Polymorphisms in the µ-opioid receptor gene (OPRM1) and the implications for alcohol dependence in humans

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Twin and adoption studies have shown that alcohol dependence contains a substantial genetic component. In attempts to identify the genetic factors involved, association studies have linked the opioid system to alcohol dependence, with a main focus on the OPRM1 gene encoding the µ-opioid receptor. Our aim was to conduct a systematic review of the literature on the associations between polymorphisms in OPRM1 and alcohol dependence. We addressed findings of 12 studies that met our inclusion criteria. All studies employed a case-control design and included alcohol dependence as a dependent outcome measure. Our review showed that clinical studies do not unequivocally support an association between polymorphisms in OPRM1 and alcohol dependence. Factors that complicate genetic research on alcohol dependence, such as gene-environment interaction, and genetic and clinical heterogeneity, are discussed.

Approximately 76.3 million people worldwide can be diagnosed with alcohol disorders [1], making alcohol dependence one of the most prevalent adult psychiatric disorders [2]. Alcohol dependence is a heterogeneous and complex disorder in which multiple genetic and environmental risk factors play a role. Twin and adoption studies indicate a substantial genetic component in the development of alcohol dependence [3–5], with heritability estimates ranging from 40 to 60% [6]. In attempts to identify genetic markers that might be involved in alcoholism association studies have examined candidate genes and their relation to alcohol dependence (substance dependence and addiction will be used interchangeably throughout this article). A pathway that has received a lot of attention in addiction studies is the endogenous opioid system (e.g., [7,8]), especially the OPRM1 gene encoding the µ-opioid receptor. A SNP in exon 1 of this gene, 118A>G (often referred to as A118G) resulting in a change in amino acid from asparagine (Asn) to aspartic acid (Asp) at position 40 of the receptor protein, is the most studied variant in this gene. It was also the polymorphism of interest that Arias and coworkers examined in their recent meta-analysis on substance dependence [9]. The authors concluded that this particular polymorphism did not appear to affect the risk for substance dependence. A possible explanation for the lack of effects could be that subjects with any type of substance dependence (e.g., alcohol, heroin, cocaine, methadone) were included in the meta-analysis, while substance use disorder is a broad, heterogeneous concept, with many different features (e.g., [10]). In addition, even within one substance use disorder different subtypes may exist. Cloninger differentiated between two types of alcoholism; Type I, characterized by later onset and little or no antisocial behavior, and Type II, illustrated by an early onset, and a more severe course with higher levels of alcohol-related problems [11]. Babor and colleagues also found two types of alcoholics in their study; Type A and B, that corresponded to Cloninger's Type I and II, respectively [12]. Early- and late-onset alcoholism was found to differ substantially in estimated heritability (70 and 30%, respectively, [13]). Hence, the aggregation of different substance disorders or even different subtypes of substance use disorders into one general concept may level off the association between one (sub)type of substance use disorder (e.g., alcoholism) or specific aspects of addiction and polymorphisms in OPRM1. Several other methodological problems may have confounded earlier studies, and will be discussed later on.

In the present review we will focus on the dependence of one specific substance: alcohol. The main aim is to provide a descriptive overview of the present literature on polymorphisms in OPRM1 and alcohol dependence in humans. Animal studies that contain relevant information will be discussed as well.

Proposed mechanism of involvement of the opioid system in alcohol dependence

One hypothetical neurobiological mechanism of addiction that provides a link between distal genes in the opioid system and a dependence
phenotype (alcoholism) refers to the mesolimbic reward system, in which the opioid system is involved. In the reward model proposed by Herz, dopamine and opioid receptors interact [14]. Alcohol consumption leads to an increase of endogenous opiates (in particular β-endorphin) that bind to the µ-opioid receptors [15], which results in heightened dopamine levels in reward areas of the brain [14,16,17]. This, in turn, may lead to reinforcing effects of alcohol, such as craving, loss of control, and feelings of intoxication and euphoria. Hence, genetic polymorphisms changing the function or regulation of the µ-opioid receptor might account for differences in response to the effects of alcohol.

Since β-endorphin is part of the processes of reward and reinforcement and often used as a biomarker of genetic risk for alcohol disorders, Froelich and coworkers examined whether hormonal responses to alcohol can be inherited [18]. Compared with other hormonal responses to alcohol, the β-endorphin response was the only highly heritable response according to findings in their twin study. In addition, Gianoulakis et al. found that ethanol increased the level of β-endorphin-related peptides, but only in high-risk individuals (with a positive family history of alcoholism) [19]. High-risk individuals also had lower basal β-endorphin levels, indicating an opioid deficiency. According to the ‘opioid-deficiency theory’, this state is related to an elevated risk for alcoholism since high-risk subjects may drink large amounts of alcohol to compensate for this deficiency [19,20]. In addition, it was found that β-endorphin gene expression was higher in rats selectively bred for alcohol preference compared with those bred for low alcohol intake, indicating that a genetic predisposition towards alcoholism may also be related to an enhanced responsiveness to alcohol of the opioid system [21,22].

Pharmacological studies provide additional evidence for the proposed link between OPRM1 and alcohol dependence. Nonselective opioid receptor antagonists (naltrexone, naloxone) decreased alcohol self-administration in rodents and monkeys [23–26], and showed beneficial effects in the treatment of alcoholism in humans [27–29], although results have not been unequivocal [30]. Opioid antagonists block the opioid receptors and, subsequently, the release of dopamine in the ventral tegmentum area, hereby influencing several aspects of alcohol dependence by reducing alcohol consumption, craving, relapse rates, and the rewarding (euphoric) effects of alcohol consumption in humans [31,32] (see for a review [33]). Interestingly, Oslin and coworkers found in their study that alcohol-dependent subjects with the Asp40 allele (118G) responded more favorably to treatment with naltrexone than subjects homozygous for the common Asn40 (118A) allele, implying that the 118A>G polymorphism moderates pharmacotherapeutic treatment [34]. However, Gelernter and coworkers could not replicate these findings [35].

The mechanism by which the specific 118A>G variant in OP RM1 influences alcohol-related phenotypes has also been the focus of extensive study. Initially it was suggested that Asn40Asp represented a gain of function variant, with the Asp-containing receptor binding β-endorphin three-times more tightly than the Asn form [7]. However, other studies failed to replicate these findings [36,37], and Zhang et al. even reported that the Asp40 SNP resulted in loss of function by reducing both mRNA and protein levels [38]. This was corroborated by in vivo studies by Heinz and colleagues, who found that alcohol-dependent patients with the G-allele (Asp40) displayed lower availability of µ-opioid receptors in the ventral striatum compared with alcohol-dependent patients without the G-allele [39].

Taken together, findings from both human and animal studies suggest a role for the opioid system in alcohol dependence. On the other hand, studies have produced contradicting results and much remains unclear about the exact biological mechanism through which the opioid system and alcohol interact [17].

Review of clinical studies in humans

A systematic literature search was carried out using Ovid Medline and Pubmed to retrieve studies reporting on the association between alcohol dependence and the µ-opioid system. Keywords used were ‘µ-opioid’, ‘OPRM1’ and ‘opiate receptor’, crossed with ‘alcohol’, ‘alcoholism’ and ‘substance’. Reference sections of the identified articles were used to find additional studies. Studies in which subjects were classified by their primary diagnosis (alcohol dependence) but had a potential prevalence of comorbid substance dependence [40,41] were included in our review, next to studies in which no information about potential comorbid substance use was provided [42–44]. As several studies used overlapping samples, we excluded the studies with the smallest samples [45–48]. In total, 12 clinical studies were selected that...
examined the association between OPRM1 polymorphisms and alcohol dependence in humans (for details of the studies, see Table 1).

Most studies focused on the 118A>G polymorphism. Outcome variable was alcohol dependence following Diagnostic and Statistical Manual of Mental Disorders (DSM) or WHO guidelines [49] in all selected studies. The vast majority of the studies were carried out in ethnically homogenous populations. All studies had a case-control design, and one study also included parents of part of the sample for a transmission disequilibrium test design [50]. Approximately a quarter of the studies excluded alcohol-dependent individuals with comorbid psychiatric disorders [51-53]. Most studies, however, did not provide information on comorbidity of the alcohol-dependent subjects. Five of the 12 studies conducted a check for psychiatric disorders in control participants [40,42,50,51,53].

A total of nine out of ten studies did not find significant differences in the frequency of the 118G allele (Asp40) between alcohol-dependent subjects and control subjects [41-44,50,52-55], although Rommelspacher et al. reported a trend towards significance (p = 0.07) for an increased frequency of the Asp40 allele in alcohol-dependent subjects [53]. In ten studies associations of genotypes containing the G-allele and alcohol dependence were also examined. Trends towards significance for an increased genotype frequency of the 118A>G polymorphism in alcohol-dependent subjects were reported (0.05 < p < 0.10) [44,52] although most researchers again failed to find significant differences in genotype frequencies containing the 118G allele between alcohol-dependent participants and controls [41-43,50,54,55]. In the studies that did find significant associations with the 118A>G variant [51,56], these associations were with the 118G genotype in the study by Bart and coworkers [51], whereas Schinka and colleagues found the 118A allele and the AA genotype significantly associated with an increased risk for alcohol dependence [56]. Bart et al. also compared individuals with early- and late-onset alcohol dependence, but did not find a significant difference in genotype frequencies between these two groups [51].

Haplotype analyses were carried out in four studies, including additional SNPs besides 118A>G, with a significant difference in haplotype frequency distributions between alcohol-dependent individuals and controls observed in the study by Zhang et al. [41], but not in studies by Bergen et al. [54], Loh et al. [43] or Luo et al. [55]. However, Luo and colleagues did find a difference in haplotype frequency between controls and ‘alcohol + opioid’-dependent patients (p = 0.004) [55].

Examination of polymorphisms in OPRM1 other than 118A>G also showed mixed results. Bergen et al. [54], Loh et al. [43] and Luo et al. [55] did not identify any associations with alcohol dependence for both exonic and intronic SNPs. Kranzler et al. [40] and Rommelspacher et al. [53] found a trend towards significance for the association of alcoholism with an intronic CA repeat polymorphism and the 17C>T SNP, respectively (p = 0.07 in both studies). Zhang et al. showed significant associations of an allele of one intronic SNP and genotypes of two intronic SNPs with alcohol dependence (p < 0.01) [41]. The authors suggested that the intronic SNPs may be involved in alternative gene splicing or transcription regulation, and thus might play a role in susceptibility to alcohol dependence.

Explanations & complicating factors
Our descriptive review on clinical studies shows inconsistent evidence for an association of the Asn40Asp polymorphism or any other polymorphisms in the µ-opioid receptor gene and alcohol dependence. Although it is possible that allelic variation in OPRM1 simply does not present a risk factor for alcohol dependence, various alternative explanations can be provided for the overall lack of positive findings.

As OPRM1 allele and genotype frequencies strongly differ between populations (e.g., [43,45]) genetic heterogeneity of the study samples can be a confounder in the association between the Asp40Asn polymorphism and alcohol dependence. However, since most of the reviewed studies controlled for population mixture by selecting ancestral homogenous samples, it is unlikely that associations have been obscured by stratification bias [9]. The most adequate way to handle stratification bias is to study trios of two parents with one affected child [57]. The family-controlled study by Franke et al., however, showed no support for preferential transmission of either allele of the 118A>G polymorphism from parents to alcohol-dependent offspring [50]. In addition, a recent study by Xuei et al. employing a sample of 219 multiplex alcohol-dependent families and a family-based test of associations also did not find associations between one or more of 18 OPRM1 SNPs and alcohol dependence [76]. One complementary explanation for the lack of significant association findings refers to the fact that the search for alcoholism-associated polymorphisms...
### Table 1. Clinical association studies of μ-opioid receptor gene (OPRM1) polymorphisms and alcohol dependence in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>Male cases (%)</th>
<th>Male control (%)</th>
<th>Polymorphism(s) in OPRM1</th>
<th>Dependent variable</th>
<th>Allele frequency (Asp40) cases*</th>
<th>Allele frequency (Asp40) controls*</th>
<th>Significant difference in allele frequencies between controls and cases (p-value)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergen et al. (1997)‡</td>
<td>Caucasian (Finnish)</td>
<td>88</td>
<td>182</td>
<td>100</td>
<td>100</td>
<td>Two SNPs§</td>
<td>Alcohol dependence</td>
<td>0.165</td>
<td>0.113</td>
<td>No (p = NR)</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>American-Indian</td>
<td>116</td>
<td>108</td>
<td>54§</td>
<td>19§</td>
<td></td>
<td></td>
<td>0.164</td>
<td>0.153</td>
<td>No (p = NR)</td>
<td></td>
</tr>
<tr>
<td>Kranzler et al. (1998)§</td>
<td>Caucasian</td>
<td>201</td>
<td>84</td>
<td>NR</td>
<td>NR</td>
<td>CA repeat</td>
<td>Alcohol dependence</td>
<td>NA</td>
<td>NA</td>
<td>p = 0.070</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>African-American</td>
<td>39</td>
<td>34</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
<td>0.118</td>
<td>0.121</td>
<td>p &gt; 0.100</td>
<td></td>
</tr>
<tr>
<td>Kranzler et al. (1998)#</td>
<td>Caucasian</td>
<td>201</td>
<td>84</td>
<td>NR</td>
<td>NR</td>
<td>CA repeat</td>
<td>Alcohol dependence</td>
<td>NA</td>
<td>NA</td>
<td>p = 0.070</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>American-Indian</td>
<td>116</td>
<td>108</td>
<td>54§</td>
<td>19§</td>
<td></td>
<td></td>
<td>0.164</td>
<td>0.153</td>
<td>No (p = NR)</td>
<td></td>
</tr>
<tr>
<td>Kranzler et al. (1998)#</td>
<td>Caucasian</td>
<td>201</td>
<td>84</td>
<td>NR</td>
<td>NR</td>
<td>CA repeat</td>
<td>Alcohol dependence</td>
<td>NA</td>
<td>NA</td>
<td>p = 0.070</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>African-American</td>
<td>39</td>
<td>34</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
<td>0.118</td>
<td>0.121</td>
<td>p &gt; 0.100</td>
<td></td>
</tr>
<tr>
<td>Rommelspacher et al. (2001)*</td>
<td>Caucasian (German)</td>
<td>327</td>
<td>340</td>
<td>86</td>
<td>NR</td>
<td>Four SNPs§</td>
<td>Alcohol dependence</td>
<td>0.107</td>
<td>0.078</td>
<td>p = 0.070</td>
<td>[53]</td>
</tr>
<tr>
<td>Schinka et al. (2002)**</td>
<td>Caucasian</td>
<td>179</td>
<td>297</td>
<td>98</td>
<td>48</td>
<td>118A&gt;G</td>
<td>Alcohol dependence</td>
<td>0.093</td>
<td>0.136</td>
<td>p = 0.035§</td>
<td>[56]</td>
</tr>
<tr>
<td>Luo et al. (2003)‡</td>
<td>European-American</td>
<td>282</td>
<td>179</td>
<td>76§§</td>
<td>56§§</td>
<td>Eight SNPs§</td>
<td>Alcohol dependence</td>
<td>0.106</td>
<td>0.137</td>
<td>No (p = NR)</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>African-American</td>
<td>16</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
<td>0.027</td>
<td>No (p = NR)</td>
<td></td>
</tr>
<tr>
<td>Loh et al. (2004)‡</td>
<td>Asian (Taiwanese)</td>
<td>158</td>
<td>149</td>
<td>94</td>
<td>99</td>
<td>Four SNPs§</td>
<td>Alcohol dependence</td>
<td>0.370</td>
<td>0.330</td>
<td>0.097 &lt; p &lt; 0.443</td>
<td>[43]</td>
</tr>
</tbody>
</table>

*From the differences in 118A>G allele frequencies one can derive genetic heterogeneity in different populations.

1 Allele, genotype and haplotype frequencies were examined in this study.

2 Including 118A>G.

§Percentages based on total sample of Southwestern American-Indians.

8Allele frequencies were examined in this study.

**Allele and genotype frequencies were examined in this study.

††Regarding differences in allele frequencies of alcohol-dependent participants and the super-restricted controls p = 0.035. Regarding differences in allele frequencies between alcohol-dependent participants and unrestricted controls p = 0.081.

§§Percentages based on total group of European-Americans and African-Americans.

##Genotype frequencies were examined in this study.

¶¶Percentages based on total group, including drug-dependent subjects.

NA: Not applicable; NR: Not reported.
Table 1. Clinical association studies of μ-opioid receptor gene (OPRM1) polymorphisms and alcohol dependence in humans (cont.).

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>N</th>
<th>M</th>
<th>F</th>
<th>118A&gt;G</th>
<th>Alcohol dependence</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al. (2004)**</td>
<td>Asian (Korean)</td>
<td>112</td>
<td>140</td>
<td>95</td>
<td>57</td>
<td>0.397</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Alcoholism</td>
<td>p = 0.105</td>
</tr>
<tr>
<td>Bart et al. (2005)**</td>
<td>Caucasian (Swedish)</td>
<td>389</td>
<td>170</td>
<td>72</td>
<td>48</td>
<td>0.125</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>Alcoholism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p = 0.007</td>
<td>(for genotype with G-allele)</td>
</tr>
<tr>
<td>Nishizawa (2006)**</td>
<td>Asian (Japanese)</td>
<td>64</td>
<td>74</td>
<td>78</td>
<td>31</td>
<td>0.523</td>
<td>0.425</td>
</tr>
<tr>
<td>Zhang et al. (2006)‡</td>
<td>European-American</td>
<td>318</td>
<td>338</td>
<td>75</td>
<td>42</td>
<td>0.120</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Alcoholism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.010</td>
<td>(for three intronic SNPs)</td>
</tr>
<tr>
<td></td>
<td>Four SNPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p = 0.023</td>
<td>(for one intronic SNP)</td>
</tr>
</tbody>
</table>

*From the differences in 118A>G allele frequencies one can derive genetic heterogeneity in different populations.
†Allele, genotype and haplotype frequencies were examined in this study.
‡Including 118A>G.
§Percentages based on total sample of Southwestern American-Indians.
#Allele frequencies were examined in this study.
**Allele and genotype frequencies were examined in this study.
‡‡Regarding differences in allele frequencies of alcohol-dependent participants and the super-restricted controls p = 0.035. Regarding differences in allele frequencies between alcohol-dependent participants and unrestricted controls p = 0.081.
¶¶Percentages based on total group of European-Americans and African-Americans.
§§Genotype frequencies were examined in this study.
*Percentages based on total group, including drug-dependent subjects.
NA: Not applicable; NR: Not reported.
in OPRM1 has been highly restricted to the 118A>G variant. It might be that other polymorphisms in the gene are related to vulnerability for alcohol dependence. However, the few haplotype analyses on alcohol dependence that were carried out also reported ambiguous results [41,43,54,55].

Prevalence estimates of alcohol-dependent individuals who seek help and subsequently receive treatment for their alcohol addiction range from approximately 8–34% at the population level [58,59]. This implies that the majority of alcoholics will not be noticed by treatment centers or hospitals. The patients in the reviewed studies were mainly recruited from addiction medical clinics, hospitals, or psychiatry wards and were often actively seeking treatment. Therefore, it is likely that a sample selection bias has occurred in many of the reviewed studies, which makes it difficult to generalize the findings to the population level. In addition, True and coworkers found that treatment seeking contains a large genetic determinant (41% [60]), which might account for a further bias of the sample. Other possible problems with the samples that might reduce generalizability and validity of the present studies include differences in male:female ratios in both case and control groups, the variable inclusion of individuals with comorbid psychiatric disorders, and the lack of controlling for nicotine dependence. The one study that used strict inclusion criteria in this regard and employed a strictly screened control group found a risk effect of the Asn40 allele on alcohol dependence [56]. To rule out all types of confounding effects, future studies should preferably be population-based, or use rigorously screened control and case groups.

One of the most likely explanations for the differences in findings could be that complex traits such as alcoholism are polygenic diseases with several genetic loci involved in the development of the disorder in a single individual [61]. This implies that effects of single genes and polymorphisms will generally be small and large study samples will be necessary to provide sufficient power to identify associations and interactions [62]. The studies reviewed here are generally small and lack sufficient statistical power to detect significant associations, at least for the 118A>G variant with its relatively low allele frequency in Caucasian populations. In addition, epistasis, the interaction between genes, is likely to affect the expression of the alcoholism phenotype. When epistasis occurs, the effect of one locus or gene is changed or concealed by effects of another locus, hereby reducing the power to find effects of the first locus [63]. To our knowledge, no studies examining the biological interactions between genes in relation to alcohol dependence have been published to date, leaving ample room for future studies.

Another important set of possible explanations is based on the phenotype definition used in the studies. Classification of psychiatric disorders is often based on DSM-IV categorization, which has been primarily developed for clinical purposes. However, alcohol dependence may be very variable in its clinical presentation (clinical heterogeneity). For example, many individuals experience comorbid disorders, besides their alcohol dependence. Behavioral phenotypes that often co-occur, such as alcoholism and depression, or alcoholism and ADHD, may share parts of the genome that are implicated in both disorders [64,65]. As such, dividing alcohol dependence into new categories, such as ‘alcoholism plus depression’ or ‘alcoholism plus ADHD’ may lead to genetically more homogeneous subtypes that could obtain stronger links in molecular-genetic studies.

Another way to deal with clinical heterogeneity issues is to focus on the mediating layer between distal gene and proximate phenotype, a layer that consists of basic biological or psychological processes that contribute to alcohol dependence [66]. An example comes from Gottesman and Gould, whose endophenotype concept is represented by latent traits that are more closely linked to the underlying genetic factors than the complex behavioral phenotype, and that can be measured at a biological, neurological or cognitive level [67]. Indeed, preclinical studies reported associations between variants in OPRM1 and psychological processes such as craving for alcohol [68–70]. It was found that G-allele carriers reported significantly higher feelings of post-alcohol intoxication, euphoria, and stimulation [69], and more craving after being exposed to alcohol [70], than individuals with only A-alleles. In a study on rhesus macaques, it was found that male macaques with the 77C>G allele (a variant that is comparable with 118A>G in humans) exhibited increased alcohol preference [68].

Neurophysiological markers such as brain oscillations have also been used as endophenotypes. For example, variations in the GABRA2 gene, coding for a subunit of the GABA A receptor, have been found to affect the β frequency
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band of the EEG [71], which in turn would predispose to alcohol dependence [72]. Endo-
phenotypes in the form of brain oscillations may thus indeed improve the power of mole-
cular genetic studies of alcohol dependence (see Porjesz et al., [73] for an overview).
Animal studies have frequently found assosi-
cations between polymorphisms in OPRM1 and alcohol self-administration [20]. Conse-
quently, it is possible that although humans may experience high levels of craving or want-
ing alcohol, they might be able to inhibit these impulses. This brings us to our last, perhaps most important, limitation of the studies under review: the lack of consideration of environmental factors. Besides the effects that a plethora of environmental factors such as peer drinking, disadvantaged family environment, and stressful life events, may have on alcohol use [74], gene and environment may also interact in the causality of alcoholism (gene-environment interaction). Accordingly, a genetic liability may express itself only in the presence of certain environmental factors. However, studies that incorporate gene-environ-
ment interactions in predicting alcohol dependence are scarce. One exception is the study by Madrid and colleagues showing that exposure to stress mediated the relationship between a polymorphism in the gene encoding the dopamine D2 receptor (DRD2) and alco-
holism [75]. To our knowledge, no studies exist that have included environmental factors in their analyses on polymorphisms in OPRM1 and alcohol dependence.

Conclusion
In conclusion, clinical studies do not unequivocally support the assumed associations between polymorphisms in the μ-opioid receptor gene and alcohol dependence. However, preclinical and animal studies reported more positive findings, and opioid-antagonists remain promising in treat-
ment for alcoholism. To enhance clarity of the findings, future studies may need to take comorbidity of patients into account, employ community-based case groups or thoroughly screened, matched case-control groups, include environmental factors, and focus on specific aspects of alcoholism or endophenotypes.

Future perspective
It is evident that research into associations between genes and phenotypes is subject to various difficulties (see also Executive summary). Clearly much work needs to be done to find out whether the opioid system harbors risk factors of alcohol dependence, and if so, through which underlying mechanisms. In tackling the problem of clinical heterogeneity, new categories of phenotypes may be constructed that are genetically more homo-
geneous and thus more relevant to molecular genetic studies than the presently used DSM categorizations. For example, one may think of comorbid alcohol dependence and depression to make up one (new) phenotype, which may show a stronger association with certain genetic loci. In addition, the endophenotypic approach is promising in providing a more proximate, intermediate layer between gene and distal phenotype, that may be able to iden-
tify new candidate genes for alcoholism, and show stronger associations with specific SNPs than the complex trait. As nature and nurture usually do not operate independently, we expect that future association studies will shift their focus to gene-environment interactions [57]. The inclusion of the environment will give signifi-
cantly more insight into the diverse factors and

Executive summary

- Our descriptive review of clinical studies showed that the evidence for associations of polymorphisms in OPRM1 and alcohol dependence in humans is inconsistent.

- Complicating factors in studies on OPRM1 polymorphisms and alcohol dependence that should be addressed in future studies are:
  - Genetic heterogeneity and population stratification;
  - Use of matched case-control groups or community-based samples;
  - Clinical heterogeneity;
  - Endophenotypes;
  - Polygenic character of the disease, with small effects of single genes;
  - Gene-environment interactions and correlations;
  - The exact biological mechanism through which the opioid system and alcohol interact.
REVIEW - van der Zwaluw, van den Wildenberg, Wiers et al.

their interactions that determine alcohol dependence. Besides the notion that pharmacotherapy with opioid antagonists continues to remain promising in the treatment of alcohol dependence, more individual-based treatment approaches that comprehend environmental factors such as stress or lifestyle of the individual in addition to genetic susceptibility will be the focus of future studies [61].

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Bibliography
Papers of special note have been highlighted as either of interest (☆) or of considerable interest (☆☆) to readers.


• Found that the 118A-G polymorphism resulted in a stronger binding of β-endorphin to the μ-opioid receptor.


• First thorough meta-analysis on the 118A-G SNP and substance dependence.


Polymorphisms in the μ-opioid receptor gene (OPRM1) and alcohol dependence - REVIEW


53. Book on gene-environment interactions that has much to offer to both laymen and specialists.
