Polymorphisms in the dopamine transporter gene (SLC6A3/DAT1) and alcohol dependence in humans: systematic review

Dopamine neurotransmission has been a key player in attempts to identify genetic factors involved in alcohol dependence. The dopamine transporter terminates dopaminergic neurotransmission, making the gene encoding the transporter (SLC6A3/DAT1) an attractive candidate in clinical studies on alcohol dependence. We conducted a systematic review of 18 studies examining associations between polymorphisms in DAT1 and alcohol dependence. The DAT1 variable number tandem repeat, the most frequent studied polymorphism in DAT1, did not show a direct association with alcohol dependence in general. Several, but not all, studies found that the DAT1 variable number tandem repeat (9-repeat allele) was associated with alcohol-withdrawal symptoms, such as seizures and delirium tremens. We discuss shortcomings, such as lack of power and disregarding moderating variables, as well as future challenges of gene association studies.

Alcoholism is one of the most prevalent psychiatric disorders. In 2001–2002, the 12-month prevalence of alcohol dependence in the USA was 3.7% [1]. Heritability estimates for alcohol dependence are high and range from 50 to 70% in both men and women, as is shown by numerous twin and adoption studies [2-6]. In attempts to discover the nature of this genetic liability for alcoholism, genes involved in dopaminergic circuits have received much attention. Alcohol is known to activate the dopaminergic system, thereby terminating the reuptake of extracellular synaptic dopamine to the fact that measurements were carried out up to several weeks after alcohol withdrawal, in which DAT levels may have already returned to normal.

Alternatively, DAT availability in the brain may be dependent on genetic variation. The DAT gene (DAT1; locus symbol SLC6A3) is localized on chromosome 5p15.3. Although many parts of the gene are highly conserved in evolutionary aspect [19], several, mainly non-functional variants have been identified in DAT1 in different populations (see e.g., [20-24]). For example, a SNP in the 3'-UTR of DAT1 consists of a G to A mutation at position 2319 in DAT cDNA (2319G>A) and has been described in several recent studies [22,25,26]. The genetic polymorphism of interest in most studies on alcohol dependence, however, is a variable number of tandem repeats (VNTR) of 40 bp. For the VNTR, numbers ranging from three to 16 have been described, with the 9- and 10-repeat...
alleles being the most common variants [19, 24]. Several studies have demonstrated ethnic differences in DAT1 VNTR frequency distributions (e.g., [27–29]). Since the VNTR is located in the 3′-UTR of the gene, outside the open reading frame of the gene, allelic variants do not result in structural or functional differences in the DAT protein. Nonetheless, research suggests that the DAT1 VNTR is able to regulate specific gene functioning by influencing levels of expression. As such, DAT expression may be influenced by alteration in the length or sequence of the DAT1 VNTR (for more info on the effects of 3′-UTRs on translation and transcription see [30]). It is also likely that the VNTR is in linkage disequilibrium with other susceptibility loci within the gene. Greenwood et al. discovered a high degree of linkage disequilibrium between SNPs in the 5′ and 3′ regions of DAT1 [31]. In addition, the VNTR polymorphism was in significant linkage disequilibrium (D′ > 0.50) with at least six SNPs within DAT1.

From the literature, it is not exactly clear whether high or low levels of DAT are likely to predispose to alcoholism. In the animal literature, one rodent study has shown that female DAT knockout mice showed decreased alcohol intake [32], indicating that low or abstinence levels of DAT result in higher levels of extracellular dopamine and subsequently in less alcohol intake. In contrast, Hall et al. found that male DAT knockout mice increased their alcohol intake and that female heterozygous mice showed increased alcohol preference [33]. In the human literature, both the 9- and the 10-repeat allele have been repeatedly associated with increased DAT expression [34–40], and as such both may be treated as risk alleles.

In the current study we give a thorough review of the clinical studies that examined the association between DAT1 and alcohol dependence either in a case–control or in a family-based design.

Method of search

A literature search was carried out using Ovid Medline and PubMed. Keywords used were ‘dopamine transporter’, ‘DAT’, ‘DAT’, combined with ‘alcohol’, ‘dependence’, and ‘alcoholism’. Studies were included if they examined the DAT1 VNTR polymorphism or the DAT1 2319G>A polymorphism, and if they applied a case–control, case only or a family-based design. Studies that were carried out on post-mortem cases (e.g., [41]) were left out of the review. A total of 18 studies were included in the present review. We first give a short, general summary of the findings of the studies. Subsequently the reviewed studies are described individually, and then in more detail, in a chronological order (Table 1 & 2). The 15 studies examining the DAT1 VNTR polymorphism are described separately from the three studies that examined the DAT1 2319G>A polymorphism, which are not included in the general summary below.

General summary

In a general case–control design, the vast majority of studies did not find significant differences in DAT1 VNTR frequency between alcohol dependent subjects and controls [23, 35, 42–49]. Only Köhne et al. found that the 9-repeat allele was significantly more frequent in German alcoholics than in controls [50]. When applying a family-based design, two studies did not show a significant transmission of one of the DAT1 VNTR alleles to alcohol dependent offspring [45, 51], while one did find a significant transmission of the 10-repeat allele [47].

Since alcohol-dependent subjects are not necessarily a homogeneous group, several researchers created subgroups, often based on the presence or absence of certain alcohol-withdrawal symptoms [42, 43, 46, 50–54]. Several studies showed that the 9-repeat allele was more often present in a subgroup of alcoholics with a variety of alcohol-withdrawal symptoms, such as withdrawal seizures and delirium tremens [43, 46, 52], paroxysmal sweats and tremors [54], and visual hallucinations [53] when compared with controls. Others did not find the 9-repeat allele to be associated with alcohol-withdrawal symptoms [50, 51]. For a more detailed overview of the studies please see below and Table 1 and 2.

Clinical studies on the DAT1 VNTR & alcohol dependence

Muramatsu and Higuchi were the first to test for differences in DAT1 VNTR allele prevalence between alcohol-dependent and control subjects [44]. In their Japanese sample, they differentiated between 80 alcoholics with the aldehyde dehydrogenase-2 (ALDH2) polymorphism, 132 alcoholics without this variant and 235 controls. Individuals with the ALDH2*2 variant display decreased ALDH2 enzyme activity, which reduces efficient breakdown of alcohol consumed [55]. This in turn creates the well-known flushing response and disproportional headaches and nausea after limited alcohol consumption. Since alcoholic persons with the ALDH2*2 allele had overcome their original genetic protection...
Table 1. Clinical association studies of DAT1 polymorphisms and alcohol dependence in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Design</th>
<th>Sample characteristics</th>
<th>Alcohol dependence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sander et al. (1997)</td>
<td>Caucasian (German)</td>
<td>Case–control</td>
<td>n_case = 293, male = 87%, n_control = 93, male = 50%; subgroups based on family history, age of onset, withdrawal symptoms, type and antisocial tendencies</td>
<td>ICD-10</td>
<td>[46]</td>
</tr>
<tr>
<td>Dobashi et al. (1997)</td>
<td>Asian (Japanese)</td>
<td>Case–control</td>
<td>n_case = 80, male = 99%, n_control = 120, male = 50%</td>
<td>DSM-III-R</td>
<td>[48]</td>
</tr>
<tr>
<td>Parsian &amp; Zhang (1997)</td>
<td>Caucasian (EA)</td>
<td>Case–control</td>
<td>n_case = 162, n_control = 29, male = 72%, n_control = 89, male = 52%; subgroups based on type 1 and 2 alcoholics</td>
<td>DSM-III-R</td>
<td>[45]</td>
</tr>
<tr>
<td>Schmidt et al. (1998)</td>
<td>German*</td>
<td>Case only</td>
<td>n_case = 48, male = 100%; subgroups based on withdrawal symptoms</td>
<td>ICD-10</td>
<td>[54]</td>
</tr>
<tr>
<td>Franke et al. (1999)</td>
<td>Caucasian (German)</td>
<td>Family based</td>
<td>n_case = 87, male = n.r.; subgroups based on withdrawal seizures and delirium</td>
<td>DSM-III-R</td>
<td>[31]</td>
</tr>
<tr>
<td>Ueno et al. (1999)</td>
<td>Asian (Japanese)</td>
<td>Case–control</td>
<td>n_case = 124, male = 95%, n_control = 107, male = 51%</td>
<td>DSM-III-R</td>
<td>[22]</td>
</tr>
<tr>
<td>Vandenbergh et al. (2000)</td>
<td>Caucasian (EA)</td>
<td>Case–control</td>
<td>n_case = 64, male = n.r., n_control = 64, male = n.r.</td>
<td>DSM-III-R</td>
<td>[23]</td>
</tr>
<tr>
<td>Heinz et al. (2000)</td>
<td>n.r.</td>
<td>Case–control</td>
<td>n_case = 14, male = 79%, n_control = 11, male = 64%</td>
<td>DSM-IV</td>
<td>[35]</td>
</tr>
<tr>
<td>Chen et al. (2001)</td>
<td>Asian (Taiwanese)</td>
<td>Case–control</td>
<td>n_case = 203, male = 83%, n_control = 213, male = 84%; subgroups based on ethnicity (4 aboriginal groups*, 1 group of Han Chinese) and severity of alcoholism</td>
<td>DSM-III-R</td>
<td>[42]</td>
</tr>
<tr>
<td>Bau et al. (2001)</td>
<td>Brazilian</td>
<td>Case–control</td>
<td>n_case = 114, male = 100%, n_control = 112, male = n.r.</td>
<td>DSM-III-R</td>
<td>[49]</td>
</tr>
<tr>
<td>Wernicke et al. (2002)</td>
<td>German*</td>
<td>Case–control</td>
<td>n_case = 351, male = 82%, n_control = 336, male = 54%; subgroups based on positive history father, positive history mother, age of onset, delirium, seizures, vegetative syndrome, type I, type II and antisocial tendencies</td>
<td>ICD-10</td>
<td>[25]</td>
</tr>
<tr>
<td>Gorwood et al. (2003)</td>
<td>Caucasian (French)</td>
<td>Case–control</td>
<td>n_case = 120, male = 100%, n_control = 65, male = 100%; subgroups based on withdrawal seizures or delirium</td>
<td>DSM-III-R</td>
<td>[52]</td>
</tr>
<tr>
<td>Limosin et al. (2004)</td>
<td>Caucasian (French)</td>
<td>Case only</td>
<td>n_case = 64, female = 100%; subgroups based on withdrawal symptoms</td>
<td>DSM-III-R</td>
<td>[53]</td>
</tr>
<tr>
<td>Köhnke et al. (2005)</td>
<td>Caucasian (German)</td>
<td>Case–control</td>
<td>n_case = 216, male = 81%, n_control = 102, male = 65% Subgroups based on withdrawal seizures or delirium</td>
<td>DSM-IV</td>
<td>[50]</td>
</tr>
<tr>
<td>Samochowiec et al. (2006)</td>
<td>Caucasian (Polish)</td>
<td>Case–control</td>
<td>n_case = 100, male offspring = 88%, n_control = 196, male = 87%; subgroups based on withdrawal seizures or delirium, or early age at onset</td>
<td>ICD-10</td>
<td>[47]</td>
</tr>
<tr>
<td>Choi et al. (2006)</td>
<td>Asian (Korean)</td>
<td>Case–control</td>
<td>n_case = 111, male = 100%, n_control = 123, male = 100%; subgroups based on family history of alcoholism</td>
<td>DSM-IV</td>
<td>[26]</td>
</tr>
<tr>
<td>Le Strat et al. (2008)</td>
<td>French*</td>
<td>Case–control</td>
<td>n_case = 250, male = 70%, n_control = 121, male = 46%; subgroups based on withdrawal seizures</td>
<td>DSM-IV</td>
<td>[43]</td>
</tr>
</tbody>
</table>

*Participants were recruited in Germany; no further information on patients’ ethnicity was given.
1The four aboriginal groups consisted of Atayal, Ami, Bunun and Paiwan participants.
2Part of this sample was examined previously for the DAT1 variable number tandem repeat [46].
380% of this French sample was of Caucasian descent. Ethnicity of the other patients was not described [43].
4DSM: Diagnostic and Statistical Manual of Mental Disorders; EA: European American; ICD: International Statistical Classification of Disease and Related Health problems; n.r: Not reported.

against alcoholism, the authors reasoned that they must share another susceptibility factor, possibly represented by a DAT1 polymorphism. No differences in DAT1 VNTR allele frequency were found between the alcoholics without the ALDH2*2 allele and controls. However, the 7-repeat allele occurred significantly more often in alcoholic persons with the ALDH2*2 allele than in controls (p < 0.05). No significant differences were found in the frequencies of the 9-repeat or 10-repeat alleles between alcoholics with the ALDH2*2 allele and controls. The authors concluded that as the search for susceptibility genes with regard to alcohol dependence is complicated by the heterogeneity of the disease, focusing on specific subpopulations might be helpful. Thus, for Japanese persons with the ALDH2 inactivity allele, an additional variation in DAT1 may increase the risk for alcoholism.

Sander et al. also formed subgroups of their sample of 293 Caucasian alcoholics [46]. They created clinically relevant subgroups based on positive family history, early age at onset, delirium, withdrawal seizures, antisocial tendencies, and type 1 and type 2 alcoholism. When compared with the 93 controls, alcoholics with an alcohol-related delirium or with withdrawal seizures showed significantly increased prevalence of the DAT1 VNTR 9-repeat allele (odds ratio [OR]: 2.49; p = .01 and OR: 2.48; p = 0.01, respectively). In addition, alcoholics homozygous for the 9-repeat allele were significantly more prevalent in the antisocial subgroup, and in the subgroup of type 2 alcoholics (OR: 3.37; p = 0.04 and OR: 4.59; p = 0.01, respectively). The entire group of alcohol-dependent individuals, or the other subgroups (positive family history, early age at onset and type 1 alcoholics) showed no difference in 9-repeat allele frequency compared with controls. The authors suggested that the 9-repeat allele might be mainly associated with more severe alcohol-induced neuro-adaptive alterations in the brain and with the expression of somatic withdrawal symptoms. In accordance with Muramatsu and Higuchi [44] further emphasized that considering alcoholics as one homogeneous group might not be the best way to examine genotype–phenotype associations [44].

Dobashi, Inada, and Hadano studied several dopamine-related genes and polymorphisms, among which the DAT1 VNTR, in a Japanese sample of 80 alcoholics and 120 control subjects [48]. Although the frequency of the 7-repeat allele was higher in alcohol-dependent patients than in controls, and the 9-repeat allele was less frequent in alcoholics than in controls, these results were only borderline significant (p < 0.10). The authors mentioned that genetic loci might be related to characteristics of personality or psychosis that predispose to addictive behavior, rather than to this behavior itself. They also pointed to the fact that ethnic differences between samples may produce different results. The DAT1 7-repeat allele has, for example, only been detected in Japanese and Chinese populations [27].

Parsian and Zhang did not find any evidence for a significant association between the DAT1 VNTR polymorphism and alcohol dependence in their Caucasian sample [45]. They compared DAT1 allele (9-, 10-, 11-repeat allele) frequencies in 162 type 1 and type 2 alcoholics with those of 89 unrelated controls. No significant differences were found between the total group of alcoholics and controls (p = 0.572), nor between type 1 and type 2 alcoholics (p = 0.347). To control for potential stratification effects caused by nonaccurate matching of cases' and control subjects' ethnicity in the case-control design, the researchers also carried out a haplotype relative-risk analysis. In this approach the frequency of transmitted haplotypes (case) is compared with the frequency of nontransmitted haplotypes (control) within a family, hereby controlling for stratification problems [56]. In 29 families, the alcoholic proband and both parents were genotyped. Haplotypes containing the 9-repeat risk allele were not transmitted more often to alcohol-dependent probands than haplotypes without this allele (p = 0.461). The authors concluded that the DAT1 VNTR polymorphism was not associated with alcoholism in their sample.

Schmidt and coworkers examined whether withdrawal symptoms in 48 chronically intoxicated, German, male alcoholics differed with the presence or absence of the DAT1 9-repeat allele [54]. The 22 patients with the 9-repeat allele reported significantly higher levels of alcohol withdrawal symptoms, such as paroxysmal sweats and tremors, than patients homozygous for the 10-repeat allele (p = 0.04). Stepwise multiple regression analysis showed that the amount of alcohol consumed in the preceding month was related to withdrawal symptoms (β = 0.42; p = 0.005), and that the DAT1 VNTR genotype showed a nearly significant trend towards association with withdrawal symptoms (β = 0.24; p = 0.10). The authors suggested that the DAT1 VNTR may play a role in the brain's capacity to adapt and compensate for long-term effects of alcohol use.
Table 2. Statistical results of clinical association studies of DAT1 polymorphisms and alcohol dependence in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases</th>
<th>Controls</th>
<th>Type of analysis</th>
<th>Polymorphism</th>
<th>( \chi^2 ) difference in VNTR allele frequency between controls and cases (total groups)</th>
<th>Extra analyses</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muramatsu &amp; Higuchi (1995)</td>
<td>0.05</td>
<td>0.92</td>
<td>( \chi^2 ), Fisher’s exact test</td>
<td>VNTR</td>
<td>n.s. ( p = n.r. )</td>
<td>7-repeat allele more frequent in cases with ALDH2*2 allele (( p &lt; 0.05 ))</td>
<td>[44]</td>
</tr>
<tr>
<td>Sander et al. (1997)</td>
<td>0.25</td>
<td>0.74</td>
<td>( \chi^2 ), Fisher’s exact test</td>
<td>VNTR</td>
<td>( p = 0.07 )</td>
<td>9-repeat allele more frequent in cases with delirium (( p = 0.01 )) and withdrawal seizures (( p = 0.01 ))</td>
<td>[46]</td>
</tr>
<tr>
<td>Dobashi et al. (1997)</td>
<td>0.03</td>
<td>0.94</td>
<td>( \chi^2 )</td>
<td>VNTR</td>
<td>.05 &lt; ( p &lt; 0.10 ) (exact ( p = n.r. ))</td>
<td>--</td>
<td>[48]</td>
</tr>
<tr>
<td>Parsian &amp; Zhang (1997)</td>
<td>0.26</td>
<td>0.72</td>
<td>( \chi^2 ), multiple regression</td>
<td>VNTR</td>
<td>( p = 0.57 )</td>
<td>haplotypes with the 9-repeat allele were not more often transmitted to AD probands (( p = 0.46 ))</td>
<td>[45]</td>
</tr>
<tr>
<td>Schmidt et al. (1998)</td>
<td>0.23</td>
<td>0.77</td>
<td>n.a.</td>
<td>VNTR</td>
<td>n.a</td>
<td>9-repeat allele carriers had more severe withdrawal symptoms than 10-allele carriers (( p = 0.04 ))</td>
<td>[54]</td>
</tr>
<tr>
<td>Franke et al. (1991)</td>
<td>0.32</td>
<td>0.68</td>
<td>n.a.</td>
<td>VNTR</td>
<td>n.a</td>
<td>the 9-repeat allele was not more frequently transmitted to AD probands, with or without delirium (( 0.35 &lt; p &lt; 0.77 ))</td>
<td>[51]</td>
</tr>
<tr>
<td>Ueno et al. (1999)</td>
<td>n.r.</td>
<td>n.r.</td>
<td>( \chi^2 ), Fisher’s exact test, Cochran Q armitage test</td>
<td>VNTR 2319G&gt;A</td>
<td>n.r</td>
<td>2319-A allele and haplotype A10( ^# ) were more frequent in cases than in controls (( p = 0.02 ) and ( p = 0.01 ), respectively). G10( ^# ) protected against AD (( p &lt; 0.01 ))</td>
<td>[22]</td>
</tr>
<tr>
<td>Vandenbergh et al. (2000)</td>
<td>n.r.</td>
<td>n.r.</td>
<td>( \chi^2 ), student’s t-test</td>
<td>VNTR ex 2 C/T, ex 9 A/G Exon 15 C/T</td>
<td>0.16 &lt; ( p &lt; 0.93 ) (exact ( p = n.r. ))</td>
<td>--</td>
<td>[23]</td>
</tr>
<tr>
<td>Heinz et al.</td>
<td>0.25</td>
<td>0.75</td>
<td>0.17</td>
<td>VNTR</td>
<td>n.s. ( p = n.r. )</td>
<td>SPECT study. Persons with 9-repeat allele showed significant reduction in DAT availability in putamen (( p &lt; 0.05 ))</td>
<td>[35]</td>
</tr>
<tr>
<td>Chen et al. (2001)</td>
<td>Atayal: 0.07</td>
<td>0.93</td>
<td>0.08</td>
<td>VNTR</td>
<td>Atayal: ( p = 1.00 )</td>
<td>No significant differences between controls and subgroups of severe cases (Atayal: ( p = 1.00 ); Ami: ( p = 0.10 ); Bunun: ( p = 0.59 ); Paiwan: ( p = 0.36 ); Han: ( p = 0.66 ))</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Ami: 0.02</td>
<td>0.98</td>
<td>0.11</td>
<td></td>
<td>Ami: ( p = 0.10 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bunun: 0.19</td>
<td>0.81</td>
<td>0.15</td>
<td></td>
<td>Bunun: ( p = 0.59 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paiwan: 0.01</td>
<td>0.99</td>
<td>0.04</td>
<td></td>
<td>Paiwan: ( p = 0.36 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Han: 0.10</td>
<td>0.90</td>
<td>0.08</td>
<td></td>
<td>Han: ( p = 0.66 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bau et al. (2001)</td>
<td>0.22</td>
<td>0.77</td>
<td>0.25</td>
<td>( \chi^2 ), regression</td>
<td>( p = 0.31 )</td>
<td>--</td>
<td>[49]</td>
</tr>
</tbody>
</table>

\( ^1 \)In some studies 9-repeat and 10-repeat allele frequencies do not add up to 1 because of rare alleles such as the 7-repeat allele.

\( ^2 \)These are approximate allele frequencies, since all alleles different from the 9-repeat allele were pooled (there were however, only five parental alleles that were neither the 9-repeat or the 10-repeat allele) [51].

\( ^{3} \)Haplotype consisting of the DAT1 VNTR 10-repeat allele and the DAT1 2319-A allele.

\( ^{4} \)Haplotype consisting of the DAT1 VNTR 10 repeat allele and the DAT1 2319-G allele.

AD: Alcohol dependence; Cases f 9: Frequency of the DAT1 VNTR 9-repeat allele in the entire group of alcoholics; Cases f 10: Frequency of the DAT1 VNTR 10-repeat allele in the entire group of alcoholics; Controls f 9: Frequency of the DAT1 VNTR 9-repeat allele in the control group; Controls f 10: Frequency of the DAT1 VNTR 10-repeat allele in the control group; DAT: Dopamine transporter; HHR: Haplotype relative risk; n.a.: Not applicable; n.r: Not reported; n.s.: Not significant; OR: Odds ratio; SPECT: Single photon emission computed tomography; TDT: Transmission disequilibrium test; VNTR: Variable number of tandem repeat.
Table 2. Statistical results of clinical association studies of DAT1 polymorphisms and alcohol dependence in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases</th>
<th>Controls</th>
<th>Type of analysis</th>
<th>Polymorphism</th>
<th>χ² difference in VNTR allele frequency between controls and cases (total groups)</th>
<th>Extra analyses</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wernicke et al. (2002)</td>
<td>see [46]</td>
<td></td>
<td>χ², OR VNTR</td>
<td>See [46]</td>
<td>2319-A allele not more frequent in cases than in controls (in total group nor in subgroups). A10* was more frequent in subgroups 2 (p = 0.02), 4 (p = 0.03) and 5 (p &lt; 0.01) (see Table 1)</td>
<td>[25]</td>
<td></td>
</tr>
<tr>
<td>Gorwood et al. (2003)</td>
<td>0.30 0.70</td>
<td>0.34 0.66</td>
<td>χ², OR, Armitage test VNTR</td>
<td>n.r.</td>
<td>9-repeat allele more frequent in cases with greater than or equal to one seizure or delirium (p = 0.03), in cases with antisocial person disorder (p = 0.03), in older cases (p &lt; 0.01), and in cases with longer history of AD (p &lt; 0.01)</td>
<td>[52]</td>
<td></td>
</tr>
<tr>
<td>Limosin et al. (2004)</td>
<td>0.34 0.65</td>
<td>n.a.</td>
<td>χ², OR, Fisher’s exact test VNTR</td>
<td>n.a</td>
<td>9-repeat allele carriers experienced more often than or equal to eight withdrawal symptoms (p = 0.04), specifically visual hallucinations (p = 0.03)</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td>Köhnke et al. (2005)</td>
<td>0.24 0.74</td>
<td>0.16 n.r.</td>
<td>Fisher’s exact test, Kruskal-Wallis test VNTR</td>
<td>p = 0.01</td>
<td>no sign. differences in allele frequencies between cases with seizures or delirium versus mild withdrawal symptoms (p = .42 and p = 0.55, respectively)</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>Samochowiec et al.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>χ², TDT VNTR</td>
<td>n.s.; p = n.r.</td>
<td>10-repeat allele was preferentially transmitted to AD offspring (p = 0.05)</td>
<td>[47]</td>
<td></td>
</tr>
<tr>
<td>Choi et al. (2006)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>χ², Fisher’s exact test 2319G&gt;A</td>
<td>n.a</td>
<td>No significant differences in allele frequencies between controls and familial alcoholics (p = 0.49) or nonfamilial alcoholics (p = 0.76)</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>Le Strat et al. (2008)</td>
<td>0.25 0.75</td>
<td>0.32 0.68</td>
<td>χ², Fisher’s exact test, logistic regression VNTR, several other SNPs</td>
<td>p = 0.166</td>
<td>9-repeat allele more frequent in cases with withdrawal seizures p = 0.02 (χ²), p = 0.03 (logistic regression)</td>
<td>[43]</td>
<td></td>
</tr>
</tbody>
</table>

*In some studies 9-repeat and 10-repeat allele frequencies do not add up to 1 because of rare alleles such as the 7-repeat allele.
*These are approximate allele frequencies, since all alleles different from the 9-repeat allele were pooled (there were however, only five parental alleles that were neither the 9-repeat or the 10-repeat allele) [51].
*Haplotype consisting of the DAT1 VNTR 10-repeat allele and the DAT1 2319-A allele.
*Haplotype consisting of the DAT1 VNTR 10-repeat allele and the DAT1 2319-G allele.
AD: Alcohol dependence; Cases f9: Frequency of the DAT1 VNTR 9-repeat allele in the entire group of alcoholics; Cases f10: Frequency of the DAT1 VNTR 10-repeat allele in the entire group of alcoholics; Controls f9: Frequency of the DAT1 VNTR 9-repeat allele in the control group; Controls f10: Frequency of the DAT1 10-repeat allele in the control group; DAT: Dopamine transporter; HHR: Haplotype relative risk; n.a.: Not applicable; n.r.: Not reported; n.s.: Not significant; OR: Odds ratio; SPECT: Single photon emission computed tomography; TDT: Transmission disequilibrium test; VNTR: Variable number of tandem repeat.
Franke et al. also used the family-based association approach of Falk and Rubinstein to avoid effects of hidden population stratification [51,56]. The authors tested whether the 9-repeat allele of the DAT1 VNTR was more frequently transmitted than other control alleles from parents to 87 Caucasian alcohol-dependent probands. The 87 Caucasian alcoholic probands consisted of 55 patients without delirium or withdrawal seizures (subgroup 1) and 32 patients with either alcohol-induced delirium or withdrawal seizures (subgroup 2). Disease probabilities for persons with the DAT1 9-repeat allele did not differ significantly from disease probabilities for persons without the DAT1 9-repeat allele (p = 0.345). No significant association was found in the subgroup between transmission of the DAT1 9-repeat allele and alcohol dependence (subgroup 1: p = 0.424; subgroup 2: p = 0.771). It was concluded that the DAT1 VNTR was not significantly associated with alcoholism as in the study of Parsian and Zhang [45], although the authors mentioned that the use of anticonvulsive medication and consequently the severity of withdrawal symptoms, might have confounded the results.

Vandenbergh et al. sequenced the entire 60,000 bp of DAT1 in 150 control subjects, 109 individuals meeting criteria for Tourette's syndrome, 15 individuals with attention deficit–hyperactivity disorder (ADHD), and in 64 individuals with alcohol dependence (all subjects were of Caucasian descent) [23]. The authors discovered a SNP in exon 15, resulting in a base substitution of C into T, which, however, did not have a functional consequence with regard to a change in amino acid. A total of 12 other, rare variants in exon 2 and exon 8 resulted in a change in the amino acid sequence, while all other SNPs in DAT1 did not. Although both the VNTR 10-repeat allele and the newly discovered exon 15 SNP were significantly more often transferred to subjects with ADHD, no difference in allele frequencies between cases and controls was found for alcoholism (0.16 < p < 0.93). Vandenbergh and colleagues stated that DAT may still be involved in alcohol dependence and other neuropsychiatric disorders, but perhaps in a more complex way, such as in genetic heterogenic processes or gene–environment interactions [23]. Since the majority of the polymorphisms found in DAT1 did not result in amino acid changes, the authors also suggested that variations in levels of expression, induced by genetic variants in promoter/enhancer regions, rather than differences in protein sequences, may explain variability in individuals’ genetic liabilities for alcoholism.

In the study by Heinz et al. single photon emission computed tomography (SPECT) was used to measure differences in DAT availability in caudate and putamen brain areas of 14 abstinent alcoholics and 11 control subjects (ethnicity was not reported) [35]. Although the 9-repeat allele was more frequent in alcohol-dependent subjects than in control subjects, the difference was not significant (p-value was not reported). DAT availability in caudate and putamen did also not significantly differ between alcoholic and control subjects (p = 0.26 and p = 0.43 respectively). Genotype did affect DAT availability in putamen in the total sample (p < 0.05), with persons with the DAT1 9-repeat allele exhibiting lower binding potential than individuals homozygous for the 10-repeat allele. No significant effect of genotype on DAT availability was found for the caudate (p = 0.11). Regardless of the small sample size, the authors also tested for several confounding variables. No significant differences were found in age, negative symptoms (anhedonia, apathy, affective flattening), occupational status, educational level, impulsivity, anxiety and depression levels between persons carrying the 9-repeat allele and those homozygous for the 10-repeat allele. It was suggested that the reduction in DAT availability in alcoholic subjects with the 9-repeat allele, resulting in decreased clearance of synaptic dopamine, may make these individuals more sensitive to drug-induced dopamine surges, especially during alcohol-withdrawal periods.

Chen and coworkers studied a group of Han Chinese and four ethnically homogeneous groups from the Taiwanese population; Atayal, Ami, Bunun, and Paiwan [42]. The 203 alcoholic subjects all reported alcohol dependence with withdrawal symptoms. A subgroup of severe alcohol-dependent subjects was created with participants who displayed impairment of liver function, peripheral neuropathy or hallucinations. The 213 control subjects were matched with the alcoholic subjects based on ethnicity and age and were excluded when they smoked at least one pack of cigarettes per day. No significant differences in DAT1 genotype distributions were found between the control and the alcoholic subjects, either for the total groups of alcoholics (Atayal: p = 1.00; Ami: p = 0.10; Bunun: p = 0.59; Paiwan: p = 0.36; Han: p = 0.66) or for the subgroups of the more severe cases (Atayal: p = 1.00; Ami: p = 0.07; Bunun: p = 0.69; Paiwan: p = 0.29;
van der Zwaluw, Engels, Buitelaar, Verkes, Franke & Scholte

Review

Dopamine transporter gene (DAT1) & alcohol dependence

Author Proof

VNTR in alcohol seeking behavior in individuals who score high on novelty seeking.

Bau and colleagues examined differences in DAT1 VNTR allele frequencies between 114 Brazilian cases and 112 Brazilian controls in a χ² test [49]. No significant differences were found (p = 0.31). However, in a regression analysis the DAT1 10/10 genotype did significantly predict levels of alcohol consumption (p = 0.04). Additionally, an interaction effect between the novelty-seeking personality trait and the DAT1 10/10 genotype was found (p = 0.04). The authors suggested that carrying the DAT1 10/10 genotype may enhance alcohol-seeking behavior in individuals who score high on novelty seeking.

Gorwood et al. studied the role of the DAT1 VNTR in alcohol-withdrawal symptoms (alcohol-withdrawal seizure and delirium tremens) by comparing 120 Caucasian male alcoholics with 65 control subjects [52]. Alcohol-dependent patients with the 9-repeat allele more frequently experienced at least one episode of alcohol-withdrawal seizure or delirium tremens, and had more often taken alcohol to reduce withdrawal symptoms than alcohol-dependent patients without the 9-repeat allele (p = 0.03 and p = 0.02, respectively). The average number of withdrawal symptoms was not significantly different between alcohol-dependent patients with and those without the 9-repeat allele (p = 0.33). The 9-repeat allele occurred more frequently in patients with antisocial personality disorder than in the rest of the alcohol-dependent sample (p = 0.03), and excluding these patients enhanced the association between the 9-repeat allele and alcoholism with alcohol withdrawal seizures and delirium tremens (p = 0.02). The 9-repeat allele was also significantly more often observed in older patients (p < 0.01) and in patients with a longer history of alcohol dependence (p < 0.01). Additionally, a principal component analysis showed that delirium tremens and alcohol-withdrawal seizures together formed one component of alcohol-withdrawal symptoms in the patient sample, suggesting that patients with seizures and delirium tremens may represent a rather homogeneous group of patients [43]. The authors further suggested that lower levels of DAT, resulting in higher levels of dopamine or hyperdopaminergic states and consequently sometimes in delusion and hallucination, may be related to delirium tremens [52] (see also [57,58]).

The above-described studies were mainly carried out in male populations. Limosin et al. examined whether the 9-repeat allele was also associated with withdrawal symptoms in 64 Caucasian, alcohol-dependent women in a case-only design [53]. The average number of withdrawal symptoms did not significantly differ between women with and without the 9-repeat allele (p = 0.22). However, women with the 9-repeat allele experienced more often at least eight (out of 11) withdrawal symptoms (p = 0.04). Further, visual hallucinations were significantly more frequent in women with the 9-repeat allele when compared with women homozygous for the 10-repeat allele (p = 0.03). Other withdrawal symptoms, including alcohol withdrawal seizures and delirium tremens were not associated with the 9-repeat allele. To explain this discordance with the results of studies on male samples [46,52,54], Limosin et al. stated that since male alcohol-dependent subjects suffer from more withdrawal symptoms than females, this may restrict the association between alcohol-withdrawal seizures and delirium tremens to male alcoholics [53]. Further, they acknowledged that the small sample size (n = 64) might have resulted in decreased power to detect differences between females with the 9-repeat allele and those without.

Köhne et al. compared Caucasian male and female alcoholic patients with severe withdrawal symptoms (alcohol-withdrawal seizure (AWS): n = 65; delirium tremens (DT): n = 83) to Caucasian alcohol-dependent patients with only mild alcohol-withdrawal symptoms (n = 97) and did not find any differences in 9-repeat allele frequencies (AWS: p = 0.42; for DT: p = 0.55) [50]. However, the 9-repeat allele was significantly more frequent in the total group of alcoholics (n = 216), when compared with healthy controls (n = 102; p = .01). This association of the 9-repeat allele with alcoholism was present for male alcoholics (p = 0.02), but not for their female counterparts (p = 0.18), although this latter lack of significance might be explained by the small sample size of female alcoholics (n = 40) and female controls (n = 36). Daily alcohol consumption was not significantly different for 9-repeat allele carriers compared with alcoholics homozygous for the 10-repeat allele.

Samochowiec and colleagues applied both a case–control and a family-based design in their study [47]. For the latter, the Transmission Disequilibrium Test was used. In this test it
is examined whether an investigated allele exceeds the 50% Mendelian chance of transmission to an alcohol-dependent proband, which would imply a role in the etiology of alcoholism. Analysis of 100 Polish families showed a significant difference in the transmission of the DAT1 VNTR 10-repeat allele, which was preferentially transmitted to affected offspring (p = 0.047). Creating homogeneous subgroups of early-onset patients and severe alcoholic patients (with seizures and/or delirium) did not result in significant differences in transmission for any of the DAT1 alleles (p = 0.34 and p = 0.66, respectively). Subsequently, 196 control subjects were included in the study. However, no significant differences in DAT1 VNTR allele frequencies were found between the alcohol-dependent participants and control subjects (p-value not reported).

Le Strat et al. genotyped the VNTR and seven SNPs in DAT1 in a sample of 250 French alcohol-dependent participants, 60 of whom had experienced withdrawal seizures [43]. When compared with 121 control subjects the 9-repeat allele was not more prevalent in the entire group of alcohol-dependent participants (p = 0.166). However, the 9-repeat allele was significantly more frequent in alcohol-dependent subjects with withdrawal seizures when compared with alcohol-dependent subjects without alcohol-withdrawal seizures, both in a χ² test (p = 0.02) and in a logistic regression analysis (p = 0.03). In the regression analysis the authors controlled for confounding variables such as age of onset of alcohol dependence and alcohol dependence severity. Two other SNPs, located close to the VNTR, were also associated with withdrawal seizures. In addition, two haplotypes showed a significant protective effect for alcohol dependence with alcohol-withdrawal seizures.

Clinical studies of the DAT1 2319G>A SNP in alcohol dependence

The three studies described below examined another polymorphism in the 3′-UTR of DAT1; 2319G>A [22]. Ueno et al. studied the prevalence of this polymorphism in a sample of Japanese subjects (n_{alcoholics} = 124; n_{controls} = 107) and found that the 2319-A allele was significantly more frequent in alcoholic subjects than in controls (OR: 1.73; p = 0.02) [22]. The risk for alcoholism increased with the number of 2319-A alleles (p = 0.046). The 2319-A allele was found only in combination with the 10-, 11-, and 14-repeat alleles of the DAT1 VNTR, and the 2319G>A polymorphism appeared to be in significant linkage disequilibrium with the VNTR polymorphism. Haplotype analyses of the 2319G>A and VNTR polymorphism were also carried out. The haplotype of the A-allele together with the 10-repeat allele of the VNTR (merged as the A10 allele) was found more often in alcohol-dependent participants than in control subjects (OR: 1.76; p = 0.01), showing a significant positive gene dose effect (p = 0.04). In contrast, the risk for alcoholism was significantly decreased for the G10 allele (OR: 0.53; p = 0.002), with a significant negative gene dose effect (p = 0.01).

Wernicke et al. examined 351 German alcoholics and 336 ethnically matched control subjects [25]. The alcohol-dependent group was divided into nine subgroups: father with a positive family history of alcohol dependence; mother with a positive history of alcohol dependence; early age at onset; delirium; withdrawal seizures; vegetative withdrawal syndrome; type 1 alcoholism; type 2 alcoholism and antisocial tendencies.

For the 2319G>A polymorphism no significant difference was detected between control subjects and alcoholic patients, neither in the total group nor in any subgroup. Two trends towards significance were found for the A/A homozygous genotype, which occurred more often in patients with withdrawal seizures (p = 0.06), and in those with an alcohol-dependent mother (p = 0.08). Regarding the VNTR, the results were already published in Sander et al. ([46] discussed before). The 2319G>A polymorphism was not in significant linkage disequilibrium with the VNTR, which was in contrast with the results of Ueno et al. [22]. When only individuals homozygous for the 10-repeat allele of the DAT1 VNTR for genotype effects of the 2319G>A polymorphism (n_{A10A10} = 183; n_{A10A10} = 186) as Ueno et al. had done and no significant differences were found in 2319G>A frequency between the total group of alcoholics and controls. However, the homozygous A10/A10 genotype was more frequently detected in patients with an alcoholic mother (OR: 5.25; p = 0.02), patients with a history of delirium (OR: 4.71; p = 0.03), and patients with a history of withdrawal seizures (OR: 7.91; p = 0.001) when compared with control subjects. The groups were also tested for differences in ethanol intake and tobacco smoking, with no significant results.

In the study by Choi et al. 111 male Korean patients with alcohol dependence were compared with 123 male Korean control subjects on several different polymorphisms, including the DAT1 2319G>A polymorphism [26].
The alcohol-dependent subjects were divided into two groups: patients with a family history of alcohol dependence (n = 43) and patients without a family history of alcohol dependence (n = 68). Genotype and allele frequencies of the 2319G>A polymorphism were not significantly different between control subjects and subjects with a family history of alcohol dependence (p = 0.49) or subjects without a family history of alcohol dependence (p = 0.76).

Discussion

One of the first things of note from the above-described studies is that a case–control design in which all alcohol-dependent individuals are treated as a single group results in a very heterogeneous phenotype with a large variety of individual symptoms and etiologies. As such, identifying subgroups in which the clinical heterogeneity of alcohol dependence is taken into account may more genuinely represent reality and increases power of finding associations, if the subgroups are large enough. Excluding moderating factors (such as withdrawal symptoms) may result in the incorrect conclusion that there is no association between genotype and phenotype, while in fact there is a relationship, but only when moderating variables are taken into account (see [59]). Munafò and Flint warn for what they call data dredging; searching for significant results in subgroups without clear a priori hypotheses [60]. Although we believe that moderating factors need to be taken into account to increase one’s ability to find genetic contributors to alcohol dependence, we would also like to stress the need for adequately powered samples and clear a priori hypotheses to prevent data dredging.

Another factor that may have caused disparity in findings consists of the differences between study samples regarding psychiatric comorbidity. Approximately one third of the reviewed studies did not, for example, give information on the exclusion or inclusion of patients with other psychiatric or substance use disorders in their study sample [23,42,44,45,47,49,53]. Others only reported having excluded patients with psychosis [22,48] or with schizophrenia or dementia [43,52]. The DAT1 VNTR has shown to be associated with ADHD (see [61] for a meta-analysis), and ADHD has in turn been related to alcohol dependence [62]. Therefore, it might be that alcoholics who also have ADHD form a subgroup that is genetically more homogeneous, resulting in stronger relations with DAT1.

Other differences in the composition of samples that might have caused a lack of univocal results include differences in ethnicity, age-range of the sample, sex differences, matching of cases to controls, recruitment of patients and the presence of withdrawal symptoms (see [63]). To complicate matters further, other genes and polymorphisms, in addition to DAT1, can also be expected to contribute to the risk of developing alcoholism, since alcoholism is a polygenic disorder. Generally, if different polymorphisms are examined in relation to alcohol dependence, their effects are analyzed separately, and not in interaction with each other (e.g., [47,64]). However, for example, Muramatsu and Higuchi revealed that the DAT1 VNTR 7-repeat allele was only significantly related to alcoholism if ALDH2 genotype was also taken into account [44]. If this interaction was not included in their analyses, it would have led to the erroneous conclusion that the DAT1 VNTR was not associated with alcohol dependence in their sample. Thus, ignoring gene–gene interactions may result in an underestimation of specific genetic associations to alcohol dependence [65]. We are not aware of other studies that have included interactions between DAT1 and other genes with respect to the link to alcohol dependence, leaving ample room for future studies.

In a similar vein, environmental factors are rarely included in gene-association studies, but have been shown to be involved in the risk for alcohol dependence. For example, social factors such as alcohol consumption of friends and family [66,67], as well as stressful life events such as bad family relationships or childhood maltreatment [64–70] are of considerable importance in later alcohol use and dependence. Genetic factors may pose a latent susceptibility for alcohol abuse, but environmental factors moderate whether this susceptibility comes to expression [71]. For instance, a gene–environment study of Madrid et al. demonstrated that dopamine D2 receptor (DRD2) genotype did not significantly differ between cases and controls [72]. However, when stress was included in the model, the DRD2A1 allele was significantly associated with alcohol dependence, but only in patients with high-stress levels (for more examples see [73–75]). As such, ignoring environmental factors may again lead to the wrong conclusion that certain genetic factors are not related to alcohol use or dependence, while in fact they are but only under specific environmental conditions [59]. The reverse is also true; environmental risk factors for alcohol use or disorders may be overlooked if they are not examined together with genetic influences.

We would like to emphasize that by highlighting gene–gene and gene–environment
interactions we do not aim at an uncontrolled, random search for all kinds of possible interactions, which would probably result in many false positives. Selecting genotypes or environmental factors based on a plausible biological mechanism or for an evidence-based reason should already decrease the chance of random gene or environment selection [76]. Genome-wide association studies should be used to provide additional information on chromosomal areas and susceptibility genes related to alcohol dependence (e.g., [77,78]). In addition, multiallelic haplotypes may aid to identify the disease locus or genetic region of interest and counter the disadvantages of multiple testing [31]. Obviously, replication is essential to decrease the possibility of reporting false positives, as are large sample sizes.

The biological mechanisms through which the DAT1 VNTR or 2139G>A polymorphisms influence alcohol-related behaviors are still largely unclear. As such, it is perhaps a step too far to already jump to associating a polymorphism with a clinical phenotype. Research that focuses on the biological or neurobiological mechanisms that underlie the supposed link between gene and phenotype are highly warranted. In addition, a way to reduce the distance between gene and phenotype is to concentrate on endophenotypes that mediate this association (e.g., [79]). Endophenotypes can consist of biological, neurological, or cognitive variables, such as craving, levels of intoxication or euphoria post-alcohol consumption, alcohol metabolism, brain waves, or expectations of alcohol consumption (for a review see [80]).

A final shortcoming of the reviewed studies is that most studies are fairly small and may lack power to accurately examine the role of DAT1 in alcoholism, hereby increasing the risk for false negatives. In addition, the DAT1 3’-UTR VNTR is a polymorphism with several different alleles, which further reduces the power of association studies. To tackle power problems and to carry out reliable replication studies very large sample sizes are needed [81]. To show that sample sizes need to be extremely large under certain circumstances, we took one reviewed study [46] as a representative for power and sample size calculations (Table 3). Systematic reviews and meta-analyses could be used to synthesize data from separate studies and counter the need for extremely large individual sample sizes [76].

The specification of subgroups of alcohol-dependent persons for which a certain polymorphism is a risk factor is only beginning to emerge, but may have great implications for the treatment of alcoholism. Further, specific subgroups may have a more homogeneous genetic cause of disease, which in turn may provide additional information on specific biological mechanisms. Although large sample sizes are needed to take so many variables into account, this would allow researchers to adopt a more individual, personalized approach, which may be very well translatable to clinical practice (see [82]).

Future perspective
Even though this description of potential difficulties in gene-association studies is not meant to be exhaustive (see also [59,63,83]), it is clear that challenges for future gene association studies are

Table 3. Example of power and sample size calculations.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Genotype relative risk’</th>
<th>Aa = 1.10</th>
<th>Aa = 1.25</th>
<th>Aa = 1.50</th>
<th>Aa = 2.00</th>
<th>Aa = 2.50</th>
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<tr>
<td>Number of cases</td>
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<td>293</td>
<td>293</td>
<td>293</td>
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<td></td>
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<td>Number of controls</td>
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<td>93</td>
<td></td>
</tr>
<tr>
<td>Risk allele (A) frequency</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
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</tr>
<tr>
<td>Prevalence of alcohol dependence*</td>
<td>0.037</td>
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<td>0.037</td>
<td>0.037</td>
<td>0.037</td>
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</tr>
<tr>
<td>Genotype relative risk Aa</td>
<td>1.10</td>
<td>1.25</td>
<td>1.50</td>
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<td>Genotype relative risk AA</td>
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<td>1.50</td>
<td>2.00</td>
<td>3.00</td>
<td>4.00</td>
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</tr>
<tr>
<td>Power* (dominant model)</td>
<td>7.2%</td>
<td>17.6%</td>
<td>45.9%</td>
<td>88.6%</td>
<td>98.3%</td>
<td></td>
</tr>
<tr>
<td>Power* (allelic test)</td>
<td>7.4%</td>
<td>18.4%</td>
<td>46.8%</td>
<td>87.2%</td>
<td>96.6%</td>
<td></td>
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<tr>
<td>Number of cases needed for power of 80%</td>
<td>12018</td>
<td>2197</td>
<td>667</td>
<td>229</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>Number of cases needed for power of 80%</td>
<td>10971</td>
<td>2061</td>
<td>651</td>
<td>239</td>
<td>160</td>
<td></td>
</tr>
</tbody>
</table>

The study of Sander and colleagues [46] was used as an example of power and sample size calculations in this table. Calculations were carried out with the case-control for discrete trait test of the Genetic Power Calculator [84].

*Prevalence of alcohol dependence was derived from the study by Grant et al. [1].

The specification of subgroups of alcohol-dependent persons for which a certain polymorphism is a risk factor is only beginning to emerge, but may have great implications for the treatment of alcoholism. Further, specific subgroups may have a more homogeneous genetic cause of disease, which in turn may provide additional information on specific biological mechanisms. Although large sample sizes are needed to take so many variables into account, this would allow researchers to adopt a more individual, personalized approach, which may be very well translatable to clinical practice (see [82]).

Future perspective
Even though this description of potential difficulties in gene-association studies is not meant to be exhaustive (see also [59,63,83]), it is clear that challenges for future gene association studies are
abundant. To tackle the lack of statistical power, larger samples are needed. In addition, creating subgroups or endophenotypes that are more closely associated with a specific genetic factor than a general alcohol-dependent phenotype, may result in more solid and more replicable results if the subgroups are of considerable size. The inclusion of specific moderating variables, such as other genetic polymorphisms or environmental risk factors may improve the specification of the phenotype with which the DAT1 gene is related. Including more individual-based variables in our gene-association studies will hopefully lead to more individual-based treatments in clinical practice [82].

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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**Executive summary**

- Generally, clinical studies showed inconsistent support for a relationship between polymorphisms in the DAT1 gene and alcohol dependence.
- Several, but not all, studies found that the DAT1 VNTR (9-repeat allele) was associated with alcohol-withdrawal symptoms, such as seizures and delirium tremens.

**Shortcomings of current studies on DAT1 and alcohol dependence include:**

- Small sample sizes, lack of power.
- Disregarding the polygenic nature of the disorder and gene–gene interactions.
- Ignoring (psychiatric) comorbidity in case and control samples.
- Overlooking clinical heterogeneity.
- Indistinct biological mechanisms.

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Papers of special note have been highlighted as:

- of interest
- of considerable interest


Dopamine transporter gene (SLC6A3/DAT1) & alcohol dependence

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