak evidence for linkage occurred <3 cM from the estimated position of *ADRB3*, one cannot, of course, exclude the possibility that a different gene in the same chromosomal region is responsible for the linkage. There are at least 47 genes known at present within the 57-cM region flanked by markers D8S382 and D8S1119. Among these is at least one potential obesity candidate gene, *CRH*, which encodes corticotropin-releasing hormone (CRH), a hypothalamic catabolic neuropeptide that contributes to energy homeostasis and that is regulated in part by leptin and insulin (25). It is also possible that a novel undiscovered gene in this region may influence obesity.

In conclusion, our analyses provide strong evidence for a gene on chromosome 8 that influences variation in BMI. Because peak evidence for linkage coincided with the location of *ADRB3*, this gene should continue to be regarded as a strong candidate for obesity. However, a role of the Trp64Arg polymorphism could not be discerned from this analysis, and it is possible that the obesity-causing mutations exist in other areas of this gene, in its regulatory sequences, or in a completely different, but tightly linked, gene altogether.

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