A GWAS top hit for circulating leptin in the leptin gene is associated with weight gain but not with leptin protein level in lithium-augmented patients with major depression

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Abstract

Lithium-treated patients often suffer from weight gain as a common adverse event. In an earlier investigation, we found an impact of two single-nucleotide polymorphisms (rs10487506 and rs2278815) at the leptin gene on weight gain but not on leptin protein levels in serum under lithium augmentation. A recent genome-wide association study identified a polymorphism at the leptin gene locus (rs10487505) associated with circulating leptin levels. To characterize potential effects of this variant in acute major depressive disorder, we investigated body mass indices from 180 lithium-augmented patients and serum concentrations of leptin protein from 89 patients using linear mixed model analyses and rs6979832, a proxy SNP of rs10487505. Body mass index was measured before and after 4 weeks of lithium augmentation, in a subsample also after 4 and 7 months. Leptin levels were measured before and during lithium augmentation. G-allele homozygotes of rs6979832 had a significantly lower body mass index increase during observation compared to A-allele hetero- and homozygotes. However, we found no influence on leptin serum levels. Joint analyses of rs6979832 with the previously investigated polymorphisms rs10487506 and rs2278815, and expressed quantitative trait data, suggest a complex interplay between SNP alleles at the leptin locus. These results strongly support our earlier findings that common genetic variation at the leptin gene locus may be involved in lithium augmentation-associated weight gain in major depressive disorder.

Introduction

Weight gain is a common adverse effect of lithium augmentation (LA) that was observed in up to 62% of psychiatric patients (Livingstone and Rampes, 2006). Leptin, an adipocyte-secreted peptide hormone, decreases body weight by both suppressing appetite and increasing energy expenditure (Ahima et al., 1996). However, increased levels of circulating leptin were found to be correlated with obesity and leptin resistance (Morris and Rui, 2009). Single-nucleotide-polymorphisms (SNPs) at the leptin gene locus (*LEP*) were reported to be associated with obesity and metabolic diseases (Yu et al., 2012), and weight gain under psychiatric medication, mainly antipsychotic-induced weight gain (Lee and Bishop, 2011). Furthermore the relation between changes in leptin levels and depression and an antidepressant-like effect of leptin are being recently discussed (Ge et al., 2018).

In an earlier investigation we found leptin serum levels positively associated with body mass index (BMI) under LA in patients with major depressive disorder (MDD; Ricken et al., 2016). Furthermore we identified an effect of two *LEP* SNPs on BMI (rs10487506, rs2278815) as well as of rs2278815 on BMI change during LA. However, we did not find support for a genotype-dependent effect on leptin serum levels (Bopp et al., 2019). New evidence for an effect of common genetic variation on leptin was provided by a genome-wide association study (GWAS) which identified several loci influencing circulating leptin levels in cohorts of European ancestry (Kilpeläinen et al., 2016). One of the top findings was a genome-wide significant SNP located 21 kb upstream of *LEP* (rs10487505). In the present study, we sought for an effect of rs10487505 on BMI as well as on leptin serum levels during LA using a prospective MDD cohort. Additionally, we analyzed potential joint effects between the GWAS SNP (rs10487505) and our candidate SNPs (rs10487506, rs2278815) on BMI and Genotype-Tissue Expression data (GTEx).

Experimental procedures

Patients and analysis of leptin protein

The cohort consists of 180 lithium-augmented patients with DSM-IV MDD. BMI data were available for all 180 patients before and after 4 weeks of LA, for 61 patients after 4 months and for 66 patients after 7 months of LA. Severity of depression, BMI (kg/m^2), and leptin protein serum concentrations (available for 89 patients; non-fasting; assessed by ELISA) were measured at baseline and after 4 weeks of LA. Follow-up data were obtained after 4 or 7 months or both. For further details on analysis of leptin protein and genotyping see Bopp et al. 2019.

Analysis of leptin polymorphisms

The proxy SNP rs6979832 was used to investigate LEP rs10487505. Pairwise linkage disequilibrium (LD) between SNP and proxy SNPs was analyzed using the application LDpair from the web-based suite LDlink (Machiela and Chanock, 2015). LEP rs10487505 showed strong LD with rs6979832, i.e. the minor allele of rs10487505 (G) was correlated with the minor allele (A) of rs6979832 and the major allele of rs10487505 (C) was correlated with the major allele of rs6979832 (G). Using LDpair (2019), we also analyzed LD patterns in Europeans between rs6979832 and the two previously investigated SNPs rs2278815 and rs10487506 (Bopp et al., 2019). We found low to moderate LD between rs6979832 and rs2278815 (R^2=0.22, D'=0.55) as well as between rs6979832 and rs10487506 (R^2=0.09, D'=0.31). These estimates were confirmed when we analyzed LD empirically in our study sample (rs6979832 and rs2278815: R^2=0.28, D'=0.63; rs6979832 and rs10487506: R^2=0.11, D'=0.34).

We used random-intercept linear mixed models to investigate SNP effects on BMI. We analyzed the effect of rs6979832 genotype on BMI with an additive genetic model and furthermore the effect of GG homozygotes versus A-allele carriers. Covariates were time, obesity before LA (Obesity was defined as a BMI ≥ 30 kg/m^2) sex, age, Hamilton Depression Rating Scale (HRDS-17 score), and psychopharmacological co-medication with a high risk of weight gain (MRWG, see Bopp et al. 2019). Then we analyzed the effect of rs6979832 on leptin serum levels. Finally, we explored whether the effects of rs6979832 on BMI are distinct

from the previously identified SNPs rs2278815 and rs10487506, by introducing the latter SNPs as covariates.

We used the Statistical Analysis System (SAS) software (version 9.4.) for the linear mixed models and SPSS (Version 21) for descriptive statistics and a paired sample t-test. The nominal significance level was set at 0.05. (for further details see Bopp et al. 2019).

Analysis of allele-specific leptin expression

Data from the GTEx project (Genotype-Tissue Expression project, 2017) were used to explore the allele-specific expression of *LEP* mRNA for rs10487505 in a multiple tissue quantitative trait locus (eQTL) comparison. The data request was executed on November 05 2020, with GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2).

Results

A total of 446 BMI measurements were obtained from 180 patients. BMI increased significantly over the observation period (see Table 1, Bopp et al., 2019; also for clinical data).

Additive genetic models

Rs6979832 (P=0.037), time (P=0.002), and the rs6979832*time interaction (P=0.011) showed significant effects on BMI. MRWG had a significant negative effect on BMI (P=0.003), whereas obesity before LA (P<0.0001) had a significant positive effect. Sex was significant (P=0.041), with males having a higher BMI.

Genotype models (AA versus G-allele carriers)

Because the courses of BMI observed in GG carriers differed from those in AA and GA carriers, we analyzed potential A-allele-specific effects on BMI by pooling GA and AA carriers for each SNP. The rs6979832 (GG/AG+AA)*time-interaction (P=0.001) had significant

negative effects on BMI, rs6979832 (GG/AG+AA) was not significant (P=0.083). The former may be interpreted as an influence of 1 or more A-alleles on BMI over time. Patients homozygous for the G-allele of rs6979832 had a significantly smaller BMI increase than patients with 1 or more A-alleles. In this model, covariates with significant positive effects were time (P=0.017) and obesity before LA (P<0.0001). MRWG had a significant negative effect (P=0.013). Sex (P=0.025) was significant, with males having a higher BMI.

The covariates HDRS-17 score and age showed no significant results in the analyses.

Detailed parameters of both statistical models, including all covariates, are shown in **Table 1**. 53 patients were AA homozygous (28.6%), 100 were GA heterozygous (54,1%) and 31 were GG homozygous (17,3%).

Influence of the genotype on leptin serum concentration

A total of 178 measurements of leptin levels from 89 patients were available for analysis. The distribution of leptin levels in blood serum was right-skewed. Therefore, we used logarithmic transformation (common logarithm) for analysis. We found no significant effect of rs6979832 or rs6979832*time on leptin levels.

rs6979832 und the previous reported LEP SNPs rs2278815 and rs10487506

The joint effects of rs6979832 and our previous findings, rs2278815 and rs10487506, were analyzed using two separate statistical models. Due to the high LD between rs2278815 and rs10487506 (see Bopp et al., 2019), effects of rs6979832 were either analyzed together with rs2278815 or rs10487506.

When rs6979832 and rs2278815 were taken together in the additive genetic model the effect of rs2278815 (P=0.004), rs6979832*time (P=0.025) and rs2278815*rs6979832*time (P=0.039) on BMI remained significant, as well as rs6979832 and rs10487506: rs10487506 (P=0.0003), rs6979832*time (P=0.047) and rs6979832*rs10487506*time (P=0.004). These results are in line with the analyzed LDs, with moderate LD between rs6979832 and rs2278815 and low LD between rs6979832 and rs10487506.

Allele-specific effects on leptin expression

In a multiple-tissue eQTL comparison, the C-allele of rs10487505 mediated a significant upregulation of LEP expression (ENSG00000174697.4) in fibroblasts (P=1.8* 10^{-10}, effect size = 0.14, GTEx variant 7_128220110_G_C_b38). The G-allele of the proxy SNP rs6979832 displayed a similar effect (P=2.7* 10^{-10}, effect size = 0.14, GTEx variant 7_128216223_A_G_b38). The eQTL comparison found no effect of either SNP in other tissues, such as human adipocytes or adult brain. See Supplementary Material, **eFigure 1.**

Discussion

In addition to our previous report on the role of SNPs rs2278815 and rs10487506 (Bopp et al., 2019), we found a significant genotype-dependent effect of LA on BMI and on BMI increase over time for rs10487505, a top SNP from a GWAS of circulating leptin levels in populations of European ancestry (Kipeläinen et al., 2016). GG-carriers of rs6979832, a nearly perfect proxy SNP of rs10487505, demonstrated a significantly lower BMI than GA- and AA-carriers, and during LA BMI increased less in GG-carriers than in GA- and AA-carriers. Furthermore, we explored potential joint effects between rs6979832 and the SNPs rs2278815 and rs10487506. This result is comparable to our previous study when taking into account the relationships of pairwise LD, with moderate LD between rs6979832 and rs2278815, and low LD between rs6979832 and rs10487506. Taken together, in the additive genetic model the SNP*SNP*time interactions showed a significant effect on BMI, but not all of the SNPs or SNP*time interactions remained significant. Based on these results, individual SNP alleles and the SNP alleles in combination seem to have specific roles on the investigated traits in patients.

In a previous investigation, we found a significant positive association of leptin levels with BMI during LA (Ricken et al. 2016), but similar to our actual finding, we could not detect associations of two leptin polymorphisms (LEP rs2278815 and rs10487506) with leptin

serum levels (Bopp et al., 2019). Earlier studies investigated the effect of leptin polymorphisms on leptin levels - mostly for rs7799039, a proxy SNP to rs10487506 - with inconclusive results regarding the effect direction and the effect alleles (e.g. Dasgupta et al., 2014; Marcello et al., 2015). As in the present dataset, the number of patients for which leptin levels were available is considerably smaller (39.9%) than those for which BMI data are available, this reduced the statistical power of our analysis. Therefore, it remains unclear whether rs10487505 also has an effect on leptin levels during LA (similar to LEP rs2278815 and rs10487506; Bopp et al., 2019).

In a meta-analysis of whole-genome association data in up to 52,126 individuals of European descent from 23 studies Kipeläinen et al. (2016) identified 5 chromosomal loci associated with circulating leptin levels. For the LEP polymorphism rs10487505 genome-significance was confirmed in the BMI-adjusted meta-analysis, with the minor G-allele as the leptin increasing allele. Kipeläinen et al. could not find an association with LEP messenger RNA expression in the omental or subcutaneous adipose tissue, liver, lymphocytes, brain or skin. This finding is in line with the result of multiple-tissue eQTL comparison from GTEx that suggests an effect of the rs10487505 C-allele (and rs6979832 G-allele) on LEP messenger RNA expression in fibroblasts, however not in other tissues. This is novel evidence since Kipeläinen et al. did not analyze this cell type or its tissue. For circulating leptin levels, Kipeläinen et al. also suggest a causal role for rs6979832, the proxy SNP for rs10487505 employed in the present study, which overlaps with predicted enhancer elements in cultured adipose cells of the Roadmap Epigenomics Project (Roadmap Epigenomics Consortium, 2015).

Here we present the first study that investigated the effect of rs10487505 on BMI increase and leptin levels during LA. So far, the mechanisms of lithium-induced weight gain are less understood. Through inhibition of the enzyme glycogen synthase kinase-3-beta lithium could activate the WNT Signaling System (Meffre et al., 2014). Activated WNT signaling was associated with inhibition of adipogenesis (Ross et al., 2000) and increased expression of LEP mRNA in adipocytes (Wabitsch et al., 1996). Evidence suggests that hypothalamic WNT

signaling is involved in energy balance regulation and glucose homeostasis (Helfer and Tups, 2016). Furthermore, data indicate that leptin may inhibit glycogen synthase kinase-3-beta and activate WNT signaling (Benzler et al., 2013). An interplay of leptin and lithium could regulate energy homeostasis.

Regarding SNP effects, the observed mutual dependence of rs10487505, rs2278815 and rs10487506 on BMI during LA suggest two noteworthy findings emerging from the analyses that take either rs2278815 or rs10487506 into account. First, when controlling for the effects of rs2278815 or rs10487506, rs10487505 appears to specifically influence BMI change over time, but not BMI alone, which may point to a specific role in lithium-induced weight gain. Furthermore, the significant three-way interactions in both models (SNP*SNP*time) strongly suggest a complex interplay of genetic factors at the LEP locus. These results emphasize the importance of SNPs at the LEP locus in lithium-induced weight gain.

Conflict of interest

Author Adli received grants / research support from the Alfred Herrhausen Society and Servier. He received speaker honoraria from Deutsche Bank, the German Federal Agency for Civic Education, ViiV, Gilead Sciences, MSD, Servier, Aristo and Lundbeck and has been a consultant to Lundbeck, Merz, mytomorrows, Deutsche Bank and MSD. Author Ricken received an unrestricted research grant from Aristo. Authors Bopp, Heilbronner, Schlattmann, Buspavanich, Mühleisen, Lang, Heinz and Schulze declare no potential conflict of interest.

Author contributions

Authors Bopp and Heilbronner contributed equally as first authors. Authors Ricken and Mühleisen contributed equally as last authors. Author Ricken had full access to all data and takes responsibility for the integrity of the data and the accuracy of the data analysis. Authors Ricken and Adli designed the study and wrote the protocol. Authors Bopp, Heilbronner, Schlattmann and Mühleisen undertook the statistical analysis. Authors Bopp, Heilbronner, Ricken and Mühleisen managed the literature searches and analyzes. Authors Bopp and Heilbronner wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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