ORIGINAL ARTICLE

Genetic variation in catechol-\(O\)-methyltransferase modifies effects of clonidine treatment in chronic fatigue syndrome

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Clonidine, an \(\alpha\)-adrenergic receptor agonist, decreases circulating norepinephrine and epinephrine, attenuating sympathetic activity. Although catechol-\(O\)-methyltransferase (COMT) metabolizes catecholamines, main effectors of sympathetic function, COMT genetic variation effects on clonidine treatment are unknown. Chronic fatigue syndrome (CFS) is hypothesized to result in part from dysregulated sympathetic function. A candidate gene analysis of COMT rs4680 effects on clinical outcomes in the Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL), a randomized double-blinded clonidine versus placebo trial, was conducted (\(N = 104\)). Patients homozygous for rs4680 high-activity allele randomized to clonidine took 2,500 fewer steps compared with placebo (\(P_{\text{interaction}} = 0.04\)). There were no differences between clonidine and placebo among patients with COMT low-activity alleles. Similar gene–drug interactions were observed for sleep (\(P_{\text{interaction}} = 0.003\)) and quality of life (\(P_{\text{interaction}} = 0.018\)). Detrimental effects of clonidine in the subset of CFS patients homozygous for COMT high-activity allele warrant investigation of potential clonidine–COMT interaction effects in other conditions.

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INTRODUCTION

Genetic variation in COMT, the locus that encodes catechol-\(O\)-methyltransferase (COMT), an enzyme that metabolizes norepinephrine, epinephrine, dopamine and catechol estrogens, has been implicated in a broad cross-section of disease and conditions from hypertension,\(^{1,2}\) preeclampsia,\(^{3}\) cardiovascular disease,\(^{4}\) psychiatric disorders,\(^{5}\) cancer\(^{6,7}\) and chronic fatigue syndrome (CFS).\(^{8}\) Furthermore, COMT has also been shown to modify clinical response to both active drugs\(^{4,9–11}\) and placebo treatments\(^{13,14}\) in randomized clinical trials. Thus, COMT is emerging as an important hub in pharmacogenomics and the development of precision medicines.

CFS is a disabling condition characterized by unexplained, long-lasting, fatigue and exertion intolerance, accompanied by pain, cognitive impairments and orthostatic intolerance.\(^{15–17}\) Elevated norepinephrine and epinephrine levels, as well as attenuated heart rate variability in adolescents with CFS,\(^{18}\) support the hypothesis that heightened sympathetic and decreased parasympathetic nervous system activity contribute to the disease process.\(^{19}\) This disabling condition affects 0.1–1.0% of children world-wide, but the pathophysiology is poorly understood and there are few treatment options.

The most well-studied single-nucleotide polymorphism (SNP) in COMT, rs4680 (val158met), encodes a three- to four-fold difference in enzymatic activity between the valine high-activity and methionine low-activity forms of the enzyme.\(^{20}\) COMT enzymatic activity has been shown to be inversely related to catecholamine levels.\(^{21}\) Thus, individuals homozygous for the high-activity valine (val/val) form of the enzyme tend to have lower catecholamine levels compared with those homozygous for the low-activity methionine (met/met) form.\(^{22–24}\) Consistent with the observation that adolescents diagnosed with CFS have elevated levels of norepinephrine and epinephrine,\(^{25}\) the COMT low-activity met/met genotype appears to be more prevalent among adolescents with CFS.\(^{8}\)

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To date, the effect of genetic variation in COMT on treatment response to clonidine has not been examined. Based on current evidence, we hypothesized that individuals homozygous for the low-activity met allele form of COMT rs4680 might benefit more from the catecholamine reduction induced by clonidine treatment than individuals homozygous for the high-activity val allele form. Furthermore, given the COMT rs4680 association with response to placebo treatment, and clonidine effects on dopamine activation in brain regions implicated in placebo response, the association between COMT and outcomes in individuals randomized to placebo treatment was examined.

MATERIALS AND METHODS
Study population and intervention
Details of the NorCAPITAL trial have been published elsewhere. Briefly, between 1 March 2010 and 15 October 2012, 120 CFS patients aged 12–18 years with 3 months of unexplained disabling, chronic/relapsing fatigue of new onset were included in a randomized clinical trial conducted at Oslo University Hospital, Norway. Patients with depression as a primary cause of fatigue were excluded from the trial. The Norwegian National Committee for Ethics in Medical Research and the Norwegian Medicines Agency approved the study. Informed consent was obtained from all participants or from parents or next of kin as required.

Clonidine doses were based on a pilot study and were designed to be in the lower range of what is considered clinically effective (25 or 50 μg for body weight < 35 or ≥ 35 kg, respectively, twice daily). The treatments were double blinded. After 8 weeks of treatment, Steps Taken, the primary outcome, de placebo treatment on the patient. Outcome, steps after 8 weeks of treatment (Figures 1a and b). This finding was consistent with a significant val/val by clonidine treatment interaction (β (s.e.) = − 0.267 (0.091); Pinteraction = 0.043). In contrast, differences between clonidine and placebo in heterozygote

Statistical methods
For primary and secondary outcomes, linear regression models were used to carry out an a priori candidate gene analysis of COMT rs4680 on the outcomes at 8 weeks. Models were adjusted for baseline, age, sex and body mass index. A per-protocol approach was adopted. The heterozygote genotype (val/met) was used as the reference, and individuals homozygous for the val (val/val) or met (met/met) alleles were assumed to be different from val/met in their response to drug and/or placebo treatment. Interaction terms for genotype by treatment allocation (drug vs placebo) were included in the model. For each outcome, we tested the interaction of clonidine with COMT genotype using a conservative 2-degree of freedom test, creating multiplicative terms for the treatment by genotype interactions (in three categories). To test interactions on the nonlinear scale, we used a COMT gene dosage model and included a quadratic term for genotype and the cross-product of treatment arm and the quadratic term. We tested the joint effect of both terms with a 2-degree of freedom test. For ease of interpretation, graphs of the change or difference in each outcome from baseline to 8 weeks of treatment (weeks 0–8) were adjusted by genotype and treatment allocation are presented here. Differences in clonidine levels in individuals randomized to clonidine treatment were analyzed by analysis of variance. The normal distributions of all the outcomes or the residuals of week 8 compared with week 0 were confirmed using the Shapiro–Wilks Test.

Using Spearman’s correlations, we examined the correlation between Steps Taken and five subjective outcome measures at 8 weeks. As this was an exploratory analysis with deliberately correlated outcomes, we did not correct for multiple testing. Hardy–Weinberg equilibrium was calculated using the Online Encyclopedia for Genetic Epidemiology studies. Statistical analyses were performed using SAS 9.3 and JMP Pro 12.0.1 (SAS Institute, Cary, NC, USA).

RESULTS
Study sample
Of the 120 participants included in the trial, data from 104 patients who were genotyped at baseline were available for primary end point evaluation at week 8. There were no differences between the individuals who completed the trial and those who dropped out by COMT genotype (P = 0.4), baseline severity for Steps Taken (P = 0.7) or any of the other outcome measures examined. The rs4680 SNP was found to be in Hardy–Weinberg equilibrium (P = 0.99), and the minor allele frequency of rs4680 (G or val) was 0.44. Patients did not vary by demographic or baseline measures as function of COMT rs4680 genotype (Table 1). There was no difference in the concentration of clonidine at steady state across the three COMT genotypes in the clonidine treatment arm (P = 0.5), mean (s.d.) μg l⁻¹: met/met = 0.25 (0.09), val/met = 0.23 (0.11) and val/val = 0.23 (0.08).

Outcomes
Steps Taken. In the unadjusted model of the primary outcome

Blood pressure. As previously described, a Task Force Monitor (TFM; Model 3040i, CNSystems Medizintechnik, Graz, Austria), with combined hardware and software device for noninvasive recording of cardiovascular variables, was used to measure blood pressure. Orthostatic tolerance was estimated as the difference between systolic blood pressure supine and standing.

Genotyping
Genotyping of the COMT SNP rs4680 was carried out by predesigned TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA), using the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were re-genotyped, and the concordance rate was 100%.
individuals and individuals homozygous for the met allele were nonsignificant. In the placebo arm, individuals homozygous for the val allele had the greatest placebo response compared with those with at least one met allele (val/met and met/met), but this difference was nonsignificant (Figure 1a). The results were essentially equivalent when the models were adjusted for age, gender and body mass index ($\beta$(s.e.) = $-2.215$ (985); $P_{\text{interaction}} = 0.027$).

**Subjective outcomes.** Table 2 shows Spearman's correlations among the five 8-week subjective outcomes and Steps Taken. Absolute correlations ranged from 0.11 to 0.74, suggesting these inventories represent complementary but incompletely overlapping domains, as expected.

Consistent with the findings for Steps Taken, the unadjusted model of KSQ also revealed a significant difference between val/val individuals treated with clonidine compared with placebo and heterozygous individuals (va/met) ($\beta$ (s.e.) = $-1.25$ (0.42), $P_{\text{int}} = 0.003$), suggesting that the sleep quality of val/val adolescents worsened with 8 weeks of clonidine versus placebo treatment (Figure 2a). The similar pattern observed in the unadjusted model for PedsQL ($\beta$ (s.e.) = $-14.2$ (5.9), $P_{\text{int}} = 0.018$) further reinforced the findings that val/val individuals had overall worse outcomes in the clonidine treatment arm compared with placebo (Figure 2b). The results were essentially equivalent when the models were adjusted for age, gender and body mass index: KSQ ($\beta$ (s.e.) = $-1.15$ (0.40), $P_{\text{int}} = 0.01$) and PedsQL ($\beta$ (s.e.) = $-12.1$ (5.7), $P_{\text{int}} = 0.04$). The direction of the COMT val/val by treatment interaction effects for CFQ ($\beta$ (s.e.) = $-2.3$ (3.0), $P_{\text{int}} = 0.43$), FDI ($\beta$ (s.e.) = $5.5$ (4.4), $P_{\text{int}} = 0.22$) and BPI ($\beta$ (s.e.) = $-0.18$ (1.00), $P_{\text{int}} = 0.86$) (Figures 2c–e) were qualitatively consistent with the other three outcomes, but these effects were nonsignificant. The strong

### Table 1. Baseline demographics and measures of all genotyped patients ($N=104$) and genotyped patients by COMT rs4680 genotype in the NorCAPITAL trial

|                          | All  | val/val | val/met | met/met | P     |
|--------------------------|------|---------|---------|---------|-------|
| **Demographics**         |      |         |         |         |       |
| $N$                      | 104  | 20      | 51      | 33      |       |
| Age (years)              | 15.4 (1.6) | 15.0 (1.7) | 15.3 (1.6) | 15.8 (1.4) | 0.16  |
| Female (%)               | 72   | 65      | 72      | 76      | 0.70  |
| BMI ($\text{kg m}^{-2}$) | 21.5 (4.1) | 20.3 (4.3) | 22.1 (4.4) | 21.3 (3.5) | 0.24  |
| **Baseline measures**    |      |         |         |         |       |
| Steps at week 0          | 4630 (2380) | 4685 (1951) | 4947 (2588) | 4120 (2240) | 0.45  |
| KSQ                      | 3.7 (0.8) | 3.7 (0.8) | 3.7 (0.9) | 3.5 (0.8) | 0.54  |
| CFQ                      | 19 (6) | 18 (6)  | 20 (6)  | 20 (6)  | 0.41  |
| FDI                      | 23 (9) | 20 (8)  | 23 (9)  | 25 (9)  | 0.14  |
| PedsQL                   | 49 (13) | 53 (12) | 50 (13) | 45 (13) | 0.07  |
| BPI                      | 4.4 (2.2) | 3.8 (2.6) | 4.2 (2) | 5.1 (2.2) | 0.07  |
| Norepinephrine (nmol/L)  | 185 (86) | 159 (80) | 189 (80) | 195 (99) | 0.33  |
| Epinephrine (nmol/L)     | 20 (12) | 17 (8)  | 22 (14) | 21 (11) | 0.31  |
| SBP supine (mm Hg)       | 113 (10) | 111 (11) | 114 (10) | 115 (9) | 0.35  |
| SBP standing (mm Hg)     | 116 (13) | 115 (14) | 115 (14) | 119 (11) | 0.35  |
| DBP supine (mm Hg)       | 64 (10) | 64 (11) | 64 (11) | 63 (7)  | 0.93  |
| DBP standing (mm Hg)     | 75 (12) | 74 (12) | 75 (12) | 76 (10) | 0.82  |

Abbreviations: BMI, body mass index; BPI, Brief Pain Inventory; CFQ, Chalder Fatigue Questionnaire; COMT, catechol-O-methyltransferase; DBP, diastolic blood pressure; FDI, Functional Disability Inventory; KSQ, Karolinska Sleep Questionnaire; NorCAPITAL, Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial; PedsQL, Pediatric Quality of Life Inventory; SBP, systolic blood pressure. Numbers are mean (s.d.) unless otherwise indicated.

Figure 1. Differences in the primary outcome Steps Taken between 8 weeks of treatment and baseline as a function of treatment allocation and COMT genotype. COMT, catechol-O-methyltransferase.
positive response of val/val individuals randomized to placebo treatment was consistent across all the secondary outcomes, whereas there was more variability among individuals with at least one met allele.

We explored the possibility of nonlinear interaction of genotype with treatment allocation across the primary and secondary end points. Although we observed no such interaction for CFQ ($P = 0.4$), FDI ($P = 0.4$) or BPI ($P = 0.7$), we found borderline or significant effects for the primary outcome Steps Taken ($P = 0.06$), KSQ ($P = 0.03$) the PedsQL ($P = 0.07$), suggesting that heterozygotes may differ from both val and met homozygotes in their responses to clonidine or placebo.

Table 2. Correlation between outcomes in the NorCAPITAL Trial at week 8 ($R$-values and $P$-values, unadjusted)

|          | Steps | KSQ | CFQ | FDI | PedsQL | BPI |
|----------|-------|-----|-----|-----|--------|-----|
| Steps    | 1.00  |     |     |     |        |     |
| KSQ      | 0.14* | 1.00|     |     |        |     |
| CFQ      | 0.15* | 0.31| 1.00|     |        |     |
| FDI      | 0.04  | 0.02| 0.34| 1.00|        |     |
| PedsQL   | 0.36  | 0.44| 0.38| 0.74| 1.00   |     |
| BPI      | 0.11  | 0.06| 0.19| 0.36| 0.42   | 1.00|

Abbreviations: BPI, Brief Pain Inventory; CFQ, Chalder Fatigue Questionnaire; FDI, Functional Disability Inventory; KSQ, Karolinska Sleep Questionnaire; NorCAPITAL, Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial; PedsQL, Pediatric Quality of Life Inventory; Steps, Steps Taken. *Correlation coefficients. **P-values.

Catecholamines and orthostatic tolerance

**Norepinephrine.** Clonidine treatment effectively reduced norepinephrine levels across all COMT genotypes compared with placebo ($\beta$ (s.e.) = $-61(18)$, $P = 0.001$) (Figure 3a). Val/val individuals randomized to clonidine treatment had the lowest levels of norepinephrine (101 ± 40 nmol) and met/met individuals the highest (126 ± 87 nmol). The val/val by treatment interaction effect was nonsignificant ($\beta$ (s.e.) = $-0.64 (47.2)$, $P_{\text{int}} = 0.99$).

**Epinephrine.** Clonidine treatment reduced epinephrine levels in all three genotype subsets ($\beta$ (s.e.) = $-6.3 (2.7)$, $P = 0.024$) relative to placebo.

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to placebo treatment (Figure 3b). Again, the val/val by treatment interaction effect was nonsignificant ($\beta$ (s.e.) = 4.2 (7.2), $P_{int} = 0.6$).

**Orthostatic tolerance.** Individuals homozygous for the val allele in the clonidine treatment arm had a decrease in orthostatic tolerance compared with those in the placebo arm ($\beta$ (s.e.) = -11.5 (5.0), $P = 0.02$) (Figure 4). The test for a nonlinear interaction of genotype with treatment allocation for orthostatic tolerance was significant ($P = 0.02$), suggesting that heterozygotes differed from both val and met homozygotes in their responses to clonidine or placebo. Individuals heterozygous for the val/met alleles had improved orthostatic tolerance, whereas there was no change in individuals homozygous for the met allele in the clonidine arm compared with placebo treatment.

**DISCUSSION**

To our knowledge, this is the first report to examine the effect of genetic variation in COMT on clonidine treatment outcomes. Furthermore, this is the first study to explore genetic effects on both drug and placebo responses. Here, we demonstrate that genetic variation at COMT rs4680 modified outcomes in both the clonidine and placebo treatment arms of the NorCAPITAL trial. Specifically with respect to the primary outcome, Steps Taken, individuals homozygous for the COMT rs4680 high-activity val allele took on average 1500 fewer steps in the clonidine treatment arm versus 1000 more steps in the placebo arm after 8 weeks of treatment. In addition, val/val subjective outcomes were consistently inferior in individuals randomized to clonidine treatment and superior in those randomized to placebo as compared with all the other participants across treatment arms and across the broad range of clinical measures assessed. This robust relationship between val/val genotype and poor clonidine versus beneficial placebo outcomes remained after controlling for age, sex and body mass index. Taken together, these findings suggest that for adolescents with the COMT val/val genotype, treatment with clonidine for CFS would not be recommended, although the possibility of modest benefit among individuals with other genotypes remains open.

As a centrally acting $\alpha_2$-adrenergic receptor agonist, clonidine is broadly used in the treatment of hypertension and often in the treatment of children with ADHD and as a mild sedative. In NorCAPITAL, the expectation was that the enhanced sympathetic nervous activity reported in adolescents with CFS, and associated higher levels of the catecholamines norepinephrine and epinephrine, would be blunted by clonidine treatment. However, the findings suggest that clonidine may not be as effective for adolescents with the COMT val/val genotype.
epinephrine compared with healthy individuals, would be restored to normal levels by the sympathetic effects of clonidine. However, despite the reduction in catecholamines observed in individuals treated with clonidine, orthostatic tolerance in individuals homozygous for the val allele worsened. Clonidine-induced decrease in the already lower epinephrine and norepinephrine levels of individuals homozygous for the COMT high-activity val allele could mechanically explain its detrimental effects on symptoms of CFS in these individuals. Alternatively, the potent negative effects of clonidine could be attributed to reduced ‘competition’ for the $\alpha_2$-adrenergic receptor by endogenous norepinephrine in individuals homozygous for the COMT high-activity val allele. In contrast, met/met and val/met adolescents did not display this negative effect, perhaps because of their higher baseline sympathetic activity. Thus, these data suggest that high levels of catecholamines might be compensatory rather than disease promoting in CFS pathophysiology.

The possibility exists that the effects observed here might be attributed to other genetic loci. Clonidine treatment effects in irritable bowel syndrome and hypertension studies were shown to be modified by genetic variation in two other genes involved in adrenergic signaling: the $\alpha_2$A-adrenergic receptor (ADRA2A) that regulates signal transduction between receptors like ADRA2A. Given that COMT is located on chromosome 22 and ADRA2A and GBNR are on chromosomes 10 and 12, respectively, the clonidine effect modification reported here is not likely related to these genes. Our results suggest that a broader examination of the effects of COMT genetic variation on clonidine in other conditions, such as hypertension, pain or attention deficit hyperactivity disorder, is warranted.

Few studies have examined drug–placebo interaction effects. Here, in the absence of a no-treatment control, interpretation of the placebo response is limited. Still, it is important to consider the mechanism underlying the consistently robust response of individuals with the val/val genotype randomized to placebo as compared with the negative responses of those randomized to clonidine. Neuroimaging studies have demonstrated that dopamine signaling is activated in response to placebo treatment, and there is evidence that genetic variation in COMT modifies individual response to placebo treatment. Clonidine has been shown to inhibit dopamine activation in brain regions implicated in placebo response, and therefore we hypothesized that clonidine might also modify the placebo response. The negative clonidine and positive placebo responses observed in val/val homozygotes in NorCAPITAL support a potential drug–placebo interaction. Thus, the findings reported here suggest exploring drug–placebo interactions and their modification by genetic variation may indeed be important to developing precision medicines.

Possibly because of the higher prevalence of the met/met genotype among adolescents with CFS, val/val subjects may have been slightly underrepresented in this study. The number of val/met subjects in the clonidine treatment arm was particularly small and therefore posed a limitation to this study. Despite this, we found clear and consistent indication for an association between the COMT val allele and reduced benefit or even side effects inherent to treatment with clonidine. By testing for clonidine levels in participants at the end of the trial, we were able to confirm that differences in outcomes were not attributed to differential adherence to treatment. The use of quantitative, physiological and subjective measures in this study make our finding that COMT is associated with clinical outcomes more robust and speak to the broad effects on symptoms and functioning of clonidine treatment in these val/val adolescents.

The findings here add to a growing list of drugs and diseases that are affected by genetic variation in COMT for benefit and potentially harm. This study further demonstrates how genetic analysis can influence clinical trial findings and highlights the important role of pharmacogenetics in gaining insight into treatment response and precision medicine, as well as shedding light on disease mechanisms. Future studies should investigate the generalizability of these findings to other treatments that involve clonidine and other drugs that may likely be influenced by genetic variation in COMT such as drugs that target $\alpha$- and $\beta$-adrenergic receptors or signaling in placebo-related pathways.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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