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Reduced Dopamine Receptor Sensitivity as an Intermediate Phenotype in Alcohol Dependence and the Role of the COMT Val158Met and DRD2 Taq1A Genotypes

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Context: Alcohol dependence is a common neuropsychiatric disorder with high heritability. However, genetic association studies on alcohol dependence are often troubled by nonreplication. The use of intermediate phenotypes may help make clear the mode of action of various candidate genes and improve the reproducibility of genetic association studies.

Objective: To test central dopamine receptor sensitivity as an intermediate phenotype for alcohol dependence, specifically evaluating the hypothesis that the dopaminergic genes COMT Val158Met and DRD2 Taq1A affect dopamine receptor sensitivity.

Design: Case-control pharmacogenetic challenge study.

Setting: Patients with alcohol dependence admitted for detoxification were compared with healthy control subjects matched for age and level of education.

Participants: Patients (n=110) were a consecutive sample, whereas controls (n=99) were recruited through advertisements in regional newspapers.

Intervention: A dopamine challenge test was subcutaneously administered using the dopamine agonist apomorphine hydrochloride (0.005 mg/kg).

Main Outcome Measures: Outcome measures were plasma growth hormone levels and results of a continuous performance task.

Results: Central dopamine receptor sensitivity is reduced in alcohol dependence, and this is modulated by dopaminergic genes. Specifically, DRD2 Taq1A genotype affected dopamine receptor sensitivity as measured by plasma growth hormone levels, and COMT Val158Met genotype affected dopamine receptor sensitivity as measured by performance on a continuous performance task. In a logistic regression analysis, reduced dopamine receptor sensitivity on both measures predicted alcohol dependence, without an additive effect of the COMT Val158Met and DRD2 Taq1A genotypes.

Conclusions: COMT Val158Met and DRD2 Taq1A may affect the intermediate phenotype of central dopamine receptor sensitivity. COMT Val158Met and DRD2 Taq1A may confer their risk of alcohol dependence through reduced dopamine receptor sensitivity in the prefrontal cortex and hindbrain, respectively.

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Alcohol dependence is considered a chronic relapsing neuropsychiatric disorder with a multifactorial origin.1,2 Heritability estimates are as high as 60%, with many genes explaining only a fraction of the heritability.3-4 Attempts to delineate genetic contributions have met with limited success. There is an ongoing search for intermediate phenotypes in alcohol dependence to aid in genetic research.5-11

Fundamental to the concept is the assumption that variation in an intermediate phenotype depends on variation of fewer genes than in the more complex disease phenotype and is therefore more tractable in genetic analysis.12 In a recent article, a plea was made to adhere to intermediate phenotypes known to be involved in the pathogenesis of a disorder to minimize the risk of pleiotropic findings.9 The selection of an intermediate phenotype should then be based on convergent evidence among various studies from human epidemiologic investigations, experimental neuroscience, and animal research.12

In alcohol dependence, evidence from various lines of research indicates that alterations in at least 2 dopamine pathways are involved in the pathogenesis.1,13-15 The first is the mesolimbic dopamine pathway, often referred to as the reward path-
way. Alcohol generates its reinforcing effect by increasing
the firing rate of dopamine neurons in the ventral tegmen-
tal area, leading to enhanced dopamine activity in the ven-
tral striatum. Reduced densities of striatal dopamine rece-
ptors have been observed in patients with alcohol depen-
dence. Reduced sensitivity to the dopamine agonist apo-
morphine hydrochloride, indexed by the growth hormone (GH) response, has repeatedly been observed in
patients with alcohol dependence. Among individuals
with addiction, reduced dopamine receptor sensitivity is
thought to decrease the sensitivity to naturally occurring
reinforcers. This may increase vulnerability to the devel-
opment of alcohol abuse because alcohol consumption
would compensate for this reward deficiency.

The second dopamine pathway involved in alcohol depen-
dence runs from the ventral tegmental area to the pre-
frontal cortex (PFC), often referred to as the inhibitory con-
trol pathway. Individuals who abuse drugs show lower
glucose metabolism in the PFC. Moreover, patients with
alcohol dependence seem to be less sensitive to the effects
of the dopamine agonist apomorphine as measured by
the performance of tasks requiring PFC functioning. It
has been suggested that PFC and mesolimbic dopamine recep-
tor dysfunction is interrelated, resulting in a double handi-
cap in alcohol dependence, including deficient reward pro-
cessing and behavioral control.

Based on this evidence, the measurement of dopa-
mine receptor sensitivity using a challenge with a dopa-
mine agonist may disclose a useful phenotype for ge-
netic studies of alcohol dependence. Indeed, studies in
animals and humans have shown that variation in dopa-
mine receptor sensitivity is highly heritable and predicts
self-administration of substances such as cocaine and alcohol. Yet, genetic studies using this dynamic
intermediate phenotype are scarce.

The dopamine receptor D2 gene (DRD2; OMIM 126450) and its genetic polymorphism Taq1A (rs1800497) are among the most extensively studied ge-
etic variants in alcohol dependence. Some studies have shown that the DRD2 Taq1A genotype is associated with a 30% reduction in dopamine receptor density in the striatum of healthy volunteers. Meta-
analyses have shown an increased risk of alcohol dependence for carriers of the A1 allele, although the odds ratios are small (range, 1.22-1.90). Some studies have tested the role of the DRD2 genotype in vivo dopamine receptor sensitivity measured by the GH response to apomorphine in patients with alcohol dependence, a genotype in linkage disequilibrium with the Taq1A genotype (AA genotype) has been found to be associated with reduced GH response to apo-
morphine. However, most studies failed to show such an association for the DRD2 Taq1A genotype itself.

Several studies have been conducted in patients with alcohol dependence, a genotype in linkage disequilibrium with the Taq1A genotype (AA genotype) has been found to be associated with reduced GH response to apomorphine. However, most studies failed to show such an association for the DRD2 Taq1A genotype itself. Second is the absence of a group of healthy control subjects in most dopamine challenge investigations. It has been suggested that dopamine receptor sensitivity is a better predictor of alcohol dependence than the presence of dopaminergic addiction-related genes.

**METHODS**

**PARTICIPANTS**

In a case-control study design, a consecutive series of male pa-
tients with alcohol dependence (diagnosed according to DSM-IV
criteria) who were admitted to our detoxification clinic were
Table 1. Demographic Characteristics Among Healthy Control Subjects and Patients With Alcohol Dependence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Subjects (n=99)</th>
<th>Patients (n=109)</th>
<th>Statistic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>39 (9)</td>
<td>41 (11)</td>
<td>F_{(1,196)}=2.6</td>
<td>.11</td>
</tr>
<tr>
<td>Educational level, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (1.0)</td>
<td>4 (3.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td>7 (7.1)</td>
<td>15 (13.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>41 (41.4)</td>
<td>46 (42.2)</td>
<td>x²=5.1</td>
<td>.27</td>
</tr>
<tr>
<td>Intermediate</td>
<td>34 (34.3)</td>
<td>28 (25.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>16 (16.2)</td>
<td>16 (14.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current substance use, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitual smoking</td>
<td>42 (42.4)</td>
<td>73 (67.0)</td>
<td>x²=14.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Habitual caffeine use</td>
<td>95 (96.0)</td>
<td>106 (97.2)</td>
<td>x²=0.9</td>
<td>.34</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitor use</td>
<td>3 (3.0)</td>
<td>11 (10.1)</td>
<td>x²=4.1</td>
<td>.05</td>
</tr>
<tr>
<td>Psychiatric comorbidity, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>NA</td>
<td>29 (26.6)</td>
<td>x²=28.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>NA</td>
<td>17 (15.6)</td>
<td>x²=16.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Obsessive-compulsive disorder</td>
<td>NA</td>
<td>2 (1.8)</td>
<td>x²=1.8</td>
<td>.18</td>
</tr>
<tr>
<td>Attention-deficit/hyperactivity disorder</td>
<td>NA</td>
<td>10 (9.2)</td>
<td>x²=9.5</td>
<td>.002</td>
</tr>
<tr>
<td>Past comorbid substance use, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulants</td>
<td>NA</td>
<td>11 (10.1)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Opioids</td>
<td>NA</td>
<td>9 (8.3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cannabis</td>
<td>NA</td>
<td>38 (34.9)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>NA</td>
<td>6 (5.8)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

a For 1 patient, the level of education was unknown.

b None is primary school with no diploma, basic is secondary school, low is lower vocational education, intermediate is higher vocational education, and high is higher professional education or university.

d Compared with healthy male control subjects who were matched for age and level of education. We selected only men to avoid potential confounding effects of the menstrual cycle on dopamine receptor functioning in women. In addition, most patients with alcohol dependence in The Netherlands who are admitted to a detoxification clinic are male. Controls were recruited through advertisements in regional newspapers. Exclusion criteria consisted of the following: non-European ancestry, language disabilities prohibiting written informed consent, any medical condition interfering with apomorphine administration (eg, allergy or recent cardiac disease), or current or past neuropsychiatric disorder related to dysfunction of the dopamine system (eg, psychosis, Korsakoff syndrome, or Parkinson disease). Controls with a history of psychiatric disorder or a family history of psychiatric disorder (including substance dependence) were excluded. In total, 110 patients and 99 controls were recruited. Table 1 gives characteristics of both study groups, and the eFigure (http://www.archgenpsychiatry.com) shows a flowchart of the study cohort selection.

To assess alcohol and drug dependence, we used section 4 of the Dutch version of the Addiction Severity Index. The Addiction Severity Index is a reliable tool for the assessment of alcohol and drug dependence in clinical samples. Lifetime occurrence of Axis I disorders was assessed using version 2.1 of the Dutch Mini-International Neuropsychiatric Interview for psychiatric disorders and the Dutch version of the ADHD (Attention-Deficit/Hyperactivity Disorder) Rating Scale.

To avoid effects of nicotine and caffeine use or withdrawal, participants were not allowed to smoke or consume caffeine-containing drinks on the morning of testing for 2 hours before measurements as assessed by self-report. Patients were tested after 1 month of controlled abstinence based on daily self-report and regular breath tests and after at least 1 week without the use of benzodiazepines.

The study was approved by the regional medical ethical board (Medisch-Ethische Toetsingscommissie Instellingen Geestelijke Gezondheidszorg) (protocol 271, P04.0388L). All participants gave written informed consent.

**Genotyping of COMT rs4680 G>A (Val158Met) and DRD2 Taq1A rs1800497 C>T**

Blood samples were obtained by venipuncture, and DNA was isolated using standard protocols. Molecular analyses were performed in a CCKL (Centrale Commissie ter bevordering van de Kwaliteit van Laboratorium onderzoek en de accreditatie van laboratoria in de gezondheidszorg)–accredited laboratory at the Department of Human Genetics, Radboud University Nijmegen Medical Centre, the Netherlands. The DRD2 Taq1A rs1800497 and COMT rs4680 polymorphisms were genotyped using TaqMan-based analysis. Genotyping was performed in a volume of 10 µL containing 10 ng of genomic DNA. For DRD2, 5 µL of TaqMan Mastermix (2×; Applied Biosystems), 0.125 µL of TaqMan assay (TaqMan assay: C_25746809_50, reporter 1, VIC-A-allele, reporter 2, FAM-A-allele, reporter 3, black-FAM-A-allele), and 3.875 µL of water were added. For COMT, 5 µL of ABgene Mastermix (2×; ABgene Ltd), 0.125 µL of TaqMan assay (TaqMan assay: C_25746809_50, reporter 1, VIC-A-allele; Applied Biosystems), and 3.875 µL of water were added. Amplification was performed on a commercially available system (7500 Fast Real-Time PCR, Applied Biosystems), starting with 15 minutes at 95°C, followed by 50 cycles of 15 seconds at 95°C and 1 minute at 60°C. Genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems).

To investigate the random genotyping error rate in the 2 assays, the laboratory included 5% duplicate DNA samples, which showed 100% consistency in genotype. In addition, 4% blanks were included, which were all negative.

**Apomorphine Challenge**

Central dopamine receptor sensitivity was measured using a challenge with subcutaneous administration of the dopamine

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agonist apomorphine hydrochloride (0.005 mg/kg). Outcome measures were the neuroendocrine response as measured by plasma GH levels and the effect on cognition as measured by performance on a continuous performance task. Samples for GH testing were obtained at baseline and at 30, 50, and 70 minutes after apomorphine administration; the cognitive task was performed at baseline and at 20 and 40 minutes after apomorphine administration.

**PLASMA APOMORPHINE AND GH MEASUREMENTS**

Blood samples (4 mL) were obtained to assess plasma apomorphine and GH levels. After centrifugation, samples of 2.0 mL were kept on ice and stored at −80°C. Apomorphine levels were assessed by high-pressure liquid chromatography with electrochemical detection. Plasma GH levels were measured by radioimmunoassay using an antiserum raised in guinea pig. One patient was excluded from analysis because of a baseline GH level above the detection limit of 1.6 ng/mL (to convert GH level to micrograms per liter, multiply by 1.0). Before January 1, 2006, we used the first (80/505) international standard for GH for the standard curve, based on a batch of ampolles (coded 80/505) containing highly purified pituitary human GH; beginning January 1, 2006, the second (98/754) international standard was used. Within and between coefficients of variation were 7.1% and 10.5%, 4.3% and 8.2%, and 5.4% and 10.9% at levels of 6.0, 10.8, and 35.2 mIU/L, respectively, before and after January 1, 2006.

**COGNITIVE TASK PERFORMANCE MEASUREMENT**

Cognition was assessed using the AX continuous performance task (AX-CPT). This task consists of single letters consecutively shown in white on a black screen with an interstimulus interval of 1300 milliseconds as described previously. Participants were instructed to give a target response (right-button press) after the probe X appeared preceded by the cue A or to give a nontarget response (left-button press) after any other combination of letters. Responses were scored in a window between 100 and 2000 milliseconds after stimulus onset. Two distracter letters were presented in red between the cue and probe; 70% of the trials were target trials. The tasks lasted 10 minutes. To reduce learning effects, participants performed a practice session before the 3 test sessions.

**STATISTICAL ANALYSIS**

Baseline differences in GH levels, cognitive task performance, and demographic characteristics between patients and controls and the 2 genotypes (DRD2 and COMT) were compared using analysis of variance for continuous variables and χ² test for categorical variables. Differences in response to apomorphine between groups (patient vs control and genotype) were analyzed using repeated-measures analysis of variance, with AX-CPT accuracy (percentage of commission errors) and plasma GH levels as dependent variables, and group (patient or control) and genotype (DRD2 or COMT) as between-subject variables. For the DRD2 genotype, 2 groups were formed (A1 carriers vs A2/A2) owing to the few A1 homozygotes. The COMT genotype was analyzed in 3 groups (Val/Val, Val/Met, and Met/Met). To test for a gene dosage effect, we used special contrast analysis, testing the hypothesis that the effect of apomorphine on cognitive task performance was twice as strong in carriers of 2 Met allele copies than in carriers of 1 Met allele compared with those homozygous for the Val allele.

Because patients often were smokers, had psychiatric comorbidity, and used selective serotonin reuptake inhibitors, these factors were added as covariates in the repeated-measures analysis of variance. To protect an overall α level of .05, Bonferroni-Holm correction was applied to the 4 independent tests, and only adjusted P values are presented.

To analyze the prediction of alcohol dependence by genotype and intermediate phenotype, logistic regression analysis was performed with group (patient or control) as the dependent variable using the forced entry method. To test a model of mediation, the intermediate phenotypes (maximum GH response and maximum cognitive response to apomorphine) were entered in block 1, and the genetic factors (DRD2 and COMT) were added in block 2.

Data were analyzed using commercially available statistical software (SPSS, version 16.0; SPSS, Inc). Two-sided α = .05 was considered statistically significant.

**RESULTS**

**BASELINE COMPARISON**

Demographics of the study population are summarized in Table 1. Patients more often were smokers, had psychiatric comorbidity, and used serotonin reuptake inhibitors. The genotype frequencies did not deviate from Hardy-Weinberg equilibrium (χ² = 2.1, P = .16 for DRD2; and χ² = 0.7, P = .40 for COMT). These are given in Table 2 and are similar to those reported in other European populations. There was no association of DRD2 Taq1A or COMT Val158Met genotype with alcohol dependence.

At baseline, patients performed worse on the AX-CPT (Table 3). Neither the DRD2 genotype nor the COMT

| Table 2. Genotype Distribution Among Healthy Control Subjects and Patients With Alcohol Dependence |
|--------------------------------------------------|------------------|------------------|------------------|------------------|
| **Genotype** | **Control Subjects (n=99)** | **Patients (n=110)** | **χ² Statistic** | **P Value** |
| DRD2 Taq1A | A1/A1 | 1.0 | 1.8 | 0.5 | .77 |
| | A1/A2 | 29.3 | 31.8 | | |
| | A2/A2 | 69.7 | 66.4 | | |
| COMT Val158 Met | Val/Val | 22.2 | 23.6 | 3.6 | .15 |
| | Val/Met | 53.5 | 40.9 | | |
| | Met/Met | 24.2 | 35.5 | | |
genotype was associated with the baseline AX-CPT task performance (the error percentages were $F_{2,95} = 1.1, P = .32$ for DRD2 and $F_{2,95} = 1.0, P = .38$ for COMT; reaction times were $F_{2,95} = 1.6, P = .21$ for DRD2 and $F_{2,95} = 0.7, P = .48$ for COMT).

**EFFECTS OF APOMORPHINE ADMINISTRATION**

There were no differences in apomorphine levels between genotype groups or between patients and controls at any time point. These results are summarized in Table 3.

Accuracy on the AX-CPT decreased after apomorphine administration (the error percentage was $F_{1,925} = 4.2, P = .02$) (Figure 1). Patients were less sensitive than controls to the effects of apomorphine on AX-CPT accuracy ($F_{1,920} = 29.4, P < .001$). Patients showed decreased GH response to apomorphine compared with controls ($F_{1,920} = 0.5, P = .88$ for response to apomorphine × DRD2).

Growth hormone levels increased after apomorphine administration ($F_{1,6,205} = 10.4, P < .001$) (Figure 2). Patients showed decreased GH response to apomorphine compared with controls ($F_{1,6,205} = 6.2, P < .001$ for response to apomorphine × group). Carriers of at least 1 DRD2 A1 allele had decreased GH response to apomorphine compared with DRD2 A2 homozygotes ($F_{1,6,205} = 4.3, P = .02$ for response to apomorphine × DRD2). There was no difference between patients and controls in the effect of the DRD2 genotype ($F_{1,6,205} = 0.2, P = .88$ for response to apomorphine × DRD2 × group). There was no difference in GH response among the 3 COMT genotypes.
PREDICTION OF ALCOHOL DEPENDENCE

Results of the logistic regression analyses are summarized in Table 4. Growth hormone response and cognitive task performance after administration of the dopamine agonist predicted alcohol dependence ($\chi^2 = 17.1$, $P < .001$ for the block 1 model [62.3% of participants were correctly predicted]). Adding DRD2 and COMT to the model did not significantly improve the prediction of alcohol dependence ($\chi^2 = 1.3$, $P = .52$ for the block 2 model [63.2% of participants were correctly predicted]). The total variance explained by the model was 11% (Nagelkerke $R^2$). Interaction between genotypes and intermediate phenotypes was not significant.

COMMENT

To our knowledge, this is the first study showing added value of apomorphine sensitivity as an intermediate phenotype in alcohol dependence. As predicted by our first hypothesis, the plasma GH level response and the disruptive effect on cognitive task performance after apomorphine administration were reduced in patients having alcohol dependence compared with healthy controls. These findings are in line with previous studies among smaller samples of patients with alcohol dependence. Our findings confirm the presence of reduced dopamine receptor sensitivity in patients with alcohol dependence, even after initial detoxification. Patients herein performed worse on the AX-CPT at baseline compared with controls. Although the data show that performance among patients with alcohol dependence can deteriorate further after administration of apomorphine, depending on the COMT genotype, a potential ceiling effect could not be ruled out completely.

In line with our second hypothesis, dopaminergic alcohol dependence genes affected central dopamine receptor sensitivity. The DRD2 genotype specifically influenced apomorphine effects on plasma GH levels. These findings suggest that the DRD2 genotype is mainly related to subcortical dopamine receptor functioning. Previous investigations have shown that the DRD2 genotype is associated with reduced dopamine receptor density in the striatum, although this was not always replicated. Because GH response is a marker for neuroinfundibular dopamine receptor function, our data suggest that dopamine receptor density may also be reduced in the neuroinfundibulum in carriers of the DRD2 Taq1A genotype. Whether the neuroinfundibular response to apomorphine is related to striatal dopamine receptor density is unknown.

The DRD2 Taq1A genotype is part of a gene cluster neighboring the ANKK1 gene cluster. Other genes within these clusters have been shown to be potential risk genes for alcohol dependence. Moreover, a study among a small sample of patients with alcohol dependence showed an association of the ANKK1 genotype with the apomorphine-induced GH response.

The COMT genotype affected the response to apomorphine as measured by the AX-CPT. Because the AX-CPT requires PFC dopamine receptor functioning, this suggests that COMT is particularly relevant to PFC dopamine. Our observations may be explained by the inverted-U hypothesis on PFC dopamine receptor functioning and cognitive task performance, indicating that both too high and too low levels of PFC dopamine are related to worse cognitive performance. Carriers of the COMT Val/Val genotype are presumed to have reduced PFC dopamine receptor functioning due to more efficient clearance of synaptic dopamine. Suboptimal PFC dopamine levels at baseline may be compensated for by dopamine receptor stimulation after apomorphine administration. However, in carriers of 1 or 2 Met alleles, administration of apomorphine may “overshoot” dopamine receptor stimulation and reduce cognitive task performance. Therefore, the effect of a phasic shift in dopamine levels on cognitive task performance after administration of a dopamine agonist may be dependent on tonic variation in dopamine levels based on variation in the COMT genotype. Future studies are needed to further confirm the inverted-U hypothesis.

In line with our third hypothesis, dopamine receptor sensitivity was a better predictor of alcohol dependence than the COMT or DRD2 genes. This shows that adding dopamine receptor sensitivity as an intermediate phenotype is a crucial step to find alcohol dependence–related risk genes. Prior evidence has suggested that the COMT genotype may be involved in impulsivity and affective processing. Impairment of executive control in carriers of the COMT Val/Val genotype may be associ-
ated with increased impulsivity or novelty seeking. However, a previous study\textsuperscript{81} did not find a correlation between apomorphine-induced GH response and personality traits, such as novelty seeking. In addition, genetic variation in COMT has been suggested to be related to affective processing. This has been formulated in the tentative “warrior-worrier” model.\textsuperscript{79} In this model, the COMT Met allele is hypothesized to be advantageous in memory and attention tasks, whereas reactivity to unpleasant stimuli is increased in COMT Met allele carriers.\textsuperscript{79,80} This increased reactivity to unpleasant stimuli might cause a lower emotional resilience against negative mood states observed in individuals with a higher COMT Met158 allele load (worrier strategy). Therefore, the COMT Met allele seems to benefit during the performance of working memory and attention-related tasks, whereas the COMT Val allele may be disadvantageous during the processing of aversive emotional stimuli (warrior strategy).

The effect of the COMT genotype was similar across patients and controls. However, in patients with alcohol dependence, improvement in cognitive task performance after apomorphine administration was seen in genotypes with at least 1 COMT Val allele, whereas this was only the case in homozygous COMT Val allele carriers among the controls. Administration of apomorphine may be less likely to result in dopamine receptor overstimulation in patients with alcohol dependence. This suggests that PFC dopamine receptor functioning may be further reduced in patients with alcohol dependence, consistent with a leftward shift of the inverted-U curve in these individuals.

Although both genetic variants were associated with dopamine receptor sensitivity, they were not predictive of alcohol dependence. The sample size may have been too small to detect an association with alcohol dependence, although it was sufficient to show an association with dopamine receptor sensitivity. This is consistent with the idea that intermediate phenotypes are genetically less complex and that their use can improve the validity of genetic association studies.

The present findings may indicate a model of pleiotropy or a causal model, with reduced dopamine receptor sensitivity mediating the genetic risk for alcohol dependence associated with the genotypes.\textsuperscript{7} However, in a model of mediation, it is to be expected that adding DRD2 and COMT to the regression model would not improve the prediction of the model based on the intermediate phenotypes alone, as is the case in the present study. In addition, both clinical and preclinical literature on the role of dopamine receptor dysfunction in the origin of alcohol dependence renders a model of mediation plausible.\textsuperscript{3,14}

Our results suggest that different genes confer their effect on disease vulnerability through different biological pathways. COMT specifically affected dopamine receptor sensitivity at the level of the PFC. This may increase vulnerability to the development of alcohol dependence through impaired response inhibition and control.\textsuperscript{7} DRD2 Taq1A affected dopamine receptor sensitivity in the hindbrain (neuroinfundibulum). Hindbrain dopamine receptor sensitivity may be related to alterations in reward processing and increase the risk of alcohol dependence.

These findings should be considered in the context of strengths and limitations of our study design. Strengths are that we used a functional intermediate phenotype of alcohol dependence by measuring the response to apomorphine administration among a large sample after initial detoxification, including a healthy control group. Furthermore, unlike previous studies using a challenge paradigm with a dopamine agonist, we measured plasma levels of the administered drug and found equal plasma apomorphine levels among patients and controls. Therefore, the differences found in the response to apomorphine between patients and controls and between the different genotype groups are likely true pharmacodynamic differences. Previous studies have shown that AX-CPT responses and plasma GH levels are stable under a placebo condition.\textsuperscript{28} Therefore, we considered it unnecessary to include a placebo condition in this larger-scale study. However, particularly with respect to AX-CPT responses, a genotype effect on habituation or a learning effect cannot be ruled out completely. It should be recognized that this study included men only. Additional work is needed to examine whether these results can be generalized to women.

It has been previously suggested that GH levels after apomorphine administration normalize after 24 hours to 8 days of abstinence.\textsuperscript{15} In our study, challenge tests were
performed after a mean of 30 days of abstinence. This suggests that reduced response to apomorphine among patients with alcohol dependence may represent a stable intermediate phenotype. If true, future studies should confirm reduced response to apomorphine among individuals at risk but without alcohol dependence (ie, unaffected family members of patients with alcohol dependence). Animal studies have shown that apomorphine sensitivity is transmitted over generations, suggesting that heritable factors are involved. However, up-to-date evidence for heritability of apomorphine sensitivity in humans is lacking. In addition, scarring effects of excessive alcohol consumption or prior detoxification on the brain remain possible, persistently reducing the sensitivity of the dopamine receptor system. Indeed, findings in monkeys have shown that reduced dopamine receptor densities in the brain are related to increased self-administration of cocaine but that cocaine administration itself further reduces dopamine receptor densities. In the present study, it cannot be excluded that reduced apomorphine sensitivity is partly a residual marker rather than an intermediate phenotype.

No neuroimaging measures were included herein. Instead of neuroimaging techniques, 2 functional measures were used based on previous findings using an apomorphine challenge paradigm. This has the advantage of measuring functional outcome measures, with good timely resolution. However, any interpretation about localization of the observed effects should be considered within this limitation. Clearly, the GH response is predominantly indicative of neuroinfundibular dopamine receptor sensitivity, and the AX-CPT response is predominantly indicative of PFC dopamine receptor sensitivity. However, the absence of an effect of COMT on the GH response does not completely rule out that COMT exerts an effect on other subcortical measures of dopamine receptor sensitivity. Similarly, despite the absence of an effect of DRD2 on the cognitive task performance, DRD2 may affect cortical dopamine receptor sensitivity when measured using different tasks.

It is likely that neurobiological pathways other than the dopamine receptor system, and additional genetic polymorphisms within those, have a role in the susceptibility to alcohol dependence (eg, within the hypothalamic-pituitary-adrenal axis or the opioid, glutamate, or serotonergic systems). The relative contributions of other such neurotransmitter systems in addition to central dopaminergic alcoholism-related genes, and (3) alcohol dependence is more robustly associated with reduced dopamine receptor sensitivity than with dopaminergic genotypes. This study emphasizes the importance of using dynamic or functional intermediate phenotypes in genetic studies on alcohol dependence.

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