GENETICS

Dopamine D2 Receptor Genotype Is Associated with Increased Mortality at a 10-Year Follow-up of Alcohol-Dependent Individuals

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Abstract — Aims: Because the TAQ1 A1 allele may be associated with alcohol-related medical illnesses, and medical illnesses in alcohol-dependent individuals are associated with increased mortality, we test the hypothesis that the TAQ1 A1 allele of the DRD2 gene is associated with increased mortality in alcohol-dependent individuals. Methods: Following an index treatment episode, a 10-year follow-up study in 366 alcohol-dependent individuals was performed. The TAQ1 A1/A2 DRD2 genotype and allele frequencies were calculated to be able to compare rates of mortality between studies with various lengths of the follow-up period. Results: The prevalence of the A1 allele differed between the deceased and living patients and the controls: 47% of the deceased were A1+, compared to 37% of the living patients and 32% of the controls. The frequency of the TAQ1 A1/A2 genotype also differed between the groups. Thus, 43% had the A1/A2 genotype in comparison with 32% in the living patients and 29% in the controls. The TAQ1 A1 allele frequency differed between the groups. The frequency of A1 allele was 25% in the deceased patients compared to 21% in the living patients and 17% in the controls. Conclusion: The TAQ1 A1 allele of the DRD2 gene (or DRD2 gene region) was associated with increased mortality over a 10-year period in alcohol-dependent individuals.

INTRODUCTION

It is well documented that alcohol use disorders (AUDs) are associated with increased mortality. Thus, Finney and Moos (1991) in their review of long-term studies of mortality in individuals treated for AUDs found that the mortality rates in these individuals in comparison with the general population was 1.6–4.7 times greater than expected. The annualized mortality rates ranged from 1.6% to 3.7% (Finney and Moos, 1991). Annualized mortality rates (i.e. overall mortality rates in percentages divided by the number of years in the follow-up period) were calculated to be able to compare rates of mortality between studies with various lengths of the follow-up period (Finney and Moos, 1991). The increased mortality in individuals treated for AUDs has been reported to be associated with male gender (Feuerlein et al., 1994) and older age (Finney and Moos, 1992; Feuerlein et al., 1994). It is also well established that the increased mortality in individuals with AUDs is associated with alcohol-related medical illnesses (Finney and Moos, 1992; Feuerlein et al., 1994). However, little, if anything, is known about the contribution of specific genes to the mortality in individuals with AUDs. One of the genes that has been extensively studied in its relationship with alcohol dependence is the dopamine D2 receptor (DRD2) gene and in particular the Taq1A polymorphism (rs1800497) of this gene (Noble, 2003). It should be noted that the TAQ1A polymorphism is found 10 kb downstream of the DRD2 gene and has recently been found to be located within a protein-encoding region of a neighbouring gene, ankyrin repeat and kinase containing 1 or ANKK1 (Neville et al., 2004). It is at present unclear whether the proposed association between DRD2 TAQ1A polymorphism and alcohol dependence is due to altered activity in the ANKK1 gene, which may encode for proteins involved in signal transduction pathways and may affect substrate-binding specificity (Neville et al., 2004). Alternatively, the TAQ1A polymorphism may be in linkage disequilibrium with upstream polymorphisms in the DRD2 gene (Connor et al., 2008). However, there are at present four recent meta-analyses (Noble, 2003; Young et al., 2004; Mufano et al., 2007; Smith et al., 2008) confirming an association between the Taq1A1 allele (rs1800497T) of the DRD2 gene and alcohol dependence, although its effect size may be small with an odds ratio of ~1.2–1.4 (Mufano et al., 2007; Smith et al., 2008). It should, however, be noted that this association has not been found in all meta-analyses (e.g. Gelemer et al., 1993). It has also been suggested that the prevalence and frequency of the TAQ1 A1 allele is increased particularly in more severe forms of alcohol dependence (Noble, 2003). It should be noted that the concept of severity varies considerably between various studies. However, when using a severity concept of alcoholism, focusing on medical illness, an increased prevalence and frequency of the TAQ1 A1 allele of the DRD2 gene has indeed been reported (Noble et al., 1994; Gorwood et al., 2000; Noble et al., 2000; Noble, 2003). Thus, since the TAQ1 A1 allele may be associated with alcohol-related medical illnesses and medical illnesses in alcohol-dependent individuals is associated with increased mortality, it seems reasonable to formulate the hypothesis that the TAQ1 A1 allele of the DRD2 gene is associated with increased mortality in alcohol-dependent individuals.

In an earlier study of ours (Berggren et al., 2006), subjects treated as in-patients for alcohol withdrawal symptoms were genotyped for the TAQ1 A polymorphism. We found that the TAQ1 A1 allele of the DRD2 gene was associated with alcohol dependence although its effect size was small with an odds ratio of 1.34. This odds ratio was thus very similar to that reported in the two meta-analyses of Mufano et al. (2007) and
Smith et al. (2008). Furthermore, in our earlier study (Berggren et al., 2006), we found that about twice as many (26% versus 14%) of the A1 carriers (genotypes A1A1 and A1A2) had been treated in somatic hospitals in comparison with the A1 non-carriers (genotype A2A2). This finding supported the notion that the TAQ1 A allele of the DRD2 gene is also associated with more severe medical illnesses in alcohol-dependent individuals as suggested by Noble (2003). In the present follow-up study, the TAQ1 A1/A2 DRD2 genotype and allele frequencies were compared between the alcohol-dependent individuals who were deceased or living at a 10-year follow-up. In addition, the genotype and allele frequencies of the alcohol-dependent individuals were compared to those of a control group of 578 individuals who had been selected as healthy control subjects for a case-control study (Berggren et al., 2006). Our hypothesis was thus that the TAQ1 A1 genotypes and/or allele frequency would be increased in the group of deceased alcohol-dependent individuals in comparison with the living alcohol-dependent individuals and/or to the otherwise healthy control subjects.

METHODS

Patients

Female and male unrelated Caucasian patients of Scandinavian origin above 20 years of age and consecutively admitted to an alcohol and drug treatment unit at the psychiatric department of a University hospital were included in this study and were the subjects reported on by Berggren et al. (2006). They had fulfilled DSM-IV criteria for alcohol dependence (American Psychiatric Association, 1994) according to diagnoses from previous hospital records and/or assessments at time after admission, i.e. they had been interviewed and evaluated for the presence of this diagnosis by an experienced psychiatrist. Discharge diagnosis rather than admission diagnosis was used. The reasons for admittance were generally long periods of heavy alcohol consumption necessitating treatment for alcohol withdrawal symptoms.

During this index treatment episode, which occurred at some occasion during 1997–1998, the patients were genotyped for the TAQ1 A polymorphism of the DRD2 gene. They were followed up after 10 years and it was determined whether the patients were alive or deceased by using information from the National Register for the Swedish population (‘Folkbokföringen’).

Control subjects

Controls in this study \( n = 578; \) women: \( n = 168; \) men: \( n = 410 \) were obtained from the INTERGENE study sample. This sample comprises randomly sampled individuals from a source population comprising all inhabitants in the region of Västra Götaland (western Sweden) at 1 April 2001, aged 25–74 at the time of sampling. The sampling continued until the end of December 2004 when 3610 members of the target population sample had been examined. The sample used in the present study was 578 individuals from the INTERGENE sample who had been selected as healthy control subjects for a case-control study. All the 578 subjects were of Scandinavian origin and their mean age ± SD was 61 ± 8 years (range: 31–75). To be included in this study, the subjects should have no histories of medical or psychiatric disorders, including substance abuse/dependence. The reported weekly alcohol consumption was 101 ± 133 g of pure alcohol (women: 53 ± 74; men: 121 ± 147).

These control subjects were used only for comparison of the DRD2 allele and genotype frequencies with those of the alcohol-dependent patients. Therefore, background data for the controls are reported only to a limited extent. For further data on the INTERGENE study see Berg et al. (2005) or www.sahlgrenska.gu.se/intergene.

Genotyping

The DRD2 TaqI PCR was performed as described by Grandy et al. (1993) with minor modifications. In short, genomic DNA was extracted from venous blood samples, and the DRD2 gene was amplified by PCR using the primers 5′CCGTGCACGGCTGGCCAAGTTGCTTA and 5′CCGTCGACCCCTTCCTGAAGTGATCATCA. The 310 bp PCR product was cleaved with TaqI, resulting in cleavage of the DRD2-A2 allele into two fragments of 180 and 130 bp, while the DRD2-A1 allele was not cleaved (Eriksson et al., 2000).

Statistical analyses

TAQ1 A DRD2 allele and genotype frequencies were compared between the three groups (deceased patients, living patients and random control subjects) by using the \( \chi^2 \) test. When the overall \( \chi^2 \) test was significant, standardized residuals (\( R \)) were used to detect a major contributor to the significant result. \( R > 1.96 \) indicates a major contribution regarding the significant result. The data are presented as mean ± standard deviation. \( P \)-values of <0.05 were considered statistically significant.

This study was approved by the Ethics Committee of the Göteborg University, Sweden, and was in compliance with the Helsinki Declaration of 1975. Informed consent was obtained from all subjects. None of the subjects was paid for their participation in the study.

RESULTS

Subjects

In total, 375 alcohol-dependent patients, consecutively admitted for treatment of alcohol withdrawal symptoms, were included in the study. At the time for the examination of the hospital records, data from nine patients had to be excluded, either due to missing data in records (\( n = 5 \)) or due to inaccurate diagnoses of alcohol dependence (\( n = 4 \)). Thus, this study sample comprised 366 patients, 67 females (18%) and 299 males (82%). The mean age of the patient group was 48 ± 11 (22–77) years [females 47 ± 9 (25–73) and males 49 ± 11 (22–77) years; ns], The mean ages for the three genotype groups were: A1/A1, 47 ± 8; A1/A2: 49 ± 10 and A2/A2: 48 ± 11. There were no significant differences in ages between the three genotype groups.

At the 10-year follow-up

One hundred and twenty-two of the 366 patients were deceased at a mean ± SD age of 51 ± 10 years. Thus, the overall mortality for the 10-year follow-up period was 33% and consequently the average annual mortality rate was 3.3% (3.5% for men and
Comparison of the TAQ I A1/A2 genotype and allele frequencies between the patient groups (deceased and living) and random control subjects

The prevalence of the A1 allele, i.e. the proportion of individuals carrying the A1 allele (genotypes A1/A1 and A1/A2) differed significantly between the deceased and living patients and the controls (χ^2 = 10.74, df = 2, P < 0.01); see Table 1. The major contributor for this significant result (R = 2.2) was the group of deceased patients who were carriers of the A1 allele, i.e. A1+. Among the deceased patients 47% were A1+ in comparison with 37% of the living patients and 32% of the random controls. Furthermore, the frequency of the TAQ1 A1/A2 genotype also differed significantly between the deceased, living patients and the controls (χ^2 = 12.12, df = 4, P < 0.02), where the major contributor for the result was the deceased patients with A1/A2 genotype (R = 2.3); see Table 2. Thus, among the deceased individuals, 43% had the A1/A2 genotype in comparison with 32% in the living patients and 29% in the random control subjects.

The TAQ 1 A1 allele frequency differed significantly between the deceased and living patients and the controls (χ^2 = 9.72, df = 2, P < 0.01), where the major contributor for this result was the deceased patients (R = 2.2); see Table 3. In the deceased patients, the frequency of A1 allele was 25% compared to 21% in the living patients and 17% in the controls.

The overall annual mortality rate was 3.3%. In the carriers of the A1 allele (genotypes A1/A1 and A1/A2), the mortality rate was 3.9% and in the non-carriers of the A1 allele (genotype A2/A2) it was 3.0%.

In the present study, we found that the TAQ I A1 allele of the DRD2 gene (or the DRD2 gene region since the TAQ I A polymorphism has been shown to be located in a neighbouring gene, i.e. ANKK1; Neville et al., 2004) was associated with increased mortality in a sample of 366 alcohol-dependent individuals. Thus, there were significantly more carriers of the A1 allele, i.e. genotypes A1/A1 and A1/A2, in the group of deceased alcohol-dependent individuals in comparison with both the groups of living alcohol-dependent individuals and random control subjects. Furthermore, the frequency of the genotype A1/A2 was significantly higher in the group of deceased alcohol-dependent individuals in comparison with both the other groups. Finally, the TAQ I A1 allele frequency was significantly overrepresented in the deceased alcohol-dependent individuals as compared with both the living alcohol-dependent individuals and random control subjects. It should be noted that there was no difference in age between the three genotype groups of the alcohol-dependent individuals. There was also no difference in mortality rates between males and females in the present study. Since there was no difference in the risk factors age and gender between the genotype groups, it was considered not necessary to use additional analysis such as logistic regression to partition out their relative contribution of their presence to the A1 allele to mortality.

These findings thus eliminate the possible confounding effects of age (Finney and Moos, 1992; Feuerlein et al., 1994) and gender (Feuerlein et al., 1994) on the mortality rate in the present study. With respect to the phenotype, the alcohol-dependent individuals in the present study had thus been treated as in-patients for alcohol withdrawal symptoms about 10 years before the present follow-up. During that index treatment episode they were also evaluated for the presence of somatic and psychiatric disorders and were found to have high co-morbidity both for somatic (∼70%) and psychiatric (∼50%) disorders. They were thus considered to have a severe form of alcohol dependence (see Berggren et al., 2006). The present finding of an association between the TAQ I A1 allele and increased mortality in alcohol-dependent individuals has not been reported earlier to our knowledge. It should, however, be noted that the first study reporting an association between the TAQ I A1 allele and alcohol-dependence was performed in deceased individuals (Blum et al., 1990). In that postmortem study, 69% of the alcohol-dependent individuals were carriers of the A1 allele in comparison with 20% in the controls who had died from non-alcohol-related causes. In the present study, 47% of the deceased alcohol-dependent individuals were
carriers of the A1 allele in comparison with 37% in the living alcohol-dependent individuals and 32% in the random control subjects. Since individuals with AUDs associated with alcohol-related medical disorders have higher mortality rates (Finney and Moos, 1992; Feuerlein et al., 1994), one reason for the association between the TAQ I A1 allele and increased mortality may thus be that the TAQ I A1 is associated with more severe concomitant medical disorders (see Berggren et al., 2006). The reason for the association between the TAQ I A1 allele and alcohol-dependence with concomitant medical disorders found in several studies (Noble et al., 1994; Gorwood et al., 2000; Noble et al., 2000; Noble, 2003) is presently unknown. It has, however, been shown that the TAQ I A1 allele is associated with the reduced number of brain dopamine D2 receptors (see Noble, 2003). It has been hypothesized that in order to compensate for the deficiency in the brain DA systems, i.e. reduced number of dopamine D2 receptors, carriers of the A1 allele may seek to stimulate the mesocorticolimbic DA pathways in the brain by using drugs, such as alcohol, which increase the activity in these pathways (Noble, 2000). Thus, carriers of the TAQ I A1 allele may have to use larger amounts of alcohol in order to override the DA deficiency in comparison with their counterparts who are non-carriers of the TAQ I A1 allele. Indeed, there are some studies showing that carriers of the TAQ I A1 allele consume larger amounts of alcohol and have an earlier onset of problem drinking (Connor et al., 2002, 2008). It seems reasonable to assume that this extreme drinking behaviour in the carriers of the TAQ I A1 allele may result in more medical complications and disorders in these individuals. This assumption is based on findings that for most medical diseases higher levels of alcohol consumption are associated with higher disease risks, possibly with some exceptions such as coronary heart disease, stroke and diabetes mellitus (Room et al., 2005). If so, this may explain the association between the A1 allele and severe alcohol dependence, when severity index is focused on concomitant medical disorders (Noble et al., 1994, 2000, 2003; Gorwood et al., 2000).

A second important finding in the present study was that the overall mortality rate in the present sample of alcohol-dependent individuals with severe alcoholism was very high. Thus, the overall SMR was about 8, i.e. the alcohol-dependent individuals were about eight times more likely to die during the 10 years follow-up period in comparison with the general Swedish population. Finney and Moos (1991) in their review of long-term studies of mortality in individuals treated for AUDs found that the mortality rates in these individuals was 1.6–4.7 times greater than expected in comparison with the general population. There are, however, some studies suggesting a very high mortality rate in certain individuals. Thus, Finney and Moos (1991), in their own study, Feuerlein et al., (1994) and Gerdner and Berglund (1997) reported increased mortality rates in the range of 8–10 times to that of controls or the general population. It therefore seems reasonable to assume that there are sub-groups of alcohol-dependent individuals, probably characterized by concomitant medical disorders and treatment resistance, which have very high mortality rates and run a substantial risk for the worst possible outcome of their alcohol dependence, i.e. premature death. In the present study, it was found that the presence of the A1 allele additionally increased (~40%; SMR 10 in the A1+ and 7 in the A1−) the already high mortality rate of the present sample of alcohol-dependent individuals. Whether this finding should lead to the implementation of genotyping for the TAQ IA polymorphism in individuals with severe forms of alcohol-dependence should be considered in the future. The findings that carriers of the A1 allele may benefit from specific pharmacological treatment, i.e. DRD2 agonist medication such as bromocriptine and speculatively also from specific psychological treatment, aimed at improving drinking refusal self-efficacy [see Connor et al. (2008) and references therein], may lend some support for this strategy. In addition, the finding of a marked reduction and even normalization of the increased mortality rate in individuals with severe alcohol dependence by achievement of long-term abstinence (Feuerlein et al., 1994; Gerdner and Berglund, 1997; Mann et al., 2005) also suggests that the treatment goal of long-term abstinence should be strongly emphasized in carriers of the A1 allele.

There are some limitations in the present study that ought to be mentioned. First, we were not able to obtain reliable and valid data on alcohol consumption before the index treatment period or the time period thereafter up to the 10-year follow-up. There was no difference in the serum bilirubin, serum enzymes and carbohydrate-deficient transferrin at baseline (start of this follow-up study) between the genotype groups [see our earlier study, Berggren et al. (2006)]. However, other severity indices such as alcohol dependence severity, other biological severity markers (such as for example Child-Pugh scores) and socio-economic status were not assessed at baseline and could therefore not be compared between the genotype groups. Possible differences in these factors between the genotype groups and their influence on mortality rates could thus not be evaluated. Furthermore, it has been suggested that the A1 allele of the DRD2 gene is associated with polysubstance abuse/dependence, psychostimulant abuse/dependence, opioid dependence, nicotine dependence and risk-taking behaviours (for review, see Noble, 2003). In the present sample of alcohol-dependent patients, there was no difference in polysubstance abuse/dependence at baseline between the genotype groups [see our earlier study, Berggren et al. (2006)]. However, it should be noted that the individuals in the present study were not assessed for tobacco use. Since tobacco use is the most prevalent drug attributed to premature death, it cannot be ruled out that the difference in mortality rate between the genotype groups was due to the difference in tobacco use between the groups. To sum up, it can therefore not with certainty be concluded that the increased mortality in the carriers of the A1 allele was due to higher levels of alcohol consumption, or more extreme drinking behaviour, or other associated behaviours.

Secondly, we were unable to obtain valid reports on causes of death for the deceased alcohol-dependent individuals. Consequently, we could not investigate whether the presence of the A1 allele was associated with specific causes of death.

Thirdly, it may be argued that the number of subjects in the present study was relatively low, particularly for the groups of alcohol-dependent individuals (deceased n = 122 and living n = 244). However, the number of random control subjects was large (n = 578) resulting in a total number of subjects of 944. It should be noted that in the earlier meta-analyses of Noble (2003) and Young et al. (2004), no individual study included more than a total number (cases and controls together) of about 500.

Finally, it should be observed that the control subjects were used only for comparison of the DRD2 allele and genotype
frequencies with those of the alcohol-dependent patients. The controls were thus not followed up for mortality. Thus, the death rate of controls with respect to DRD2 allele or genotype frequency has not been studied. It should therefore be underscored that it is at present unclear whether the association of the A1 allele of the DRD2 gene and increased mortality is present only in alcohol-dependent individuals or if it is also present in the general population.

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