DEPRESSIVE DISORDER MODERATES THE EFFECT OF THE *FTO* GENE ON BODY MASS INDEX

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Abstract

There is evidence that obesity related disorders are increased among people with depression. Variation in the *FTO* (fat mass and obesity associated) gene has been shown to contribute to common forms of human obesity. This study aimed to investigate the genetic influence of polymorphisms in *FTO* in relation to Body Mass Index (BMI) in two independent samples of major depressive disorder (MDD) cases and controls.

We analysed 88 polymorphisms in the *FTO* gene in a clinically ascertained sample of 2442 MDD cases and 809 controls (Radiant Study). Eight of the top 10 SNPs showing the strongest associations with BMI were followed-up in a population-based cohort (PsyCoLaus Study) consisting of 1292 depression cases and 1690 controls.

Linear regression analyses of the *FTO* variants and BMI yielded 10 SNPs significantly associated with increased BMI in the depressive group but not the control group in the Radiant sample. The same pattern was found in the PsyCoLaus sample. We found a significant interaction between genotype and affected status in relation to BMI for 7 SNPs in Radiant ($p<0.0057$), with PsyCoLaus giving supportive evidence for 5 SNPs (p-values between 0.03-0.06) which increased in significance when the data were combined in a meta-analysis.

This is the first study investigating *FTO* and BMI within the context of MDD, and the results indicate that having a history of depression moderates the effect of *FTO* on BMI. This finding suggests that *FTO* is involved in the mechanism underlying the association between mood disorders and obesity.

Keywords: Depression; *FTO* gene; obesity; BMI; Body Mass Index

Introduction

In addition to unipolar major depressive disorder (MDD) being a major public health problem in its own right[1] there is growing evidence suggesting that rates of physical disease, such as obesity and diabetes, are elevated amongst people with depression.[2,3] The study by Farmer et al. demonstrated a striking relationship between recurrent clinically sampled depression and increased BMI, type II diabetes, coronary heart disease and hypertension. Regression analyses showed that the association of depression with this cluster of disorders (sometimes referred to a 'metabolic syndrome') was largely accounted for BMI.[2] A recent World Health Survey (WHS) study involving 245404 participants from 60 countries in regions all over the world found that the number of participants with one or more chronic physical disease, and comorbid depression, ranges from 9.3 to 23%.[3] Furthermore, recent studies support the hypothesis that there may be shared aetiological factors, including genetic factors, between recurrent unipolar depression, obesity and physical disorders.[2,4]

The fat mass and obesity associated gene, *FTO*, on chromosome 16q has been reported multiple times to contribute to common forms of human obesity.[5] In 2007, two groups independently identified a common single nucleotide polymorphism (SNP) in *FTO* (rs9939609) that was associated with BMI and an increased risk for adult obesity.[6,7] In the same year, a third group performing a genome-wide association (GWA) study of BMI also found variants in *FTO* strongly associated with BMI and obesity-related traits.[8]

Recently the *FTO* association with BMI has also been replicated in two large GWA studies for obesity.[9,10] These two studies strongly support the association between *FTO* variants and obesity.

Animal studies reported that *FTO* is widely expressed in the brain, with high expression in hypothalamic nuclei regulating energy balance.[11] Human studies have also found that the *FTO* variant (rs9939609) risk allele is associated with an increased energy intake[12,13] and diminished satiety,[14] implicating *FTO* in the regulation of appetite.

This study explores the hypothesis that shared genetic aetiological factors exist between depression and obesity. Specifically, we aimed to investigate the influence of polymorphisms in *FTO* in relationship with BMI on a sample of depressed patients and psychiatrically healthy controls and follow-up the SNPs showing the strongest associations in an independent population-based cohort.

Materials and methods

Clinical Sample

1. Radiant Study

The depression case sample included 2442 individuals (740 men, 1702 women; mean age ± s.d.: 45.25±12.15) and was sourced from several studies: the Depression Case-Control (DeCC) study[15], Depression Network (DeNT) study[16,17] and the Genome Based Therapeutic Drugs for Depression (GENDEP) study.[18] The DeCC sample comprises subjects with recurrent unipolar depression (minimum two episodes) of at least moderate

severity as defined by DSM-IV or ICD-10 criteria, recruited from three UK sites (London, Cardiff and Birmingham).[15] Probands from the DeNT affected sibling pair linkage study consisting of cases with recurrent unipolar depression of at least moderate severity collected at seven European sites and one US site.[16,17] The GENDEP study includes individuals with an episode of depression of at least moderate severity recruited from nine European centres,[18] not necessarily recurrent. Either DSM-IV or ICD-10 diagnosis of depression were ascertained using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview in all three studies.[19] Individuals were excluded if they, or a first-degree relative, had a history of mania, hypomania, schizophrenia or bipolar disorder, or if the depression occurred in relation to alcohol, substance misuse or a medical illness or medication.

Eight hundred and nine control individuals from the UK (313 men, 496 women; mean age ± s.d.: 39.9±13.71) were screened for lifetime absence of any psychiatric disorder using a modified version of the Past History Schedule.[20] Participants were excluded if they, or a first-degree relative, ever fulfilled the criteria for depression, bipolar disorder or schizophrenia or if they scored 10 or above on the Beck Depression Inventory.[21]
All cases and controls were of white European ancestry.

2. PsyCoLaus Study

The sample is part of the PsyCoLaus study which focused on psychiatric symptoms in a population-based cohort originally assessed for cardiovascular risk factors (CoLaus). CoLaus is a cross-sectional study including 6188 extensively phenotyped Caucasian

individuals randomly selected from the list of residents of the city of Lausanne (Switzerland). All 35 to 66 year-old subjects of the CoLaus sample were invited to participate in the psychiatric evaluation for the PsyCoLaus sub-study (see[22,23] for a detailed description). The PsyCoLaus sample included 1296 cases (431 men, 862 women; mean age ± s.d.: 49.69±8.68) who fulfilled lifetime ever criteria for MDD according to DSM-IV based on assessment using the Diagnostic Interview for Genetics Studies (DIGS[24]). The 1690 PsyCoLaus subjects (974 men, 724 women; mean age ± s.d.: 50.59±8.94) who have never fulfilled criteria for MDD were included as controls.

All studies were approved by the local ethical committees and informed written consent was obtained from all participants.

Table 1 summarizes the characteristics of the subjects in both studies.

Phenotypic data

Body Mass Index (BMI) was defined as weight (kilograms) / height (metres)^2. In the Radiant study self-reported height and weight were obtained during the SCAN interview for cases and during telephone interview for controls. In the PsyCoLaus sample weight and height were measured with participants standing without shoes in light indoor clothes.[22] In both samples the distribution of BMI was positively skewed. We therefore transformed the data to $\log_{10}$(BMI) to achieve a closer approximation to normal distribution.

Genotyping

1. Radiant Study

Depression cases and controls were genotyped using the Illumina HumanHap610-Quad BeadChips by the Centre National de Génotypage (CNG) as previously described.[25] After applying stringent quality control criteria for missing genotypes, departure from Hardy-Weinberg equilibrium and low minor allele frequency, a total of 88 SNPs spanning *FTO* (+/- 20 Kb either side, chr16: 52,295,376-52,705,882. UCSC Mar.2006) were included in the analyses.

2. PsyCoLaus Study

Genotyping for depressive cases and controls was performed using the Affymetrix 500 K SNP chip. The genotyping are described in detail in the CoLaus[22] and PsyCoLaus[23] studies. IMPUTE v0.2 was used to impute the SNPs used in the Radiant study based on the HapMap CEU (Phase II, release 21) population.

Statistical analyses

T-Test analyses were carried out to test the association between BMI and affected status in both studies.

In Radiant study linear regression models for quantitative traits assuming an additive genetic model were performed for each SNP to test for association between LogBMI and *FTO* variants. The analyses were first carried out in the whole sample and then separately in the depressive cases and controls. Following the same procedures the most significant SNPs associated with LogBMI in the Radiant sample were analysed in PsyCoLaus.

Affected status, gender and age were included as covariates in the regression analyses in both studies. Principal components were used to control for possible population stratification. In the Radiant study we included centre of ascertainment as a covariate. We also tested for the interaction between *FTO* polymorphisms and affected status for an effect on LogBMI.

The number of effective/independent tests in the Radiant data was calculated using the method of Galwey et al.[26] The significance was assessed by dividing 0.05 by the number of effective tests (calculated to be 44.8115), giving threshold of $p< 0.0011$.

The statistical analyses were performed using the statistical package PLINK v1.06[27] and the software Quicktest[28] in the Radiant and PsyCoLaus studies, respectively.

Since the most strongly associated *FTO* polymorphism reported in literature so far is rs9939609, we used Beagle[29] to impute the genotypes for this SNP in the UK subsample of the Radiant study (1361 cases; 813 controls) using HapMap CEU samples as a reference panel. Two genotyped SNPs (rs8050136, rs3751812) are in absolute linkage disequilibrium (LD) ($r^2=1$) with rs9939609, allowing imputation with high certainty. (For LD structure of the variants most significantly associated see supplementary Figure 1). Analysis of imputed data was performed using gene dosage values. The regression analyses with the imputed variant were performed using R software.

Finally, we performed an interaction analysis between the most significant SNPs associated with LogBMI and affected status in the combined Radiant and PsyCoLaus samples.

Results

BMI and Depressive status

In Radiant, as previously reported in a subset of the same data[2], there was a significant association between BMI and affected status. Depressed patients had significantly higher BMI values in comparison with controls in both men and women (Men: t=-5.049, p=0.007; Women: t=-8.755, p<0.0001). These results differ in the PsyCoLaus sample where there were no significant differences in BMI values between depressed cases and controls (Men: t=-1.57, p=0.117; Women: t=-0.11, p=0.913) (Table 1).

FTO and LogBMI

All SNPs tested were in Hardy Weinberg Equilibrium (Radiant: p> 0.01; PsyCoLaus: p> 10^{-4}) in depressive cases and controls.

1. Radiant Study

Following linear regression between the 88 *FTO* polymorphisms and LogBMI, 9 of these SNPs located in the first intron of the gene were the most significantly associated with BMI using affected status, gender, age and principal components as covariates. Although these SNPs are mainly in high LD with each other (Figure 1), conditional analyses conducted on the most significant SNP (rs3751812) suggested that they represent two independent signals. One signal includes SNPs rs7205986 and rs6499640, and the second

signal includes the rest of the SNPs. Linear regression carried out with the imputed rs9939609 SNP in the UK subsample, also correcting for affected status, gender, age and principal components yielded a significant association with LogBMI (ß=-0.006, p=0.0149).

Linear regression in the depressive group with gender, age, principal components and study as covariates presented evidence of association for the same 9 SNPs that were associated with LogBMI in the combined sample. Moreover, the association between these SNPs and BMI was stronger when analysing the depressive group alone versus the combined sample. Seven remained statistically significant following multiple testing correction (see Table 2).

None of the SNPs tested for association between *FTO* and LogBMI reached significant p-values in the control group alone. Results for the combined, case and control analyses are shown in Figure 1.

Controlling for gender, age and principal components, rs9939609 was associated with LogBMI in the depressive group (ß=-0.011, p=0.0007) but not in the control group (ß=0.003, p=0.39).

2. PsyCoLaus Study

Eight of the top 10 SNPs associated with BMI in the Radiant study had very high imputation accuracy (rSqHat > 0.9) and were included in association analyses. Linear

regression analyses in combined depressive cases and controls yielded 6 SNPs significantly associated with LogBMI when affected status, gender, age and principal components were used as covariates. Results carried out in the depressive group alone with gender, age and principal components co-varied in the regression model yielded the same 6 SNPs significantly associated with LogBMI (Table 3). As in the Radiant study, the analyses in the control group alone showed no significant associations with logBMI (Table 3).

Interaction between FTO and affected status

1. Radiant Study

We found a significant interaction between genotype and affected status in relationship to LogBMI, and found no significant interaction with gender (data not shown). An interaction between genotype and affected status was observed in 7 SNPs ($p<0.0057$) (Table 2).

We also found an interaction between the previously reported rs9939609 (the polymorphism most commonly associated with BMI in previous studies) and affected status in the UK Radiant subsample (ß=-0.01, $p=0.0047$) taking gender, age and principal components as covariates in the model.

2. Radiant and PsyCoLaus combined analysis

Five of the seven SNPs with high imputation quality genotypes in PsyCoLaus, showed evidence (p-values between 0.03-0.06) of interaction between genotype and MDD affected status in their effect on LogBMI (Table 3). Additionally, these SNPs were

statistically significant in a meta-analysis of both cohorts (Table 4). The Cochran Q p-values from the test of heterogeneity of effects in each cohort were non significant indicating that estimates were similar across the two cohorts and justifying the effects meta-analysis (data not shown).

Discussion

We have investigated the influence of variants in *FTO* on BMI in a large clinical sample of depressed patients and controls (Radiant) to explore the genetic mechanism underlying the reported association between BMI or obesity and psychiatric disorders.[30-32] We have then followed up our findings in a population-based cohort (PsyCoLaus).We found associations between several SNPs in *FTO* and BMI that are in keeping with previous reports. However an intriguing novel finding in the Radiant sample is that the association observed in the whole sample could be attributed to the depressive group, with none of the SNPs being significantly associated with BMI in the control sample alone, and the association strengthening when analysing the patients alone. A similar pattern was found in the PsyCoLaus sample, strongly arguing against this being a chance finding.

There are two potential limitations of our study. The first is in the Radiant sample self-reported data were used to calculate BMI. Against this, height and weight were actually measured in the replication sample (PsyCoLaus) and the same pattern of results was found. The second limitation is that *FTO* polymorphism most frequently reported to be associated with BMI (rs9939609) in previous studies was here imputed rather than

genotyped directly. Against this, the imputation was based on two genotyped SNPs (rs8050136, rs3751812) that are in absolute LD (r^2=1) with rs9939609.

In the Radiant study evidence that the observed *FTO*-BMI association is specific to the depressive sample is further supported by the interaction between *FTO* variants and affected status. This interaction was found in 7 of the SNPs associated with BMI and indicates that the failure to observe an association in the control sample is unlikely to be a consequence of a lack of power. This suggests that the effect of the *FTO* gene on BMI is modified by having a depressive disorder. Furthermore, the interaction between *FTO* variants and affected status found in the Radiant data was corroborated by the combined results obtained from the Radiant and PsyCoLaus samples.

As this was the first study of its kind concerning the relationship between BMI and depression we decided to interrogate the entire *FTO* gene. However the evidence for the association with BMI was, as in previous studies not involved with depression, concentrated in a group of SNPs located in the first intron of the gene. Previous studies report association with SNPs located in the same region.[6-10] Interestingly, 5 associated SNPs (rs6499640, rs8050136, rs3751812, rs7190492, rs8044769) in our sample have also been found to be genome-wide significantly associated with variation in BMI in the study by Thorleifsson et al.[9] Two of the most significant SNPs associated with BMI in Radiant and PsyCoLaus studies (rs3751812, rs8050136) are in absolute LD (r^2=1) with the *FTO* variant rs9939609. The results obtained with this imputed variant in both studies are in the same direction and confirm previous findings.[6-8,33-37]

In the Radiant study depressed patients had higher BMI than controls and this could be attributable to a side effect of antidepressant treatment, or because of people who are depressed carry out less physical activity and/or have an increased food intake. These effects are impossible to tease apart in the Radiant sample because all of the cases had at some point received antidepressants. Also, our control sample has been screened so as to have no history of psychiatric disorder in themselves or a first-degree relative. This is something previous studies investigating BMI, obesity and *FTO* have not done and could further explain why no association is observed in our control group.

In contrast, in the PsyCoLaus sample there were no statistically significant differences in BMI between depressed cases and controls. These differences possibly reflect the fact that Radiant is a clinically ascertained sample of mainly recurrent depression and all of the cases recruited have received antidepressant treatment, whereas the PsyCoLaus cases were recruited from the community and were an arguably less severe and more broadly defined group, where only one episode was required for inclusion and in which only 37.5% of cases had ever received treatment with antidepressants. However, it is noteworthy that the regression of BMI on antidepressant status showed no hint of association ($p = 0.573$) nor was there any effect when antidepressant status was included as a covariate in the regression of BMI on *FTO* SNPs. These findings indicate that moderating effect of depressive disorder on the BMI/*FTO* association is unlikely to be simply a result of taking antidepressants.

An alternative but not necessarily exclusive explanation is that depression and obesity involve pathophysiologically overlapping mechanisms. The hypothalamic-pituitary-adrenocortical (HPA) system governs the stress response and has been implicated in the aetiology of depression.[38] *FTO* is highly expressed in the hypothalamus, pituitary and adrenal glands indicating a possible role in the HPA axis which is involved in body weight regulation.[39]

Although several studies have investigated the influence of *FTO* variants on BMI, and the association between obesity and psychiatric disorders separately, to the authors' knowledge this is the first study investigating the relationship between *FTO*, BMI and psychiatric disorders (depression) concurrently. Our research suggests that although an association is observed in the whole sample, having a history of depression does in fact moderate the effect of *FTO* on BMI. The results found initially in the large clinical sample of depressed cases and controls (Radiant) have been replicated in a population-based cohort (PsyCoLaus). In addition, since milder forms of depression and depressive symptoms are very common in the general population[40] it is possible that experiencing such symptoms moderates the effect of *FTO* in the population as a whole and partly determines whom of those carrying ' at risk' *FTO* variants go on to become overweight or obese.

Conflict of interest

Aitchison, Farmer and McGuffin have received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies including GlaxoSmithKline.

Aitchison's declares interests through Advisory Boards for Johnson & Johnson, Lundbeck, Roche Diagnostics, and Bristol-Myers Squibb; membership of Bristol-Myers Squibb UK Steering group 2003 to present; consultancy work for Roche Diagnostics, Johnson & Johnson Pharmaceutical Research and Development, Lundbeck, and Bristol-Myers Squibb Pharmaceuticals Limited; grants awarded by Johnson & Johnson Pharmaceutical Research and Development, Bristol-Myers Squibb Pharmaceuticals Limited, and E Merck Pharmaceuticals. Maier is member of the Advisory Boards/ has received fees for speaking from: Lilly, Lundbeck. Tozzi was full time employee of GlaxoSmithKline at the time when the work was performed. All other authors declare no conflicts of interest.

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Supplementary information is available at *Molecular Psychiatry's* website.

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Table 1. Demographic characteristics of the Radiant and PsyCoLaus samples.

	Radiant Study		PsyCoLaus Study	
	Depression sample	Control sample	Depression sample	Control sample
Sample size	n=2442	n=809	n=1296	n=1698
Gender (%) (male/female)	30.3 / 69.7	38.7 / 61.3	33.49 / 66.51	57.36 / 42.64
Mean age (years) ± s.d	45.25 ± 12.15	39.90 ± 13.71	49.69 ± 8.68	50.59 ± 8.94
Mean BMI ± s.d. (male/female)	26.62 ±4.32 / 26.31±5.96	25.18 ±4.31 / 23.78±4.53	26.04 ± 3.84 / 24.83 ± 5.06	26.39 ± 3.80 / 24.86 ± 4.86

Table 2. Association analyses between *FTO* and LogBMI and the interaction with affected status in the Radiant study.

SNP	Position[a]	Allele[b]	MAF	p-values			
				Combined	Cases	Controls	Interaction[c]
rs7205986	52312647	A/G	0.48 (A)	0.0024	0.002	0.518	0.1641
rs6499640	52327178	G/A	0.38 (G)	0.0006	0.0014	0.149	0.5219
rs9930333	52357478	G/T	0.44 (G)	0.0032	0.0001	0.166	0.0014
rs10852521	52362466	T/C	0.48 (T)	0.0016	$7.5x10^{-5}$	0.272	0.0019
rs8050136	52373776	A/C	0.41 (A)	0.0018	0.0001	0.317	0.0031
rs3751812	52375961	T/G	0.41 (T)	0.0013	$6.8x10^{-5}$	0.337	0.0028
rs9941349	52382989	T/C	0.43 (T)	0.0013	$7.4x10^{-5}$	0.399	0.0037
rs7190492	52386253	A/G	0.37 (A)	0.0092	0.001	0.309	0.0057
rs8044769	52396636	T/C	0.48 (T)	0.0011	$7x10^{-5}$	0.454	0.0045

[a] Position (chr 16) according to the HapMap database.

[b]Risk Alleles are underlined.

[c] Interaction between *FTO* SNPs and affected status.

MAF: minor allele frequency. Minor allele is in brackets.

Table 3.Association analyses between *FTO* and LogBMI and the interaction with affected status in the PsyCoLaus study.

SNP	Position[a]	Allele[b]	MAF	p-values			
				Combined	Cases	Controls	Interaction[c]
rs9930333	52357478	G/T	0.44 (G)	0.00096	0.00066	0.1633	0.0640
rs10852521	52362466	T/C	0.46 (T)	0.0856	0.136	0.3242	0.5468
rs8050136	52373776	A/C	0.41 (A)	0.0047	0.00099	0.4063	0.0336
rs3751812	52375961	T/G	0.41 (T)	0.0057	0.0014	0.4047	0.0408
rs9939609	52378028	A/T	0.41 (A)	0.0058	0.0016	0.3915	0.0444
rs9941349	52382989	T/C	0.43 (T)	0.0026	0.0010	0.2737	0.0530
rs7190492	52386253	A/G	0.33 (A)	0.025	0.0164	0.4032	0.1390
rs8044769	52396636	T/C	0.46 (T)	0.16	0.157	0.5371	0.4311

[a] Position (chr 16) according to the HapMap database.

[b]Coded Alleles are underlined.

[c] Interaction between *FTO* SNPs and affected status.

MAF: minor allele frequency. Minor allele is in brackets.

Table 4.Interaction analyses between *FTO* and affected status in the combined sample.

SNP	Position[a]	Coded Allele	ß	SE	p-value
rs9930333	52357478	G	0.01	0.0029	$7x10^{-4}$
rs10852521	52362466	T	-0.007	0.0029	0.0199
rs8050136	52373776	A	0.01	0.03	$5x10^{-4}$
rs3751812	52375961	T	0.01	0.03	$6x10^{-4}$
rs9939609	52378028	T	-0.01	0.03	0.001
rs9941349	52382989	T	0.01	0.029	0.001

[a] Position (chr 16) according to the HapMap database.

SE is the standard error.

Figure 1. Plot showing –log p-values for the SNPs analysed in *FTO* gene in the Radiant sample.

Supplementary Figure 1. LD plot structure for the most significant SNPs in *FTO* gene. Red represents very high LD (r^2) between SNPs.

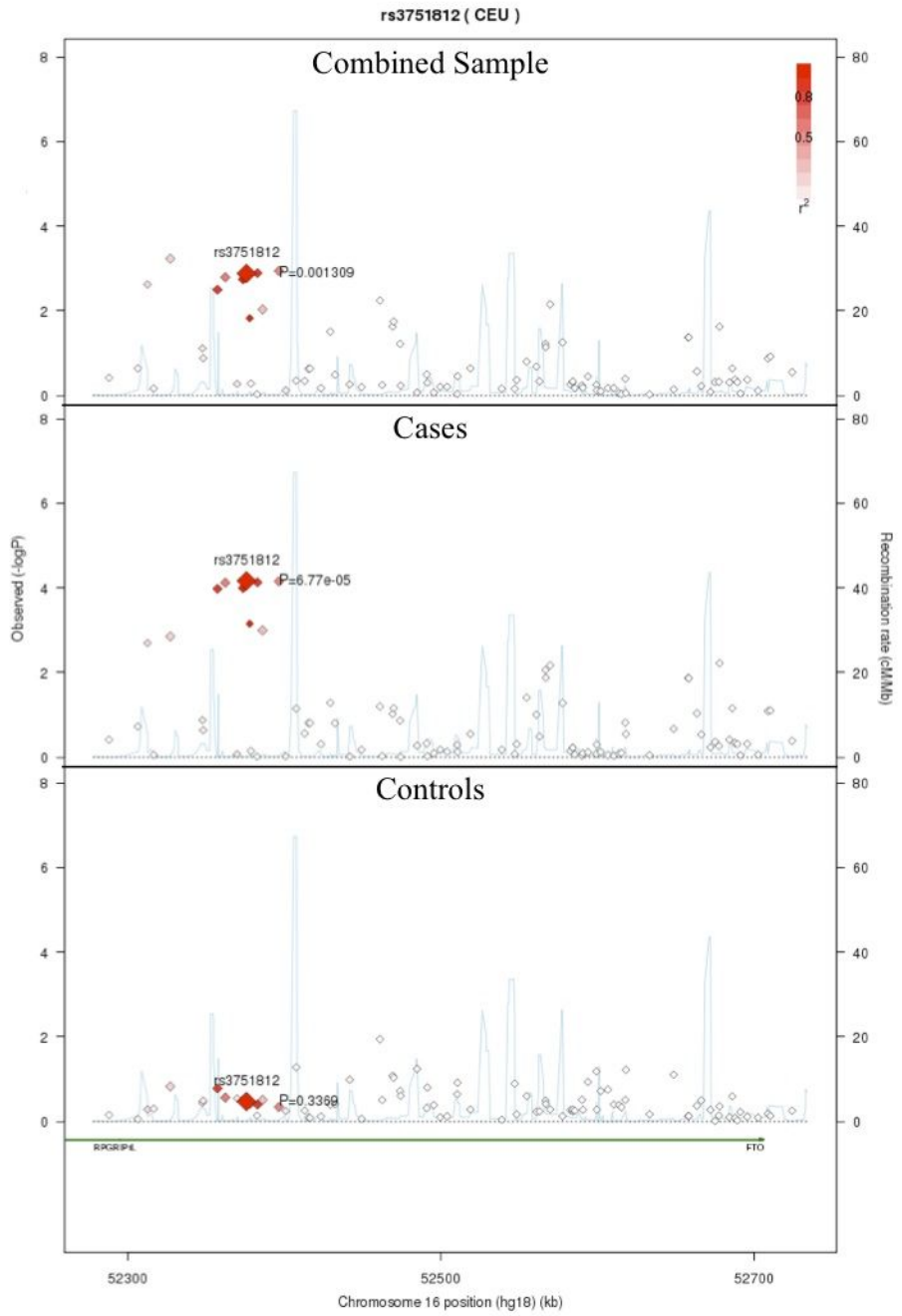

Figure 1. Plot showing –log p-values for the SNPs analysed in FTO gene in the Radiant sample.