

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 trac-

table in genetic analysis.¹¹ 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-*

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way. Alcohol generates its reinforcing effect by increasing the firing rate of dopamine neurons in the ventral tegmental area, leading to enhanced dopamine activity in the ventral striatum.¹⁶ Reduced densities of striatal dopamine receptors have been observed in patients with alcohol dependence.^{1,14} Reduced sensitivity to the dopamine agonist apomorphine hydrochloride, indexed by the growth hormone (GH) response, has repeatedly been observed in patients with alcohol dependence.¹⁷⁻²¹ Among individuals with addiction, reduced dopamine receptor sensitivity is thought to decrease the sensitivity to naturally occurring reinforcers. This may increase vulnerability to the development of alcohol abuse because alcohol consumption would compensate for this reward deficiency.^{1,13,14,21-23}

The second dopamine pathway involved in alcohol dependence runs from the ventral tegmental area to the prefrontal cortex (PFC), often referred to as the *inhibitory control pathway*.^{14,24,25} Individuals who abuse drugs show lower glucose metabolism in the PFC.^{1,14} 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.²⁰ It has been suggested that PFC and mesolimbic dopamine receptor dysfunction is interrelated, resulting in a double handicap in alcohol dependence, including deficient reward processing and behavioral control.^{1,14,26,27}

Based on this evidence, the measurement of dopamine receptor sensitivity using a challenge with a dopamine agonist may disclose a useful phenotype for genetic studies of alcohol dependence.²⁸ Indeed, studies in animals and humans have shown that variation in dopamine receptor sensitivity is highly heritable²⁹⁻³¹ and predicts self-administration of substances such as cocaine and alcohol.^{15,30} 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 genetic variants in alcohol dependence. Some studies^{32,33} have shown that the *DRD2* Taq1A genotype is associated with a 30% reduction in dopamine receptor density in the striatum of healthy volunteers. Meta-analyses³⁴⁻³⁶ 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 in vivo dopamine receptor sensitivity measured by the GH response to the dopamine agonist 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 apomorphine.³⁷ However, most studies^{18,37-39} failed to show such an association for the *DRD2* Taq1A genotype itself. Several issues with respect to these studies should be taken into consideration. First is the frequent low statistical power. Sample sizes vary between 18 and 97 participants, with only the latter representing enough power to detect any genotype effect.^{18,38,39} Second is the absence of a group of healthy control subjects in most dopamine challenge investigations.^{18,38} It has been suggested that genes for which expression is altered by chronic excessive alcohol intake encode proteins that affect apo-

morphine-induced GH release.^{38,40} Any association between genes and dopamine receptor sensitivity in patients with alcohol dependence should be confirmed in a healthy control group. Third is the timing of measurements with respect to alcohol detoxification.^{18,38} Previous investigations have shown a strong influence of detoxification on the GH response to apomorphine in patients with alcohol dependence.¹⁸ An intermediate phenotype should ideally be independent of the state of the disorder (eg, intoxication or detoxification).^{7,9,11} In the only study¹⁹ that showed an association between *DRD2* Taq1A and reduced GH response to apomorphine, measurements were performed on day 1 of detoxification among patients with alcohol dependence. Therefore, the usefulness of the GH response to apomorphine as an intermediate phenotype for alcohol dependence relative to *DRD2*, including a healthy control group, has yet to be confirmed in a substantially large sample after conclusion of initial detoxification.

Another dopaminergic gene that has been frequently studied in alcohol dependence is the catechol O-methyltransferase gene (*COMT*; OMIM +116790). The *COMT* enzyme encoded by this gene metabolizes dopamine, adrenalin, and noradrenalin and is the main factor controlling dopamine levels in the PFC. A common functional single-nucleotide polymorphism in the coding region of *COMT* induces a valine to methionine substitution (Val158Met [rs4680]) and is associated with a 40% reduction in enzyme activity.^{41,42} The *COMT* genotype affected reactivity to the administration of dopamine agonists among healthy volunteers in tasks that rely on PFC functioning.⁴³ The *COMT* genotype has been associated with subtypes of alcohol dependence, with a higher frequency of the Met allele in patients with type 1 alcohol dependence (ie, early-onset alcohol dependence with presumed high heritability).⁴⁴⁻⁴⁶ However, negative association results have also been reported.⁴⁷⁻⁵⁰ Therefore, the *COMT* genotype is a notable candidate to study relative to the effects of apomorphine on cognitive task performance in alcohol dependence.

The present study was designed to assess the functional consequences of risk alleles along the dopamine pathway, as measured by plasma GH levels and results of a continuous performance task after a challenge with the dopamine agonist apomorphine, to disclose functional in vivo intermediate phenotypes for alcohol dependence. The hypotheses of the study were as follows: (1) patients with alcohol dependence show reduced reactivity to the dopamine agonist apomorphine compared with healthy control subjects, (2) genetic variation in *DRD2* and *COMT* affects the response to apomorphine, and (3) 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 patients 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

| Characteristic | Control Subjects (n=99) | Patients (n=109) ^a | Statistic | P Value |
|--|----------------------------|----------------------------------|----------------|---------|
| Age, mean (SD), y | 39 (9) | 41 (11) | $F_{1,98}=2.6$ | .11 |
| Educational level, No. (%) ^b | | | | |
| None | 1 (1.0) | 4 (3.7) | $\chi^2=5.1$ | .27 |
| Basic | 7 (7.1) | 15 (13.8) | | |
| Low | 41 (41.4) | 46 (42.2) | | |
| Intermediate | 34 (34.3) | 28 (25.7) | | |
| High | 16 (16.2) | 16 (14.7) | | |
| Current substance use, No. (%) | | | | |
| Habitual smoking | 42 (42.4) | 73 (67.0) | $\chi^2=14.0$ | <.001 |
| Habitual caffeine use | 95 (96.0) | 106 (97.2) | $\chi^2=0.9$ | .34 |
| Selective serotonin reuptake inhibitor use | 3 (3.0) | 11 (10.1) | $\chi^2=4.1$ | .05 |
| Psychiatric comorbidity, No. (%) | | | | |
| Depression | NA | 29 (26.6) | $\chi^2=28.4$ | <.001 |
| Anxiety disorder | NA | 17 (15.6) | $\chi^2=16.7$ | <.001 |
| Obsessive-compulsive disorder | NA | 2 (1.8) | $\chi^2=1.8$ | .18 |
| Attention-deficit/hyperactivity disorder | NA | 10 (9.2) | $\chi^2=9.5$ | .002 |
| Past comorbid substance use, No. (%) | | | | |
| Stimulants | NA | 11 (10.1) | NA | NA |
| Opioids | NA | 9 (8.3) | NA | NA |
| Cannabis | NA | 38 (34.9) | NA | NA |
| Hallucinogens | NA | 6 (5.6) | NA | NA |

Abbreviation: NA, not applicable.

^aFor 1 patient, the level of education was unknown.

^bNone 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.

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.⁵¹⁻⁵⁵ 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.⁵⁶ The Addiction Severity Index is a reliable tool for the assessment of alcohol and drug dependence in clinical samples.^{57,58} 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.⁵⁹⁻⁶¹

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.^{62,63} 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 Geeste-

lijke 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 bevoordering 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.⁶⁴ Genotyping was performed in a volume of 10 μ L containing 10 ng of genomic DNA. For *DRD2*, 5 μ L of TaqMan Mastermix (2; Applied Biosystems), 0.125 μ L of TaqMan assay (TaqMan assay: C_7486676_10, reporter 1, VIC-A-allele, reverse assay; Applied Biosystems), and 3.875 μ L of water were added. For *COMT*, 5 μ L of ABgene Mastermix (2 \times , ABgene Ltd), 0.125 μ L of TaqMan assay (TaqMan assay: C_25746809_50, reporter 1, VIC-A-allele; Applied Biosystems), and 3.875 μ L 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

Table 2. Genotype Distribution Among Healthy Control Subjects and Patients With Alcohol Dependence

| Genotype | % | | χ^2 Statistic | P Value |
|-----------------------|-------------------------|------------------|--------------------|---------|
| | Control Subjects (n=99) | Patients (n=110) | | |
| <i>DRD2</i> Taq1A | | | | |
| A1/A1 | 1.0 | 1.8 | 0.5 | .77 |
| A1/A2 | 29.3 | 31.8 | | |
| A2/A2 | 69.7 | 66.4 | | |
| <i>COMT</i> Val158Met | | | | |
| Val/Val | 22.2 | 23.6 | 3.6 | .15 |
| Val/Met | 53.5 | 40.9 | | |
| Met/Met | 24.2 | 35.5 | | |

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 -30°C . Apomorphine levels were assessed by high-pressure liquid chromatography with electrochemical detection.⁶⁵ 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 ampoules (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.⁶⁶ 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.^{66,67} 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 con-

trols and the 2 genotypes (*DRD2* and *COMT*) were compared using analysis of variance for continuous variables and χ^2 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, time as a within-subject variable, 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 $\alpha = .05$ 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 ($\chi^2=2.1$, $P=.16$ for *DRD2*; and $\chi^2=0.7$, $P=.40$ for *COMT*). These are given in **Table 2** and are similar to those reported in other European samples.⁶⁸ 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 3. Endophenotype Measures in Response to Apomorphine Hydrochloride Administration Among Healthy Control Subjects and Patients With Alcohol Dependence

| Variable | Mean (SD) | | F Score | P Value |
|------------------------------------|---------------------------|--------------------|--------------------|---------|
| | Control Subjects (n = 99) | Patients (n = 110) | | |
| Plasma growth hormone level, ng/mL | (n = 87) | | | |
| Baseline | 0.5 (1.7) | 0.6 (2.0) | $F_{1,195} = 0.3$ | .60 |
| 30 min | 25.7 (25.7) | 14.4 (22.4) | $F_{1,195} = 10.8$ | .001 |
| 50 min | 27.0 (21.1) | 21.1 (19.4) | $F_{1,195} = 4.1$ | .045 |
| 70 min | 20.0 (18.1) | 16.5 (15.2) | $F_{1,195} = 2.1$ | .15 |
| Apomorphine level | (n = 92) | (n = 90) | | |
| 10 min | 1.59 (0.94) | 1.48 (1.22) | $F_{1,180} = 0.5$ | .48 |
| 30 min | 0.80 (0.64) | 0.94 (0.65) | $F_{1,180} = 2.4$ | .12 |
| 50 min | 0.50 (0.51) | 0.60 (0.34) | $F_{1,180} = 2.5$ | .11 |
| Error percentage on AX-CPT task | | | | |
| Baseline | 3.0 (0.3) | 7.4 (0.7) | $F_{1,207} = 29.4$ | <.001 |
| 20 min | 5.1 (0.6) | 7.2 (0.9) | $F_{1,207} = 4.0$ | .046 |
| 40 min | 4.9 (0.6) | 7.0 (0.9) | $F_{1,207} = 4.0$ | .047 |
| Reaction time on AX-CPT task, ms | | | | |
| Baseline | 330 (58) | 406 (130) | $F_{1,207} = 28.4$ | <.001 |
| 20 min | 350 (90) | 412 (146) | $F_{1,207} = 13.2$ | <.001 |
| 40 min | 350 (84) | 410 (121) | $F_{1,207} = 17.4$ | <.001 |

Abbreviation: AX-CPT, AX continuous performance task.

SI conversion factor: To convert growth hormone level to micrograms per liter, multiply by 1.0.

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 $F_{1,9,205} = 4.2$, $P = .02$) (**Figure 1**). Patients were less sensitive than controls to the effects of apomorphine on AX-CPT accuracy ($F_{1,9,205} = 5.3$, $P = .005$ for response to apomorphine \times group). Carriers of 1 or 2 *COMT* Met alleles were more sensitive than noncarriers of a Met allele to the detrimental effects of apomorphine on AX-CPT accuracy, with homozygous Met allele carriers being most sensitive ($F_{3,8,205} = 3.1$, $P = .048$ for response to apomorphine \times *COMT*). The special contrast analysis for a gene dosage effect fell just short of being significant (the contrast value was $F_{4,205} = 0.061$, $P = .06$). There was no difference in the effect of the *COMT* genotype between patients and controls ($F_{4,93} = 1.6$, $P = .17$ for response to apomorphine \times *COMT* \times group). The *DRD2* genotype did not affect sensitivity to the effect of apomorphine on AX-CPT accuracy ($F_{1,6,205} = 0.5$, $P = .58$ for response to apomorphine \times *DRD2*).

Growth hormone levels increased after apomorphine administration ($F_{1,6,205} = 104.6$, $P < .001$) (**Figure 2**). Patients showed decreased GH response to apomorphine compared with controls ($F_{1,6,205} = 6.2$, $P < .001$ for response to apomorphine \times group). Carriers of at least

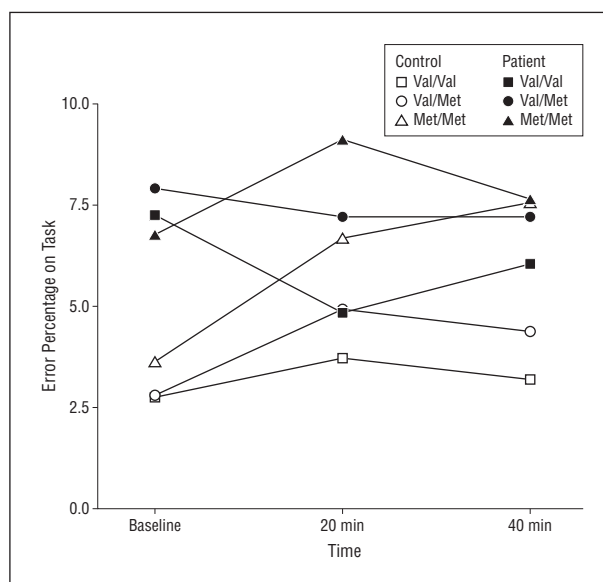


Figure 1. Effect of the *COMT* genotype (*COMT* Val158Met) on the cognitive endophenotype (AX continuous performance task [AX-CPT] response to apomorphine hydrochloride administration) in healthy control subjects and in patients with alcohol dependence. Among controls and patients, carriers of the *COMT* Met/Met genotype made more errors on the AX-CPT after apomorphine administration. The deterioration in AX-CPT response after apomorphine administration was less in carriers of a *COMT* Val allele at both 20 minutes ($F_{2,205} = 6.5$, $P = .01$) and 40 minutes ($F_{2,205} = 3.1$, $P = .049$).

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 \times *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 \times *DRD2* \times group). There was no difference in GH response among the 3 *COMT* genotypes

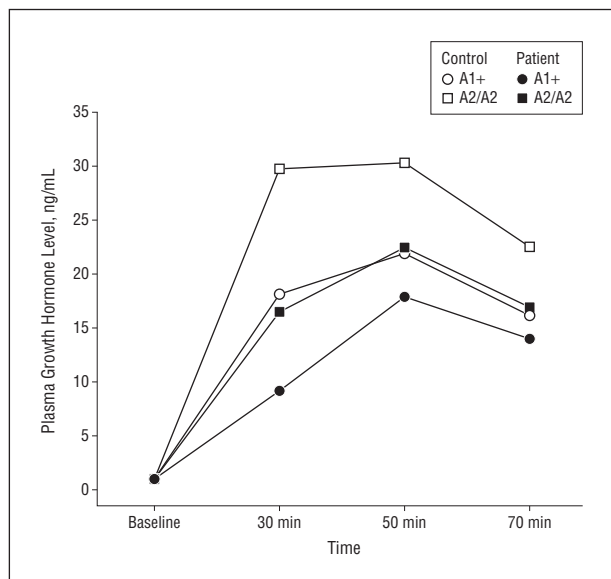


Figure 2. Effect of the *DRD2* genotype (*DRD2* Taq1A) on the neuroendocrine endophenotype (plasma growth hormone response to apomorphine hydrochloride administration) in healthy control subjects and in patients with alcohol dependence. Among controls and patients, the *DRD2* genotype affects the response to apomorphine, with carriers of a *DRD2* A1 allele showing a blunted response, particularly at 30 minutes ($F_{1,205}=6.5$, $P=.01$ at 30 minutes; $F_{1,205}=3.7$, $P=.06$ at 50 minutes; and $F_{1,205}=2.4$, $P=.12$ at 70 minutes). In addition, patients show a blunted response at 30 minutes and at 50 minutes ($F_{1,205}=2.9$, $P<.001$ at 30 minutes; $F_{1,205}=5.2$, $P=.02$ at 50 minutes; and $F_{1,205}=2.8$, $P=.10$ at 70 minutes). To convert growth hormone level to micrograms per liter, multiply by 1.0.

($F_{3,1,205}=0.4$, $P=.90$ for response to apomorphine \times *COMT*).

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^{20,21,69} among smaller samples of patients with alcohol dependence. Our findings confirm the presence of reduced dopamine receptor sensitivity in patients with alcohol depen-

dence, 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.³³ 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.⁷⁰⁻⁷² Other genes within these clusters have been shown to be potential risk genes for alcohol dependence.⁷³ Moreover, a study¹⁹ 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.⁷⁴⁻⁷⁷ 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.⁷⁸ 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.⁴³ 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^{79,80} 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-

Table 4. Prediction of Alcohol Dependence Based on Response to Apomorphine Hydrochloride Administration (Intermediate Phenotype) and Genotype

| Factor Included | Block 1 ^a | | | Block 2 ^b | | |
|---------------------------------|----------------------|---------|------------------|----------------------|---------|------------------|
| | β (SE) | P Value | OR (95% CI) | β (SE) | P Value | OR (95% CI) |
| Intermediate phenotype | | | | | | |
| Maximum growth hormone response | -0.02 (0.01) | .008 | 0.98 (0.97-0.99) | -0.02 (0.01) | .009 | 0.98 (0.97-0.99) |
| Maximum AX-CPT response | 0.06 (0.02) | .01 | 1.06 (1.01-1.11) | 0.06 (0.02) | .01 | 1.06 (1.02-1.11) |
| Genotype | | | | | | |
| <i>COMT</i> Val158Met | NA | NA | NA | -0.23 (0.20) | .26 | 0.80 (0.54-1.18) |
| <i>DRD2</i> Taq1A | NA | NA | NA | -0.01 (0.31) | .99 | 1.00 (0.54-1.84) |

Abbreviations: AX-CPT, AX continuous performance task; NA, not applicable; OR, odds ratio.

^aRegression analysis with only the intermediate phenotype as a predictor of alcohol dependence.

^bRegression analysis with the intermediate phenotype and the genotype as predictors of alcohol dependence, showing no additive effect of the genotype predictor.

ated with increased impulsivity or novelty seeking. However, a previous study⁸¹ 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.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.^{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 be beneficial during the performance of working memory and attention-related tasks, whereas the *COMT* Val allele may be advantageous 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.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.^{2,14}

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.¹ *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.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.¹⁷ 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 studies³⁰ 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.^{18,30,31,38,82,83} 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.⁸⁴ 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 dopamine receptor sensitivity remain to be studied.

In summary, the present study provides evidence that (1) dopamine receptor sensitivity is lower in patients with alcohol dependence than in matched healthy controls, (2) dopamine receptor sensitivity is associated with 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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