Examining the Causal Role of Leptin in Alzheimer Disease: A Mendelian Randomization Study

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Abstract

Background: Observational evidence regarding the role of leptin in Alzheimer disease (AD) is conflicting. We sought to determine the causal role of circulating leptin and soluble plasma leptin receptor (sOB-R) levels in AD using a separate-sample Mendelian randomization study. **Methods:** Single nucleotide polymorphisms (SNPs) independently and solely predictive of log-transformed leptin (rs10487505 [LEP], rs780093 [GCKR], rs900400 [CCNL1], rs6071166 [SLC32A1], and rs6738627 [COBLL1]) and of sOB-R (rs1137101 [LEPR], rs2767485 [LEPR], and rs1751492 [LEPR]) levels (ng/mL) were obtained from 2 previously reported genome-wide association studies. We obtained associations of leptin and sOB-R levels with AD using inverse variance weighting with fixed effects by combining Wald estimates for each SNP. Sensitivity analyses included using weighted median and MR-Egger methods and repeating the analyses using only SNPs of genome-wide significance. **Results:** Using inverse variance

weighting, genetically predicted circulating leptin levels were not associated with AD, albeit with wide confidence intervals (CIs): odds ratio (OR) 0.99 per log-transformed ng/mL; 95% CI 0.55–1.78. Similarly, the association of sOB-R with AD was null using inverse variance weighting (OR 1.08 per log-transformed ng/mL; 95% CI 0.83–1.41). Results from our sensitivity analyses confirmed our findings. **Conclusions:** In this first Mendelian randomization study estimating the causal effect of leptin on AD, we did not find an effect of genetically predicted circulating leptin and sOB-R levels on AD. As such, this study suggests that leptin is unlikely to be a major contributor to AD, although the wide CIs preclude a definitive assessment.

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Introduction

There are only limited treatment options for Alzheimer disease (AD), and drug development for the disease has been marred by an exceedingly high failure rate, with nearly all drug candidates failing phase 3 trials [1]. Pre-clinical and observational studies associating lower circu-

lating leptin levels with AD have garnered substantial scientific and commercial enthusiasm for exploring the potential of leptin as both a therapy and a biomarker for AD [2]. Observational studies suggest that circulating leptin levels are negatively associated with AD and other types of dementia [3–10], but with inconsistent associations with clinical measures of AD, such as cognition and imaging features. Similarly, animal studies have suggested a functional role of leptin, relevant to AD, including in amyloid- β pathology [11, 12] and in the CA1 region of the hippocampus relevant to learning and memory [13, 14]. As one might expect, observational studies finding no significant association of leptin with AD or dementia in general have been reported less frequently in the literature, but a number do exist [15–19].

Despite the large volume of reports on leptin and AD, it is important to recognize that all observational studies are subject to unmeasured confounding and, because of this potential for bias, cannot solely be relied upon for evidence [20]. Oftentimes, only randomized controlled trials can elucidate this bias and, in some cases, may reveal completely different findings. No randomized controlled trials of leptin have been reported for AD or other dementias, but clinical development of leptin for AD is being pursued [21]. Given the high failure rate of AD drug development, it seems prudent to first determine if leptin has a causal role in AD and as such has genetic validation as a target of intervention.

An increasingly popular nonexperimental technique to provide causal evidence of an exposure on an outcome, as well as genetic validation, is Mendelian randomization, which leverages the natural random assignment of genotypes at conception [22]. In principle, Mendelian randomization uses a genetic variant as a proxy of the exposure of interest to provide an unbiased estimate of association with the outcome of interest. This variant must be associated with the exposure, must be independent of confounders of the exposure-outcome relationship, and must only affect the outcome through the exposure (i.e., no pleiotropy). This technique may be particularly useful to elucidate causal effects when observational evidence is conflicting and experimental evidence lacking. Furthermore, it can be a useful tool to prioritize or deprioritize investment in randomized controlled trials [23]. To clarify the potential role of leptin in AD, we conducted a separate-sample Mendelian randomization study to determine the causal effect of genetically predicted circulating leptin and soluble plasma leptin receptor (sOB-R) levels on AD in the largest publically available case-control study of AD (cases, 17,008; controls, 37,154).

Materials and Methods

Single nucleotide polymorphisms (SNPs) were identified by first identifying all published genome-wide association studies (GWASs) for leptin and sOB-R. We identified 1 GWAS for circulating leptin (log-transformed ng/mL) [24] and 1 GWAS for sOB-R (log-transformed ng/mL) [25]. All SNPs reported by the authors as having a p value of $<5 \times 10^{-5}$ after adjusting for age, sex (if applicable), and body mass index (BMI) were considered for inclusion.

The GWAS for leptin identified 5 SNPs predictive of leptin levels in 32,161 individuals of European descent from 23 different studies with men ($n = 13,363$) and women ($n = 18,698$) analyzed separately because of sex differences in circulating leptin [24]. Loci from this first stage were then examined in a sample of 19,979 individuals of European descent from 13 studies. All studies measured either plasma or serum leptin and included morning or fasting samples. Genotype associations were adjusted for age, age squared, sex, BMI, and any necessary study-specific covariates. Because BMI does not differentiate between lean and fat body mass, a sensitivity analysis using a subsample of studies that measured body fat confirmed no marked differences in effect size relative to BMI-adjusted estimates. The GWAS for sOB-R identified SNPs among 1,504 women of European ancestry from the Nurses' Health Study (mean age $\pm$ SD, 56.2 $\pm$ 6.9 years) and then replicated them in an independent sample of 875 young healthy men from the Cyprus army (mean age $\pm$ SD, 18.4 $\pm$ 0.6 years) [25]. The β coefficients and standard errors from the Cyprus army cohort were used because of their greater associated precision. These estimates were adjusted for age and BMI.

Pleiotropic effects on AD for each SNP were identified from the Ensembl project (<http://www.ensembl.org>). Ensembl is a comprehensive, curated cross-reference from genotype to phenotype. Correlation between SNPs (linkage disequilibrium) on the same chromosome was determined using SNP Annotation and Proxy Search (<http://www.broad.mit.edu/mpg/snap/ldsearchpw.php>).

Genetic associations with AD came from a case-control study from the International Genomics of Alzheimer's Project (IGAP), which is described in detail elsewhere [26]. IGAP is a large 2-stage study based upon GWAS of individuals of European ancestry. In the data source used for this analysis, IGAP genotyped and imputed data on 7,055,881 SNPs from 4 major consortia which were meta-analyzed together to give summary estimates for 17,008 AD cases and 37,154 controls. Cases were diagnosed using internationally accepted criteria with clinical or autopsy confirmation.

We first obtained overall estimates of the effect of circulating leptin and sOB-R levels on AD using inverse variance weighting with fixed effects to combine Wald estimates for each SNP (i.e., the β coefficient of the SNP for the disease divided by the β coefficient of the SNP for the exposure) with the variance of each Wald estimate obtained using an approximation to Fieller's theorem [27] (i.e., the standard error of the β coefficient for the disease squared divided by the β coefficient for the exposure squared). Three sensitivity analyses were done: (1) a weighted median method [28], which may generate correct estimates if up to 50% of SNPs are invalid instrumental variables (e.g., due to pleiotropy); (2) MR-Egger regression [29], which may generate correct estimates if up to 100% of SNPs are invalid instrumental variables; and (3) re-analysis using only SNPs of genome-wide significance. Finally, to give an overview of the likely functional relevance of leptin and

Table 1. Characteristics of SNPs predictive of circulating leptin levels and soluble leptin receptor levels

SNP	Proximal gene (chromosome)	Effect allele ^a	Effect allele frequency ^a	<i>p</i> value for effect allele for association with exposure of interest ^a	Pleiotropic effects ^b
<i>Circulating leptin levels</i>					
rs10487505	<i>LEP</i> (7)	G	0.50	1.99×10^{-12c}	no data available breast size, blood pressure, calcium levels, cholesterol levels, Crohn disease, fasting glucose, glucose homeostasis traits, palmitic/palmitoleic acid plasma levels, sex hormone-binding globulin levels, triglyceride levels, uric acid levels, waist circumference
rs780093	<i>GCKR</i> (2)	C	0.61	3.80×10^{-10c}	
rs900400	<i>CCNL1</i> (3)	T	0.60	1.17×10^{-7}	
rs6071166	<i>SLC32A1</i> (20)	C	0.37	1.75×10^{-8c}	
rs6738627	<i>COBLL1</i> (2)	A	0.37	1.92×10^{-6}	no data available
<i>Soluble leptin receptor levels</i>					
rs1137100	<i>LEPR</i> (1)	A	0.11	3.83×10^{-6}	leptin receptor polymorphism
rs1137101	<i>LEPR</i> (1)	A	0.33	2.05×10^{-8c}	leptin receptor polymorphism
rs2767485	<i>LEPR</i> (1)	T	0.30	6.27×10^{-7}	no data available
rs1751492	<i>LEPR</i> (1)	T	0.14	9.37×10^{-7}	soluble leptin receptor levels

SNP, single nucleotide polymorphism. ^a Data from genome-wide association studies for circulating leptin and soluble leptin receptor levels. ^b Phenotype data from Ensembl.org. ^c Genome-wide significance ($p < 5 \times 10^{-8}$).

sOB-R to AD, we assessed if the distribution of *p* values for the associations of SNPs in the relevant genes, *LEP* and *LEPR*, with AD differed from the uniform distribution expected by chance accounting for linkage disequilibrium using a GATES test [30].

All statistical analyses were conducted using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All data sources were de-identified and publically available, and thus, no ethical committee approval was required.

Results

Five SNPs were reported in a published GWAS to be predictive of log-transformed leptin levels after adjustment for BMI and other covariates: rs10487505 (*LEP*), rs780093 (*GCKR*), rs900400 (*CCNL1*), rs6071166 (*SLC32A1*), and rs6738627 (*COBLL1*) (Table 1) [24]. Since all of these SNPs were on different chromosomes, none were excluded from the analysis because of linkage disequilibrium. No pleiotropic effects relevant to AD were identified.

Four SNPs were reported in a published GWAS to be predictive of log-transformed sOB-R levels after adjustment for BMI and other covariates: rs1137100 (*LEPR*), rs1137101 (*LEPR*), rs2767485 (*LEPR*), and rs1751492 (*LEPR*) (Table 1) [25]. When assessing linkage disequilibrium

Table 2. Linkage disequilibrium (r^2 values) among single nucleotide polymorphisms on the *LEPR* gene

	rs1137100	rs1137101	rs2767485	rs1751492
rs1137100	1	0.521	0.065	0.836
rs1137101	0.521	1	0.126	0.344
rs2767485	0.065	0.126	1	0.078
rs1751492	0.836	0.344	0.078	1

between pairs of SNPs on the *LEPR* gene (Table 2), only SNPs rs1137100 and rs1751492 were highly correlated ($r^2 = 0.836$), so rs1137100 was discarded because it had a less significant *p* value. No pleiotropic effects relevant to AD were identified.

Using inverse variance weighting, the estimated effect of leptin on AD was null, albeit with wide confidence intervals (CIs): odds ratio (OR) 0.99 per log-transformed ng/mL; 95% CI 0.55–1.78. Figure 1 shows the overall and SNP-specific estimates for leptin. Associations were similar using the weighted median (OR 1.39; 95% CI 0.65–2.95) and MR-Egger (OR 0.92; 95% CI 0.20–4.32) methods. When using only the 3 SNPs with genome-wide significance (rs10487505, rs780093, and rs6071166), the association remained null using inverse variance weight-

Fig. 1. Overall and single nucleotide polymorphism (SNP)-specific associations of circulating leptin levels with Alzheimer disease (AD). Forest plot of odds ratios (ORs) and 95% confidence intervals for overall and SNP-specific associations of leptin with AD per log ng/mL increase in genetically predicted circulating leptin level. FE, fixed effects.

ing (OR 1.62; 95% CI 0.80–3.30), the weighted median method (OR 1.74; 95% CI 0.77–3.94), and the MR-Egger method (OR 1.72; 95% CI 0.01–246.29), although with unreliable confidence limits for the latter.

Similarly, the estimated effect of sOB-R on AD was null using inverse variance weighting (OR 1.08 per log-transformed ng/mL; 95% CI 0.83–1.41). Figure 2 shows the overall and SNP-specific estimates for sOB-R. Associations were similar using the weighted median (OR 1.09; 95% CI 0.82–1.46) and MR-Egger (OR 1.05; 95% CI 0.13–8.65) methods. Only 1 SNP had genome-wide significance (rs1137101), which had a similar estimate to the main analysis using inverse variance weighting (OR 1.07; 95% CI 0.70–1.65). Because there was only 1 SNP with genome-wide significance, we could not repeat weighted median and MR-Egger methods.

Finally, the GATES test had no indication that the *LEP* or *LEPR* genes were associated with AD. The gene-based *p* values for these associations with AD were 0.27 for *LEP* and 0.44 for *LEPR*.

Discussion

In this first Mendelian randomization study assessing the causal effect of leptin on AD, we failed to determine a causal effect of genetically predicted circulating leptin levels on AD, consistent with some observational studies [15, 18, 19]. We also failed to determine a causal effect of genetically predicted sOB-R on AD. sOB-R is the major leptin-binding protein in the circulation [31] and has an inverse relationship with circulating leptin levels [32].

Fig. 2. Overall and single nucleotide polymorphism (SNP)-specific associations of soluble leptin receptor levels with Alzheimer disease (AD). Forest plot of odds ratios (ORs) and 95% confidence intervals for overall and SNP-specific associations of soluble plasma leptin receptor (sOB-R) levels with AD per log ng/mL increase in genetically predicted circulating leptin level. FE, fixed effects.

Thus, these consistent findings provide further support for lack of a causal relation of leptin with AD. The results from the weighted median and MR-Egger methods confirmed the robustness of this lack of causal effect, as results were generally similar to the main analysis using inverse variance weighting. Finally, we found no evidence of gene-level associations of *LEP* or *LEPR* with AD. As such, this study suggests that higher leptin levels are unlikely to be protective against AD, although the wide CIs preclude a definitive assessment.

This was the first study to examine the causal relation of leptin and sOB-R levels with AD using a very large sample and a method that may allow for unbiased estimates. However, some limitations warrant consideration. First, not all of the SNPs included in the analysis had achieved genome-wide significance in their respective GWASs, raising the risk that they were false-positive predictors of leptin or sOB-R levels. However, our 3 sensitivity analyses all supported the results from our main analysis and helped rule out this possibility. Second, no information was available on pleiotropic effects for some of the SNPs included, which leaves the possibility that some effects relevant to AD might exist but have not yet been identified. However, since the results from our sensitivity analysis were similar to our main analysis, we can assume that assumptions regarding lack of relevant pleiotropy were not violated. Third, we did not have individual-level data on leptin or sOB-R levels, so we could not examine if the relation of leptin and sOB-R with AD differed by specific levels or thresholds of these biomarkers. However, dose response is a key attribute of causality, so we would not expect a causal relationship to appear only when a specific

level of an exposure is achieved. Fourth, we were not able to conduct separate analyses for men and women since we did not have sex-stratified genetic associations for AD. Women have higher leptin levels than men [33], but this does not imply that the relation of leptin with AD differs by sex because other factors may explain the sex differences in both leptin levels and AD prevalence. Fifth, the underlying studies concern individuals of European descent, so we could not test our hypothesis in racially and ethnically diverse populations. If leptin were truly a causal factor in AD, we would not expect it to have different effects by baseline levels, sex, or race/ethnicity. In fact, leptin levels have moderate heritability (~30%), independent of age, sex, and adiposity, and consistent heritability among black and white racial groups [34]. Sixth, IGAP included prevalence case-control studies of an elderly population with AD, so we could not determine if leptin influenced disease progression or severity. The risk of survivor bias is also relevant, although it is likely less pervasive for IGAP than for other prevalence case-control studies, such as those for cardiovascular disease. Survivor bias is attenuated by the fact that AD does not have the lethality of a discrete event like myocardial infarction, despite being associated with accelerated mortality. Therefore, we would not expect major differences between people included in IGAP and the general population of patients with AD. Another relevant concern related to survivor bias is if lower leptin indeed caused greater mortality. This would be expected to bias our results to the null; however, the observational evidence on leptin and mortality is inconsistent [35, 36], making it difficult to rule this possibility in or out. Seventh, we did not have data on cognitive impairment, so we could not determine if leptin was causal of cognitive impairment, independent of AD. Finally, our results were near null, whereas previous observational studies have suggested a much larger effect of leptin on AD, for example, a hazard ratio of 0.60 per log ng/mL leptin in the Framingham cohort [3]. Although we did not have power to detect very small effects of leptin on AD, we had power to detect larger ones. Specifically, with 80% power and an α of 0.05, our analysis would have the power to detect an OR as low as about 0.89 or as high as about 1.12 per standard deviation of an exposure, assuming 5% variance explained by the SNPs on leptin [37]. Another limitation of Mendelian randomization is its lower statistical power in general, given the limited strength of SNPs to explain variation in intermediate traits.

Because leptin is a product of fat cells, its circulating levels are strongly correlated with adiposity among the healthy population [38] and also in the obese population

[39], who are thought to have developed resistance to its effects on satiety [40]. Observational evidence supports an association with adiposity and AD that varies by age [41], so, leptin might theoretically be a mediator in the adiposity-AD pathway. However, recent Mendelian randomization analyses found no evidence of a causal association of BMI with AD [42], and the present study found no causal association of leptin with AD, independent of BMI. Unintentional weight loss is a hallmark of AD [43], beginning years before the onset of symptoms [44], so measures of adiposity are expected to be confounders in observational studies examining the relationship between leptin and AD. Although adiposity explains a large degree of the variance in leptin levels [39], leptin secretion is influenced by a variety of endocrine factors [45–47] that may also be confounders.

The apparent lack of a causal relation of leptin with AD is supported by the observation that cognitive impairment, let alone pathologic features of AD, does not seem to be a hallmark of congenital leptin or leptin receptor deficiency disorders. In these very rare genetic disorders, most reports have only described the profound adverse metabolic changes that these people experience [48, 49]. In 1 case report, leptin replacement was reported to improve some neurocognitive domains in a developmentally delayed child with congenital leptin deficiency, in addition to dramatic weight reduction and resolution of hypertension, dyslipidemia, and hyperinsulinemia [50]. However, there were few neurocognitive improvements after 1 year of leptin treatment, and most improvements occurred after 2 years of treatment, which coincided with the child finishing his first year of school. Although this case report has been widely cited, including by articles suggesting that leptin might be a potential treatment for AD [2, 6], its relevance to AD or dementia is speculative at best. Prospective surveillance of people with congenital leptin or leptin receptor deficiency may provide important insights into the possible etiological role of leptin in other diseases.

In conclusion, our findings do not support a causal role of leptin in AD. Although they do not automatically invalidate the body of research on leptin and AD, they do provide evidence casting doubt as to whether leptin is a genetic target or would be an effective disease-modifying treatment for AD.

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The authors report no conflicts of interest.

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Author Contributions

Matthew L. Romo: study concept and design, statistical analysis, interpretation of data, and manuscript drafting. C. Mary Schooling: study concept and design, oversight of statistical analysis, interpretation of data, and critical revision and approval of manuscript.

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