ASSOCIATION STUDY OF 37 GENES RELATED TO SEROTONIN AND DOPAMINE NEUROTRANSMISSION AND NEUROTROPHIC FACTORS IN COCAINE DEPENDENCE

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ABSTRACT

Cocaine dependence is a neuropsychiatric disorder in which both environmental and genetic factors are involved. Several processes, that include reward and neuroadaptations, mediate the transition from use to dependence. In this regard, dopamine and serotonin neurotransmission systems are clearly involved in reward and other cocaine-related effects, whereas neurotrophic factors may be responsible for neuroadaptations associated with cocaine dependence. We examined the contribution to cocaine dependence of 37 genes related to the dopaminergic and serotoninergic systems, neurotrophic factors and their receptors through a case-control association study with 319 Single Nucleotide Polymorphisms (SNPs) selected according to genetic coverage criteria in 432 cocaine-dependent patients and 482 sex-matched unrelated controls. Single marker analyses provided evidence for association of the serotonin receptor *HTR2A* with cocaine dependence (rs6561333; nominal P-value adjusted for age = 1.9e-04, OR = 1.72 (1.29-2.30)). When patients were subdivided according to the presence or absence of psychotic symptoms, we confirmed the association between cocaine dependence and *HTR2A* in both subgroups of patients. Our data show additional evidence for the involvement of the serotoninergic system in the genetic susceptibility to cocaine dependence.

INTRODUCTION

Cocaine dependence is a neuropsychiatric disorder characterized by persistent, compulsive and uncontrolled use of cocaine. Both environmental and genetic factors underlie this disorder. Although some studies have estimated a heritability of cocaine dependence around 60-70% (Kendler *et al.* 2000; Kendler & Prescott 1998), genetic susceptibility factors are not well understood. Several processes, such as reward and neuroadaptations, are involved in the transition from the first cocaine use to compulsive use, loss of control and development of dependence.

The dopaminergic and serotoninergic neurotransmission systems play an important role in cocaine reward and other drug effects. Cocaine inhibits dopamine (DA) and serotonin (5-HT) reuptake and increases their synaptic levels (reviewed by Filip 2005). Dopamine neurotransmission has been established as the main mediator of cocaine reward and its pleasurable effects and responses (Kuhar *et al.* 1991; Spealman 1993; Volkow *et al.* 1997), while serotonin neurotransmission plays an important role in its hyperlocomotor, reinforcing and discriminative effects and sensitization (Filip 2005; Filip *et al.* 2004; Spealman 1993). Positive associations have been described in dopaminergic genes (*DAT1* and receptors *DRD2*, *DRD3* or *DRD4*), although some of these studies display conflicting results (Ballon et al., 2007; Bloch et al., 2009; Comings et al., 1999; Fernandez-Castillo et al., 2010; Freimer et al., 1996; Gelernter et al., 1999; Guindalini et al., 2006; Lohoff et al., 2010; Messas et al., 2005; Noble et al., 1993). On the other hand, genetic studies focusing on the serotoninergic receptors *HTR1B, HTR3A and HTR3B* failed to detect association with cocaine dependence (Cigler et al., 2001; Enoch et al., 2010) and controversial results were obtained for the *SERT* gene (Enoch et al., 2010; Mannelli et al., 2005; Patkar et al., 2002; Patkar et al., 2001). Other genes encoding enzymes involved in DA and 5-HT synthesis and degradation (*DBH, TPH2*

and *COMT*) have been also studied in cocaine dependence, showing positive associations in the *COMT* gene and also in the *DBH* gene, although the latter was only associated with cocaine-induced paranoia (Cubells *et al.* 2000; Lohoff *et al.* 2008; Dahl *et al.* 2006; Guindalini *et al.* 2008).

In addition to its reward and reinforcing effects, cocaine also induces neuroplasticity changes that may be involved in mechanisms underlying dependence, such as reward-related learning and memory as well as drug-induced neuroadaptations (Thomas *et al.* 2008). Some neurotrophins, which are neurotrophic factors implicated in neuronal survival, differentiation, function and plasticity, may be involved in these cocaine-induced neuroadaptive changes (Berhow *et al.* 1995; Thomas *et al.* 2008). Neurotrophic factors play important roles in brain cells involved in the action of psychostimulants, such as dopaminergic or serotoninergic neurons, and participate in activity-dependent plasticity and modulation of cocaine-induced biochemical changes in the mesolimbic dopamine system (Angelucci *et al.* 2009; Berhow *et al.* 1995). For instance, studies performed in animal models showed that neurotrophin-3 (NT-3) has been related to cocaine sensitization (Freeman & Pierce 2002; Pierce *et al.* 1999), and brain-derived neurotrophic factor (BDNF) is involved in cocaine-seeking behaviour, relapse, sensitization and cocaine-conditioned cues (Bahi *et al.* 2008; McGinty *et al.* 2010). Interestingly, decreased levels of BDNF and nerve growth factor (NGF) have been identified in serum of cocaine abusers (Angelucci *et al.* 2007). Except for a work by Lohoff et al. (2009), which failed to detect association with several SNPs in the recently described Cerebral Dopamine Neurotrophic Factor (*CDNF*), to our knowledge no association studies focusing on candidate genes for cocaine dependence have considered neurotrophic factors or their receptors.

In this study we aimed to examine SNPs covering 37 genes related to serotoninergic and dopaminergic neurotransmission (receptors, transporters and enzymes involved in DA and 5-HT synthesis and degradation), as well as several neurotrophic factors, including neurotrophins, and their receptors, in a Spanish sample of 432 cocaine-dependent patients and 482 sex-matched unrelated controls.

MATERIALS AND METHODS

Subjects

The clinical sample consisted of 432 patients (mean age ± S.D.: 34.5 ± 7.6 years, gender frequency: 82.6% males (n = 357)) recruited and evaluated according to the Diagnostic and Statistical Manual of Mental Disorders criteria for cocaine dependence (DSM-IV TR) between 2005 and 2010 at the Psychiatry Department of the Hospital Universitari Vall d'Hebron and at the Mental Health Division of the Fundació Althaia de Manresa, Barcelona, Spain. The Structured Clinical Interview (SCID) was administered and volunteers with current DSM-IV diagnosis of cocaine dependence were included in the study. The vast majority of the patients self-administered the drug by nasal insufflation (94%), although other routes of administration were also reported, in most cases combined with nasal insufflation (8.7% injected and 7.5% smoked). Seventy-nine per cent of the patients were evaluated for the presence (n = 195) or absence (n = 145) of psychotic symptoms. Seventy-six per cent (n = 329) reported age at the initial consumption (mean age ± S.D.: 22.8 ± 7.5 years) as well as age at dependence onset (mean age ± S.D.: 27.7 ± 7.5 years). The time to develop dependency, calculated as the time between the first cocaine consumption and the onset of the dependence, was less than two years in almost half of the patients (32% reported less than one year; 11% one year; 17% 2 years; 14% between 3 and 5 years; 12% between 6 and 9 years and 14% between 10 and 25 years). About 75% of the patients had been dependent to cocaine during more than six years when they were included in the study (25.6% had a length of dependency between 0 and 5 years; 31% between 6 and 10 years; 34.6% between 11 and 20 years and 9% above 20 years).

The control sample consisted of 482 unrelated blood donors (mean age ± S.D.: 43.3 ± 14.4 years; gender frequency: 82.6% males (n = 398)) recruited at the Blood and

Tissues Bank of the Hospital Universitari Vall d'Hebron, Barcelona. None of them had injected drugs intravenously.

Patients and controls were Spanish, Caucasian and sex-matched.

The study was approved by the Ethics committee of both institutions and written informed consent was obtained from all cases and controls.

DNA isolation and quantification

Genomic DNA was isolated from peripheral blood lymphocytes by the salting-out procedure (Miller *et al.*, 1988). A few DNA samples (<0.5%) were isolated from saliva using the Oragene DNA Self-Collection Kit (DNA Genotek, Kanata, Ontario, Canada). The double-stranded concentrations of all samples were determined on a Gemini XPS fluorometer (Molecular Devices, Sunnyvale, CA, USA) using the PicoGreen dsDNA Quantitation Kit (Molecular Probes, Eugene, OR, USA), following the manufacturer's instructions.

SNP selection

We initially selected a total of 38 candidate genes encoding 14 serotonin receptors (*HTR1A, HTR1B, HTR1D, HTR1E, HTR1F, HTR2A, HTR2B, HTR2C, HTR3A, HTR3B, HTR4, HTR5A, HTR6* and *HTR7*), five dopamine receptors (*DRD1, DRD2, DRD3, DRD4 and DRD5*), the serotonin and dopamine transporters (*SLC6A4* and *SLC6A3*, respectively), enzymes involved in serotonin and dopamine synthesis and degradation (*TH, TPH1, DDC, MAOA, MAOB, COMT* and *DBH*), five neurotrophic factors (*NGF, BDNF, NTF3, NTF4* and *CNTF*) and their receptors (*NTRK1, NTRK2, NTRK3, NGFR*, and *CNTFR*) (Supplementary Table S1), although finally *DRD4* was not included in the study (see below for more details). We used information on the CEPH panel from the

HapMap database (www.hapmap.org; release 20, January 2006) to select tagSNPs. To minimize redundancy of the selected markers and ensure full genetic coverage of candidate genes, we used the LD-select software (droog.gs.washington.edu/ldSelect.html) to evaluate the linkage disequilibrium (LD) patterns of the region spanning each candidate gene plus 3 to 5 kb of flanking sequences. A total of 400 SNPs were initially selected at an r^2 threshold of 0.85. From all SNPs within serotoninergic or dopaminergic genes, a minor allele frequency (MAF) > 0.15 was considered for genes with less than 15 tagSNPs and MAF > 0.25 for those with more than 15 tagSNPs, whereas we considered a MAF > 0.10 for genes encoding neurotrophic factors or their receptors as they were part of a previous design that followed distinct criteria (Ribases *et al.* 2008; Supplementary Table S1). Additionally, the non-synonymous SNP rs6265 (*BDNF*, exon 2) was included in our selection since it had been described to have a functional effect, and four non-synonymous SNPs were also included as potentially functional variants: rs1058576 (*HTR2A*, exon 3), rs6318 (*HTR2C*, exon 4), rs2228673 (*SLC6A4*, exon 5) and rs1007211 (*NTRK1*, exon 1). To discard population stratification we included 48 unlinked genome-wide SNPs located at least 100 Kb a part from any known gene (Sanchez *et al.* 2006).

Plex Design, Genotyping, and Quality Control

Of the initially selected 400 SNPs, 31 did not pass through the SNPlex design pipeline (ms.appliedbiosystems.com/snplex/snplexStart.jsp), resulting in a design rate of 92% (Supplementary Table S1). Eight SNPlex genotyping assays were designed including a total of 369 SNPs: four for the serotoninergic and dopaminergic systems (48, 47, 46 and 45 SNPs) and four for the neurotrophic factors and their receptors (45, 48, 46 and 44 SNPs). We also added an additional SNPlex assay of 48 unlinked genome-wide SNPs to

discard population stratification, as mentioned above. Genotyping was performed using the SNPlex technology. Two CEPH DNA samples (NA11992 and NA11993) were included in the different genotyping assays, and a concordance rate of 100% with HapMap data was obtained. Replicates were also included in all plates as an additional quality control, and genotype concordance was also 100%. All SNPs included in the study presented a sample call rate >85%, and all samples had a SNP call rate >70%. SNPs showing departure from Hardy-Weinberg Equilibrium (HWE) ($P < 0.01$) or low MAF (<0.1) in the control sample were excluded.

Statistical analyses

All statistical analyses were performed as described previously (Ribases *et al.* 2009). The minimal statistical power was calculated *post hoc* with the Genetic Power Calculator software (pngu.mgh.harvard.edu/~purcell/gpc/cc2.html), under a codominant model and assuming an odds ratio (OR) of 1.5, significance level of 0.05, the lowest MAF value of 0.10 and a prevalence for cocaine dependence of 0.0133 (8.3% of adult Spanish consumers, from which 16% develop dependence (EMCDDA 2010 Annual Report; Wagner & Anthony 2002).

Population stratification: Potential genetic stratification was tested in our sample by analyzing the 44 SNPs successfully genotyped and in Hardy-Weinberg equilibrium (HWE) from an initial 48 anonymous SNP set following three different approaches: 1) Structure software (version 2.3.3; Pritchard *et al.* 2000) under the admixture model, with a length of the burning period and a number of MCMC repeats of 100,000 and performing five independent runs at each K value (from 1 to 5), in which K refers to the number of groups to be inferred; 2) the Fst coefficient calculated with the Weir and Cockerham approach using the FSTAT software and a 95% confidence interval (CI)

determined by bootstrapping (Goudet 1995); and 3) the Pritchard and Rosenberg method (Pritchard & Rosenberg 1999) considering codominant, dominant, and recessive models.

Single-Marker Analysis: The analysis of HWE (threshold set at $P < 0.01$) and the comparison of genotype frequencies between cases and controls under a codominant model were performed using the SNPassoc R library (Gonzalez *et al.* 2007). Dominant (11 vs. 12 + 22), recessive (11 + 12 vs. 22) and overdominant (12 vs. 11 + 22) models were only considered for those SNPs in which nominal association was detected under a codominant model. Differences in age at assessment between cases and controls were evaluated using the non-parametric Mann–Whitney U-test with the statistical package SPSS 15.0 (SPSS Inc., Chicago, USA).. Significant differences in age were detected, so it was considered as a covariate in all statistical tests. All those SNPs that remained nominally significant after age correction were evaluated for their possible effect on the time between initial and regular consumption considering the most significant model for each SNP in the single-marker analysis and using a non-parametric Mann–Whitney U-test using SPSS 15.0. For multiple testing corrections we used the Q-value R package (Storey 2002) to apply a False Discovery Rate (FDR) of 10%, that corresponded to a significance threshold of $P \leq 1.9e\text{-}04$ after considering all the tests performed. We also applied Spectral Decomposition (SpD), a within-gene correction method for multiple testing: On the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNPs within each gene, we obtained a new significance threshold for each of the 37 genes included in the study (Nyholt 2004).

The SNPs that remained significant after applying these methodologies for multiple testing correction were assessed in cocaine-dependent patients according to the presence or absence of cocaine-induced psychotic symptoms using the statistical

package SPSS 15.0. The web utility SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpfunc.htm) was used to detect potential functional effect of associated variants (alteration of a transcription factor binding site, changing of splicing pattern or efficiency by disruption of splice sites, exonic splicing enhancers or silencers, regulation of protein translation by affecting microRNA binding sites or conservation scores).

Multiple-Marker Analysis: To minimize multiple testing and type I errors, we decided a priori to restrict the haplotype-based association study to those genes associated with cocaine dependence in the single-marker analysis after correction for multiple testing (FDR and SpD). Rather than restricting the study to physically contiguous SNPs in the gene, the best two-marker haplotype from all possible combinations was identified, and afterwards additional markers (up to four) were added in a stepwise manner to the initial two-SNP haplotype. Significance was estimated by a permutation procedure with 10,000 permutations using the UNPHASED software. Because the expectation-maximization (EM) algorithm implemented in the UNPHASED software does not accurately estimate low haplotype frequencies, haplotypes with frequencies < 0.05 were excluded.

RESULTS

We aimed to study tagSNPs in 38 candidate genes related to dopaminergic and serotoninergic neurotransmission, neurotrophic factors and their receptors in 432 cocaine-dependent patients and 482 controls. Of the 369 SNPs included in the assays, 50 were excluded for several reasons (35 showed >15% missing genotypes, three were monomorphic, 11 had a MAF < 0.10 and one showed HWE departure in the control sample) (Supplementary Table S1). Finally, a total of 319 SNPs within 37 genes (the two SNPs in the *DRD4* gene failed) with an average call rate of 97% (SD = 1.94) were assessed in a case-control association study. The minimal statistical power for the χ^2 test, considering the SNP with the lowest MAF (0.10), was 63.3%.

Population stratification was excluded with the STRUCTURE software, observing that the probability of more than one population inferred in our sample is extremely low (P < 1e-50) (Supplementary Table S2). Stratification was also excluded using two alternative methods: the Fst coefficient (Θ = 0 with a 95% CI of (-0.001, 0.000)) and the Pritchard and Rosenberg method (P = 0.568).

The comparison of genotype frequencies between cocaine-dependent patients and controls under a codominant model showed nominally significant differences for 12 SNPs. After adjusting by age, eight SNPs located in seven genes remained significant (*HTR1E*, *HTR2A*, *DBH*, *TH*, *NTRK2*, *NTRK3* and *CNTFR;* Table 1 and Supplementary Table S3), All 8 SNPs were evaluated for their possible effect on the time between the first consumption and the onset of cocaine dependence and no significant differences were observed (Supplementary Table S4).

Of those 8 SNPs nominally associated four of them overcame the within-gene correction by SpD. When correcting by multiple testing using FDR, a single SNP in the

HTR2A gene, rs6561333, remained associated with cocaine dependence after applying 10% FDR (Table 1). When we subdivided patients according to the presence or absence of cocaine-induced psychotic symptoms, significant differences in genotype frequencies for rs6561333 were detected in the two groups of patients separately (psychotics versus controls, P = 7.2e-03, OR = 1.5 (1.1-2.2); non-psychotics versus controls, P = 2.6e-03, OR = 1.74 (1.2-2.5)). Consistently, no differences in genotype frequencies for this SNP were detected between the two clinical subgroups (psychotics versus non-psychotics, P = 0.79).

The multiple-marker analysis considering the 21 genotyped SNPs within *HTR2A* showed no evidence of association between cocaine dependence and any allelic combination within the gene after applying corrections by permutation.

DISCUSSION

Several lines of evidence indicate that genes of the serotoninergic and dopaminergic neurotransmission systems and neurotrophic factors represent strong candidates to be involved in the susceptibility to cocaine dependence. To our knowledge, this is the first comprehensive candidate gene study that investigates exhaustively SNPs across genes related to these pathways in drug dependence, with the exception of previous Genome-Wide Association Studies (GWAS) (e.g. Drgon *et al*. 2010; Johnson *et al*. 2011; Liu *et al*. 2006; Uhl *et al*. 2008). Our data provide preliminary evidence of association between *HTR2A* and cocaine dependence. Several strengths of our study reinforce the validity of the observed results: 1) cases and gender-matched control subjects were carefully selected from the same geographical area around Barcelona, Spain; 2) The possible presence of genetic stratification was discarded using a specific set of markers and three statistical methods; 3) Different methodologies for multiple testing correction were applied. However, a replicate study is warranted to confirm the association finding in an independent population.

The serotoninergic system regulates processes related to reward and reinforcement and is also important for maintaining plasticity at the cellular level, which is the basis for behavioural adaptation. Clinical and pre-clinical studies have provided evidence that 5-HT and its receptors modulate the mechanism of action of cocaine, and that different subtypes of 5-HT receptors contribute to cocaine sensitization, self-administration, conditioned place preference, reinstatement of seeking behaviour and withdrawal symptoms in animal models (Filip 2005; Filip *et al.* 2010; Muller & Huston 2006).

The serotonin receptor 5-HT2A (encoded by the *HTR2A* gene), which is found in abundance in the mesolimbocortical dopaminergic system, appears to modulate behavioural effects of cocaine. Antagonists of 5-HT2A receptors inhibit cocaine-induced hyperlocomotion and behavioural sensitization, while agonists enhance the locomotor stimulant effect of cocaine (Davidson *et al.* 2002; Filip *et al.* 2004). Pre-treatment with 5-HT2A antagonist also block cue-induced and reinstatement of cocaine-seeking behaviour in rats, without modifying its reinforcing effects in rats and non-human primates (Fantegrossi *et al.* 2002; Filip *et al.* 2006; Fletcher *et al.* 2002; Nic Dhonnchadha *et al.* 2009). The results obtained from these animal studies point to the gene encoding this serotonin receptor as a good candidate to be involved in cocaine dependence in humans.

The rs6561333 SNP in *HTR2A* that we found associated with cocaine dependence is located in intron 2 of the gene. This SNP is not predicted to have a potential functional effect and is in high LD with another SNP (rs9567737, $r^2 = 0.97$) which shows a weak resemblance to regulatory elements (potential regulatory score = 0.081). Moreover, the nominally associated SNP rs6561332 (in moderate LD with SNP rs6561333, $r^2 = 0.57$), which is also located in intron 2 of the gene, also showed a low regulatory score (0.062). Although predictions show that none of these SNPs seem to have a relevant functional effect, they could be in LD with other yet unknown susceptibility variants directly responsible for the genetic predisposition to cocaine dependence.

While no previous association studies in cocaine dependence have tested the *HTR2A* gene, others assessed its possible contribution to heroin and alcohol dependence, displaying contradictory results (Nakamura *et al.* 1999; Saiz *et al.* 2008; Saiz *et al.* 2009). The two SNPs considered in those previous studies (rs6311 and rs6313) were not

directly genotyped here, but are in high LD with the tagSNP rs9526246 (supplementary Table S1), which was not found associated in our sample (P = 0.4, supplementary Table S3).

In addition, consistently with our results, previous studies that focused on other serotoninergic receptors, such as *HTR1B*, *HTR3A* or *HTR3B,* found no association with cocaine dependence (Cigler *et al.* 2001; Enoch *et al.* 2010). Several SNPs genotyped in those studies have also been genotyped here (rs1176744, rs3782025) or can be inferred by other tagSNPs in high LD (rs3758987, rs2276302, rs1176713, rs2276307; Supplementary Table 1). Other polymorphisms, however, were not included but since our study was designed under genetic coverage criteria, *HTR1B* and *HTR3A* achieved 100% coverage, whereas *HTR3B* had a 67% coverage (Supplementary Table S1).

We also identified nominal associations of cocaine dependence with other genes of the serotoninergic (*HTR1E*) and dopaminergic (*DBH* and *TH*) systems or with neurotrophic factor receptors (*NTRK2, NTRK3,* and *CNTFR*) (Table 1). These genes may deserve further attention, as the limited sample size of our cohort (n=432 cases) could have prevented overcoming of multiple testing thresholds.

Previous association studies on drug dependence also focused on dopaminergic genes, such as *DRD2, DRD3, DRD4, DAT1, DBH, TPH2* and *COMT*, or neurotrophic factors and often displayed conflicting results (Ballon *et al.* 2007; Bloch *et al.* 2009; Comings *et al.* 1999; Cubells *et al.* 2000; Dahl *et al.* 2006; Fernandez-Castillo *et al.* 2010; Freimer *et al.* 1996; Gelernter *et al.* 1999; Guindalini *et al.* 2006; Guindalini *et al.* 2008; Lohoff *et al.* 2010; Lohoff *et al.* 2008; Messas *et al.* 2005; Noble *et al.* 1993; Lohoff *et al.* 2009; Uhl *et al.* 2001). Discrepant results between studies may be due to limited sample size, clinical heterogeneity (e.g. differences in comorbidities) or the presence of population stratification in some of these studies, among other reasons.

Although we discarded the presence of population stratification in our sample, its size is still limited. These issues underscore the need for more extensive association studies in large and homogeneous cohorts and with adequate genetic coverage of the regions of interest. We selected SNPs under genetic coverage criteria and achieved adequate coverage for most of them (average coverage of 84%). Gaps, however, still exist in 17 genes because of genotyping failure of 35 SNPs and, thus, their contribution to cocaine dependence cannot be discarded. Specifically, the *DRD4* gene could not be evaluated because of genotyping failure. Also, cocaine dependence was not discarded in the control sample, which could lead to false negative findings in the present association study.

In conclusion, we identified association between *HTR2A* and cocaine dependence, consistently with several animal studies that show its involvement in cocaine-seeking behaviour. Although further studies in independent cohorts are required to confirm our finding, this result supports the involvement of serotoninergic neurotransmission in the genetic component that underlies the predisposition to cocaine addiction.

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Table 1. Single-marker analysis: Nominal associations identified in 432 cocaine-dependent patients and 482 controls from Spain.

			Cases N (%)				Controls N (%)						Genotype 11 vs 12+22		Genotype 22 vs 11+12		Genotype 12 vs 11+22	
Gene	**SNP**		**11**	**12**	**22**	**Sum**	**11**	**12**	**22**	**Sum**	**P**	**P***	**OR (95% CI)***	**P***	**OR (95% CI)***	**P***	**OR (95% CI)***	**P***
Serotonergic system																		
HTR1E	rs1408449	T>A	172 (42.0)	202 (49.3)	38 (8.8)	412	187 (39.6)	207 (43.9)	78 (16.5)	472	2.3e-03	0.010[&]	_	NS	1.92 (1.22-3.03) [α]	3.7e-03[&]	_	NS
HTR2A	rs6561332	G>T	125 (29.6)	231 (54.6)	67 (15.8)	423	148 (30.8)	222 (46.2)	111 (23.1)	481	9.2e-03	4.2e-03	_	NS	1.72 (1.19-2.44) [α]	3.0e-03	1.48 (1.12-1.97)	6.0e-03
	rs6561333	C>T	119 (29.4)	221 (54.6)	65 (16.0)	405	179 (38.0)	205 (43.5)	87 (18.5)	471	4.1e-03	9.1e-04[&]	1.55 (1.15-2.10)	4.3e-03	_	NS	1.72 (1.29-2.30)	1.9e-04[&&]
Dopaminergic system																		
DBH	rs6479643	G>C	163 (38.3)	215 (50.5)	48 (11.3)	426	173 (36.0)	222 (46.2)	85 (17.7)	480	0.022	4.8e-03[&]	_	NS	1.92 (1.30-2.94) [α]	1.2e-03[&]	_	NS
TH	rs2070762	A>G	92 (21.7)	236 (55.8)	95 (22.5)	423	130 (28.1)	215 (46.4)	118 (25.5)	463	0.017	0.027	_	NS	_	NS	1.47 (1.10-1.95)	8.0e-03[&]
Neurotrophic factors																		
NTRK2	rs1147193	T>C	244 (57.7)	160 (37.8)	19 (4.5)	423	273 (57.7)	159 (33.6)	41 (8.7)	473	0.029	0.037	_	NS	1.96 (1.07-3.57) [α]	0.011	_	NS
NTRK3	rs12595249	G>C	183 (43.5)	182 (43.2)	56 (13.3)	431	205 (43.6)	227 (48.3)	38 (8.1)	470	0.030	NS						
	rs744994	C>T	147 (39.3)	179 (47.9)	48 (12.8)	374	231 (48.0)	205 (42.6)	45 (9.4)	481	0.027	0.011	1.52 (1.13-2.04)	5.2e-03	_	NS	_	NS
	rs998636	A>G	238 (56.5)	144 (34.2)	39 (9.3)	421	268 (55.8)	187 (39.0)	25 (5.2)	480	0.037	NS						
CNTFR	rs7036351	C>T	311 (74.2)	97 (23.2)	11 (2.6)	419	321 (67.0)	148 (30.9)	10 (2.1)	479	0.032	0.041	1.41 (1.03-1.92) [α]	0.030	_	NS	1.49 (1.09-2.04) [α]	0.013
NTF3	rs4073543	A>G	138 (33.3)	181 (43.7)	95 (22.9)	414	159 (33.1)	242 (50.3)	80 (16.6)	481	0.037	NS						
NGFR	rs534561	C>G	187 (44.4)	165 (39.2)	69 (16.4)	421	208 (43.3)	219 (45.6)	53 (11.0)	480	0.031	NS						

NS: Not significant
SNP variants are shown in the + strand. The major allele is encoded as 1 and the minor allele as 2.
*Adjusted by age.
[α] When odds ratio <1, the inverted score is shown.
[&] Statistically significant P-values after applying Spectral Decomposition (SpD) correction in each gene: P<0.011 (*HTR1E*), P<2.6e-03 (*HTR2A*), P<5e-03 (*DBH*), P<0.025 (*TH*), P<1.2e-03 (*NTRK2*), P<1e-03 (*NTRK3*) and P<3.9e-03 (*CNTFR*).
[&&]Statistically significant P-values after applying Spectral Decomposition (SpD) correction and 10% False Discovery Rate (P<1.9e-04).