DRD2 C957T and *Taq*IA genotyping reveals gender effects and unique low-risk and high-risk genotypes in alcohol dependence

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ABSTRACT

Aims: As recent conflicting reports describe genetic association between both the C- and the T-alleles of the dopamine D2 receptor (*DRD2*) C957T polymorphism (rs6277) in alcohol dependent subjects, our aim was to examine this polymorphism and *Taq*IA (rs1800497) in Australian alcohol dependent subjects. **Methods:** The C957T polymorphism was genotyped in 228 patients with alcohol dependence (72 females and 156 males) and 228 healthy controls. **Results:** The C-allele and C/C genotype of C957T was associated with alcohol dependence whereas the *Taq*IA polymorphism was not. When analysed separately for C957T, males showed even stronger association with the C-allele and females showed no association. C957T and *Taq*IA haplotyping revealed strong association with alcohol dependence and double genotype analysis (combining C957T and *Taq*IA genotypes) revealed the relative risk of different genotypes varied by up to 27-fold with the TT/A1A2 having an 8.5-fold lower risk of alcohol dependence than other genotypes. **Conclusions:** Decreased DRD2 binding associated with the C-allele of the *DRD2* C957T polymorphism is likely to be important in the underlying pathophysiology of at least some forms of alcohol dependence and this effect appears to be limited to males only.

INTRODUCTION

Alcohol dependence is a common, chronic, disabling illness that is one of the most costly public health problems (Grant *et al.*, 2004). Twin and adoption studies indicate that heritable factors account for 50% to 60% of the risk for developing alcoholism and potential susceptibility genes have been identified by association and linkage studies (Quickfall and el-Guebaly, 2006). Several lines of evidence implicate the *DRD2* gene as a candidate gene for susceptibility. Alcohol induced drug reward is largely mediated via DRD2 (Blum *et al.*, 2000) and neuroimaging evidence indicates that patients with alcohol dependence have decreased striatal DRD2 density (Repo *et al.*, 1999) . Conversely, unaffected offspring of alcohol dependent fathers have increased striatal DRD2 density compared to those without a family history of this disorder, indicating that higher levels of DRD2 availability may protect against the development of alcohol dependence (Volkow *et al.*, 2006). Finally, animal data reveal that DRD2 over expression in the nucleus accumbens core reduces alcohol consumption in both alcohol preferring and non-preferring rats (Thanos *et al.*, 2004).

In vivo positron emission tomography studies have found that the C-allele of C957T (rs6277), a polymorphism in the *DRD2* gene is associated with lower striatal DRD2 binding potential than the T-allele in healthy subjects (Hirvonen *et al.*, 2004). Contrary findings in an *in vitro* study reported an increase in DRD2 mRNA stability and protein translation associated with the C-allele (Duan *et al.*, 2003). A recent within-family association study reported association of the T-allele and alcohol dependence, but they could not demonstrate a population level association (Hill *et al.*, 2008).

Another association study of alcohol dependent patients (Berggren *et al.*, 2006) confirmed earlier findings that a polymorphism in the *DRD2* region, *Taq*IA, is associated with alcohol

dependence (Blum *et al.*, 1990). This association has also been confirmed by several meta-analyses (Young *et al.*, 2004; Munafo *et al.*, 2007; Smith *et al.*, 2008; Le Foll *et al.*, 2009) but it has not always been reproduced by individual studies (Anghelescu *et al.*, 2001; Haberstick *et al.*, 2007). *Taq*IA is a polymorphism in the ankyrin repeat and protein kinase domain-containing protein 1 gene (*ANKK1*) that is found immediately downstream from *DRD2*. In a pharmacogenetic study, *Taq*IA was associated with treatment response to bromocriptine, a pre-synaptic DRD2 agonist (Lawford *et al.*, 1995). The greatest improvement in craving and anxiety occurred in patients receiving bromocriptine and carrying the *Taq*IA A1-allele. A recent study found that both the C957T C-allele and the *Taq*IA A1-allele were associated with alcohol dependence, although the C957T polymorphism appeared to have a stronger effect (Ponce *et al.*, 2009).

As C957T and *Taq*IA have previously been implicated in DRD2 function, dopamine response and alcohol dependence, we investigated the role of these polymorphism in a case-control genetic association study of a treatment seeking alcohol dependent population.

METHODS

Subjects

A total of 228 alcohol dependent subjects were recruited from large public hospitals in Brisbane, Australia. All subjects met DSM IV (Diagnostic and Statistical Manual of Mental Disorders IV) criteria for Alcohol Dependence. There were 72 females and 156 males in this group with a mean age of 40.7 years (s.d. 10.4 years; range 20-68 years). All were hospital inpatients and represented a spectrum of disease severity. A significant proportion (n = 65) of these patients were diagnosed with two or more alcohol related medical conditions such as pancreatitis, cirrhosis, hepatitis or peripheral neuropathy. Alcohol dependent patients were excluded from the study if they had dementia, delirium, psychosis, or any other condition that would affect their ability to provide informed consent.

The control group included 228 subjects (94 females and 134 males) with a mean age of 36.8 years (s.d. 12.8 years; range 18-66 years). The control group was composed of volunteers from the general public, hospital staff, university staff and university students. Formal screening for alcohol dependence or other psychological disorders was not undertaken in the control population. To minimise population stratification bias, both control and clinical subjects were recruited in the Brisbane region (a city of approximately 2 million inhabitants on the East Coast of Australia). All subjects reported being of British or European descent.

Each participant gave written informed consent. Ethics approval was obtained from all institutions involved (QUT ethics number 0700000611).

Genotyping

Genotyping of the C957T polymorphism was performed by allele-specific kinetic real-time PCR (Lawford *et al.*, 2005) using the Applied Biosystems 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Sequence specific primers were designed for the C-allele (5'-ATGGTCTCCACAGCACTCTC-3'), the T-allele (5'-ATGGTCTCCACAGCACTCTT-3') and a common reverse primer (5'-CATTGGGCATGGTCTGGATC-3'). A total of 5-10 ng of genomic DNA was amplified in 1 x SYBR green PCR master mix (Applied Biosystems) containing 0.4 μM of allele specific forward primer and 0.4 μM of common reverse primer in a 25 μL volume. Amplification conditions were: step 1, 50°C for 2 min; step 2, 95°C for 10 min; step 3, 95°C for 15 s; step 4, 60°C for 1 min, steps 3 and 4 were repeated by 40 cycles. A cycle time (Ct) value was obtained by setting the threshold during geometric phase of amplification and scored relative to the ΔCt generated between the matched and mismatched primer pairs.

*Taq*I A genotyping was performed by restriction fragment length polymorphism (RFLP) analysis of PCR products. A genomic sequence of 501 bp of the coding region of *ANKK1* was amplified by PCR using the forward primer 5′-GCACGTGCCACCATACCC-3′ and the reverse primer 5′-TGCAGAGCAGTCAGGCTG -3′. A total of 5-10 ng of genomic DNA was amplified in a PCR master mix containing 0.2 μM of forward primer and 0.2 μM of reverse primer, 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1 unit of Platinum *Taq* DNA Polymerase (Invitrogen) in a 25 μL volume. Amplification conditions were: step 1: 94°C for 4 min, step 2: 94°C for 30 s, step 3: 68°C for 30 s, step 4: 72°C for 30 s, steps 2-4 were repeated by 40. Amplified PCR fragments were digested with *Taq*I restriction enzyme (New England Biolabs) and digested fragments were visualized via agarose gel electrophoresis.

Statistical analysis

For each polymorphism, a homogeneity χ^2 analysis was employed to test the hypothesis of homogeneity of allele frequency distributions between alcohol dependent and control populations. An odds ratio with 95% confidence interval (CI) was also calculated. Genotype frequencies were similarly compared between alcohol dependent and control populations. An extended Mantel-Haenszel test was performed to test for a trend to alcohol dependence with increasing copy number (0, 1, or 2) of the allele. Odds ratios were calculated relative to the homozygote for the non-associated allele and population attributable fractions calculated. Genotype data were entered into JLIN v1.6.0 (Carter *et al.*, 2006) to estimate haplotype frequencies. Haplotype frequencies were compared between alcohol dependent and control groups using a homogeneity χ^2 analysis. Each haplotype with a significant association was analysed in isolation by partitioning the χ^2 accordingly. Generated haplotypes were analysed for linkage disequilibrium (LD) measures (D' and r^2) using the JLIN (Carter *et al.*, 2006). The calculations for attributable risk were performed using genotypes, counting the presence of the C-allele as a risk factor. Statistical tests were performed using the COMPARE2 program from the WinPepi suite of epidemiology programs (Abramson, 2004) and SPSS version 16. Hardy-Weinberg equilibrium (HWE) was computed using Utility Programs for Analysis of Genetic Linkage (Ott, 1988). The analysis of genotypes under a recessive model involved pooling genotypes and comparing frequencies using a two-by-two contingency table. For example, for the C957T polymorphism, the T/T and C/T genotypes were pooled for both groups and compared with the C/C genotype.

RESULTS

C957T genotyping

In order to find genetic associations between alcohol dependence and polymorphisms in the *DRD2* region, the *DRD2* C957T polymorphism was genotyped in 228 unrelated alcohol

dependent subjects and 226 unrelated Caucasian healthy control subjects. The frequency of the C957T alleles in the alcohol dependent and control groups (Table 1) revealed that the C-allele was found more frequently in the alcohol dependent group (50% of alleles) compared with the control group (42% of alleles) with an odds ratio (OR) of 1.37 (P = 0.019). The genotype frequencies of both groups are displayed in Table 2. The C/C genotype was over represented in the alcohol dependent group when compared with the control sample, with an OR of 1.9 (P = 0.034). A simple comparison of the genotypes between alcohol dependent and control subjects just achieved significance (χ^2 = 6.33, P = 0.042) but this association became more obvious when analysed using a Mantel-Haenszel test for trend (χ^2 = 5.23, P = 0.022). The OR for the C/T genotype (1.27) was not significantly different from the T/T genotype (OR = 1) indicating that the C-allele appears to behave as a recessive allele. When analysed under a C-allele recessive model, the C/C genotype is almost twice as likely to be found in those with alcohol dependence (OR = 1.75, 95% CI, 1.09 to 2.82; P = 0.014). The attributable fraction, which is the proportion of cases in the study population that would not have occurred had the risk factor (risk allele) not been present, is a useful measure to quantify the impact of the allele on the development of alcohol dependence. Analysis of the genotype data revealed that 13% (95% CI, 3.37 to 22.09%, P = 0.004) of the susceptibility to alcohol dependence could be attributed to having the C/C genotype of C957T polymorphism. Both alcohol dependent and control groups were shown to be in Hardy-Weinberg equilibrium based on the respective genotype frequencies of each group (controls, χ^2 = 0.077, P = 0.78; alcohol dependence, χ^2 = 1.42, P = 0.23).

Gender differences

There has been widespread discussion of sexual dimorphism in alcohol dependence (Devaud and Prendergast, 2009) so we investigated association of C957T separately in male and

female alcohol dependent subjects (Table 2). Despite decreased power due to reduced numbers, the strength of association in male alcohol dependent subjects was increased (Mantel-Haenszel test for trend $\chi^2 = 6.72$, $P = 0.009$). Indeed, male subjects with the C/C genotype were 2.2 times as likely to have alcohol dependence (OR = 2.17, $P = 0.014$). In contrast, the female subjects showed no indication of association with this polymorphism (Mantel-Haenszel test for trend $\chi^2 = 0.497$, $P = 0.481$), although there were only 72 female subjects in the study.

TaqIA genotyping

As the *Taq*IA polymorphism has repeatedly been seen associated with alcohol dependence (Lawford *et al.*, 1997), it was also genotyped in the same samples (one control and two alcohol dependent samples failed to genotype). No association was found at the allele ($\chi^2 = 1.94$, $P = 0.163$; Table 1) or genotype level ($\chi^2 = 1.88$, $P = 0.39$; Table 2). The A1-allele of *Taq*IA is reported to be inherited dominantly (Lawford *et al.*, 1997) so the genotypes were examined under a partial and complete dominance model. No trend in susceptibility to alcohol dependence was found when analysed under a partially dominant model (Mantel-Haenszel $\chi^2 = 1.86$, $P = 0.173$; Table 2). When analysed under an A1-dominant model, the A1-containing genotypes (A1/A1 and A1/A2) also showed no association with alcohol dependence ($\chi^2 = 1.768$, $P = 0.184$).

Haplotype analysis

The C957T polymorphism showed association with alcohol dependence in our study and *Taq*IA has previously been associated with alcohol dependence so haplotype analysis was also performed for both polymorphisms. Haplotype frequencies for the two polymorphisms are shown in Table 3. Overall haplotype frequency was highly associated with alcohol

dependence (χ^2 = 25.11, *P* = 0.00001). This association was due to the over representation of the 957C/*Taq*I A1 haplotype in alcohol dependent patients. The 957C/*Taq*I A2, 957T/*Taq*I A2 and the 957T/*Taq*I A1 haplotypes were not significantly associated with alcohol dependence. The 957C/*Taq*I A1 haplotype was approximately twice as likely to be associated with alcohol dependence compared to the 957T/*Taq*I A2 haplotype. The 957T/*Taq*I A1 haplotype appears to be protective as it has an OR of 0.284, i.e. this haplotype is about 3.5 times less likely to be found associated with alcohol dependence than the 957T/*Taq*I A2 haplotype (*P* = 0.002) and almost seven times less likely to be found associated with alcohol dependence than the 957C/*Taq*I A1 haplotype (OR = 6.782, *P* = $4.5x10^{-6}$).

Linkage disequilibrium

The *Taq*I A1 and the 957C alleles were in partial linkage disequilibrium (LD) in both the control population *D´* = 0.321 and r^2 = 0.029 and the alcohol dependent population *D´* = 0.824 and r^2 = 0.173. While LD exists in the control population it is significantly larger in the alcohol group (Goodness-of-fit χ^2 = 13.35, *P* = 0.001) suggesting a functional relationship between alleles not just LD as a consequence of genetic proximity, i.e. the polymorphisms are inherited together more strongly in cases because both polymorphisms have a (functional) role in disease, not just the C957T polymorphism.

Combined C957T and TaqIA genotypes

The combined genotypes of both the C957T polymorphism and the *Taq*IA polymorphism were scored for each individual, e.g. if a person was homozygous C/C for C957T polymorphism and heterozygous for the *Taq*IA polymorphism their combined genotype was recorded as CC/A1A2. Two alcohol dependent patients failed to genotype for *Taq*IA so 226

combined alcohol genotypes were obtained. One control sample failed to genotype for C957T and a different control sample failed to genotype for *Taq*IA so 226 combined control genotypes were obtained (Table 4). As there are 9 possible combined genotypes, the counts for each genotype are quite low and only two reached significance, the CC/A1A2 genotype and the TT/A1A2 genotype. Because of the very low frequency of the 957T/ *Taq*I A1 haplotype (about 7% in controls and 2% in alcohol dependent samples), no controls or alcohol dependent samples had the TT/A1A1 combined genotype.

The TT/A2A2 combined genotype was chosen as the OR = 1 low-risk or 'normal' genotype. Examination of the ORs of each genotype reveals that there are 5 genotypes with a similar OR to TT/A2A2, i.e. close to 1.0. The OR of TT/A2A2, CC/A2A2, CT/A1A1, CT/A1A2 and CT/A2A2 vary between 0.808 and 1.25. These can be considered 'normal' risk genotypes. The CC/A1A1 genotype has an OR of 1.75 and the CC/A1A2 genotype has an OR of 3.4 so these genotypes can be considered high-risk genotypes. The TT/A1A2 genotype has an OR of 0.125 meaning that people with this genotype are eight times less likely to develop alcohol dependence than people with a normal-risk genotype. Compared to people with the high-risk CC/A1A2 genotype, those with the TT/A1A2 genotype are almost 30-times less likely to develop alcohol dependence (OR = 27.2, $\chi^2 = 24.608$, $P = 5x10^{-6}$).

When the high-risk CC/A1A1 and CC/A1A2genoypes were combined and compared with the remaining genotypes, people with these genotypes were more than three times as likely to have alcohol dependence (OR = 3.36, 95% CI = 1.72 to 6.87, $\chi^2 = 15.68$, $P = 0.00008$,) and the attributable fraction in the population was 12.7% (95% C.I. = 6.4% to 18.6%).

DISCUSSION

Analysis of individual polymorphisms identified modest association between the presence of the C-allele of the *DRD2* C957T polymorphism and alcohol dependence. The A1 allele of the *Taq*IA polymorphism has previously been associated with alcohol dependence and we observed that it was more abundant in alcohol dependent patients but this observation did not reach significance in our study. This may be because the A1-allele has a relatively low allele frequency resulting in a small effect size (Berggren *et al.*, 2006). Consequently, the number of subjects in this study might have been too small to detect association. However, when the data from both polymorphisms was analysed together by haplotype and combined genotype analysis the observed association was much stronger. This observation lends support to the argument that both polymorphisms are important in alcohol dependence and confirms the *DRD2* gene and adjacent region (*ANKK1*) as an alcohol dependence susceptibility gene.

While the functional role of *DRD2* has been well defined in addiction and other reward related disorders, it is less clear what role might be played by the *Taq*IA polymorphism in the *ANKK1* gene. ANKK1 is a serine/threonine kinase that is likely to be involved in signal transduction. The *Taq*IA polymorphism causes a non-conservative (Glu to Lys) amino acid change in the 11th ankyrin repeat of ANKK1. This might affect substrate binding but it is unlikely to affect the structural integrity of the protein (Neville *et al.*, 2004). While it is plausible that ANKK1 is involved in transduction of signals in the dopaminergic reward pathway, the failure to detect its expression in whole brain homogenates does not support this hypothesis (Neville *et al.*, 2004). The most plausible explanation is that the *Taq*IA polymorphism is only associated with alcohol dependence because it is in disequilibrium with C957T, especially since the association seen for *Taq*IA is not as strong as the C957T polymorphism. However, the linkage disequilibrium between C957T and *Taq*IA seen in

alcohol dependent cases is almost three times higher than in controls ($D´ = 0.824$ in cases compared to $D´ = 0.321$ in controls), suggesting that *Taq*IA has a role in disease independent of the C957T polymorphism. An alternative explanation for previously observed association with *Taq*IA is that the *ANKK1* gene has no direct role in addiction but the proximity of *Taq*IA to C957T in the *DRD2* gene (they are only separated by about 12.5 kilobases) may result in a change in the regulation of the *DRD2* gene.

DRD2 is present as both presynaptic autoreceptors and postsynaptic receptors(Grigoriadis and Seeman, 1984). Dopaminergic activation of presynaptic autoreceptors decreases both release and synthesis of dopamine and increase dopamine reuptake (Tissari *et al.*, 1983). It is known that low DRD2 binding is associated with increased drug reinforcing effects (Volkow *et al.*, 1999a) and the intensity of this reinforcement is proportional to dopamine release (Volkow *et al.*, 1999b). Reduced density of presynaptic DRD2 associated with the C/C genotype of C957T may result in decreased autoreceptor activity, thereby resulting in increased dopaminergic transmission and increased drug reward.

Abstinent alcohol dependent patients have decreased DRD2 availability. Low DRD2 availability is also associated with increased craving for alcohol and increased risk of relapse (Heinz *et al.*, 2005). Furthermore, alcohol dependent patients exhibit diminished dopamine release following amphetamine challenge, reflecting decreased dopaminergic neurotransmission (Martinez *et al.*, 2005). Animal studies also show lower density of DRD2 in alcohol-preferring rodents than in non-alcohol preferring animals (Boehme and Ciaranello, 1981; Stefanini *et al.*, 1992; McBride *et al.*, 1993) and that the administration of DRD2 agonists reduces alcohol consumption in alcohol-preferring rats (Dyr *et al.*, 1993).

The C/C genotype of *DRD2* C957T has been associated with increased cigarette smoking in response to negative mood, consistent with an enhanced risk for addictive behaviour, at least in a sub-group of smokers (Perkins *et al.*, 2008). We have also recently found association between C957T and *Taq*IA genotypes and haplotypes in nicotine dependent subjects (Voisey *et al.*, 2012). Conversely, homozygotes for the T-allele have shown enhanced avoidance learning compared with those carrying the C-allele, indicating enhanced learning from error in this sub-group (Frank *et al.*, 2007). Impaired capacity to learn from experience is frequently seen in those with substance misuse.

Our findings are supported by a recent study that also found a strong association between the C957T C-allele and alcohol dependence (Ponce *et al.*, 2008). They also found a weaker association with the *Taq*IA A1-allele. However, two reports conflict with our observations. It has been reported that the C-allele results in higher mRNA stability *in vitro* (Duan *et al.*, 2003), which could translate into higher levels of receptor. However, it is possible that individual cells in the striatum may not respond to RNA instability elements in the same manner *in vivo*. Also, while we observed an association between the C-allele of C957T and alcohol dependence, a recent report found association of the T-allele in a family study but they could not demonstrate a population level association using limited numbers of subjects (81 cases and 78 controls)(Hill *et al.*, 2008).

When we analysed male and female subjects separately we found an even stronger association between C957T and alcohol dependence in males despite decreased power due to reduced numbers, while the female subjects showed no sign of any association. It is well recognised that incidence and risk of developing alcohol dependence varies greatly between men and women (Limosin *et al.*, 2002; Lopez-Castroman *et al.*, 2009). This may be due to

significant environmental differences or the impact of those environmental differences on different susceptibility genes. Alternatively, a more biological difference in brain function between the sexes may be a more salient contributor. In this respect, there is growing evidence for *in vivo* gender differences in D2 dopamine receptor characteristics. In a positron emission tomography study, women had higher D2 dopamine binding potentials than men in the frontal cortex (Kaasinen *et al.*, 2001) and lower D2 dopamine binding potentials in striatal regions (Pohjalainen *et al.*, 1998). It is suggested (Limosin *et al.*, 2002; Lopez-Castroman *et al.*, 2009) that these differences may be reflected in gender-associated differences found in alcohol dependence and in the present study.

Our study is one of only a few to demonstrate clear gender differences for particular susceptibility genes. Some studies have examined male or female risk alone but few studies have directly compared genetic risk for particular polymorphisms in both men and women with alcohol dependence. However, in one Spanish study of alcohol dependent subjects, an interleukin-1 receptor polymorphism was associated in male subjects but not female subjects (Saiz *et al.*, 2009).

By comparing the odds ratios of the combined genotypes of the C957T and *Taq*IA polymorphisms we observed five genotypes (representing about 87% of controls and 81% of alcohol cases) that had a neutral risk status for alcohol dependence, i.e. an odds ratio of about one. There were two high-risk genotypes (representing about 6% of controls and 18% of alcohol cases) that had about 3.5 times the chance of having alcohol dependence and, interestingly, we found one novel low-risk genotype (representing about 7% of controls and less than 1% of alcohol cases) that had an 8.5 times lower risk of having alcohol dependence.

While our data shows strong association between *DRD2* genotypes and alcohol dependence the number of subjects is still modest by current expectations. In addition, the control population was not formally screened for alcohol dependence or other psychological disorders which would be expected to decrease the power to detect associations.

Our results and the data of others suggest that the D2 receptor is centrally involved in the development of alcohol dependence and this has implications for pharmacological intervention strategies. Our findings support the conclusion that the dopamine D2 receptor and the dopamine pathway is important in defining genetic susceptibility to alcohol dependence but only in males.

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CONFLICT OF INTEREST

All other authors declare that they have no conflicts of interest.

CONTRIBUTORS

All authors made a substantial contribution to drafting the manuscript or reviewing it critically gave final approval of the version of the article to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper. In addition: Young, Lawford, Swagell, van Daal, Morris - made a substantial contribution to the

conception and design, the acquisition of data and the analysis and interpretation of the data; Hughes - made a substantial contribution to the analysis and interpretation of the data; Connor, Feeney and Noble - made a substantial contribution to the acquisition of the data.

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Table 1. Allele frequencies of C957T and *Taq*IA polymorphisms in patients with alcohol dependence compared to controls

		Allele frequency		χ^2	*P*	Odds Ratio
C957T (rs6277)	*N*	C (%)	T (%)			(95% CI*)
Control	454	193 (42.5)	261 (57.5)	5.47	0.019	1.37
Alcohol dependence	456	229 (50.2)	227 (49.8)			(1.04-1.79)
*Taq*IA (rs1800497)		A1** (%)	A2 (%)			
Control	454	77(17.0)	377 (83.0)	1.942	0.163	1.27
Alcohol dependence	452	93 (20.6)	359 (79.4)			(0.90-1.80)

* Fisher's exact confidence interval
** The A1 allele is T and the A2 allele is C

Table 2. Genotype frequencies of C957T, in patients with alcohol dependence compared to controls

	N	Genotype frequency			χ^2 (*P*)
C957T (rs6277)		C/C (%)	C/T (%)	T/T (%)	
Control	227	40 (17.6)	113 (49.8)	74 (32.6)	6.33* (0.042)
Alcohol dependence	228	62 (27.2)	105 (46.0)	61 (26.8)	5.23** (0.022)
Odds Ratio (*P*)		1.88 (0.034)	1.27 (1.00)	1.00	
Alcohol dependent Males	156	47 (30.1)	69 (44.2)	40 (25.7)	8.37* (0.015) 6.72** (0.009)
Odds Ratio (*P*)		2.17 (0.014)	1.13 (1.00)	1.00	
Alcohol dependent Females	72	15 (20.8)	36 (50.0)	21 (29.2)	0.505* (0.777) 0.497** (0.481)
Odds Ratio (*P*)		1.32 (0.955)	1.12 (1.00)	1.00	
*Taq*IA (rs1800497)		A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	
Control	227	8 (3.5)	61 (26.9)	158 (69.6)	1.88* (0.390) 1.86** (0.173)
Alcohol dependence	226	11 (4.9)	71 (31.4)	144 (63.7)	
Odds Ratio (*P*)		1.88 (0.034)	1.14 (1.00)	1.00	

* Likelihood-ratio χ^2 test

** Mantel-Haenszel χ^2 test for trend

Table 3. Haplotype analysis of C957T and *Taq*I A polymorphisms in patients with alcohol dependence

Haplotype*	Controls	Alcohol	Odds Ratio** (*P*)	χ^{2*}***	*P*
957T/*Taq*I A2	230	216	1	0.868	0.352
957T/*Taq*I A1	30	8	0.284 (0.002)	14.124	0.0002
957C/*Taq*I A2	145	143	1.05 (1.000)	0.020	0.886
957C/*Taq*I A1	47	85	1.926 (0.004)	12.967	0.0003
TOTAL	452	452		25.11	0.00001

* Haplotype frequencies were estimated using JLIN. Note, haplotypes with a missing genotype were omitted from the analysis (2 control and 2 alcohol dependence samples)

** Odds ratio with respect to 957T/*Taq*I A1 haplotype which is more commonly associated with a normal phenotype

*** Likelihood ratio χ^2 test comparing haplotype frequency between groups when all other haplotypes were pooled

Table 4. Combined genotype frequencies of the C957T and *Taq*I A polymorphisms in patients with alcohol dependence

Genotype	Control	Alcohol	OR	χ^2*	*P*
CC/A1A1	4	7	1.750	0.758	1.000
CC/A1A2	10	34	3.400	10.219	0.01
CC/A2A2	26	21	0.808	0.380	1.000
CT/A1A1	4	4	1.000	0.0	1.000**
CT/A1A2	35	35	1.000	0.0	1.000
CT/A2A2	73	65	0.890	0.212	1.000
TT/A1A1	0	0			
TT/A1A2	16	2	0.125	10.93	0.007
TT/A2A2	58	58	1		
Total	226	226		28.053	0.0002

* Likelihood-ratio Chi-square

** At least one cell has an expected frequency < 5