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Genetic polymorphisms associated with constipation and anticholinergic symptoms in patients receiving clozapine

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Running title: Pharmacogenetics of clozapine

Abstract

Background

Clozapine impairs gastrointestinal motility owing to its anticholinergic and antiserotonergic properties. This commonly leads to constipation, and potentially to more severe complications such as bowel obstruction and ischemia. The aim of this study is to determine whether or not genetic variations in the genes encoding muscarinic and serotonergic receptors (*CHRM2*, *CHRM3*, *HTR2*, *HTR3*, *HTR4*, and *HTR7*) explain the variations in incidence of constipation and anticholinergic symptoms during clozapine treatment. Genes associated with opiate-induced constipation were also included in this analysis (*TPH1*, *OPRM1*, *ABCB1*, and *COMT*).

Procedures

Blood samples from 176 clozapine-treated, Finnish, Caucasian patients with schizophrenia were genotyped. Constipation and anticholinergic symptoms were rated using the Liverpool University Neuroleptic Side Effect Rating Scale self-report questionnaire. In total, 192 single-nucleotide polymorphisms (SNPs) were detected, and grouped to formulate a weighted genetic-risk score (GRS).

Results

No significant associations between individual SNPs or GRSs and constipation or laxative use were observed. A GRS of 19 SNPs in *CHRM2*, *CHRM3*, *HTR3C*, *HTR7*, *ABCB1*, *OPRM1* and *TPH1* was associated with anticholinergic symptoms in a generalized linear univariate (GLM) model with body-mass index, clozapine monotherapy and GRS as explaining variables (permuted $p = 0.014$). GLM analysis performed on the opiate-induced constipation associated SNPs and a single *CHRM3* SNP revealed an association between anticholinergic symptoms and a score of 8 SNPs (adjusted $p = 0.038$, permuted $p = 0.002$).

Conclusions

Two GRSs are able to predict the risk of anticholinergic symptoms in patients receiving clozapine, and possibly an increased risk of gastrointestinal hypomotility.

Keywords: clozapine; antipsychotics; polymorphism; adverse effects; constipation

Introduction

Constipation is a common adverse effect of clozapine that is reported to occur in 14–60% of patients receiving treatment with this drug (1, 2), often leading to increased use of laxative medication (3). The impairment of gastrointestinal motility associated with clozapine can also result in severe complications such as dysphagia, ileus, intestinal obstruction, bowel ischemia, and megacolon. Among clinicians, these risks seem to be less well known than clozapine-induced agranulocytosis, despite clozapine-induced gastrointestinal hypomotility and agranulosytosis having similar prevalences (4.0–8.0 ‰ versus 3.8–8.0 ‰). Furthermore, clozapine-induced gastrointestinal hypomotility is associated with a greater risk of mortality, and careful monitoring of patients receiving clozapine for symptoms of gastrointestinal hypomotility is recommended. (4, 5)

Clozapine-induced constipation seems to be associated with the anticholinergic and antiserotonergic properties of this agent (5, 6). In the absence of clozapine, acetylcholine stimulates muscarinic receptors in both smooth muscle cells and the interstitial cells of Cajal (ICC) of the gastrointestinal tract, the latter of which function as the pacemaker cells of the gastrointestinal tract (7). Muscarinic receptors (of the M_2 and M_3 subtypes) are expressed in ICCs and smooth muscle cells of the gastrointestinal tract (8). However, other anticholinergic agents with an efficacy similar to that of clozapine have much lower risks of gastrointestinal hypomotility. For example, the risk of constipation associated with use of clozapine is three times greater than that associated with chlorpromazine (9). Treatment with clozapine is also associated with a higher risk of ileus than that of other antipsychotics, as demonstrated by data from a large European study (10). This increase in risk is proposed to be caused by the antiserotonergic effects of clozapine (5). Clozapine is an antagonist of several 5-hydroxytryptamine receptors (5-HT_2, 5-HT_3, 5-HT_6, and 5-HT_7) (11). Serotonin also has a complex and crucial but still controversial role in gut function. Data from many studies have suggested that 5-HT receptors, especially 5-HT_3 and 5-HT_4, have an important role in

the regulation of gut motility. (5, 12). 5-HT_2 receptors have been suggested to modulate visceral sensation and 5-HT_7 receptors might be involved in mediating relaxation of gastrointestinal smooth muscle. Furthermore, inhibition of 5-HT_3 receptors has been reported reduce the rate of colon transit, inhibit gastrocolic reflexes, increase the level of colonic compliance and possibly reduce intestinal sensitivity to distension. (5)

The aim of this study is to determine the influence of genetic variations in different subtypes of muscarinic and serotonergic receptors, and whether such variations can explain the variable incidence of constipation or anticholinergic symptoms during clozapine treatment. Interactions between such variations and the risk of constipation or anticholinergic symptoms caused by antipsychotic medications have not been studied previously. All muscarinic and serotonergic subtypes that have been previously suggested to have a role in regulating gut motility were included in this study. Single-nucleotide polymorphisms (SNPs) in the genes encoding the M_2 and M_3 muscarinic receptors (*CHRM2*, *CHRM3*) and the 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 receptors (HTR2, HTR3, HTR4, and HTR7) were extracted. Additional analyses were performed, including SNPs in *TPH1*, *OPRM1*, *ABCB1*, *CHRM3* and *COMT*, which have all previously been suggested to contribute to the variability in incidence of constipation among patients with cancer receiving treatment with opioids (13). *TPH1* encodes tryptophan 5-hydroxylase 1, which catalyzes the first and rate-limiting step in the biosynthesis of serotonin (14). TPH1 transcript levels are increased in patients with chronic constipation (15). *OPRM1* encodes the mu-type opioid receptor. *ABCB1* encodes Multidrug resistance protein 1, a protein that transports various molecules across extracellular and intracellular membranes. Furthermore, *ABCB1* is widely expressed in various tissues, including the bowel, blood-brain barrier, liver and kidneys (16). *COMT* encodes catechol O-methyltransferase, an enzyme that catalyzes the degradation of catecholamines such as dopamine, epinephrine and norepinephrine (14).

Materials and Methods

Patients

The sample was screened from 256 patients, of which 19 declined to participate in this study. Samples were collected from three hospital districts in western Finland (Satakunta, Pirkanmaa, and Seinäjoki). Inclusion criteria included a current F2-group diagnosis, according to the International Classification of Diseases, Tenth Revision (ICD-10), and a stabilized clozapine treatment. Patients with organic brain diseases were excluded from the analysis. The study population comprised 237 patients receiving clozapine (136 men, 101 women, with a mean age 42.5 ± 11.0 years) who were diagnosed with either schizophrenia (*n* = 223, 94.1%), schizoaffective disorder or delusional disorder. The mean elapsed time from first hospitalization owing to a psychotic episode was 17.3 years (± 10.0 years). All patients were adults (≥18 years of age), Caucasian and of Finnish origin. Blood samples were obtained from 190 patients, of which 176 genotyped patients were eligible to remain in the study after quality controls. Patients gave written, informed consent prior to participation. The study was approved by the Ethics Committee of Satakunta Hospital District on the 23rd of April 2008 and was conducted in accordance with the Tenets of the Declaration of Helsinki. More detailed information on the data collection process and the clinical and demographic characteristics of the study population is provided in our previous studies (17, 18).

Of the 176 patients whose samples were genotyped, 57 (32.4 %) were receiving a combination of at least two antipsychotics, including clozapine, and 119 patients (67.6%) were receiving clozapine monotherapy. All doses of antipsychotic medication were converted to chlorpromazine equivalents (19). The mean daily dose of clozapine received was 403 mg (SD ± 152 mg) or 806 mg (SD ± 304 mg) expressed as a chlorpromazine equivalent dose. The mean total antipsychotic dose received was 912 mg (SD ± 304 mg) as a chlorpromazine equivalent dose. More than half of all patients had received clozapine treatment for >5 years and none for <3 months at the time of study enrollment.

The demographics of the genotyped patients are presented in Table 1. Eleven patients were also receiving anticholinergic medications, of which five were for the control of extrapyramidal symptoms (biperiden or similar) and six were for bladder functional disorders (oxybutynin, or trospium chloride).

Patients completed the Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS) (20), a self-assessment questionnaire that consists of a list of 51 symptoms. Patients were asked to rate the severity of each listed adverse effect on a scale from 0 to 4 (0 = not at all, 1 = very little, 2 = a little, 3 = quite a lot and 4 = very much). Question number 10 "constipation" was used as a dichotomized variable with two groups: those with no constipation at all (LUNSERS scale 0, $n = 49$) versus those who reported very little to very much constipation (LUNSERS scale 1–4, $n = 127$). A more-specific dichotomized variable was also used as a response variable, which included groups of patients who reported having quite a lot, to very much constipation (LUNSERS scale 3–4) or were using laxative medications ($n = 95$) versus those who reported having no constipation at all to a little constipation and were not receiving laxative medications ($n = 81$). To study patients' anticholinergic symptoms, a factor was formed by summing the severity of the following five items from LUNSERS: "Dry mouth", "Constipation", "Palpitations", "Difficulty of passing water" and "Blurred vision".

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using an QIAamp DNA Blood Midikit and an automated biorobot M48 extraction (Qiagen, Hilden, Germany). Samples were genotyped using an Illumina Infinium HumanCoreExome-12 DNA Analysis Beadchip, version 1.0., according to the manufacturer's instructions at Helmholtz Zentrum, München, Germany. The following quality control filters were applied: GenCall score <0.15, GenTrain score <0.20, sample and an SNP call rate <0.95, Hardy–Weinberg equation p-value <10−6, excess heterozygosity, cryptic

relatedness (pi-hat >0.2), gender check and multidimensional scaling (MDS). After quality control, 176 samples and 531,983 SNPs were available, of which 192 SNPs were in the genes of interest (*CHRM2*, *CHRM3*, *HTR2*, *HTR3*, *HTR4*, *HTR7*, *TPH1*, *OPRM1*, *ABCB1* and *COMT*). SNPs with a minor allele frequency (MAF) of <0.01 were excluded from the study.

Statistical methods

Values for comparisons between each group are presented as mean ± standard deviation of the mean, unless otherwise stated. Pearson correlations, chi-squared tests and t-tests were used in exploratory analyses of the relationships between constipation, use of laxatives and anticholinergic factor, and potential explanatory variables such as use of clozapine dose, clozapine and its metabolite norclozapine (*N*-desmethylclozapine) concentrations, body-mass index (BMI), smoking status, age, and use of benzodiazepines or selective-serotonin reuptake inhibitors (SSRIs). Reliability analysis for anticholinergic factor was performed using a principal components analysis for ordinal data, using a one-component solution in which all five items were loaded (>0.5). The Cronbach's alpha value was 0.60. Generalized linear univariate models (GLMs) were used to analyze the explanatory factors: SNPs encoded separately with additive, dominant and recessive coding and covariates for response variables. The response variables analyzed with GLM were dichotomized constipation (binominal distribution), dichotomized constipation taking laxative use into account (binominal distribution) and the anticholinergic factor (Gaussian distribution). Covariates in the models were chosen primarily according to literature and secondarily according to the results of exploratory bivariate analyses. Finally, the best fitting model was used. In the GLM analysis, the SNPs were analyzed by entering them into the model one at a time. To adjust significance levels for multiple testing, the false-discovery rate (FDR) was used. This accounts for the number of SNPs and different modes of inheritance in the GLM. SNPs were coded with additive, dominant and recessive models. Details of

the coding are described in our previous study (18). The statistical significance level was set at $p < 0.05$.

Statistical analyses were performed using R statistical analysis software (version 3.1.2, 2014, The R Foundation for Statistical Computing), SPSS (version 22, IBM inc.), gPLINK (version 2.050), PLINK (version 1.07) and Haploview analysis software (version 4.2).

Genetic risk score (GRS)

Genetic risk scores (GRSs) were calculated for each patient as a weighted sum of the risk alleles in relation to the studied phenotype. SNPs that had an unadjusted level of statistical significance ($p < 0.05$) in the GLM models were selected for further analysis. These SNPs were inserted into the same model with the other coefficients and a stepwise analysis was performed using Akaike information criteria (AIC) (21). AIC is a method of model-based genetic mapping (22). Data obtained using AIC is used to identify the influential tag SNPs of the SNPs preselected with GLM by also taking into account the extent of linkage disequilibrium (LD) between the SNPs. SNPs that remained significant, as indicated by AIC, were used in the GRS. The model returned by AIC was analyzed with stepwise variance inflation factor (VIF) to examine collinearity between the SNPs in the GRS. The corresponding beta estimate value (β) from the GLM for each SNP was used to adjust the weighting of individual effects. The formed GRS was then used as an explanatory variable in the final GLM, together with other explanatory variables.

Permutation test

Linear combinations of genetic effects estimated using GLMs might overfit data and cause inflated type I errors. An estimation of the null distribution of the GRS test statistics is required to validate the GRS results (23). A permutation test was performed, in which the patient identification numbers

from the phenotype data were sampled. This resulted in SNP genotypes that were independent of the studied phenotypes. The permutation was conducted 999 times using the same statistical methods as described above, but with sampled data. P-values were collected for GRSs from each model in each sampled dataset. The result was considered statistically significant if the p-value derived from the actual data analysis was <5 % of the p-values derived from the permutated data.

Results

In exploratory analyses, patients in the group who had more severe constipation or used laxatives were significantly older than those who had less-severe constipation and required no laxatives (41.34 ± 10.6 years of age versus 44.52 ± 11.6 years of age, t-test p = 0.035). No statistically significant associations were observed using analyses of the correlations between dichotomized constipation or anticholinergic factor and age, BMI, smoking status, gender, clozapine dose, or clozapine and norclozapine concentrations. Use of laxatives was associated with BMI (30.4 ± 6.1 kg/m^2 versus. 28.2 ± 6.3 kg/m^2, t-test p = 0.032), with patients with a lower BMI more likely to be using laxatives. Use of laxatives was also associated with use of benzodiazepines (chi-square, p = 0.020), with patients with benzodiazepine medication also more likely to be using laxatives. Anticholinergic factor was also associated with use of benzodiazepines (4.45 ± 3.40 versus 5.38 ± 3.36, t-test p = 0.042) and with use of SSRIs (4.59 ± 3.49 versus 5.70 ± 3.13, t-test p = 0.015). No correlation between anticholinergic medication use and anticholinergic factor was observed (r = 0.063). Dichotomized constipation was not associated with use of benzodiazepines or SSRIs.

In the GLM analyses, with constipation or use of laxative as the dependent variable, SNPs in *CHRM2*, *CHRM3*, *HTR2*, *HTR3*, *HTR4* and *HTR7* with, or without the inclusion of additional SNPs in *TPH1*, *OPRM1*, *ABCB1* and *COMT*, no statistically significant associations were observed level in any model with varying combinations of additional explaining variables (age, BMI, gender, use of benzodiazepines or SSRIs). With anticholinergic factor as the dependent variable and BMI and SNPs as the explaining variables, following analysis SNPs in *CHRM2*, *CHRM3*, *HTR2*, *HTR3*, *HTR4*, and *HTR7*, only rs685548 remained statistically significant after FDR-adjustment (dominant coding p = 0.038, additive coding p = 0.044). However, this association became statistically insignificant following FDR-adjustment when SNPs associated with opiate-induced constipation were added to this analysis (dominant coding p = 0.060, additive coding p = 0.071).

In the GRS analysis, no significant associations between GRS and constipation or use of laxatives were observed. When only SNPs in *CHRM2*, *CHRM3*, *HTR2*, *HTR3*, *HTR4* and *HTR7* were analyzed, associations between the formed GRS and the anticholinergic factor remained statistically insignificant after permutation. When SNPs in genes associated with opiate-induced constipation (*ABCB1*, *OPRM1*, *TPH1* and *COMT*) were added to the analysis, a score of 19 SNPs in *CHRM2*, *CHRM3*, *HTR3C*, *HTR7*, *ABCB1*, *OPRM1* and *TPH1* (table 2) was found to be associated with anticholinergic factor in a GLM model with BMI and GRS as explaining variables ($p = 0.017$). GLM analysis performed only on SNPs associated with opiate-induced constipation and a single SNP in *CHRM3* that was associated with anticholinergic factor resulted in a score of 7 SNPs ($p = 0.0023$, table 2). In both models, no significant collinearity was observed between the SNPs or other predictor variables (VIF <10).

Discussion

To the best of our knowledge, no previous study has examined the association between clozapine-induced gastric hypomotility or anticholinergic symptoms related to antipsychotic medication and genetic polymorphisms. The main findings of this study are the two GRS (table 2) and one single SNP in *CHRM3* (rs685548) that are associated with anticholinergic symptoms in patients with schizophrenia who are receiving clozapine. This effect is a result of SNPs in various genes, including genes linked with cholinergic (*CHRM2* and *CHRM3*) and serotonergic neurotransmission (*HTR3C*, *HTR7*, and *TPH1*), but also with genes associated with opiate-induced constipation (*ABCB1* and *OPRM1*). This finding might reflect individual genetic variations in response to clozapine or in the functionality of the autonomic nervous system. No statistically significant associations between SNPs in serotonergic or muscarinic receptors, or SNPs in genes related to opiate-induced constipation and constipation or laxative use were observed in this study.

The model with the 19-SNP-score, BMI and clozapine monotherapy explained 43% of the variability in anticholinergic symptoms in clozapine-treated patients. The effect was confirmed using a permutation test ($p = 0.014$), but the high adjusted R^2 value and very low p-value in the GLM ($<2 \times 10^{-16}$) might contain a type I error owing to overfitting in the GRS analysis. The rs685548 SNP located in *CHRM3* is an intron variant with a MAF of 0.36, with no other SNPs with a level of LD >0.8 r^2 present in this cohort (Table 3). No significant associations between any phenotype and *CHRM3* rs685548 have been reported previously. Furthermore, no items were found in the PubMed database describing any of the SNPs included in the GRSs or SNPs with a level of LD >0.8 r^2. SNPs in *ABCB1* have been widely investigated. SNPs in this gene have been studied in relation to patient's responses to clozapine and plasma clozapine levels (24, 25), but not in relation to anticholinergic symptoms. The current study cannot propose a single mechanism explaining the increased risk of anticholinergic symptoms during clozapine treatment, but rather suggests that the genetic effect is cumulative, owing to alterations in muscarinic and serotonergic receptor function (in *CHRM2*, *CHRM3*, *HTR3C*, and

HTR7), the serotonin-related enzyme *TPH1*, and in *ABCB1* and *OPRM1* function. rs1045642 in *ABCB1*, which was also included in the GRS, might affect the absorption of clozapine from the gut, as previously reported (24), however, in this study, no significant association between serum clozapine and norclozapine concentration and anticholinergic symptoms was observed. The variability in anticholinergic symptoms might also be related to the functions of ABCB1 in other tissues. Important functional roles of ABCB1 also has an important function maintaining the blood-brain barrier and blood-cerebral spinal fluid barrier (16).

As Palmer et al. (2008) state clozapine induced gastric hypomotility is underrecognized and potentially life-threatening adverse effect of clozapine treatment (5). Objective evidence of gastrointestinal hypomotility in clozapine-treated patients has been shown by using a colonic transit test (26). Recent study reported that median colonic transit times are over four times longer than those on other antipsychotics or healthy controls and four fifths of patients using clozapine had colonic hypomotility (27). Furthermore, older age, female sex, treatment with clozapine, tricyclic antidepressants, anticholinergics and opioids are reported to be associated with an increased risk of ileus (28). Patients with a greater incidence of anticholinergic symptoms could have an elevated risk of gastric hypomotility and/or bowel complications such as ileus. Thus, the GRSs reported in this study could also be used as markers for the risk of these symptoms. The genes investigated in this study were originally selected based upon the hypothesized pharmacological effects of clozapine on the muscarinic and serotonergic receptors that regulate gut motility.

Many confounding factors could not be taken into account by this study, such as variations in diet or amount of exercise, although these can both be expected to influence BMI. In this study cohort, age and gender were both correlated with BMI, which highlights the importance of including BMI in the models used. A total of 192 SNPs were selected for analysis. The ability to detect small or moderate

effects associated with these SNPs is limited by the relatively small sample size of the study cohort; however, the well-defined hypothesis and focus only on specific genes reduces the need for a larger sample. A similar statistical approach was used in three of our earlier reports, which included the same patient sample (18, 29, 30). Limitations of this study include the reliance upon self-assessment of the severity of constipation and anticholinergic symptoms by each patient using the LUNSERS questionnaire. No information on patients' bowel movement frequencies or stool consistencies, with the help of stool charts, for example, was available. LUNSERS is, nevertheless, a reliable and validated method of measuring the adverse effects of antipsychotic treatments (31). Another limitation is that anticholinergic factor, as used to examine the severity of anticholinergic symptoms is a sum variable with only a moderate level of internal consistency (Cronbach's alpha = 0.6). However, the symptoms included in the anticholinergic factor (dry mouth, constipation, palpitations, difficulty of passing water and blurred vision) are all commonly recognized anticholinergic symptoms (32).

In conclusion, this study suggests that the risk of anticholinergic symptoms during clozapine treatment is associated with a GRS consisting of SNPs in *CHRM2*, *CHRM3*, *HTR3C*, *HTR7*, *TPH1*, *ABCB1* and *OPRM1* genes, in addition to rs685548 in *CHRM3*. No significant associations between the genes studied in this analysis and constipation or laxative use were found.

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Table 1. Demographics of the genotyped patients (n = 176)

Age mean (sd) years	43.5 (10.9)
Gender n (%)	
Men	104 (59.1 %)
Women	72 (40.9 %)
BMI mean (sd) kg/m2	29.8 (6.38)
Clozapine treatment duration n (%)	
3 months to 1 year	4 (2.3 %)
1 to 5 years	51 (29.0 %)
Over 5 years	106 (60.2 %)
Unknown	15 (8.5%)
Sum of clozapine and norclozapine concentrations, mean (sd) µmol/l	2.40 (1.28)
Regular smoking n (%)	
Non-smoking	85 (48.3 %)
Smoking	89 (50.6 %)
Unknown	2 (1.1 %)
Laxative in use n (%)	46 (26.1 %)
Quite a lot or very much constipation or laxative in use n (%)	81 (46.0 %)

Table 2. Results (test score, beta coefficients, significance levels and permutation rankings) of two general linear models with genetic risk scores, consisting of listed SNPs, explaining the risks of anticholinergic adverse effects summarized as 'anticholinergic factor', including BMI and clozapine monotherapy as confounding factors.

Genes selected for GRS analysis	**Formed GRS**	**BMI**			**Clozapine monotherapy**			**GRS**			**Adjusted R^2**	**Permutation ranking**
		t	β	p	t	β	p	t	β	p		
CHRM2 CHRM3 HTR2 HTR3 HTR4 HTR7 ABCB1 OPRM1 TPH1 COMT	CHRM2 ADD rs10228048* CHRM2 DOM rs12535371** CHRM3 DOM rs685548 CHRM3 REC rs11577797*** CHRM3 ADD rs6691263 CHRM3 REC rs10925910 CHRM3 DOM rs2841037 HTR4 REC rs7723153 HTR7 DOM rs7074715 HTR7 ADD rs7074715 ABCB1 ADD rs10276036 ABCB1 REC rs2235023 ABCB1 REC rs9282564 ABCB1 DOM rs10260862 ABCB1 DOM rs10248420 ABCB1 ADD rs2032582 ABCB1 ADD rs3789243 OPRM1 REC rs2272381 TPH1 ADD rs10832876	3.3	0.20	0.00045	-3.9	-1.7	0.00015	10.1	0.65	$< 2x10^{-16}$	0,43	14th out of 1 000 permutations (p = 0.014)
CHRM3 ADD rs685548 (single SNP) ABCB1 OPRM1 TPH1 COMT	CHRM3 ADD rs685548 OPRM1 REC rs2272381 OPRM1 REC rs34427887 ABCB1 ADD rs1002204 ABCB1 ADD rs10260862 ABCB1 REC rs2235023 ABCB1 REC rs9282564 TPH1 DOM rs10832876	1.9	0.067	0.056	-3.8	-1.8	0.00018	8.6	0.79	$6.1x10^{-15}$	0,36	2nd out of 1 000 permutations (p = 0.002)

* ADD = additive coding, ** DOM = dominant coding, *** REC = recessive coding

Table 3. SNPs in the two GRSs explaining anticholinergic symptoms, including SNPs with a level of LD >0.8 R^2, annotations of the SNPs and the number of items referring to these SNPs in the PubMed database.

Gene	SNP	SNPs in > 0.8 LD (r^2)	Annotation	Number of citations of the SNP in Pubmed
CHRM2	rs10228048	-	intron	-
	rs12535371		intron, LOC349160 (ncRNA)	-
		rs1364405 (0.83)	intron	-
CHRM3	rs685548		intron	-
		rs497576 (0.80)	intron	-
	rs11577797		intron	-
		rs6678395 (0.98)	intron	-
	rs6691263		intron	-
		rs12090480 (0.86)	intron	-
	rs10925910	-	intron	-
	rs2841037		intron	-
		rs626694 (1)	intron	-
HTR4	rs7723153		intron	-
HTR7	rs7074715	-	intron	-
ABCB1	rs10276036	-	intron	10
	rs2235023	-	intron	3
	rs9282564	-	missense, frameshift	22
	rs10260862	-	intron	2
	rs10248420	-	intron	9
	rs2032582	-	missense	237
	rs3789243	-	intron	27
	rs1002204		intron	1
		rs1045642 (0.91)	cds-synon	365
OPRM1	rs2272381	-	intron	2
	rs34427887	-	STOP-GAIN	-
TPH1	rs10832876		intron	-
		rs7943884 (0.85)	< 5000 base pairs before gene reading region, no annotation	-