

## PAPER

# A meta-analytic investigation of linkage and association of common leptin receptor (LEPR) polymorphisms with body mass index and waist circumference

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**METHOD:** We analyzed data pooled from nine studies on the human leptin receptor (LEPR) gene for the association of three alleles (K109R, Q223R and K656N) of LEPR with body mass index (BMI; kg/m<sup>2</sup>) and waist circumference (WC). A total of 3263 related and unrelated subjects from diverse ethnic backgrounds including African-American, Caucasian, Danish, Finnish, French Canadian and Nigerian were studied. We tested effects of individual alleles, joint effects of alleles at multiple loci, epistatic effects among alleles at different loci, effect modification by age, sex, diabetes and ethnicity, and pleiotropic genotype effects on BMI and WC.

**RESULTS:** We found that none of the effects were significant at the 0.05 level. Heterogeneity tests showed that the variations of the non-significant effects are within the range of sampling variation.

**CONCLUSION:** We conclude that, although certain genotypic effects could be population-specific, there was no statistically compelling evidence that any of the three LEPR alleles is associated with BMI or WC in the overall population.

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**Keywords:** body mass index; waist circumference; meta-analysis; leptin receptor polymorphism; linkage; association

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## Introduction

The role of homozygosity for inactivating mutations of the leptin receptor (LEPR) in producing extreme obesity syndromes in laboratory animals is established.<sup>1</sup> Heterozygosity for *Lepr* mutations in mice and rats also results in increased in fat stores.<sup>2,3</sup> Additionally, a small number of extremely

obese humans from consanguineous pedigrees have been identified who are obese due to homozygosity for inactivating mutations of *LEPR*.<sup>4</sup> However, instances of obesity due to inactivating mutations of *LEPR* in humans are quite rare. The question of whether more common polymorphisms of the *LEPR* gene confer increased susceptibility to obesity remains an open and important issue in the molecular physiology of human body weight. A relatively large number of studies<sup>5–24</sup> have now addressed this question (see Table 1). As shown, there are marked differences among these studies in the

conclusions reached. A few studies have detected significant effects on adiposity-related phenotypes in the primary sample or sub-samples, but others have not. In such situations, pooling data from several studies, which can include raw data pooling or meta-analysis of summary statistics from individual studies, can be useful for assessing the likelihood and magnitude of the association between the allelic variants and phenotypes of interest. To the extent that the non-significant results are due to type 2 errors, pooling data from multiple studies greatly increases power and reduces the

**Table 1** Leptin receptor (*LEPR*) in humans: linkage and association studies

Reference	Linkage Ethnicity	Linkage Sample	Association		BMI		Waist	
			Sample	Control	Linkage	Association	Linkage	Association
Considine et al, 1996 <sup>5</sup>	African-American		8 obese males (BMI = 36.9 ± 1.5)	7 lean males (BMI = 23.3 ± 0.9)	N/A	0	N/A	N/A
Norman et al, 1996 <sup>6</sup>	Pima Indians	217 families: 716 siblings			0	N/A	N/A	N/A
Echwald et al, 1997 <sup>7</sup>	Danish		156 obese males (BMI ≥ 31)	205 lean males (BMI = 21.5)	N/A	0	N/A	N/A
Francke et al, 1997 <sup>8</sup>	French	101 families: 286 obese sibs, 121 non-obese sibs, 107 parents			0	0	N/A	N/A
Gotoda et al, 1997 <sup>9</sup>	British		190 obese males (BMI > 28)	132 lean males (BMI < 22)	N/A	0	N/A	N/A
Matsuoka et al, 1997 <sup>10</sup>	Japanese		47 obese subjects (BMI = 35 ± 6.5)	68 lean subjects (BMI = 21.6 ± 2.2)	N/A	0	N/A	N/A
Silver et al, 1997 <sup>11</sup>	Caucasian		175 obese subjects (BMI = 36.7 ± 9.6)	107 lean subjects (BMI = 21 ± 1.4)	N/A	0	N/A	N/A
Hasstedt et al, 1997 <sup>12</sup>	European	42 families: 616 individuals			0	N/A	N/A	N/A
Thompson et al, 1997 <sup>13</sup>	Pima Indians		10 obese subjects (body fat 40 ± 5%)	10 lean subjects (body fat = 23 ± 5%)	N/A	2 <sup>a</sup>	N/A	N/A
Oksanen et al, 1998 <sup>14</sup>	Finnish		249 obese subjects (BMI = 42.8 ± 7.1)	138 lean subjects (BMI = 22.6 ± 1.8)	N/A	0	N/A	N/A
Rolland et al, 1998 <sup>15</sup>	French		343 obese subjects (BMI = 47 ± 7)	79 lean subjects (BMI < 27)	N/A	0	N/A	N/A
Norman et al, 1998 <sup>16</sup>	Pima Indians	127 families: 362 sib-pairs			N/A	N/A	0	N/A
Roth et al, 1998 <sup>17</sup>	German	130 children (BMI = 33.9 ± 6.5), both parents			2 <sup>b</sup>	N/A	N/A	N/A
Chagnon et al, 1999 <sup>18</sup>	QFS	169 families (QFS): 314–325 sib-pairs	141 obese subjects (BMI ≥ 27)	167 lean subjects (BMI < 27)	2	0	N/A	N/A
De Silva et al, 1999 <sup>19</sup>	Nauruan		232 obese males (BMI = 37)		N/A	0	N/A	N/A
Bray et al, 1999 <sup>20</sup>	Mexican	59 Mexican-American families: 159 obese sibs (BMI = 35.3 ± 4.5) 97 non-obese sibs (BMI = 26.1 ± 3) 46 parents (BMI = 31.4 ± 6.2)			0	N/A	0	N/A
Chagnon et al, 2000 <sup>21</sup>	African-American Caucasian	115 AA families: 319 AA subjects 99 Caucasian families: 522 subjects			1	1	N/A	N/A
Endo et al, 2000 <sup>22</sup>	Japanese		90 obese children	463 lean children	N/A	0	N/A	N/A
Del Giudice et al, 2000 <sup>23</sup>	Italian	48 pairs of discordant twins			0	N/A	N/A	N/A
van der Kallen et al, 2000 <sup>24</sup>	Dutch	18 families: 198 subjects			2	N/A	0	N/A

0 = not significant; 1 = significant only in a subgroup; 2 = overall significance; N/A = not assessed.

<sup>a</sup>body fat percentage result;

<sup>b</sup>transmission disequilibrium test.

probability of such an error. Alternatively, to the extent that some of the apparently significant results are simply due to type 1 errors resulting from a large number of independent studies being done on a related question, data pooling potentially reduces the type 1 error rate by conducting overall tests for all studies combined. Data pooling can be an especially useful for objectively and quantitatively addressing whether study-to-study variations in results are simply due to random sampling error or to deterministic factors across studies. In the latter case, data pooling may identify which factors (eg age, sex, ethnic background of subjects, etc) influence the outcome of the constituent studies.<sup>25</sup>

We report a pooled analysis/meta-analysis of the association of common *LEPR* polymorphisms with body mass index (BMI; kg/m<sup>2</sup>) and waist circumference (WC). In conducting the analysis, we pooled raw data from studies in which we have collaborated. This approach has two advantages. First, by relying only on raw data we avoid many of the statistical problems related to pooling summary statistics.<sup>26</sup> Second, because studies were selected for inclusion on the basis of having been conducted by collaborating groups, rather than on the basis of having been published, publication bias is avoided.<sup>27</sup> In the process of conducting this meta-analysis, many complex statistical challenges arose, which are described in a separate paper on methodology.<sup>28</sup> The present paper focuses on the substantive findings of biological relevance that may be important to obesity researchers and clinicians, keeping methodological details to a minimum.

## Methods

### Samples

A total of 3263 individuals were included in this meta-analysis (Table 2). Sixty-two percent are related to one or more subjects in the data set. The largest number of generations among the family pedigrees in the pooled data was two. The subjects were ethnically diverse, ie African-American, Caucasian, Danish, Finnish, French Canadian and Nigerian; approximately 50% were female (Table 3).

**Table 2** Data sets for the pooled analysis

Study <sup>a</sup> and authors	Number of subjects	Ethnicity
Heritage Family Study (HFS), Chagnon et al, 2000 <sup>21</sup>	857 related subjects from 215 families	Caucasian, African-American
Quebec Family Study (QFS), Chagnon et al, 1999 <sup>18</sup>	687 related subjects from 175 families	Caucasian
African-American (AfAm) Study, unpublished data	228 unrelated	African-American
Nigerian Study, Tanizawa et al, 1994 <sup>37</sup>	221 unrelated	African
Finnish Study 2, Oksanen et al, 1998 <sup>14</sup>	112 unrelated	Caucasian
Baltimore Study, Silver et al, 1997 <sup>11</sup>	251 unrelated	Caucasian, African-American, Hispanic <sup>b</sup>
Finnish Study 1, Oksanen et al, 1998 <sup>14</sup>	65 unrelated	Caucasian
Muscatine Iowa Family Study (MIFS), Donohoue et al, 2000 <sup>38</sup>	482 related subjects from 112 families <sup>c</sup>	Caucasian
Danish Study, Echwald et al, 1997 <sup>7</sup>	360 unrelated	Caucasian
Total	3263	

<sup>a</sup>These are not necessarily official names.

<sup>b</sup>There were only two Hispanic subjects, who were excluded from the present analysis.

<sup>c</sup>The number of families with an obese child from over 400 families in this study.

### Genotyping

Genomic DNA samples were prepared from whole blood in the laboratories providing each dataset. While several *LEPR* allelic variants have previously been described, three exonic polymorphisms that result in amino acid substitutions in the *LEPR* gene comprise the combined data set used in the present analysis: *K109R* (coding exon 2); *Q223R* (coding exon 4); and *K656N* (coding exon 12). Detection of polymorphisms was by PCR-restriction fragment length polymorphism analysis or SSCP screening (see the individual referenced data sets in Table 2 for the methods used).

### Statistical methods

The statistical methods are described in detail in a separate paper.<sup>28</sup> In brief, we tested for association of the *LEPR* polymorphisms with the phenotypes using both univariate and multivariate tests. We conducted association tests and joint tests for both linkage and association using transmission disequilibrium tests for quantitative traits.<sup>29</sup> Finally, we tested for linkage using the new and old versions of the genetic model-free Haseman–Elston test<sup>30</sup> using identity in state (IIS) rather than identity by descent (IBD) because we were interested in the effects of the specific polymorphisms rather than in general linkage to the genetic region of *LEPR*.

**Table 3** Descriptive statistics from the 3263 observations

Continuous variables	Mean ± s.d.	Min, max	Percentage missing
Age (y)	38.2 ± 15.8	3, 94	2.4
BMI (kg/m <sup>2</sup> )	28.2 ± 7.2	14, 77	1.2
WC (cm)	90.2 ± 18.4	33, 178	22.9
Discrete variables	Category	Percentage <sup>a</sup>	Percentage missing
Sex	Female	50.9	< 0.05
Diabetes	Yes	24.4	64.0
Ethnicity	Caucasian	74.7	None
	African-American	18.4	
	African	6.8	
	Hispanic	0.1	

<sup>a</sup>Out of non-missing observations.

In each situation, the individual and joint effects of each polymorphism were assessed. Both main effects of the polymorphisms, and their interactions (ie epistasis), were examined. Incomplete data were handled in two ways: (1) data were analyzed excluding cases with incomplete data for the analysis in question; and (2) analyses were repeated handling the incomplete information by multiple imputation.<sup>31</sup> For the association studies involving related individuals, non-independence of observations was accommodated by the use of the ASSOC program in the S.A.G.E. software.<sup>32</sup>

All of these analyses were adjusted for covariates such as age, its polynomials and sex essentially through residual analyses. To test significance of heterogeneity among the estimates, we employed the chi-square *Q*-test statistic of Hedges and Olkin.<sup>33</sup> Funnel plot of standard errors (s.e.) vs estimates from all studies and all loci for each of BMI and WC was used for visual exploration of potential publication bias. In addition, Kendall's rank correlation between the estimates and the standard errors was used as a formal test of publication bias as suggested by Begg and Mazumdar.<sup>34</sup>

## Results

Descriptive statistics are presented in Table 3. The range of age is large, as are the ranges of BMI and WC. The estimated

allele frequencies of the alleles at exon 2, exon 4 and exon 12 were 0.23, 0.48 and 0.20, respectively. The maximum likelihood test<sup>35</sup> for departure from Hardy–Weinberg equilibrium showed no evidence for a departure for alleles at any exon: exon 2 ( $P=0.285$ ); exon 4 ( $P=0.597$ ); and exon 12 ( $P=0.537$ ). The multiple imputation resulted in little change in statistical significance: exon 2 ( $P=0.960$ ); exon 4 ( $P=0.770$ ); and exon 12 ( $P=0.374$ ). However, as expected, the three exons are all in significant in pair-wise linkage disequilibrium regardless of the imputation: exon 2 vs exon 4 ( $P<0.001$ ); exon 2 vs exon 12 ( $P<0.001$ ); and exon 4 vs exon 12 ( $P<0.001$ ).

Results of the ASSOC analyses are presented in terms of differences of the estimated effects of the two genotypes, the 'wild-type' homozygote and the heterozygote, on BMI and WC, separately, from those of the 'mutant' homozygous genotypes after adjusting for age and sex (see Table 4). For example, subjects heterozygous (*K109R*) for the exon 2 allele had a meta estimate effect size of 0.03 on BMI when compared to subjects with *K109K* genotype. No single effect was significant from either the individual studies or the meta-analysis. We also assessed the significance of the phenotypic variation due to genotypic variation, by exon (data not shown). The effects were also not significant for either phenotype for any exon

**Table 4** Estimated effects and standard errors (with reference to mutant homozygotes) of each genotype on body mass index (BMI) and waist circumference (WC) at each locus adjusted for age and sex

Study	Exon 2		Exon 4		Exon 12	
	<i>K109R</i> (s.e.)	<i>R109R</i> (s.e.)	<i>Q223R</i> (s.e.)	<i>R223R</i> (s.e.)	<i>K656N</i> (s.e.)	<i>N656N</i> (s.e.)
<b>BMI</b>						
Finnish 1	0.39 (0.529)	0.90 (0.574)	0.40 (0.612)	1.03 (0.637)	-0.24 (0.517)	-0.55 (1.308)
Finnish 2	0.90 (0.757)	0.44 (1.147)	0.12 (0.929)	0.96 (1.012)	-0.24 (0.821)	0.27 (3.775)
QFS	0.12 (0.499)	-0.15 (0.933)	-0.83 (0.541)	-0.30 (0.745)	0.45 (0.545)	1.13 (1.137)
HFS	0.07 (0.407)	-0.20 (0.818)	0.65 (0.422)	0.73 (0.548)	0.24 (0.399)	-0.86 (1.123)
MIFS	-0.51 (0.625)	-1.45 (1.426)	-0.46 (0.654)	-0.32 (0.860)	-0.14 (0.590)	1.06 (1.969)
Baltimore	—	—	0.77 (1.448)	0.76 (1.766)	-0.98 (1.300)	0.84 (3.585)
Danish	-0.90 (0.678)	1.17 (1.974)	0.35 (0.670)	1.58 (0.892)	-0.24 (0.639)	0.64 (1.239)
Nigerian	-0.52 (— <sup>a</sup> )	0.00 (— <sup>a</sup> )	-0.76 (0.996)	-0.53 (1.017)	0.55 (0.710)	3.12 (1.796)
AfAm	0.13 (1.045)	5.24 (5.043)	1.80 (1.187)	-0.06 (1.251)	0.07 (0.988)	-1.51 (1.858)
Meta est. <sup>b</sup>	0.03 (0.22), $P=0.892$	0.33 (0.37), $P=0.372$	0.13 (0.23), $P=0.571$	0.50 (0.28), $P=0.074$	0.06 (0.21), $P=0.775$	0.31 (0.51), $P=0.543$
<i>Q</i> <sup>c</sup>	4.46, d.f.=6, $P=0.615$	4.37, d.f.=6, $P=0.626$	8.76, d.f.=8, $P=0.363$	5.85, d.f.=8, $P=0.664$	2.64, d.f.=8, $P=0.955$	5.68, d.f.=8, $P=0.683$
<b>WC</b>						
Finnish 1	—	—	—	—	—	—
Finnish 2	3.92 (2.023)	1.63 (3.045)	0.46 (2.469)	3.34 (2.700)	0.50 (2.223)	2.21 (10.079)
QFS	0.65 (1.224)	-0.73 (2.248)	-0.84 (1.317)	-0.28 (1.808)	1.05 (1.336)	2.51 (2.764)
HFS	0.67 (1.057)	0.03 (2.111)	1.07 (1.102)	1.70 (1.416)	0.72 (1.040)	-0.76 (2.987)
MIFS	-1.14 (1.593)	-4.98 (3.623)	-1.71 (1.666)	-3.88 (2.180)	-0.84 (1.513)	4.58 (5.007)
Baltimore	—	—	-1.52 (3.216)	-3.58 (3.913)	-0.91 (2.908)	-2.36 (7.412)
Danish	—	—	—	—	—	—
Nigerian	-0.67 (— <sup>a</sup> )	0.00 (— <sup>a</sup> )	-3.94 (2.630)	-3.46 (2.676)	0.23 (1.885)	10.02 (4.528)
AfAm	—	—	—	—	—	—
Meta est. <sup>b</sup>	0.70 (0.67), $P=0.296$	-0.56 (1.28), $P=0.662$	-0.40 (0.68), $P=0.556$	-0.23 (0.86), $P=0.789$	0.37 (0.63), $P=0.557$	2.48 (1.67), $P=0.137$
<i>Q</i> <sup>c</sup>	3.87, d.f.=3, $P=0.276$	2.09, d.f.=3, $P=0.554$	4.56, d.f.=5, $P=0.472$	8.60, d.f.=5, $P=0.126$	1.21, d.f.=5, $P=0.944$	4.55, d.f.=5, $P=0.473$

<sup>a</sup>Failed to converge and excluded from meta-analysis.

<sup>b</sup>Meta pooled estimate weighted by standard errors.<sup>33</sup>

<sup>c</sup> $\chi^2$  statistics for testing heterogeneity of effect sizes.<sup>33</sup>

from individual studies or from the meta-analysis;  $P$ -values ranged from 0.10 to 0.95.

Potential effect modifications were evaluated by testing significance of interaction effects among genotypes and main covariates using backward elimination ordinary least squares (OLS) regression. Only the interaction effect between *R109R* at exon 2 and sex was significant ( $P=0.027$ ) for BMI, implying that male subjects with *R109R* genotype at exon 2 have significantly higher BMI than the other subjects. However, the contribution of this interaction effect to the variations of BMI is minimal (increase in  $r^2 < 0.01\%$ ). The non-significant allele-by-environment interaction effects suggest that the genotypic effects, if any, are not significantly modified by the main covariates such as diabetes, sex, age and ethnicity. Finally, multivariate analyses for pleiotropic effects of the alleles on BMI and WC adjusted for sex, ethnicity, diabetes and age polynomials indicated that the polymorph-

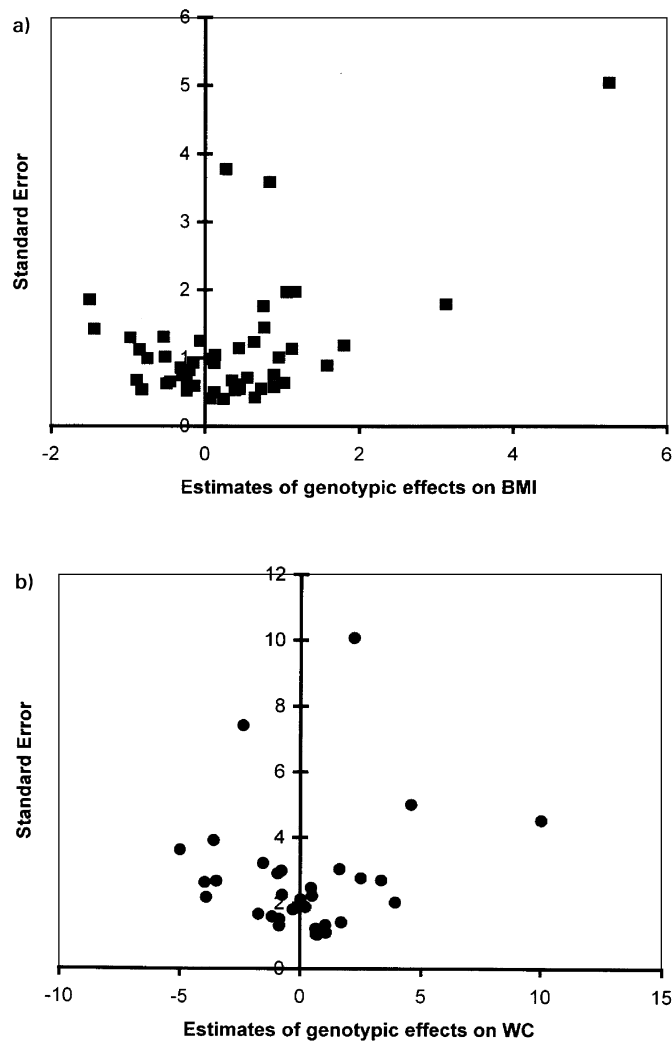
isms had no statistically significant simultaneous effects on BMI and WC (see Heo *et al*<sup>28</sup> for details).

Results of the Hedges–Olkin chi-square test statistic  $Q$  are listed in Table 4. The non-significant heterogeneity indicates that study-to-study variation in outcome is consistent with simple random sampling variation.

The funnel plots (Figure 1) for both BMI and WC show that the estimates are fairly symmetric about 0 and fanning out, as the s.e.s get larger. Furthermore, Kendall's rank correlation did not support publication bias for either BMI (0.058,  $P=0.553$ ) or WC ( $-0.060$ ,  $P=0.627$ ).

## Discussion

The lack of association between the *LEPR* amino acid substitutions and obesity indices, despite a large sample, suggests that the substitutions do not affect these phenotypes.



**Figure 1** Funnel plot of standard errors vs estimates of genotypic effects on (a) BMI and (b) WC obtained from all studies and loci (Table 4).

While amino acid substitutions may result in either non-functioning or poorly functioning proteins or even functional proteins (if the effect is 'silent'), it is important to note that among complex traits with multiple pathways, such as obesity, the absence of association does not necessarily indicate a lack of effect. It may simply be that persons with the amino acid substitution compensated by other means, or that additional genotypic factors may be involved and need to be taken into account before the phenotype becomes manifest. Another possibility is 'hyper' functioning, ie a protein that has greater functional activity than 'wild-type'. This is a less common result of mutation, but is a formal possibility with precedent. Thus, we might have detected a hyper-functioning 'leanness' allele, although there was no strong evidence for this.

We acknowledge that the Hedges–Olkin chi-square test statistic is often less powerful than a direct test of heterogeneity as a function of a specific measured moderator variable. However, we believe that the results of the tests (Table 4) are relatively strong in suggesting that the variation due to sources (such as different populations and genotyping in different laboratories) is not substantial.

With respect to possible 'collaboration' bias, although it is theoretically possible, it is not obvious to us exactly how such bias would operate or that it would be likely, given that our collaborators were not selected on the basis of the results of their *LEPR* studies. However, the patterns on the funnel plots (Figure 1) and the non-significant Kendall's rank correlations imply that there are no substantial biases due to 'collaboration' and/or unpublished reports.

The lack of association, of course, does not rule out the possibility that the three alleles may influence intermediate traits, or phenotypes, not examined as part of these analyses. Moreover, these results might have been very different if we had examined *LEPR* along with other genes known to influence energy homeostasis (eg *CART*, *LEP*, *MC4R*). This sort of contingent/epistatic analysis (see Horikawa *et al*<sup>36</sup> for an example) is required if we are to fully understand the genetics of complex traits such as human weight homeostasis.

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