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Differential role of serotonergic polymorphisms in alcohol and heroin dependence

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ABSTRACT

Background: Twin studies suggest that genetic factors account for 40–60% of the variance in alcohol dependence. It has been stated that different drug dependencies may have unique genetic influences. Alterations in serotonin availability and function can affect drinking behaviour. This study aimed to investigate whether three serotonergic polymorphisms (HTR2A A-1438G (rs6311), and SCL6A4 5-HTTLPR and STin2 VNTR) were associated with alcohol dependence, and, whether the serotonergic polymorphisms played a similar role in conferring vulnerability in alcohol and heroin dependence.
Methods: 165 alcohol dependent patients, 113 heroin dependent patients, and 420 healthy controls from a homogeneous Spanish Caucasian population were genotyped using standard methods.
Results: Genotypic frequencies of the A-1438G, 5-HTTLPR, and STin2 VNTR polymorphisms did not differ significantly across the three groups. None of the three polymorphisms contributed to distinguishing alcoholic patients from healthy controls. There was an excess of −1438G and 5-HTTLPR L carriers in alcoholic patients in comparison to the heroin dependent group (OR (95% CI) = 1.98 (1.13–3.45) and 1.92 (1.07–3.44), respectively). The A-1438G and 5-HTTLPR polymorphisms also interacted in distinguishing alcohol from heroin dependent patients (Wald (df) = 10.21 (4), $p = 0.037$). The association of −1438A/G with alcohol dependence was especially pronounced in the presence of 5-HTTLPR S/S, less evident with 5-HTTLPR L/S and not present with 5-HTTLPR L/L. SCL6A4 polymorphism haplotypes were similarly distributed in all three groups.
Conclusions: Our data do not support a role of serotonergic polymorphisms in alcohol dependence but suggest a differential genetic background to alcohol and heroin dependence.

1. Introduction

It is accepted that alcohol dependence is influenced by environmental and genetic factors (Edenberg and Foroud, 2006). Twin epidemiological studies show that genetic factors account for about 40–60% of the overall variance in alcohol dependence (Dick and Beirut, 2006). However, predisposition to addiction may be due both to genetic variants that are common to all addictions and to those specific to a particular addiction (Kreek et al., 2005).

Alterations in serotonin (5-HT) availability and function can affect drinking behaviour (Johnson and Ait-Daoud, 2000). The serotonin 2A receptor (HTR2A) antagonists have been shown to reduce alcohol intake (Overstreet et al., 1997), and recent data suggest a lower HTR2A receptor binding in the prefrontal cortex (PFC) of alcohol dependent patients with a positive family history of alcoholism (Underwood et al., 2008). The serotonin 2A receptor (HTR2A) gene is located on chromosome 13q14-q21. Two polymorphisms of this gene, T102C (rs6313) and A-1438G (rs6311), have been described as being in complete linkage disequilibrium in different populations (Saiz et al.,

Abbreviations: bp, Base pair; CI, confidence interval; df, Degrees of freedom; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders-IV; EuropASI, European addiction severity index; HTR2A, serotonin receptor type 2A; 5-HT, serotonin; 5-HTT, serotonin transporter; 5-HTTLPR, serotonin transporter promoter polymorphism; IPDE–CIE 10, International Personality Disorders Examination–CIE 10; LD, linkage disequilibrium; LRT, likelihood ratio test; MINI, Mini-International Neuropsychiatric Interview; OR, odds ratio; PFC, prefrontal cortex; SD, standard deviation; SERT, serotonin transporter gene; SLC6A4, serotonin transporter gene; SPSS, Statistical Package for Social Sciences; STin2 VNTR, serotonin transporter intron 2 variable number of tandem repeats polymorphism; χ^2, Chi-square test.

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2008a). Recent findings suggest that the A-1438G polymorphism might have functional effects on expression of the HTR2A receptor in the brain (Parsons et al., 2004; Myers et al., 2007). On the other hand, the 102T allele has been suggested to be associated with alterations in the amount of HTR2A receptor concentration in the PFC (Turecki et al., 1999; Underwood et al., 2008). Association between these polymorphisms and alcoholism has been reported in two prior case-control association studies (Nakamura et al., 1999; Hwu and Chen, 2000).

One of the most important mechanisms related to the control of the synaptic 5-HT concentration is the functionality of the 5-HT transporter (5-HTT). The 5-HTT gene (also known as SLC6A4 or SERT) is mapped on chromosome 17q11.1-q12. A functional polymorphism of this gene (5-HTTLPR) involving two common L (44-base pair insertion) and S (deletion) alleles is related to the differential expression of 5-HTT binding sites in cell lines (Lesch et al., 1996). Several case-control association studies and even two meta-analyses (Gorwood et al., 2004; Feinn et al., 2005) suggest an association between the low activity variant (S allele) and alcohol dependence (Sander et al., 1997; Konishi et al., 2004a,b) or the risk of relapse in abstinent alcohol dependent patients (Pinto et al., 2008). Nevertheless, other studies have described an association of the long allele of this polymorphism with alcohol dependence (Heinz et al., 2000; Hu et al., 2005; Kweon et al., 2005; Bleich et al., 2007; Gokturk et al., 2008; Johnson et al., 2008). Several negative studies have also been published (Gorwood et al., 2000; Thompson et al., 2000; Kranzler et al., 2002; Foley et al., 2004; Choi et al., 2006; Köhnke et al., 2006; Dick et al., 2007; Mokrović et al., 2008; Samochowiec et al., 2008).

Another functional polymorphism of the SLC6A4 is a 17 base pair (bp) variable number of tandem repeats (termed STin2 VNTR), located in intron 2, involving two major alleles (termed STin2.10 and STin2.12) that correspond to 10- or 12-repeat units of 17 VNTR. The STin2 polymorphism appears to modulate the gene's transcription in an allele-dependent manner (Hranilovic et al., 2004). The 10/10 genotype and STin2.10 allele has been associated with alcoholism in one case-control association study (Mokrović et al., 2008), but not in another (Thompson et al., 2000).

Finally, linkage studies of alcohol dependence have identified several chromosomal regions, including chromosomes 13 and 17 (Agrawal et al., 2008; Gelernter et al., 2009).

On the other hand, prior studies also suggest a possible contribution of the above mentioned polymorphisms towards susceptibility to heroin dependence (Tan et al., 1999; Gerra et al., 2004; Saiz et al., 2008b), as well as, a linkage between heroin dependence and markers on the long arm of chromosome 17 (Gelernter et al., 2006; Glatt et al., 2006).

Finally, associations have been suggested between aggressive and impulsive behaviours and genes related to the serotonergic system (Preuss et al., 2001; Giegling et al., 2006; Brezo et al., 2008). However, high levels of impulsivity and aggressiveness have been also related to substance abuse and dependence (Cuomo et al., 2008).

In this study, we aimed to investigate first whether three serotonergic polymorphisms (HTR2A A-1438G (rs6311), and SCL6A4 5-HTTLPR and STin2 VNTR) were associated with alcohol dependence, and second, whether the serotonergic polymorphisms played a similar role in conferring vulnerability in alcohol and heroin dependence.

2. Methods

2.1. Patient population

The total sample was composed of one hundred and sixty-five unrelated alcohol dependent outpatients (mean age (SD) = 47.78 (9.08) years; 84.8% males), one hundred and thirteen unrelated heroin dependent outpatients (mean age (SD) = 31.65 (6.30) years; 88.5% males), and four hundred and twenty unselected healthy controls (mean age (SD) = 40.6 (11.3) years; 51.4% males), all from the North of Spain. All individuals were of Caucasian Spanish origin.

Alcohol dependent patients were consecutively admitted to an outpatient detoxification unit with a diagnosis of alcohol dependence. Only active drinkers with an alcohol intake of at least 210 g/week for men and 140 g/week for women during the past month were enrolled. Alcoholic patients with a current diagnosis of dependence or abuse of other substances except nicotine were excluded from the study (Florez et al., 2008). Enrollment of heroin dependent patients has been previously described by Saiz et al. (2008b). Severity of the addiction was measured using the European Addiction Severity Index (EuropASI) (Kokkevi and Hartgers, 1995). Only alcohol or heroin dependent patients without DSM-IV, Axis I diagnoses were included in the study.

Absence of Axis I diagnoses was determined by experienced psychiatrists based on DSM-IV criteria and clinical records. In addition, the Spanish version of the Mini-International Neuropsychiatric Interview (MINI, DSM-IV criteria) was used as a diagnostic interview in the two groups of patients, as well as in the healthy control group. Only healthy controls without a history of drug or alcohol abuse or dependence and without a personal or first-degree family history of psychiatric disorders were enrolled in the study.

Written informed consent was obtained from all subjects included in the study. The study was subject to and in compliance with Spanish national legislation, was conducted according to the provisions of the World Medical Association Declaration of Helsinki, and received institutional approval (World Medical Association, 1989).

This clinical sample had at least 80% statistical power to detect genetic effects with an odds ratio of 2, assuming that the frequency of minor alleles was at least 35% in our sample of healthy controls. Similar frequencies have been previously described in other Spanish Caucasian samples (Mata et al., 2004).

2.2. Genotyping

Briefly, genomic DNA was extracted from peripheral white blood cells obtained from each participant according to standard protocols (Miller et al., 1988). HTR2A and SLC6A4 gene polymorphisms were identified according to previously published methods (Florez et al., 2008). Patients and controls were analyzed side by side to eliminate errors in genotyping. The genotypes were determined by researchers who were blind to patient information.

2.3. Data analysis

The genotype and allele distribution as well as the presence of Hardy–Weinberg equilibrium were tested by chi-square (χ^2) tests. A Bonferroni correction coefficient of 3 (3 genetic markers were under study) was applied to p values to control for multiple comparisons. To assess whether the polymorphisms distinguished alcohol dependent patients from either healthy controls or heroin dependent patients, two series of logistic regression models were estimated. First models were estimated with only one polymorphism as the independent variable. Genotypes with similar associations with the dependent variable were merged in order to form more efficient models. Polymorphisms with crude associations beyond the 5% level of statistical significance were entered into a multivariate logistic regression model in order to assess whether each polymorphism independently distinguished alcohol dependent patients from the other group. Whether the multivariate model distinguished alcohol dependent patients from the other group was assessed by the likelihood ratio test with Bonferroni adjustment (i.e. $p<0.25$ for a model with two independent variables and $p<0.17$ for a model with three independent variables). In addition, models were estimated to investigate whether two-way interactions between genetic variants were associated with alcohol dependence. SPSS versions 15.0 and 16.0

Table 1
Demographic and clinical characteristics of heroin and alcohol dependent patients.

	Heroin patients	Alcohol patients[a]
Characteristic	Mean (SD)	Mean (SD)
Age, years	31.6 (6.3)	47.8 (9.1)
Males age, years	31.9 (6.3)	47.4 (9.1)
Females age, years	29.7 (6.5)	49.8 (8.6)
Evolution time of dependence, years	9.3 (4.2)	+1 year
Dose of heroin (mg/day)/ethanol (g per /week)	766.7 (604.7)	1042.5 (351.5)
EuropASI scores		
Medical	0.4 (1.2)	6.2 (2.6)
Employment/resources	2.5 (3.1)	3.3 (3.5)
Alcohol	0.8 (1.7)	7.8 (0.5)
Other drugs	2.9 (1.9)	0 (0.0)
Legal	1.4 (2.5)	1.1 (2.5)
Family/social	2.2 (2.4)	6.9 (1.8)
Psychiatric	2.1 (2.5)	7.4 (1.0)
	n (%)	*n* (%)
Gender		
Male	100 (88.5)	140 (84.8)
Female	13 (11.5)	25 (15.2)
Route of heroin administration		
Smoked	103 (91.2)	
Intravenous	10 (8.8)	
Past-month abuse/dependence		
Alcohol	8 (7.1)	
Cannabis	24 (21.2)	
Cocaine	28 (24.8)	
Hallucinogens	3 (2.7)	
Ecstasy and analogues	2 (1.8)	
Methadone	15 (13.3)	
Benzodiazepines	28 (24.8)	
Patients with personality disorders[b]	48 (42.5)	41 (24.8)
Type of personality disorder (IPDE–CIE 10)		
Paranoid	5 (4.4)	4 (2.4)
Schizoid[b]	13 (11.5)	1 (0.6)
Dissocial[b]	16 (14.2)	2 (1.2)
Emotional unstable[b]	27 (23.9)	21 (12.7)
Histrionic[b]	9 (8.0)	1 (0.6)
Anankastic[b]	5 (4.4)	0 (0.0)
Anxious	11 (9.7)	17 (10.3)
Dependent	6 (5.3)	8 (4.8)

IPDE–CIE 10: International Personality Disorders Examination–CIE 10.
[a] Alcohol dependent patients with a current diagnosis of dependence or abuse of other substances except nicotine were excluded.
[b] Inter-group differences $p<0.05$.

(SPSS Inc., Chicago, Illinois, USA) and EPI Info version 6 (Centre for Disease Control, Atlanta, Georgia, USA) were used for the statistical analyses. The SHEsis Program (Shi and He, 2005) was used to calculate the linkage disequilibrium (LD) between all pairs of markers, to estimate the frequencies of the SCL6A4 polymorphism haplotypes in all three groups and to test for between-group differences using a likelihood ratio test (LRT).

Table 3
Effects of polymorphisms of the serotonin 2A receptor gene and serotonin transporter gene in distinguishing alcohol dependent patients from healthy controls.

		OR[a]	(95% CI)
A-1438G	A/A	1.00	(Ref. group)
	A/G	1.22	(0.76–1.98)
	G/G	0.96	(0.56–1.64)
5-HTTLPR	L/L	1.00	(Ref. group)
	L/S	1.29	(0.85–1.97)
	S/S	0.82	(0.47–1.42)
STin2 VNTR[b]	12/12	1.00	(Ref. group)
	12/10	0.97	(0.65–1.44)
	10/10	0.89	(0.52–1.55)

OR: odds ratio; CI: confidence intervals; VNTR: variable number of tandem repeats.
[a] Crude odds ratio.
[b] Rare genotypes excluded.

3. Results

Sociodemographic and clinical data of alcohol and heroin dependent patients are included in Table 1.

All groups showed Hardy–Weinberg equilibrium for the analyzed genetic variability. Some degree of LD was found between 5-HTTLPR and STin2 VNTR polymorphisms (Cramer's $V=0.248$, $p<0.00001$; $D'=0.351$; $r^2=0.061$), but LD was not complete enough to warrant not genotyping both polymorphisms.

After Bonferroni correction, no significant differences were found between the three groups in the genotypic frequencies of the A-1438G, 5-HTTLPR, and STin2 VNTR polymorphisms (Table 2).

None of the three polymorphisms contributed significantly to distinguishing between alcoholic patients and healthy controls (Table 3) and there was no evidence of interaction between pairs of polymorphisms in this respect (A-1438G × 5-HTTLPR: Wald (df) = 3.45 (4), $p=0.485$; A-1438G × STin2 VNTR: Wald (df) = 4.99 (4), $p=0.288$; 5-HTTLPR × STin2 VNTR: Wald (df) = 5.98 (4), $p=0.201$).

The A-1438G and 5-HTTLPR polymorphisms contributed to distinguishing alcohol dependent patients from patients who were heroin dependent (Table 4). This was the case when each polymorphism was considered alone and when included together in a multivariate model. The statistical significance of the multivariate model containing both polymorphisms was 0.007. The −1438G allele carriers (−1438A/G and −1438G/G) and the 5-HTTLPR L allele carriers (5-HTTLPR L/L and 5-HTTLPR L/S) were overrepresented in alcoholic patients compared to heroin dependent patients. The strength of their association with alcohol dependence over heroin

Table 2
Genotype frequencies of polymorphisms of the serotonin 2A receptor gene and serotonin transporter gene.

		Alcohol dependent patients		Heroin dependent patients		Healthy controls		χ^2 (df)	*p* value
		n	(%)	*n*	(%)	*n*	(%)		Uncorrected (corrected)
A-1438G	A/A	31	(19.1%)	36	(31.9%)	88	(21.0%)	9.67 (4)	0.046 (0.138)
	A/G	87	(53.7%)	53	(46.9%)	202	(48.0%)		
	G/G	44	(27.2%)	24	(21.2%)	130	(31.0%)		
5-HTTLPR	L/L	44	(26.8%)	24	(20.4%)	124	(29.5%)	8.25 (4)	0.083 (0.249)
	L/S	93	(56.7%)	59	(52.2%)	203	(48.3%)		
	S/S	27	(16.5%)	31	(27.4%)	93	(22.2%)		
STin2 VNTR	12/12	70	(42.4%)	54	(47.8%)	174	(41.4%)	5.51 (6)	0.480 (>1)
	12/10	69	(41.8%)	38	(33.7%)	177	(42.1%)		
	10/10	23	(13.9%)	17	(15.0%)	64	(15.2%)		
	Others	3	(1.9%)	4	(3.5%)	5	(1.2%)		

df: Degrees of freedom; VNTR: variable number of tandem repeats.

Table 4
Effects of polymorphisms of the serotonin 2A receptor gene and serotonin transporter gene in distinguishing alcohol dependent patients from heroin dependent patients.

		OR[a]	(95% CI)	OR[b]	(95% CI)
A-1438G	A/A	1.00	(Ref. group)	1.00	(Ref. group)
	A/G or G/G	1.98*	(1.13–3.45)	1.95*	(1.11–3.42)
5-HTTLPR	L/L or L/S	1.92*	(1.07–3.44)	1.86*	(1.03–3.36)
	S/S	1.00	(Ref. group)	1.00	(Ref. group)
STin2 VNTR[c]	12/12	1.00	(Ref. group)		
	12/10	1.40	(0.82–2.38)		
	10/10	1.04	(0.51–2.15)		

*$p<0.05$.
OR: odds ratio; CI: confidence intervals; VNTR: variable number of tandem repeats.
[a] Crude odds ratio.
[b] Adjusted odds ratio.
[c] Rare genotypes excluded.

dependence was almost identical (−1438G allele OR = 1.98 and 5-HTTLPR L allele OR = 1.92, respectively).

In addition, there was some evidence of interaction between A-1438G and 5-HTTLPR (Wald (df) = 10.21 (4), $p = 0.037$) in respect of distinguishing alcoholics from heroin dependent patients. The association of −1438A/G with alcohol dependence was especially pronounced in the presence of 5-HTTLPR S/S, it was less evident in the presence of 5-HTTLPR L/S and it was not evident at all in the presence of 5-HTTLPR L/L. There was no evidence of interaction between A-1438G and STin2 VNTR (Wald (df) = 0.64 (4), $p = 0.958$) and between 5-HTTLPR and STin2 VNTR (Wald (df) = 4.07 (4), $p = 0.396$).

Haplotype analyses on SCL6A4 polymorphisms revealed no significant differences in the frequency of haplotypes (6 different haplotypes by group with non-zero frequencies) between alcohol dependent patients and both healthy controls (Global χ^2 (3) = 5.51, $p = 0.138$) and heroin dependent patients (Global χ^2 (3) = 3.88, $p = 0.275$).

4. Discussion

In the present study, we found no association between the studied serotonergic polymorphisms and alcohol dependence. However, more importantly, we found an excess of −1438G allele (A/G + G/G) and 5-HTTLPR L allele (L/L + L/S) carriers in the alcohol dependent patients compared to the heroin dependent group (the minimum statistical power when comparing these groups of patients was 75% to detect a relative risk of 2.2), suggesting a different genetic background for both addictions.

Prior case-control association studies on the role of the HTR2A polymorphisms in alcoholism found an association between the −1438G allele and aldehyde dehydrogenase 2 family deficient alcohol dependence in a Japanese population (Nakamura et al., 1999) as well as a lower frequency of the 102T allele (the T102C polymorphism has been described in complete LD in different populations) in Chinese male alcohol abusers (Hwu and Chen, 2000). Discrepancies with our findings may be due to the fact that genotypic and allelic frequencies differ between different ethnic groups (Abdolmaleky et al., 2004). Our reported genotypic and allelic frequencies are different from those reported by Nakamura et al. (1999), and Hwu and Chen (2000) in their Asian samples ($p<0.001$).

Most studies looking at an association between SCL6A4 and alcohol dependence have focused on the 5-HTTLPR polymorphism, with inconclusive results. Several case-control association studies support our negative findings (Gorwood et al., 2000; Thompson et al., 2000; Kranzler et al., 2002; Foley et al., 2004; Choi et al., 2006; Köhnke et al., 2006; Dick et al., 2007; Mokrović et al., 2008; Samochowiec et al., 2008). However, higher frequencies of the S/S genotype (Sander et al., 1997), or the S allele (Konishi et al., 2004a,b) in alcohol dependent patients have been reported and these results are supported by case-control based meta-analytic evidences showing that the S allele could be a risk factor for a phenotype related to alcohol dependence (Gorwood et al., 2004; Feinn et al., 2005), specially among alcohol dependent patients with psychiatry comorbidity, early-onset, or greater severity (Feinn et al., 2005). Conversely, an excess of the L allele associated with alcoholism in males (Hu et al., 2005; Kweon et al., 2005), with compulsive craving in alcohol dependent male patients (Bleich et al., 2007), with more chronic and severe pattern of drinking (Johnson et al., 2008), or even with a greater risk of alcohol induced neurotoxic damage to the 5-HTT (Heinz et al., 2000) has been reported in ethnically different populations. In the same way, an excess of the L/L genotype has been found in females without co-morbid psychiatric disorders (Gokturk et al., 2008). Discrepant findings or failures to replicate candidate genes when studying psychiatric disorders have been attributed to several causes, mainly, sample size, diagnosis and population heterogeneity. Recently, the 5-HTTLPR polymorphism has been reported to be functionally triallelic (resulting from an A→G substitution in the L allele), and the L_G allele is similar to the S allele in its effect on gene expression, whereas the L_A allele is the highest expressing allele (Hu et al., 2006). It is possible that unrecognized L_G alleles in L/L and L/S genotypes could minimize differences between groups thereby leading prior studies to inconclusive results.

Regarding the STin2 VNTR polymorphism we have found two prior reports in the literature. The first one (Thompson et al., 2000) is in agreement with our negative findings, whereas Mokrović et al. (2008) have reported an excess of both the 10/10 genotype and STin2.10 allele in alcohol dependent males compared with healthy controls. In vivo data show that the STin2 VNTR polymorphism acts as a transcriptional regulator and has allele-dependent differential enhancer-like properties (STin2.12 seemed to be significantly stronger than the STin2.10 allele) within the rostral hindbrain (MacKenzie and Quinn, 1999).

To our knowledge, this is the first report showing a different genetic background in alcohol and heroin dependent patients. The previously reported association between the A-1438G polymorphism and heroin dependence (Saiz et al., 2008b) was not replicated in alcohol dependent patients. Alcohol dependent patients differ in allelic and genotypic frequencies with the heroin dependent patients, but not with the healthy control group. Genetic vulnerability to dependence has been shown to be drug-specific (Tsuang et al., 1998), and this specificity might explain, at least in part, inconclusive results found in case-control association studies when evaluating candidate genes in different drug categories (Kreek et al., 2005). Nevertheless, case-control association studies in substance dependence do not include other drug dependence categories as a second control group, limiting the understanding of unshared genetic influences. However, identifying the allelic variants that contribute to vulnerability to alcohol dependence and comparing them to the variants that predispose to other addictions can improve understanding of human addictions and assist efforts to match vulnerable individuals with the prevention and treatment strategies most likely to work to them (Johnson et al., 2006).

In this study, only Caucasians of Spanish origin were included, allele and genotypic frequencies of all studied polymorphisms were similar to those previously found in other samples of healthy controls of Caucasian Spanish origin (Mata et al., 2004), and a fairly rigorous Bonferroni correction for multiple testing was applied. This makes our finding less likely to be a type I error. Nevertheless, some methodological limitations should be taken into account. Firstly, it is possible that due to the small sample size our study had insufficient statistical power to detect associations with a small effect size. Secondly, the exclusion criteria used in alcohol dependent patients configured a group of patients more similar to the healthy controls (no physical or mental illness, not living alone, not taken other drugs). It is well known that the serotonergic system has been associated with several Axis I disorders. Therefore, in order to avoid this influencing

factor we decided to include a more homogeneous population free of Axis I comorbidity. However, we acknowledge that the lack of generalization and the biased population selected are indeed limitations of the study. Thirdly, more than one third of the heroin dependent patients were also users of other drugs. However, in all cases, the main consumed drug was heroin and these data are comparable to those reported in other studies (Torrens et al., 1996). On the other hand, the different rates of personality disorders could have influenced the aforesaid results. Nevertheless, the real influence is yet unknown as prior data by Preuss et al. (2001), even suggest that associations between the serotonergic system and alcohol dependence may be independent of the presence of antisocial and borderline personality disorders. Finally, related to the 5-HTTLPR polymorphism, due to limited availability of DNA for genotyping, we were unable to examine the A→G substitution within the L allele in our samples.

5. Conclusions

In contrast with some prior studies, our data do not support a role of serotonergic polymorphisms in alcohol dependence. However, they provide evidence of a differential genetic background to alcohol and heroin dependence. Further research is required in other settings in order to replicate our findings using larger samples that address the issue of Axis I and II comorbidity and that include the triallelic 5-HTTLPR polymorphism.

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