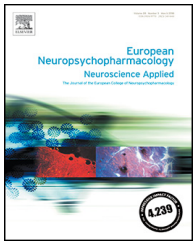
European Neuropsychopharmacology (2019) 29, 211–221

www.elsevier.com/locate/euroneuro



Leptin gene polymorphisms are associated with weight gain during lithium augmentation in patients with major depression

Sandra K. Bopp[a,1], Urs Heilbronner[b,1], Peter Schlattmann[c], Thomas W. Mühleisen[d,e], Tom Bschor[f,g], Christoph Richter[h,a], Bruno Steinacher[i], Thomas J. Stamm[a,n], Angela Merkl[a,j], Stefan Herms[e,k], Stephan Köhler[a], Philipp Sterzer[a], Rainer Hellweg[a], Andreas Heinz[a], Sven Cichon[d,e,o], Undine E. Lang[l], Thomas G. Schulze[b,m], Mazda Adli[a,j,1], Roland Ricken[a,1,*]

[a] *Department of Psychiatry and Psychotherapy, Charité University Medicine Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany*
[b] *Institute of Psychiatric Phenomics and Genomics (IPPG), University Hospital, LMU Munich, Germany*
[c] *Department of Statistics, Informatics and Documentation, Friedrich-Schiller-University Jena, Jena, Germany*
[d] *Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich, Germany*
[e] *Human Genomics Research Group and Division of Medical Genetics, Department of Biomedicine, University of Basel, Basel, Switzerland*
[f] *Department of Psychiatry, Schlosspark Hospital Berlin, Berlin, Germany*
[g] *Department of Psychiatry and Psychotherapy, Technical University of Dresden Medical School, Dresden, Germany*
[h] *Department of Psychiatry and Psychotherapy, Vivantes Hospital, Kaulsdorf, Berlin, Germany*
[i] *Department of Psychiatry and Psychotherapy, Vivantes Hospital Wenckebach, Berlin, Germany*
[j] *Department of Psychiatry and Psychotherapy, Fliedner Hospital Berlin, Berlin, Germany*
[k] *Institute of Human Genetics, University of Bonn, Bonn, Germany*
[l] *Department of Psychiatry and Psychotherapy, University Psychiatric Clinics (UPK),University of Basel, Switzerland*
[m] *Department of Psychiatry and Psychotherapy, University Medical Center, Georg-August-University, Göttingen, Germany*

* Corresponding author.
E-mail address: roland.ricken@charite.de (R. Ricken).
[1] These authors contributed equally to the manuscript.

https://doi.org/10.1016/j.euroneuro.2018.12.006
0924-977X/

[n] *Medical School Brandenburg Theodor Fontane, Neuruppin, Germany*
[o] *Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland*

Received 16 May 2018; received in revised form 29 November 2018; accepted 1 December 2018

KEYWORDS
Lithium;
Depression;
Weight gain;
Leptin;
SNP

Abstract
Weight gain is a common adverse effect of lithium augmentation. Previous studies indicate an impact of genetic variants at the leptin gene on weight gain as a consequence of psychopharmacological treatment. The primary aim of our study was to identify variants at the leptin locus that might predict lithium-induced weight gain. The secondary aim was to investigate if these variants modulate leptin levels. In 180 patients with acute major depressive disorder, body mass index was measured before and after 4 weeks of lithium augmentation, in a subsample also after 4 and/or 7 months. In a subsample of 89 patients, leptin serum concentrations were measured before and during lithium augmentation. We used linear mixed model analyzes to investigate the effects of 2 polymorphisms at the leptin locus (rs4731426 and rs7799039, employing the respective proxy SNPs rs2278815 and rs10487506) on changes in body mass index and leptin levels. For both polymorphisms, which are in high linkage disequilibrium, body mass index was significantly lower in homozygous A-allele carriers than in carriers of other genotypes at baseline. Over the follow-up period, body mass index increased less in homozygous A-allele carriers of rs4731426 than in carriers of other genotypes. This was not the case for rs7799039. Neither polymorphism modulated leptin protein expression. Our study strongly supports the hypothesis that genetic variability at the leptin locus is involved in lithium augmentation-associated weight gain in major depressive disorder. Furthermore, Genotype-Tissue Expression data provide strong evidence that rs4731426 influences the expression of leptin messenger ribonucleic acid in fibroblasts.

1. Introduction

Lithium augmentation (LA) is an effective strategy in the treatment of major depressive disorder (MDD) (Bschor, 2014). Weight gain is a common adverse effect of lithium treatment that occurs in 25%—62% of patients (Livingstone and Rampes, 2006). Weight gain leads to obesity, increases the risk of developing metabolic disorders and is a serious reason for treatment interruption and a decreased quality of life (Vestergaard and Licht, 2001).

Leptin, an anorexigenic protein hormone, is primarily secreted by white adipose tissue. Through its hypothalamic receptor, leptin seems to influence several biological functions involved in the pathophysiology of obesity (e.g. Chen et al., 2016). It decreases body weight by both suppressing appetite and increasing energy expenditure (Morris and Rui, 2009).

Different polymorphisms in the leptin gene (LEP) are discussed to be involved in obesity, weight gain, and metabolic diseases (Dahlman and Arner, 2007; Yu et al., 2012). Over the past years, numerous studies were undertaken to discover genetic variants that may be involved in weight gain triggered by psychopharmacological treatment. However, the majority of studies focused on antipsychotic-induced weight gain (AIWG). Research on LEP polymorphisms focused mainly on rs7799039 (−2548 G/A) and produced inconsistent results (Lee and Bishop, 2011), although several studies found an association of the G allele of rs7799039 with AIWG (Ellingrod et al., 2007; Brandl et al., 2012). A small number of studies also analyzed other polymorphisms in LEP, e.g. rs4731426 and rs10954173, and reported an association with AIWG (Brandl et al., 2012; Srivastava et al., 2008).

We found only 1 earlier study that explored polymorphisms in genes and their influence on weight gain during lithium treatment (Zill et al., 2003). This study found no association between polymorphisms in the alpha-subunit of the G(olf) gene and weight gain in 149 bipolar patients on lithium treatment.

In a previous study, we found an effect of leptin serum levels on weight gain during LA (Ricken et al., 2016). Genotype-dependent effects on leptin serum levels are described in the literature (Ben Ali et al., 2009; Kloiber et al., 2013). On the basis of these previous findings and the pharmacogenetic knowledge gap regarding lithium-induced weight gain, we investigated in a prospective cohort study the effect of polymorphisms in LEP on body mass index (BMI) and leptin serum levels during LA. The primary aim of our study was to identify polymorphisms in LEP that are associated with lithium-induced weight gain. The secondary aim was to investigate whether these variants modulate serum leptin levels.

2. Experimental procedures

2.1. Participants

A total of 185 patients with MDD were recruited between December 2008 and December 2012 at 12 psychiatric departments of the

Berlin Research Network on Depression, Berlin, Germany. BMI data were available for all 185 patients before and after 4 weeks of LA, for 61 patients after 4 months of LA and for 66 patients after 7 months of LA. Leptin serum levels were available for 89 patients before and after 4 weeks of LA. Inclusion criteria were as follows: Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV diagnosis MDD, age older than 18 years, indication for antidepressant pharmacotherapy, insufficient response to previous adequate antidepressant treatment, clinical indication for LA and Hamilton Depression Rating Scale (HDRS-17) score ≥ 12 (Hamilton, 1960). The DSM-IV diagnosis was confirmed by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998). To control for potential population stratification, we analyzed the genome-wide data of the 185 participants together with data from the third phase of The International HapMap project (The International HapMap project, 2017). We carried out principal components analysis (PCA) with smartpca (version 13,050) to calculate ancestry components (Patterson et al., 2006). On the basis of a visualization of the first 2 principal components, we excluded 5 participants with high genetic divergence from our sample. The local ethics committee approved the study and written informed consent was obtained from all participants.

2.1.1. Procedures

Severity of depression, BMI (kg/m^2), and leptin serum concentrations (non-fasting) were measured at baseline and after 4 weeks of LA, and follow-up data were obtained after 4 or 7 months or both. Blood samples were collected by peripheral venipuncture. Obesity was defined as a BMI $\geq 30\,kg/m^2$. All patients received individual doses of lithium carbonate and 1 or more antidepressants (see Table 1).

2.2. Laboratory analysis

DNA was extracted according to the manufacturer's recommendations (Chemagen, Baesweiler, Germany) and genotyped genome-wide by Infinium assays (Illumina, San Diego, CA, USA) for BeadChips Human660W-Quad and HumanOmni1-Quad.

Leptin levels were measured in the re-thawed serum samples with the RayBioHuman Leptin ELISA kit, according to the manufacturer's instructions.

2.3. Single nucleotide polymorphisms

Based on a literature search on LEP SNPs associated with obesity and psychopharmaceutical-induced weight gain, the marker content of the genome-wide assays, and the linkage disequilibrium (LD) patterns in Europeans between SNPs identified by the literature search, we selected three SNPs to be analyzed in the present study: rs7799039, rs10954173 and rs4731426.

These were investigated using proxy SNPs (rs10487506 for rs7799039, distance = 628 bp, $r^2 = 1.000$, D' = 1.000; rs11760956 for rs10954173, distance = 353 bp, $r^2 = 1.000$, D' = 1.000; and rs2278815 for rs4731426, distance = 219 bp, $r^2 = 1.000$, D' = 1.000.

We analyzed LD patterns in Europeans between these SNPs and found moderate LD between rs11760956 and both rs10487506 ($r^2 = 0.487$, D' = 0.898) and rs2278815 ($r^2 = 0.664$, D' = 0.917), but fairly high LD between rs2278815 and rs10487506 ($r^2 = 0.766$, D' = 1.000). Proxy search and LD between SNPs were determined using the SNAP proxy search (SNAP Proxy Search of the Broad Institute, 2016) with the 1000 Genomes Pilot 1 as reference. The SNPs were extracted from genome-wide genotype data. The positions of rs2278815 and rs10487506 (the SNPs that showed significant associations with BMI in the screening analyzes; see below) are visualized in Supplementary Material, Fig. 1.

When LD between rs2278815 and rs10487506 was determined empirically in our study sample, high LD between both markers ($r^2 = 0.740$, D' = 1.000) was confirmed.

Minor allele frequencies in our sample (rs2278815 (G): 0.467, rs10487506 (A): 0.458) were similar to those identified in the European population (rs2278815 (G): 0.446, rs10487506 (A): 0.442; using LDlink (2018)). Experimental clusterplots of the proxy variants rs2278815 and rs10487506 are visualized in Supplementary Material, Fig. 2.

2.4. Analysis of allele-specific expression

Data from the Genotype-Tissue Expression (GTEx) project (Genotype-Tissue Expression project, 2017) were used to evaluate the allele-specific expression of LEP mRNA for rs4731426 and rs2278815 in a multiple tissue quantitative trait locus (eQTL) comparison. The data request was executed on June 6, 2017, with GTEx Analysis Release V6p (dbGaP Accession phs000424.v6.p1).

2.4.1. Statistical analysis

We used random-intercept linear mixed models to investigate SNP effects on BMI. First, we performed a screening analysis to investigate the effect of each SNP on BMI. Second, we analyzed the effect of genotype on BMI with an additive genetic model for the 2 A/G SNPs that showed an association in the screening analysis (rs2278815 and rs10487506). Covariates were obesity before LA, sex, age, HRDS-17 score, and psychopharmacological co-medication with a high risk of weight gain (see Table 1). This last covariate also included antipsychotics known to be associated with weight gain, allowing us to correct for their effect on BMI. Third, we analyzed the effects on BMI of the AA versus the G allele. The covariates were the same as in the additive model. As a final step, we analyzed the effects of both SNPs on leptin serum concentration, with leptin level as the dependent variable and time, SNP, and SNP*time interaction as covariates. We used a Referencing Type III test of fixed effects as an overall test for the respective term in the model. The results of this test, for example the overall effect of time at different time points, are presented in the Results section. Tables 2 and 3 show the estimates of the model's individual coefficients, for example the change in BMI at the individual measurement points compared with baseline. Model regression coefficients are reported together with their standard error (SE) estimates and a 95% confidence interval (CI). The Kolmogorov-Smirnov test was used to assess potential deviation from Gaussian distribution. The nominal significance level was set at 0.05 in all analyzes except in the screening analysis, for which it was set at 0.1. To control Type-I error cumulation in the separate linear mixed model analyzes of rs2278815 and rs10487506 (the SNPs that showed significant associations with BMI in the screening analyzes; see below) we used the software Single Nucleotide Polymorphism Spectral Decomposition (Nyholt, 2004). We determined an experiment-wide significance threshold required to keep Type-I error rate at 0.05 of 0.040. 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.

3. Results

3.1. Clinical characteristics

The demographic and clinical data are summarized in Table 1. A total of 446 BMI measurements were obtained from 180 patients. BMI increased significantly over the observation period. Allele distributions of the 3 SNPs did not

Table 1 Clinical data and results.

	n (%)								
Patients	185 (100)								
Female	117 (63.2)								
Male	68 (36.8)								
Obesity before LA	31 (16.8)								
MRWG	87 (47.0)								
		Minor allele	MAF						
rs2278815		G	0.47						
AA homozygous	53 (28.3)								
AG heterozygous	88 (47.1)								
GG homozygous	44 (23.5)								
rs10487506		A	0.46						
AA homozygous	42 (22.5)								
AG heterozygous	83 (44.4)								
GG homozygous	60 (32.1)								
Psychotropic comedication									
Antidepressants									
SSRI	91 (49.2)								
SNRI	54 (29.2)								
TCA	17 (9.2)								
Bupropione	10 (5.4)								
Agomelatine	33 (17.8)								
Mirtazapine	12 (14.1)								
MAO-I	5 (2.7)								
Atypical antipsychotics	51 (27.6)								
Antiepileptic drugs	14 (7.6)								
Benzodiazepines	43 (23.2)								
Low-potency antipsychotics	9 (4.9)								
		Mean (SD)*	***n* (%)**	**Mean (SD)***	***n* (%)**	**Mean (SD)***	***n* (%)**	**Mean (SD)***	
Age	185 (100)	49.04 (13.63)							
Lithium serum level (mmmol/ml) after four weeks of LA	159 (85.9)	0.705 (0.167)							
		Before LA		**After LA**		**First follow up**		**Second follow up**	***P* value** **
BMI	185 (100)	25.71 (5.45)	185 (100)	26.16 (5.42)	61 (33.0)	25.89 (4.22)	66 (35.7)	27.21 (5.60)	<0.05
HDRS-17 score	184 (99.5)	21.46 (5.40)	185 (100)	12.31 (7.08)		-	-	-	<0.05
Leptin serum level (in pg/ml)	89 (49.4)	265.78 (366.79)	89 (49.4)	304.73 (463.63)		-	-	-	<0.05

Legend: MAF = minor allele frequency; LA = Lithium augmentation; MRWG = Comedication with a risk for weight gain; SSRI = Serotonin Reuptake Inhibitors; SNRI = Serotonin and Norepinephrine Reuptake Inhibitor; TCA: Tricyclic Antidepressant; MAO-I = Mono Amine Oxidase Inhibitor (Tranylcypromine); SD = standard deviation; HDRS-17 = Hamilton Depression Rating Scale; BMI = body mass index.

* result of descriptive statistics.

** result of paired sample *t*-test, for BMI the value before lithium augmentation was compared each with the value after 4 weeks (after lithium augmentation), after 4 months (first follow up) and after 7 months (second follow up); for HDRS-17 score and leptin serum level the value before lithium augmentation was compared each with the value after 4 weeks (after lithium augmentation).

Table 2 The effect of rs2278815 and rs10487506 genotypes and covariates on the course of BMI during LA.

SNP	Covariate	Genotype	Time*	Estimate**	Estimate***	95%- CI		*t* value	*P* value
						Lower	Upper		
rs2278815									
			1	23.14		20.57	25.71	17.76	<0.0001
			2		0.16	−0.27	0.57	0.70	0.486
			3		−0.37	−1.09	0.35	−1.02	0.309
			4		0.61	−0.06	1.27	1.80	0.074
		GA	1		1.28	0.005	2.56	1.98	0.049
		GG	1		1.72	0.17	3.27	2.18	0.030
		AA	1		0	.	.	.	.
		GA	2		0.18	−0.28	0.63	0.77	0.440
		GG	2		0.40	−0.15	0.94	1.44	0.151
		AA	2		0	.	.	.	.
		GA	3		1.18	0.36	2.00	2.84	0.005
		GG	3		1.58	0.63	2.52	3.29	0.001
		AA	3		0	.	.	.	.
		GA	4		0.81	−0.01	1.63	1.94	0.053
		GG	4		0.88	−0.05	1.81	1.87	0.063
		AA	4		0	.	.	.	.
	MRWG				−1.36	−2.43	−0.29	−2.51	0.013
	Sex: female				.	.	.	.	.
	male				1.20	0.01	2.32	2.12	0.035
	Age				0.01	−0.04	0.05	0.26	0.792
	HDRS-17				10.82	9.38	12.27	14.78	<0.0001
	Obesity before LA				−0.008	−0.03	0.02	−0.66	0.511
rs10487506									
			1	23.01		20.28	25.73	16.66	<0.0001
			2		0.17	−0.29	0.63	0.72	0.470
			3		−0.13	−0.93	0.67	−0.32	0.746
			4		0.67	−0.01	1.35	1.93	0.054
		GA	1		1.84	0.45	3.23	2.61	0.010
		GG	1		1.49	−0.06	3.03	1.90	0.059
		AA	1		0	.	.	.	.
		GA	2		0.20	−0.29	0.70	0.80	0.425
		GG	2		0.20	−0.33	0.74	0.77	0.448
		AA	2		0	.	.	.	.
		GA	3		0.96	0.04	1.87	2.06	0.040
		GG	3		0.93	0.003	1.85	1.97	0.049
		AA	3		0	.	.	.	.
		GA	4		0.99	0.12	1.86	2.25	0.025
		GG	4		0.45	−0.43	1.34	1.01	0.313
		AA	4		0	.	.	.	.
	MRWG				−1.46	−2.55	−0.38	−2.66	0.009
	Sex: female				.	.	.	.	.
	male				1.23	0.12	2.35	2.18	0.030
	Age				0.01	−0.03	0.05	0.29	0.769
	HDRS-17				−0.01	−0.03	0.02	−0.64	0.526
	Obesity before LA				10.80	9.35	12.24	14.75	<0.0001

Example of how to read the table: The estimated BMI for rs10487506 AA carriers at time 3 is calculated as follows: (estimated BMI at time 1 in AA: 23.01) + (estimated BMI at time 3 in AA: - 0.13) = 22.88

Legend: LA = Lithium augmentation; MRWG = Comedication with a risk for weight; HDRS-17 = Hamilton Depression Rating Scale; BMI = body mass index; CI = confidence intervall.

* duration LA: 1 = baseline (before LA), 2 = after 4 weeks of LA (study endpoint), 3 = after 4 months of LA (first follow up visit), 4 = after 7 months of LA (second follow up visit).

** effect of time displayed for AA-genotype as reference-genotype in BMI in kg/m^2.

*** difference in BMI compared to the reference-value of AA.

Table 3 The effect of the AA genotype and G allele genotypes of rs2278815 and rs10487506 and covariates on the course of BMI during LA.

SNP	Covariate	Genotype	Time*	Estimate**	Estimate***	95%- CI		*t* value	*P* value
						Lower	Upper		
rs2278815									
			1	23.25		22.70	25.80	17.97	<0.0001
			2		0.15	−0.27	0.57	0.70	0.482
			3		−0.37	−1.09	0.34	−1.02	0.308
			4		0.60	−0.06	1.27	1.80	0.072
		AA* **	1		0	.	.	.	.
		GG or GA	1		1.41	0.20	2.62	2.30	0.023
		AA	2		0	.	.	.	.
		GG or GA	2		0.25	−0.18	0.67	1.14	0.254
		AA	3		0	.	.	.	.
		GG or GA	3		1.31	0.53	2.08	3.33	0.001
		AA	4		0	.	.	.	.
		GG or GA	4		0.83	0.08	1.58	2.17	0.031
	MRWG				−1.36	−2.43	−0.29	−2.51	0.013
	Sex: female				.	.	.	.	.
	male				1.20	0.08	2.32	2.12	0.036
	Age				0.003	−0.04	0.04	0.16	0.873
	HDRS-17				−0.01	−0.03	0.02	−0.65	0.514
	Obesity before LA				10.85	9.41	12.29	14.84	<0.0001
rs10487506									
			1	22.82		20.17	25.47	16.98	<0.0001
			2		0.17	−0.29	0.72	0.69	0.472
			3		−0.13	−0.93	0.66	−0.33	0.742
			4		0.66	−0.01	1.34	1.93	0.054
		AA* **	1		0	.	.	.	.
		GG or GA	1		1.71	0.41	3.02	2.59	0.010
		AA	2		0	.	.	.	.
		GG or GA	2		0.20	−0.26	0.66	0.87	0.385
		AA	3		0	.	.	.	.
		GG or GA	3		0.94	0.10	1.78	2.21	0.028
		AA	4		0	.	.	.	.
		GG or GA	4		0.73	−0.04	1.50	1.88	0.062
	MRWG				−1.42	−2.48	−0.35	−2.63	0.009
	Sex: female				.	.	.	.	.
	male				1.23	0.12	2.35	2.18	0.030
	Age				0.01	−0.03	0.05	0.29	0.769
	HDRS-17				−0.01	−0.03	0.02	−0.65	0.515
	Obesity before LA				10.80	9.36	12.24	14.80	<0.0001

Example of how to read the table: The estimated BMI for patients with the rs10487506 G allele at time 3 is calculated as follows: (estimated BMI at time 1 in AA: 22.82) + (estimated BMI at time 3 in AA: −0.13) + (estimated BMI at time 3 in GG or GA: 0.94) = 23.63

Legend: LA = Lithium augmentation; MRWG = Comedication with a risk for weight; HDRS-17 = Hamilton Depression Rating Scale; BMI = body mass index; CI = confidence interval.

* Duration LA: 1 = baseline (before LA), 2 = after 4 weeks of LA (study endpoint), 3 = after 4 months of LA (first follow up visit), 4 = after 7 months of LA (second follow up visit).

** effect of time displayed for AA-genotype as reference-genotype in BMI in kg/m^2.

*** difference in BMI compared to the reference-value of AA.

deviate significantly from the Hardy-Weinberg equilibrium ($P > 0.05$).

3.1.1. Screening analysis

We found significant effects of rs2278815 ($P = 0.025$) and rs10487506 ($P = 0.028$) on BMI. However, rs11760956 did not influence BMI ($P = 0.124$) and was therefore not included in further analyzes. The effects of rs2278815 and rs10487506 were analyzed in separate statistical models.

3.2. Additive genetic models

Fig. 1 displays the course of BMI during the study period, stratified according to the genotypes of rs2278815

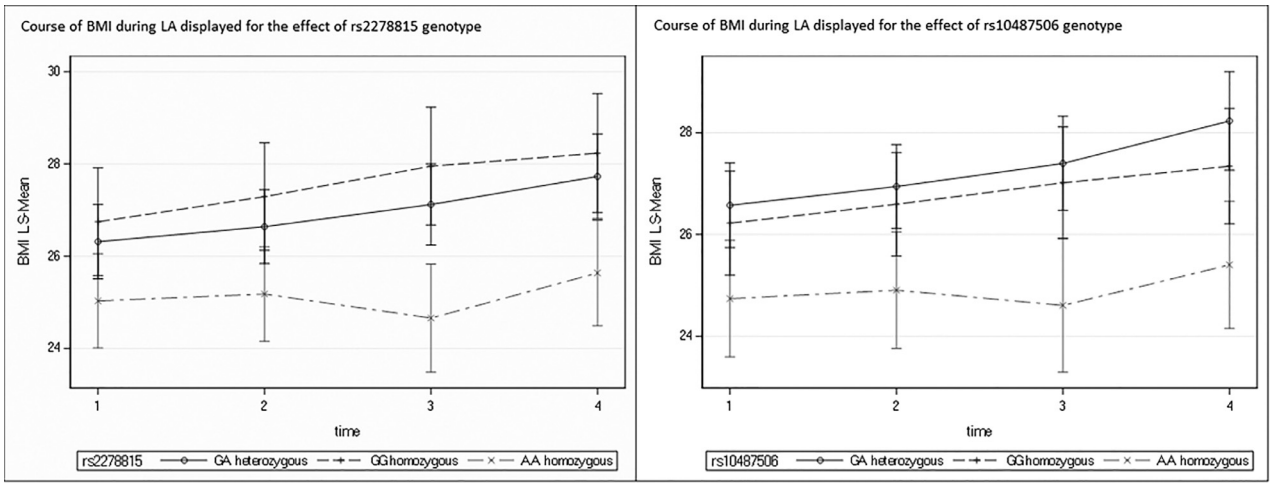


Fig. 1 LA = lithium augmentation; BMI = body mass index (kg/m^2) time: 1 = baseline (before LA), 2 = after 4 weeks of LA (study endpoint); 3 = after 4 months of LA (first follow up visit), 4 = after 7 months of LA (second follow up visit)
For values of 95%-confidence intervals see Table 2.

and rs10487506. The detailed parameters of both statistical models, including all covariates, are displayed in Table 2.

Rs2278815 ($P = 0.004$), time ($P < 0.0001$), and the rs2278815*time interaction ($P = 0.030$) showed significant effects on BMI. Medication with a risk for weight gain (MRWG) had a significant negative effect on BMI ($P = 0.013$), whereas obesity before LA ($P < 0.0001$) had a significant positive effect. Sex was significant ($P = 0.035$), with males having a higher BMI, whereas HDRS-17 score ($P = 0.511$) and age ($P = 0.792$) were not.

Both rs10487506 ($P = 0.004$) and time ($P < 0.0001$) showed significant effects on BMI, whereas the rs10487506*time interaction did not ($P = 0.217$). Again in this model, MRWG had a significant negative effect on BMI ($P = 0.009$), and obesity before LA ($P < 0.0001$) had a significant positive effect. Sex was significant ($P = 0.037$), with males having a higher BMI, whereas HDRS-17 score ($P = 0.526$) and age ($P = 0.879$) were not.

3.3. Genotype models (AA versus G allele carriers)

Because the courses of BMI observed in AA carriers differed from those in GG and GA carriers, we analyzed potential G allele-specific effects on BMI by pooling GA and GG carriers for each SNP. Fig. 2 displays the course of BMI during the study period for rs2278815 and rs10487506, each stratified according to allele (AA versus G allele). Detailed parameters of both statistical models, including all covariates, are shown in Table 3.

Both rs2278815 (AA/AG + GG; $P = 0.001$) and the rs2278815 (AA/AG + GG)*time-interaction ($P = 0.006$) had significant negative effects on BMI; the latter may be interpreted as an influence of 1 or more G alleles on BMI over time. Patients homozygous for the A allele of rs2278815 had a significantly lower BMI and smaller BMI increase than patients with 1 or more G alleles. In this model, covariates with significant positive effects were time ($P < 0.0001$) and obesity before LA ($P < 0.0001$). MRWG had a significant negative effect ($P = 0.013$). Sex ($P = 0.036$) was significant, with males having a higher BMI, whereas HDRS-17 score ($P = 0.514$) and age ($P = 0.873$) were not.

Rs10487506 (AA/AG + GG) had a significant negative effect on BMI ($P = 0.001$). The rs10487506 (AA/AG + GG)*time-interaction ($P = 0.087$) was not significant. In this model, time ($P < 0.0001$) and obesity before LA ($P < 0.0001$) had a significant positive effect. MRWG showed a significant negative effect ($P = 0.009$). Sex ($P = 0.030$) was significant, with males having a higher BMI, whereas HDRS-17 score ($P = 0.514$) and age ($P = 0.769$) were not.

3.4. 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 effect of rs2278815 ($P = 0.554$), rs2278815*time ($P = 0.462$), rs10487506 ($P = 0.242$), or rs10487506*time ($P = 0.435$) on leptin levels.

3.5. Allele-specific effects on leptin expression

In a multiple-tissue eQTL comparison, the A allele of rs2278815 mediated a significant upregulation of LEP expression (ENSG00000174697.4) in fibroblasts ($P = 6.86^* 10^{-9}$, effect size = 0.19, GTEx variant 7_127,881,851_G_A_b37). See Supplementary Material, Table 1 and Fig. 3.

The direction of effect by the A allele of rs4731426 was similar and significant. The eQTL comparison found no effect of either SNP in other tissues, such as human adipocytes or adult brain.

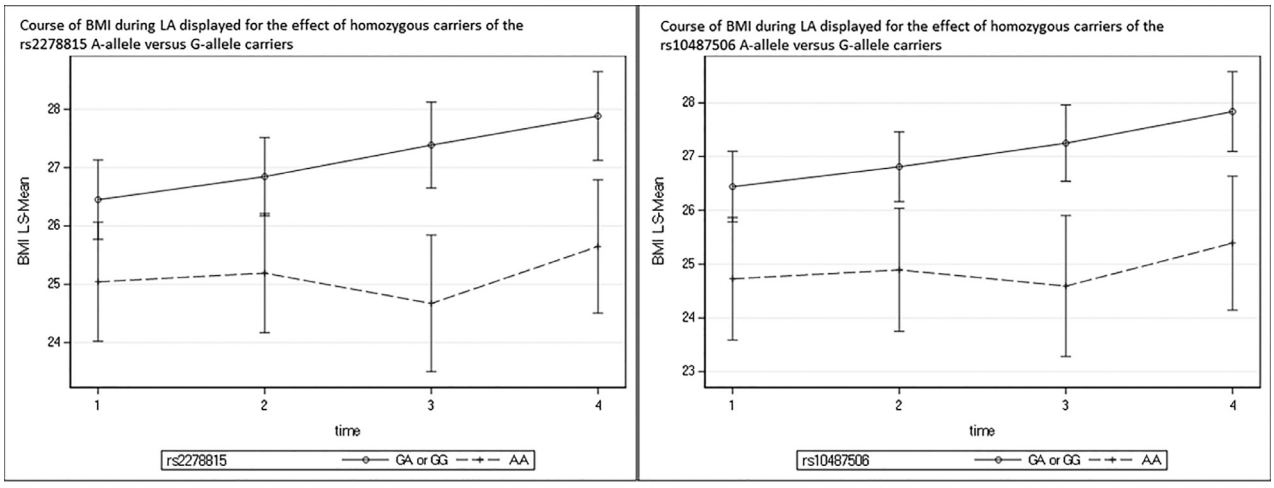


Fig. 2 LA = lithium augmentation; BMI = body mass index (kg/m^2) time: 1 = baseline (before LA), 2 = after 4 weeks of LA (study endpoint); 3 = after 4 months of LA (first follow up visit), 4 = after 7 months of LA (second follow up visit)
For values of 95%-confidence intervals see Table 3.

4. Discussion

We studied the influence of polymorphisms in LEP on weight gain and leptin serum levels during LA in patients with MDD. We found a significant genotype-dependent effect of LA on BMI for rs2278815 and rs10487506. For both SNPs, AA carriers had a significantly lower BMI than GA and GG carriers. Moreover, rs2278815 showed a significant effect on the course of BMI during LA: BMI increased less in AA carriers than in GA and GG carriers.

Because genotype data of rs4731426 were not available for our sample, we determined rs2278815 as a proxy marker for rs4731426. In a 6-week open-label study of olanzapine performed in North India, Srivastava et al. genotyped 14 polymorphisms, including both rs2278815 and rs4731426, in 154 patients with schizophrenia and found that the minor allele of rs4731426 (G) was associated with weight gain. The group also analyzed the effect of rs2278815 on weight gain but did not find a significant association (Srivastava et al., 2008). Given the strong LD between the genotypes of rs4731426 and those of rs2278815 in Europeans, we were surprised that the result of the study by Srivastava et al. was inconsistent with ours, especially because a similar pattern of high LD has been observed in the North Indian Gujarati population (LDlink, 2017). Srivastava et al. found a low LD ($r^2 = 0.199$) between rs4731426 and rs2278815 in their patient sample, however, which seems to explain this apparent discrepancy.

Dasgupta et al. studied 304 obese individuals and 309 controls in the South Indian population and found that rs4731426 was associated with BMI (Dasgupta et al., 2014). However, the study reported that the C allele was both the minor and the effect allele of rs4731426 associated with higher BMI. These results are not in line with the study by Srivastava et al. As argued by Dasgupta (personal communication), differences in minor allele frequencies between the 2 study samples may be due to differences between the North and South Indian sub-populations (Juyal et al., 2014). These putative population differences are not reflected in the South Indian Telugu population, where G was also found to be the minor allele of rs4731426, but may exist nevertheless. The observation of different effect alleles at 1 locus, as in the studies by Srivastava et al. and Dasgupta et al., is not uncommon, even in similar ethnic groups. These so-called "flip-flop effects" may indicate that the SNP in question is correlated with a causal variant at another locus (Lin et al., 2007). Rs4731426 may therefore not be the causal variant mediating the observed effect of lithium on weight gain. In support of this interpretation, rs2278815 and rs4731426 do not lead to known sequence variation in the LEP transcripts and are not known to affect splicing (Erez et al., 2011). Therefore, putative effects on leptin levels may be secondary to variants in high LD to rs2278815 or rs4731426. We may have been unable to detect a genotype effect on circulating leptin levels also because of the tissue specificity of leptin. GTEx data suggest that rs2278815 functions as a fibroblast-specific eQTL, i.e. the A allele mediates an upregulation of LEP mRNA expression. Although leptin protein secretion and expression of LEP mRNA have been shown in human fibroblasts (Glasow et al., 2001), multiple-tissue eQTL comparison data did not find this effect in other tissues. Genotype-specific leptin secretion by fibroblasts may thus contribute to total leptin in plasma (Glasow et al., 2001). However, its effects may be small and thus harder to detect, which might partially account for the disruptions in the relationship between BMI and leptin levels observed in our study.

In our study, AA carriers of rs10487506, a proxy SNP for rs7799039, had a significantly lower BMI than G allele carriers but showed only a trend towards a smaller BMI increase during LA. The A allele in rs10487506 corresponds to the A allele in rs7799039, given local and worldwide minor allele distributions. Studies of rs7799039 identified an association with AIWG (Lee and Bishop, 2011), but the direction of the allelic association is unclear (Templeman et al., 2005; Calarge et al., 2009; Perez-Iglesias et al., 2010). Rs7799039 is a functional SNP located in the promoter region of LEP and may regulate the rate of mRNA transcription and leptin

secretion. In 39 unmedicated, non-obese females, Hoffstedt et al. found a 50% higher rate of leptin secretion and 60% more LEP mRNA transcription in rs7799039 AA carriers than in G carriers (Hoffstedt et al., 2002). Other studies also found higher leptin levels in rs7799039 AA carriers (Dasgupta et al., 2014; Marcello et al., 2015). However, in the present study we did not find an association between rs10487506 and leptin levels. Two earlier studies also did not find an association of leptin levels with rs7799039 in 190 long-term clozapine-treated patients with schizophrenia (Klemettilä et al., 2015) or with rs10487506 in 3 consanguineous families with obesity (Fourati et al., 2013). The reasons for these discrepant findings are hard to pinpoint and may be due to many factors, such as differences in medication (lithium versus antipsychotics), low statistical power of the present study because of missing data, and participant characteristics (patients versus general population), including ethnicity.

Lithium acts as a direct (Klein and Melton, 1996) and indirect (Li et al., 2007) inhibitor of the enzyme glycogen synthase kinase 3 beta (GSK3B). Through inhibition of GSK3B, lithium activates the Wnt signaling system (Meffre et al., 2014). Activated Wnt signaling was shown to inhibit adipogenesis (Ross et al., 2000; Longo et al., 2004). Furthermore, Wnt signaling activation through insulin injection is associated with increased expression of LEP mRNA in adipocytes in both mice (Chen et al., 2015) and humans (Wabitsch et al., 1996), providing a putative molecular interface for the interaction of lithium and leptin. Increased plasma leptin levels were observed in patients with non-insulin-dependent diabetes mellitus and healthy people after insulin infusion (Malmström et al., 1996). In an earlier study, our group found increased leptin serum levels in 89 patients after 4 weeks of LA (Ricken et al., 2016). This finding is in line with 1 earlier study that found higher leptin levels after lithium treatment (Atmaca et al., 2002) but not with other research (Baptista et al., 2000; Himmerich et al., 2005; Soeiro-de-Souza et al., 2014). As demonstrated in the present study, genetic variation of LEP is able to modulate the relationship between weight gain and lithium treatment. However, an integrative model has yet to be developed.

In conclusion, AA carriers of rs2278815 may be less susceptible to weight gain during LA, which makes this SNP a promising biomarker for clinical risk assessment and prediction of adverse effects. Further studies are needed to confirm our findings in independent replication samples and to formulate a comprehensive model of the interaction of LEP and leptin levels in lithium treatment in affective disorders.

Conflict of interest

Author Köhler received speaker honoraria from Aristo pharma. Author Hellweg received speaker honoraria from Bristol-Myers Squibb, Janssen-Cilag, Lundbeck, Merz, and Otsuka. He is a consultant to Lundbeck and Otsuka. 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, Mühleisen, Bschor, Richter, Steinacher, Stamm, Merkl, Herms, Sterzer, Heinz, Cichon, Lang and Schulze declare no potential conflict of interest.

Author contributions

Authors Bopp and Heilbronner contributed equally as first authors. Authors Ricken and Adli 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, Heilbronner and Ricken wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Role of the funding source

The study was funded by sources of the Mood Disorders Research Unit of Charité University Medicine, Berlin, Department of Psychiatry and Psychotherapy, Campus Charité Mitte (CCM) and by grants from the Deutsche Forschungsgemeinschaft (DFG): www.kfo241.de: SCHU 1603/5-1 and www.PsyCourse.de: SCHU 1603 /7-1 . Neither the Mood Disorders Research Unit of Charité University Medicine nor the DFG had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report and in the decision to submit the paper for publication.

Acknowledgments

We thank Jacquie Klesing, freelance Board-certified Editor in the Life Sciences (ELS) and Thomas Furlong, who provided editorial support for this manuscript.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2018.12.006.

References

Atmaca, M., Kuloglu, M., Tezcan, E., Ustundag, B., 2002. Weight gain and serum leptin levels in patients on lithium treatment. Neuropsychobiology 46 (2), 67–69.

Baptista, T., Lacruz, A., de Mendoza, S., Guillén, M.M., Burguera, JL., de Burguera, M., Hernández, L., 2000. Endocrine effects of lithium carbonate in healthy premenopausal women: relationship with body weight regulation. Prog. Neuropsychopharmacol. Biol. Psychiatry 24 (1), 1–16.

Ben Ali, S., Kallel, A., Ftouhi, B., Sediri, Y., Feki, M., Slimane, H., Jemaa, R., Kaabachi, N., 2009. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. Clin. Biochem. 42 (7-8), 584–588.

Brandl, E.J., Frydrychowicz, C., Tiwari, A.K., Lett, T.A., Kitzrow, W., Büttner, S., Ehrlich, S., Meltzer, H.Y., Lieberman, J.A., Kennedy, J.L., Müller, D.J., Puls, I., 2012. Association study of polymorphisms in leptin and leptin receptor genes with antipsychotic-induced body weight gain. Prog. Neuropsychopharmacol. Biol. Psychiatry 38 (2), 134–141.

Bschor, T., 2014. Lithium in the treatment of major depressive disorder. Drugs 74 (8), 855–862.

Calarge, C.A., Ellingrod, V.L., Zimmerman, B., Acion, L., Sivitz, W.I., Schlechte, J.A., 2009. Leptin gene -2548 G/A variants predict risperidone-associated weight gain in children and adolescents. Psychiatr. Genet. 19 (6), 320–327.

Chen, Z.L., Shao, W.J., Xu, F., Liu, L., Lin, B.S., Wie, X.H., Song, Z.L., Lu, H.G., Fantus, I.G., Wenig, J.P., Jin, T.R., 2015. Acute Wnt pathway activation positively regulates leptin gene expression in mature adipocytes. Cell Signal. 27 (3), 587–597.

Chen, H., Fajol, A., Hoene, M., Zhang, B., Schleicher, E.D., Lin, Y., Calaminus, C., Pichler, B.J., Weigert, C., Häring, H.U., Lang, F., Föller, M., 2016. PI3K-resistant GSK3 controls adiponectin formation and protects from metabolic syndrome. Proc. Natl. Acad. Sci. U. S. A. 113 (20), 5754–5759.

Dahlman, I., Arner, P., 2007. Obesity and polymorphisms in genes regulating human adipose tissue. Int. J. Obes. (Lond.) 31 (11), 1629–1641.

Dasgupta, S., Salman, M., Siddalingaiah, L.B., Lakshmi, G.L., Xaviour, D., Sreenath, J., 2014. Genetic variants in leptin: determinants of obesity and leptin levels in south Indian population. Adipocyte 4 (2), 135–140.

Ellingrod, V.L., Bishop, J.R., Moline, J., Lin, Y.C., Miller, D.D., 2007. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. Psychopharmacol. Bull. 40 (1), 57–62.

Erez, G., Tirosh, A., Rudich, A., Meiner, V., Schwarzfuchs, D., Sharon, N., Shpitzen, S., Blüher, M., Stumvoll, M., Thiery, J., Fiedler, G.M., Friedlander, Y., Leiterstdorf, E., Shai, I., 2011. Phenotypic and genetic variation in leptin as determinants of weight regain. Int. J. Obes. (Lond.) 35 (6), 785–792.

Fourati, M., Mnif, M., Kharrat, N., Charfi, N., Kammoun, M., Fendri, N., Sessi, S., Abid, M., Rebai, A., Fakhfakh, F., 2013. Association between Leptin gene polymorphisms and plasma leptin level in three consanguineous families with obesity. Gene 527 (1), 75–81.

Glasow, A., Kiess, W., Anderegg, U., Berthold, A., Bottner, A., Kratzsch, J., 2001. Expression of leptin (Ob) and leptin receptor (Ob-R) in human fibroblasts: regulation of leptin secretion by insulin. J. Clin. Endocrinol. Metab. 86 (9), 4472–4479.

Hamilton, M., 1960. A rating scale for depression. J Neurol Neurosurg. Psychiatry 23, 56–62.

Himmerich, H., Koethe, D., Schuld, A., Yassouridis, A., Pollmächer, T., 2005. Plasma levels of leptin and endogenous immune modulators during treatment with carbamazepine or lithium. Psychopharmacology (Berl) 179 (2), 447–451.

Hoffstedt, J., Eriksson, P., Mottagui-Tabar, S., Arner, P., 2002. A polymorphism in the leptin promoter region (-2548G/A) influences gene expression and adipose tissue secretion of leptin. Horm. Metab. Res. 34 (7), 355–359.

Juyal, G., Mondal, M., Luisi, P., Laayouni, H., Sood, A., Midha, V., Heutink, P., Bertranpetit, J., Thelma, B.K., Casals, F., 2014. Population and genomic lessons from genetic analysis of two Indian populations. Hum. Genet. 133 (10), 1273–1287.

Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. U.S.A. 93 (16), 8455–8459.

Klemettilä, J.P., Kampman, O., Seppälä, N., Viikki, M., Hämäläinen, M., Moilanen, E., Mononen, N., Lehtimäki, T., Leinonen, E., 2015. Association study of the HTR2C, leptin and adiponectin genes and serum marker analyzes in clozapine treated long-term patients with schizophrenia. Eur. Psychiatry 30 (2), 296–302.

Kloiber, S., Ripke, S., Kohli, M.A., Reppermund, S., Salyakina, D., Uher, R., McGuffin, P., Perlis, R.H., Hamilton, S.P., Pütz, B., Hennings, J., Brückl, T., Klengel, T., Bettecken, T., Ising, M., Uhr, M., Dose, T., Unschuld, P.G., Zihl, J., Binder, E., Müller-Myhsok, B., Holsboer, F., Lucae, S., 2013. Resistance to antidepressant treatment is associated with polymorphisms in the leptin gene, decreased leptin mRNA expression, and decreased leptin serum levels. Eur. Neuropsychopharmacol. 23 (7), 653–662.

LDlink, 2017. https://analysistools.nci.nih.gov/LDlink/ Accessed March 11, 2017.

LDlink, 2018. https://analysistools.nci.nih.gov/LDlink/ Accessed November 5, 2018.

Lee, A.K., Bishop, J.R., 2011. Pharmacogenetics of leptin in antipsychotic-associated weight gain and obesity-related complications. Pharmacogenomics 12 (7), 999–1016.

Li, X., Friedman, A.B., Zhu, W., Wang, L., Boswell, S., May, R.S., Davis, L.L., Jope, R.S., 2007. Lithium regulates glycogen synthase kinase-3beta in human peripheral blood mononuclear cells: implication in the treatment of bipolar disorder. Biol. Psychiatry 61 (2), 216–222.

Lin, P.I., Vance, J.M., Pericak-Vance, M.A., Martin, E.R., 2007. No gene is an island: the flip-flop phenomenon. Am. J. Hum. Genet. 80 (5), 1002.

Livingstone, C., Rampes, H., 2006. Lithium: a review of its metabolic adverse effects. J. Psychopharmacol. 20 (3), 347–355.

Longo, K.A., Wright, W.S., Kang, S., Gerin, I., Chiang, S.H., Lucas, P.C., Opp, M.R., MacDougald, O.A., 2004. Wnt10b inhibits development of white and brown adipose tissues. J. Biol. Chem. 279 (34), 35503–35509.

Malmström, R., Taskinen, M.R., Karonen, S.L., Yki-Järvinen, H., 1996. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. Diabetologia 39 (8), 993–996.

Marcello, M.A., Calixto, A.R., de Almeida, J.F., Martins, M.B., Cunha, L.L., Cavalari, C.A., Etchebehere, E.C., da Assumpção, L.V., Geloneze, B., Carvalho, A.L., Ward, L.S., 2015. Polymorphism in LEP and LEPR may modify leptin levels and represent risk factors for thyroid cancer. Int. J. Endocrinol. 2015, 173218.

Meffre, D., Grenier, J., Bernard, S., Courtin, F., Dudev, T., Shackleford, G., Jafarian-Tehrani, M., Massaad, C., 2014. Wnt and lithium: a common destiny in the therapy of nervous system pathologies. Cell Mol. Life Sci. 71 (7), 1123–1148.

Morris, D.L., Rui, L., 2009. Recent advances in understanding leptin signaling and leptin resistance. Am. J. Physiol. Endocrinol. Metab. 297 (6), E1247–E1259.

Nyholt, D.R., 2004. A simple correction for multiple testing for SNPs in linkage disequilibrium with each other. Am. J. Hum. Genet. 74 (4), 765–769.

Patterson, N., Price, A.L., Reich, D., 2006. Population structure and eigenanalysis. PLOS Genet. 2 (12), e190.

Perez-Iglesias, R., Mata, I., Amado, J.A., Berja, A., Garcia-Unzueta, M.T., Martínez García, O., Arranz, M.J., Vazquez-Barquero, J.L., Crespo-Facorro, B., 2010. Effect of FTO, SH2B1, LEP, and LEPR polymorphisms on weight gain associated with antipsychotic treatment. J. Clin. Psychopharmacol. 30 (6), 661–666.

Ricken, R., Bopp, S., Schlattmann, P., Himmerich, H., Bschor, T., Richter, C., Stamm, T.J., Bauer, F., Heinz, A., Hellweg, R., Lang, U.E., Adli, M., 2016. Leptin serum concentrations are associated with weight gain during lithium augmentation. Psychoneuroendocrinology 71, 31–35.

Ross, S.E., Hemati, N., Longo, K.A., Bennett, C.N., Lucas, P.C., Erickson, R.L., MacDougald, O.A., 2000. Inhibition of adipogenesis by Wnt signalling. Science 289 (5481), 950–953.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), 22–33 quiz 34-57.

SNAP Proxy Search of the Broad Institute, 2016. http://www.broadinstitute.org/mpg/snap/index.php Accessed December 20, 2016.

Soeiro-de-Souza, M.G., Gold, P.W., Brunoni, A.R., de Sousa, R.T., Zanetti, M.V., Carvalho, A.F., Gattaz, W.F., Machado-Vieira, R., Teixeira, A.L., 2014. Lithium decreases plasma adiponectin levels in bipolar depression. Neurosci. Lett. 564, 111–114.

Srivastava, V., Deshpande, S.N., Nimgaonkar, V.L., Lerer, B., Thelma, B., 2008. Genetic correlates of olanzapine-induced weight gain in schizophrenia subjects from north India: role of metabolic pathway genes. Pharmacogenomics 9 (8), 1055–1068.

Templeman, L.A., Reynolds, G.P., Arranz, B., San, L., 2005. Polymorphisms of the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenet. Genom. 15 (4), 195–200.

The Genotype-Tissue Expression project, 2017. The GTEx Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. https://www.gtexportal.org/home/documentationPage Accessed June 6, 2017.

The International HapMap project, 2017. http://www.sanger.ac.uk/resources/downloads/human/hapmap3.html Accessed March 12, 2017.

Vestergaard, P., Licht, R.W., 2001. 50 Years with lithium treatment in affective disorders: present problems and priorities. World J. Biol. Psychiatry 2 (1), 18–26.

Wabitsch, M., Jensen, P.B., Blum, W.F., 1996. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45 (10), 1435–1438.

Yu, Z., Han, S., Cao, X., Zhu, C., Wang, X., Guo, X., 2012. Genetic polymorphisms in adipokine genes and the risk of obesity: a systematic review and meta-analysis. Obes. (Silver Spring) 20 (2), 396–406.

Zill, P., Malitas, P.N., Bondy, B, Engel, R., Boufidou, F., Behrens, S., Alevizos, B.E., Nikolaou, C.K., Christodoulou, G.N., 2003. Analysis of polymorphisms in the alpha-subunit of the olfactory G-protein Golf in lithium-treated bipolar patients. Psychiatr. Genet. 13 (2), 65–69.