Citation for published version (APA):
Association of \textit{CRTC1} polymorphisms with obesity markers in subjects from the general population with lifetime depression

Lina Quteineh, Martin Preisig, Margarita Rivera, Yuri Milaneschi, Enrique Castelao, Mehdi Gholam-Rezaee, Frederik Vandenberghe, Nuria Saigi-Morgui, Aurélie Delacrétaz, Jean-René Cardinaux, Gonneke Willemsen, Dorret I. Boomsma, Brenda W.J.H. Penninx, Ana Ching-López, Philippe Conus, Chin B. Eap

PII: S0165-0327(15)31310-0
DOI: http://dx.doi.org/10.1016/j.jad.2016.03.031
Reference: JAD8118

To appear in: \textit{Journal of Affective Disorders}

Received date: 23 November 2015
Revised date: 10 February 2016
Accepted date: 9 March 2016

Cite this article as: Lina Quteineh, Martin Preisig, Margarita Rivera, Yuri Milaneschi, Enrique Castelao, Mehdi Gholam-Rezaee, Frederik Vandenberghe, Nuria Saigi-Morgui, Aurélie Delacrétaz, Jean-René Cardinaux, Gonneke Willemsen, Dorret I. Boomsma, Brenda W.J.H. Penninx, Ana Ching-López, Philippe Conus and Chin B. Eap, Association of \textit{CRTC1} polymorphisms with obesity markers in subjects from the general population with lifetime depression, \textit{Journal of Affective Disorders}, http://dx.doi.org/10.1016/j.jad.2016.03.031

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Association of CRTC1 polymorphisms with obesity markers in subjects from the general population with lifetime depression.

Lina Quteineh, MD, PhD, Martin Preisig, MD, MPH, Margarita Rivera, PhD, Yuri Milaneschi, PhD, Enrique Castelao, MSc, Mehdi Gholam-Rezaee, PhD, Frederik Vandenberghhe, PharmD, MSc, Nuria Saigi-Morgui, PharmD, MSc, Aurélie Delacrétaz, MSc, Yuri Milaneschi, PhD, Enrique Castelao, MSc, Mehdi Gholam-Rezaee, PhD, Frederik Vandenberghhe, PharmD, MSc, Nuria Saigi-Morgui, PharmD, MSc, Aurélie Delacrétaz, MSc, Jean-René Cardinaux, PhD, Gonneke Willemsen, PhD, Dorret I Boomsma, PhD, Brenda WJH Penninx, PhD, Ana Ching-López, Philippe Conus, MD, Chin B Eap, PhD*

1Unit of Pharmacogenetics and Clinical Psychopharmacology, Centre for Psychiatric Neuroscience, Department of Psychiatry, Lausanne University Hospital, Prilly, Switzerland.
2Centre of Psychiatric Epidemiology and Psychopathology, Department of Psychiatry, Lausanne University Hospital, Prilly, Switzerland.
3Instituto de Investigación Biosanitaria ibs.GRANADA. Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain.
4CIBERSAM-University of Granada and Institute of Neurosciences Federico Olóriz, Centro de Investigación Biomédica, University of Granada, Spain.
5MRC Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, United Kingdom.
6Department of Psychiatry, EMGO Institute for Health and Care Research and Neuroscience Campus Amsterdam, VU University Medical Center/GGZ inGeest, Amsterdam, The Netherlands.
7Centre for Psychiatric Neuroscience, Department of Psychiatry, Lausanne University Hospital, Prilly, Switzerland.
Abstract:

Background:

Psychiatric disorders have been hypothesized to share common etiological pathways with obesity, suggesting related neurobiological bases. We aimed to examine whether CRTC1 polymorphisms were associated with major depressive disorder (MDD) and to test the association of these polymorphisms with obesity markers in several large case-control samples with MDD.

Methods:
The association between CRTC1 polymorphisms and MDD was investigated in three case-control samples with MDD (PsyCoLaus $n_1=3,362$, Radiant $n_2=3,148$ and NESDA/NTR $n_3=4,663$). The effect of CRTC1 polymorphisms on obesity markers was then explored.

**Results:**

CRTC1 polymorphisms were not associated with MDD in the three samples. CRTC1 rs6510997C>T was significantly associated with fat mass in the PsyCoLaus study. In fact, a protective effect of this polymorphism was found in MDD cases ($n=1,434$, $\beta=-1.32\%$, 95% CI -2.07 to -0.57, $P<0.001$), but not in controls. In the Radiant study, CRTC1 polymorphisms were associated with BMI, exclusively in individuals with MDD ($n=2,138$, $\beta=-0.75$ kg/m$^2$, 95% CI -1.30 to -0.21, $p=0.007$), while no association with BMI was found in the NESDA/NTR study.

**Limitations:**

Estimated fat mass using bioimpedance that capture more accurately adiposity was only present in the PsyCoLaus sample.

**Conclusions:**

CRTC1 polymorphisms seem to play a role with obesity markers in individuals with MDD rather than non-depressive individuals. Therefore, the weak association previously reported in the population-based samples was driven by cases diagnosed with lifetime MDD. However, CRTC1 seems not to be implicated directly in the development of psychiatric diseases.

**Keywords:** Obesity, major depressive disorder, psychiatric disorders, psychotropic drugs, pharmacogenetics, genetic polymorphisms.
Introduction:

Based on a growing body of evidence, it has been hypothesized that psychiatric disorders, such as schizophrenia and mood disorders, are causally related to or share common etiological pathways with obesity, suggesting that comorbid obesity and psychiatric disorders have related neurobiological bases (Farmer et al., 2008; Forty et al., 2014). Increased appetite, weight gain and obesity are common symptoms of depression (Blaine, 2008; Luppino et al., 2010). On the other hand, obese individuals were also found to have higher prevalence of depression (Luppino et al., 2010). Shared genetic factors were previously suggested (Afari et al., 2010; Comings et al., 1996; Rivera et al., 2012; Samaan et al., 2013), however, due to the pathological complexity of both conditions and the polygenic factors affecting them, many genetic factors are still to unravel. Additionally, due to heterogeneity of depression, genetic association studies could give conflicting results. One example is the fat mass and obesity-associated gene (FTO) for which the common rs9939609-A variant previously associated with increased body mass index (BMI) and obesity in several studies (Frayling et al., 2007; Qi et al., 2008; Scuteri et al., 2007) was shown to be negatively associated with depression in a meta-analysis of four large studies (Samaan et al., 2013). On the other hand, the same variant was found to be positively associated with depression in a recent large case-control study (Milaneschi et al., 2014), but this association was entirely driven by the atypical depression subtype (subtype characterized typically by increased appetite, weight gain and hypersomnia).
We previously showed an association between a coding single nucleotide polymorphism (SNP) within the CREB-regulated transcription coactivator 1 (CRTC1) gene (rs3746266A>G) and BMI in 3 independent psychiatric samples (brief description of these samples is given in the Supplementary data and is described in details in ref (Choong et al., 2013)). Carriers of the rs3746266-G variant showed significantly protective effect against obesity compared to non-carriers. The sex-stratified analysis in the 3 combined psychiatric samples showed a protective effect for the G allele both in men and women. However, the strongest and most clinically relevant association was observed in women younger than 45 years old. Although the effect was weaker, we also found a protective effect of the T allele of the CRTC1 rs6510997C>T (a proxy of rs3746266A>G, \( r^2=0.7 \)) on fat accumulation in a large population-based sample (CoLaus), with the strongest association again in premenopausal women (Choong et al., 2013).

In animal models, we previously showed that mice lacking CRTC1 exhibit neurobehavioral endophenotypes related to mood disorders, depression-related behavior and a blunted behavioral response to antidepressants (Breuillaud et al., 2012). Crtc1 knockout mice also developed obese features, including obesity-related metabolic complications, under normal diets (Altarejos et al., 2008; Breuillaud et al., 2009). Altogether, these findings suggest that the Crtc1 gene might play a common role in obesity and depression-related behavior.

In the present study, we aimed to examine whether CRTC1 SNPs are associated with major depressive disorder (MDD), and to test the association of CRTC1 SNPs with obesity markers in several large case-control samples with MDD. The same CRTC1 SNPs (rs3746266A>G and rs6510997C>T) investigated previously (Choong et al., 2013) were used in the current study.
Patients and methods:

The associations between \textit{CRTC1} polymorphisms and obesity markers were first tested in the PsyCoLaus sample and tested for replication in the Radiant and NESDA/NTR study. Supplementary Table S1 presents the main similarities and differences between these samples.

**Discovery cohort: CoLaus|PsyCoLaus**

Participants aged 35 to 75 years in this population-based cohort study (CoLaus; n=6,734) were recruited between June 2003 and May 2006 in Lausanne, Switzerland as previously described (Firmann et al., 2008). The assessments at baseline and at the first follow-up included cardiovascular risk factors such as BMI, fat mass, blood pressure, glucose and lipid profiles. In addition, a genome-wide association study was performed in all Caucasians (91% of the sample). All participants of CoLaus were asked to also participate in the psychiatric evaluations (PsyCoLaus (Preisig et al., 2009)), which included the semi-structured Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994; Preisig et al., 1999). The assignment of the lifetime diagnosis of MDD and the subtyping into atypical and non-atypical MDD was performed according to DSM-IV criteria. Complete physical and psychiatric data were available for 3,354 participants aged 35 to 65 year-old who completed the psychiatric evaluation either at the baseline or the first follow-up (n\textsubscript{total}=3362). 1434 cases of lifetime depression were compared with 1,920 controls with no previous history of depression. Genotyping for the CoLaus/PsyCoLaus subjects was performed using the Affymetrix
GeneChip® Human Mapping 500K array set. The CRTC1 rs6510997C>T SNP was directly genotyped (Choong et al., 2013). The CRTC1 rs7257846T>C, which is in linkage disequilibrium (LD) with rs3746266A>G ($r^2=0.93$), was imputed and analyzed ($r^2$ hat value was 0.70).

The Institutional Ethics Committee of the University of Lausanne approved the CoLaus and subsequently the PsyCoLaus study. All participants signed a written informed consent to participate in the study.

- **The Radiant study**

  The Radiant study was founded from three studies: the Depression Case–Control (DeCC) study, Depression Network (DeNT) study and the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. The DeCC study is a multicentre case-control study conducted over three investigative sites in UK (London, Cardiff and Birmingham)(Cohen-Woods et al., 2009). The DeNT study is a family-based study that recruited sibling pairs affected with unipolar depression from eight European clinical sites and one in the USA (Farmer et al., 2004; McGuffin et al., 2005). Participants in the DeCC and DeNT studies were included if they had lifetime diagnosis of two or more episodes of MDD of at least moderate severity. Subjects in the GENDEP study were recruited from nine European centers if they experienced at least one depressive episode of at least moderate severity (Uher et al., 2009).

  Diagnosis of MDD was defined as of illness fulfilling ICD–10 and/or DSM–IV criteria and was ascertained using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview in all three studies (Wing et al., 1990). Control subjects were recruited via the Medical Research Council general practice research framework (Sham et al., 2000). They were screened for lifetime absence of any psychiatric disorder using a modified version of the Past History Schedule(McGuffin et al., 1986). Self-reported height and weight were obtained from participants in the MDD group via the SCAN interview and from the controls via telephone.
interview. The reliability of self-report of height and weight was assessed in the GENDEP dataset (n=811) where also height and weight were measured. The correlations for measured versus self-reported height, weight and BMI were 0.97, 0.95 and 0.95, respectively (Hung et al., 2014; Hung et al., 2015). All cases and controls were of white European ancestry. Genotyping for the Radiant subjects was performed using the Illumina HumanHap610-Quad BeadChips by the Centre National de Génotypage (CNG), Evry, France, as previously described (Lewis et al., 2010). CRTC1 rs3746266A>G was successfully imputed using the Beagle Program (BEAGLE Version 3.0 Copyright (c) 2007–2010 Brian L Browning), with imputation quality >0.8. The CRTC1 rs2075017T>C, which is in complete linkage disequilibrium (LD) with the rs6510997C>T, was also selected for the association analyses. Approval was obtained from the local research ethics committees/institutional research boards of all of the participating sites. All participants provided written informed consent.

- NESDA/NTR study:

This sample consisted of 4,663 unrelated participants of European ancestry from the Netherlands Study of Depression and Anxiety (NESDA)(Penninx et al., 2008) (1,636 cases and 425 controls) and from the Netherlands Twin Registry (NTR)(Boomsma et al., 2006) (132 cases and 2,470 controls). The diagnosis of lifetime and/or current MDD according to DSM-IV was ascertained using the Composite Interview Diagnostic Instrument (WHO WMH-CIDI, 2015). Weight and height were measured at the study clinic during the visit for NESDA (Penninx et al., 2008), and during the home visit for NTR-Biobank (Willemsen et al., 2010). Genotyping was performed on multiple chip platforms in (partially overlapping) different subsets of the total sample (Affymetrix-Perlegen 5.0, Illumina 370K, Illumina 660K, Illumina Omni 1M and Affymetrix 6.0) and data were imputed using the 1000 Genomes phase 1
INTEGRATED RELEASE version 3 ALL panel ($r^2$ hat value was 0.99 for rs6510997C>T and 0.77 for rs3746266A>G).

The NESDA/NTR study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam, and all subjects provided written informed consent.

**Statistical analysis:**

For association studies, chi-square (Chi$^2$) or Fisher exact tests for binomial variables were used. Differences in genotype frequencies as well as deviation from Hardy-Weinberg equilibrium were assessed using Chi$^2$ test. All genetic analyses for CRTC1 SNPs were performed using a dominant model (wild type vs. variant allele carriers), the same model used for the previously mentioned psychiatric samples (Choong et al., 2013).

Multivariable regression analyses were used to test the association between the CRTC1 SNPs and MDD adjusting for age, sex and BMI. For PsyCoLaus data, a Generalized Linear Model (GLM) adjusted for age and sex was used to test the association between CRTC1 rs6510997C>T polymorphism and obesity markers (BMI, fat mass and waist circumference) using SAS 9.3 version (SAS Institute Inc., Cary, NC, USA). In a first step, we tested for the interaction between CRTC1 SNPs and MDD status for an effect on obesity markers. For the Radiant and NESDA/NTR studies BMI was the only available obesity marker. For the data of these studies, linear regression models adjusted for age, sex and principal components were used to test the association between CRTC1 polymorphisms and BMI.

**Results:**

Demographic characteristics of the study samples:
General characteristics of the PsyCoLaus sample are presented in Supplementary Table S2. The Radiant study included 3148 subjects (2,338 cases and 810 controls), mean age±standard deviation (s.d.) was 43.9±12.8 years. Females represent nearly 68% of the study sample. Mean BMI in the total study sample was 25.9 kg/m$^2$ (s.d. 5.4). The NESDA/NTR study included 4,663 subjects, mean age±s.d. was 42.7±14.0 years. Females represent nearly 64% of the study sample. Mean BMI in the total study sample was 25.0 kg/m$^2$ (s.d. 4.5).

**CRTC1 polymorphisms and MDD:**

Genotype distribution in the overall study samples and among MDD cases and controls are presented in Table 1. Genotype distribution did not differ between MDD cases and controls in any of the three studies (Table 1). Multivariate analyses adjusted for age and sex confirmed the absence of an association between **CRTC1** polymorphisms and MDD in the three studies (data not shown).

**CRTC1 polymorphisms and obesity markers:**

**PsyCoLaus**

**CRTC1 rs6510997C>T** was significantly associated with fat mass in the global PsyCoLaus sample (Table 2). We observed interactions between **CRTC1 rs6510997C>T** and sex (p=0.03) as well as between this SNP and MDD status (p=0.01) regarding the fat mass, indicating a significantly stronger effect of this genetic variant in females and in subjects with MDD. Indeed, this SNP was significantly associated with lower fat mass in females but not in males. Similarly, this SNP was significantly associated with lower fat mass in subjects with MDD but not in controls (Table 2). Additionally, the 3-way interaction between **CRTC1 rs6510997C>T**, sex and MDD regarding the fat mass was not significant (p=0.54). In females, we did not find...
an interaction between the \textit{CRTC1 rs6510997C>T SNP} and menopausal status and among depressed subjects there was no interaction between this SNP and the atypical depression subtype to affect the fat mass. Regarding the BMI and waist circumference there was no evidence of an interaction between \textit{CRTC1 rs6510997C>T} and sex or MDD status and the SNP was not associated with these obesity markers (Table 2).

No difference on the associations between \textit{CRTC1 rs6510997C>T} and fat mass was noticed by adjusting the GLM model for other available co-variables that could affect the gene association with obesity markers, like socioeconomic status, drug dependence, alcohol consumption, former and current tobacco consumer (Supplementary Table S3). Nearly the same associations with obesity markers were observed in PsyCoLaus for \textit{CRTC1 rs7257846T>C} (in strong LD with \textit{rs3746266A>G}, $r^2=0.93$)(Supplementary Table S4).

\textbf{The Radiant and NESDA/NTR studies:}

The \textit{CRTC1 rs3746266A>G SNP} was associated with BMI in the total Radiant sample and exclusively in females (Table 3). By stratifying MDD cases and controls, the protective effect of the \textit{rs3746266G-allele} was only observed in MDD cases, while no association was observed in controls. A weak association was observed between \textit{CRTC1 rs2075017T>C} (in complete LD with the \textit{rs6510997C>T}) and BMI in the Radiant sample (Table 3). Regarding the NESDA/NTR study, \textit{CRTC1 SNPs} were not associated with BMI (Table 4).

\textbf{Discussion:}

We previously showed a protective effect of the variant \textit{CRTC1 rs3746266G-allele} on increased BMI in three independent psychiatric samples (Choong et al., 2013). The same protective effect was also observed in a population-based sample (CoLaus) between the
rs6510997T-allele (in a strong LD with the rs3746266G-allele) and fat mass. However, the effect of the SNP was weaker compared to the psychiatric samples (Choong et al., 2013). In the present study, we were able to show in the psychiatrically evaluated sub-sample of CoLaus (PsyCoLaus) that the effect of CRTC1 rs6510997C>T on fat mass was restricted to subjects with a lifetime history of MDD. In these subjects, the CRTC1 rs6510997T-allele showed a significant protective effect for the fat mass. Although there was no significant interaction between the CRTC1 SNPs and MDD, similar trend regarding the BMI was observed in the Radiant sample (no fat mass data available in this sample), which included more severe treated cases with lifetime recurrent depression. The CRTC1 rs3746266A>G SNP only reached the level of statistical significance in MDD cases. However, these results could not be replicated in a third case-control sample with lifetime depression, the NESDA/NTR study.

CRTC1 rs6510997C>T was associated with fat mass exclusively among depressive cases but not unaffected individuals in the PsyCoLaus sample. This association in depressed subjects did not differ in function of the depression subtypes (atypical versus others). Such an association with the BMI was not observed in the whole PsyCoLaus sample, but CRTC1 SNPs were associated with BMI in the Radiant study. BMI may less accurately capture adiposity than estimated fat mass using bioimpedance (Marques-Vidal et al., 2009; Prentice and Jebb, 2001). Using the whole CoLaus sample, previous work established that fat mass enables capture of 3 times more subjects with high cardiovascular risk than BMI (Marques-Vidal et al., 2009) and we also showed that this sample is better powered to detect an association of the rs6510997C>T SNP with fat mass than with BMI (Choong et al., 2013). In the psychiatric samples, based on subjects at high risk of metabolic abnormalities because of the disorder
and of the psychotropic medications, an association between CRTC1 SNPs and BMI could be observed (Choong et al., 2013). Accordingly, the association between CRTC1 SNPs and BMI observed in the Radiant study could be explained by the higher degree of severity of the disorder in this sample compared to that in the PsyCoLaus. Interestingly, post hoc analysis also revealed a significant protective effect of the CRTC1 rs6510997C>T SNP on BMI and waist circumference in PsyCoLaus subjects with MDD (but not in the overall sample) which would be in line with our hypothesis that the impact of CRTC1 variants on adiposity markers is restricted to affected subjects.

CRTC1 SNPs were associated with obesity markers in both PsyCoLaus and Radiant studies, but the association was entirely attributable to MDD cases. On the other hand, CRTC1 SNPs were not associated with BMI in the NESDA/NTR study, neither in the whole sample, nor in the stratified MDD case-control analyses. Previously, PsyCoLaus and DeCC (from the Radiant study) samples detected a protective effect of the FTO rs9939609-A variant on MDD (Samaan et al., 2013), while in the NESDA/NTR study the SNP was positively associated with MDD, especially in the atypical depression subtype (Milaneschi et al., 2014). Therefore, an inconsistency was already observed before for these studies which could be partly explained by the heterogeneity of depression in genetic association studies.

Obesity results from an imbalance between energy intake and energy expenditure. Recent studies have shown that mice lacking the Crtc1 gene eat more and have less energy expenditure than wild-type mice, thus developing an obese feature, including obesity-related metabolic complications, under normal diets (Altarejos et al., 2008; Breuillaud et al., 2009). These results suggest that CRTC1 is playing a major role in the hypothalamic control
of food intake. CRTC1 is mainly expressed in the brain (Altarejos et al., 2008; Breuillaud et al., 2009; Conkright et al., 2003; Kovacs et al., 2007; Wu et al., 2006) where it may modulate leptin anorexic effect in the hypothalamus. In the cell, the inactive phosphorylated form of CRTC1 is sequestered in the cytoplasm, and its migration to the nucleus requires the concomitant activation of the phosphatase calcineurin and the inactivation of kinases of the 5’ adenosine monophosphate-activated protein kinase (AMPK) family (Altarejos and Montminy, 2011). Interestingly, psychotropic drugs may increase weight by selective and potent stimulation of hypothalamic AMPK (Minokoshi et al., 2004) which has been shown to regulate food intake and reverse the actions of the anorexigenic hormone leptin (Kim et al., 2007).

In the PsyCoLaus sample, an interaction between CRTC1 SNP and sex was observed regarding the fat mass and the protective effect of the SNP was mainly observed in females from the global sample. The same protective effect of CRTC1 SNP on BMI was also observed in females from the global Radiant sample. This is in line with our previous results in the psychiatric and CoLaus samples (Choong et al., 2013). This stronger association found in females compared to males could be caused by a differential role of the leptin mediating satiety pathway in the enhancement of CRTC1 activity. Women have much higher leptin levels than men (Rosenbaum et al., 1996) and female sex was found to predict stronger weight gain during psychotropic treatment (Gebhardt et al., 2009). A hypothetical mechanism of the effect of CRTC1 and its interaction with sex hormones was published previously (Choong et al., 2013). Additionally, a meta-analysis of GWAS reported an association of an intronic SNP of CRTC1 (rs10423674A>C) with the age of menarche (Elks et al., 2010), supporting a potential interaction between sex hormones and CRTC1.
The CRTC1 SNPs were not associated with depression in our three MDD case-control studies. Animal models lacking the Crtc1 gene showed behavioral abnormalities and depression-related behavior (Breuillaud et al., 2012), but this finding could not be translated in our study. However, the functional activities of these SNPs in humans are still unknown and possibly the complete absence of the CRTC1 gene could lead to the observed behavioral disturbances in animal models, which is probably not the case for effect of the tested SNPs on CRTC1 expression. On the other hand, the observed effect of the CRTC1 SNPs on obesity markers was uniquely detected in subjects with MDD. The lack of association between CRTC1 SNPs and obesity markers in controls from the PsyCoLaus and the Radiant samples explains the weak association previously found in the population-based sample (Choong et al., 2013). It also confirms our hypothesis in which psychiatric illness and/or treatment with potentially weight gain-inducing psychotropic drugs could play a role in genetically mediated energy homeostasis and that the effect of CRTC1 variations on obesity markers was unmasked in this group of subjects. Interestingly, a study in animal models (Mastronardi et al., 2011) showed that rats exposed to a high-fat diet after stress and treatment for a short period with antidepressants, to have more body weight and size from 17 to 22 weeks following antidepressant discontinuation compared to rats with the same conditions but treated with saline (Mastronardi et al., 2011). These findings support our concept of persistent, long-term effects of pharmacological–environment interactions on body weight regulation, even if the exposure to these medications was in any period in their lifetime.

This study has several limitations. Estimated fat mass using bioimpedance was only measured in the PsyCoLaus sample while only BMI was the only adiposity marker measured
in the replication samples. Additionally, height and weight were self-reported in the Radiant study, which might influence the accuracy of BMI measurements in this sample, although the reliability of self-report of height and weight was assessed in the GENDEP dataset. Psychotropic medications were not available in the Radiant sample, therefore, associations between \textit{CRTC1} SNPs and BMI in treated vs. untreated cases could not be calculated. Both \textit{CRTC1} SNPs ($rs6510997\text{C}>T$ and $rs3746266\text{A}>G$) were not always available in our three case-control samples; however, SNPs with nearly complete LD ($r^2>0.9$) were selected and used as proxies, and results from these proxies could be generalized to our 2 \textit{CRTC1} SNPs (meaning that the genotype present at one locus of the SNPs in LD is independent of the genotype at the second locus, both SNPs having the same allele frequency and could represent each other). On the other hand, SNPs were not always directly genotyped and different imputation methods with different imputation qualities were used which can potentially influence the results on the associations between different imputed \textit{CRTC1} SNPs and obesity markers in our studies.

In conclusion, we showed in this study that \textit{CRTC1} polymorphisms play no role in obesity markers in the general population and that the weak effect previously reported was driven by cases diagnosed with lifetime MDD. \textit{CRTC1} seems to play an important role in the high prevalence of overweight and obesity specifically in psychiatric samples, a population at risk of developing obesity because of the disease itself and/or the medications. However, \textit{CRTC1} seems not to be implicated directly in the development of psychiatric diseases.
References:

2015. The world health organization world mental health composite international diagnostic interview (WHO WMMH-CIDI).


Wing, J.K., Babor, T., Brugha, T., Burke, J., Cooper, J.E., Giel, R., Jablenski, A., Regier, D., Sartorius, N., 1990. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry 47, 589-593.

Table 1: Genotype distribution in the total study samples, among MDD subjects and controls

<table>
<thead>
<tr>
<th>Study</th>
<th>rs6510997C&gt;T</th>
<th>rs3746266A&gt;G</th>
<th>rs2075017T&gt;C</th>
<th>rs3746266A&gt;G</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PsyCoLaus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3347</td>
<td>2842</td>
<td>2160</td>
<td>4663</td>
</tr>
<tr>
<td>MDD cases</td>
<td>1431</td>
<td>2142</td>
<td>1352</td>
<td>1768</td>
</tr>
<tr>
<td>Controls</td>
<td>1916</td>
<td>700</td>
<td>808</td>
<td>2895</td>
</tr>
<tr>
<td><strong>The Radiant study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3347</td>
<td>2173</td>
<td>1357</td>
<td>3235</td>
</tr>
<tr>
<td>MDD cases</td>
<td>1431</td>
<td>1637</td>
<td>849</td>
<td>1234</td>
</tr>
<tr>
<td>Controls</td>
<td>1916</td>
<td>536</td>
<td>508</td>
<td>501</td>
</tr>
<tr>
<td><strong>NESDA/NTR study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4663</td>
<td>3235</td>
<td>1357</td>
<td>3235</td>
</tr>
<tr>
<td>MDD cases</td>
<td>1768</td>
<td>1234</td>
<td>485</td>
<td>485</td>
</tr>
<tr>
<td>Controls</td>
<td>2895</td>
<td>2001</td>
<td>821</td>
<td>821</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2128 (63.6)</td>
<td>1092 (32.6)</td>
<td>127 (3.8)</td>
<td>1224 (69.4)</td>
<td>1522 (32.6)</td>
<td>189 (4.1)</td>
</tr>
<tr>
<td>MDD cases</td>
<td>912 (63.7)</td>
<td>468 (32.7)</td>
<td>51 (3.6)</td>
<td>1234 (69.8)</td>
<td>567 (32.1)</td>
<td>77 (4.4)</td>
</tr>
<tr>
<td>Controls</td>
<td>1216 (63.5)</td>
<td>624 (32.6)</td>
<td>76 (3.9)</td>
<td>2001 (69.1)</td>
<td>955 (33.0)</td>
<td>112 (3.9)</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium
MDD: Major depressive disorder, N: number, HWE: Hardy-Weinberg Equilibrium

$ Both SNPs are in complete linkage disequilibrium ($r^2=1$)

Table 2: Association between CRTC1 rs6510997C>T polymorphism and fat mass, BMI and waist circumference in the PsyCoLaus sample, among sex-stratified and MDD subjects and controls

<table>
<thead>
<tr>
<th></th>
<th>Fat mass$^\dagger$</th>
<th>BMI$^\ddagger$</th>
<th>Waist circumference$^\ddagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Estimates (95% C.I.)</td>
<td>p-value</td>
</tr>
<tr>
<td>All subjects</td>
<td>3362</td>
<td>-0.66 (-1.28 to -0.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>Males</td>
<td>1576</td>
<td>-0.03 (-0.56 to 0.51)</td>
<td>0.92</td>
</tr>
<tr>
<td>Females</td>
<td>1786</td>
<td>-1.08 (-1.81 to -0.34)</td>
<td>0.004</td>
</tr>
<tr>
<td>Subjects with MDD</td>
<td>1434</td>
<td>-1.32 (-2.07 to -0.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>1920</td>
<td>-0.01 (-0.60 to 0.57)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Models were adjusted for age and sex (when appropriate).

BMI: body mass index, C.I.: confidence interval, MDD: Major depressive disorder
$^\dagger$ CRTC1 rs6510997C>T and MDD status interaction regarding fat mass: p=0.014
$^\ddagger$ CRTC1 rs6510997C>T and sex interaction regarding fat mass: estimate: p=0.026

Table 3: Association between CRTC1 polymorphisms and BMI in the total, sex-stratified sample and in depressive cases and controls of the Radiant study sample:

<table>
<thead>
<tr>
<th></th>
<th>rs3746266A&gt;G$^\dagger$</th>
<th>rs2075017T&gt;C$^\ddagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Estimates (95% C.I.)</td>
</tr>
<tr>
<td>All subjects$^\dagger$</td>
<td>2822</td>
<td>-0.67 (-1.13 to -0.21)</td>
</tr>
<tr>
<td>Male</td>
<td>909</td>
<td>-0.38 (-1.05 to 0.30)</td>
</tr>
<tr>
<td>Female</td>
<td>1916</td>
<td>-0.79 (-1.38 to -0.21)</td>
</tr>
<tr>
<td>Subjects with MDD</td>
<td>2138</td>
<td>-0.75 (-1.30 to -0.21)</td>
</tr>
<tr>
<td>Controls</td>
<td>684</td>
<td>-0.41 (-1.20 to 0.39)</td>
</tr>
</tbody>
</table>

Models were adjusted for age and sex (when appropriate) and principal components.

C.I.: confidence interval, MDD: Major depressive disorder
$^\dagger$ Available subjects with clinical and genetic data.
$^\ddagger$ CRTC1 rs3746266A>G and MDD status and/or sex interactions regarding BMI: p>0.05

$^\dagger$ CRTC1 rs2075017T>C and MDD status and/or sex interactions regarding BMI: p>0.05
**Table 4:** Association between *CRTC1* polymorphisms and BMI in the total, sex-stratified sample and in depressive cases and controls of the NESDA/NTR study sample:

<table>
<thead>
<tr>
<th></th>
<th>rs3746266A&gt;G Estimates (SE)</th>
<th>rs6510997C&gt;T Estimates (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>P</td>
</tr>
<tr>
<td><strong>All subjects</strong></td>
<td>4663</td>
<td>-0.047 (0.130)</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>1676</td>
<td>0.072 (0.202)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>2987</td>
<td>-0.121 (0.179)</td>
</tr>
<tr>
<td><strong>Subjects with MDD</strong></td>
<td>1768</td>
<td>0.038 (0.256)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>2895</td>
<td>-0.076 (0.150)</td>
</tr>
</tbody>
</table>

Model were adjusted for age and sex (when appropriate) and principal components
SE: standard error, MDD: Major depressive disorder
Highlights:

- Psychiatric disorders may share common etiological pathways with obesity.
- CRTC1 polymorphisms were not associated with major depression disorder (MDD) in three case-control samples with MDD.
- In PsyCoLaus, CRTC1 rs6510997C>T was significantly associated with lower fat mass in females but not in males.
- In PsyCoLaus, CRTC1 rs6510997C>T was significantly associated with lower fat mass in subjects with MDD but not in controls.
- CRTC1 polymorphisms were associated with BMI in MDD cases from the Radiant study, but not from the NESDA/NTR study.
- CRTC1 polymorphisms seem to play a role with obesity markers only in individuals with MDD but not in non-depressive individuals.