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

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Abstract

Clonidine, an α_2 -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 to placebo ($p_{\text{interaction}}=0.04$). There were no differences between clonidine and placebo amongst patients with *COMT* low-activity

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alleles. Similar gene-drug interactions were observed for sleep ($p_{\text{int}}=0.003$) and quality of life ($p_{\text{int}}=0.018$). 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.

Introduction

Genetic variation in *COMT*, the locus that encodes catechol-O-methyltransferase, an enzyme which 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, 9–12) 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.

Chronic fatigue syndrome (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 to 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 amongst adolescents with CFS (8).

Clonidine is a centrally acting α_2 -adrenergic receptor agonist which attenuates sympathetic nervous activity, decreasing circulating norepinephrine and epinephrine levels while increasing parasympathetic nervous activity (26). Clonidine has also been shown to inhibit dopamine activation in the brain (27, 28). The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL), was a randomized placebo controlled trial (RCT) designed to study the effects of short-term low-dose clonidine treatment for CFS among adolescents (29). In NorCAPITAL low-dose clonidine was found to reduce catecholamine levels and attenuate markers of sympathetic outflow and systemic inflammation; however clonidine treatment failed to provide greater benefit than placebo (30).

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. Further, 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 Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL) trial have been published elsewhere (29). Briefly, between March 1, 2010, and October 15, 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. Written informed consent was obtained from all participants or from parents or next of kin as required.

Clonidine doses were based on a pilot study (31), and were designed to be in the lower range of what is considered clinically effective (25 µg or 50 µg for body weight <35 kg or >35 kg, respectively twice daily). The treatments were double-blinded. After 8 weeks of treatment, Steps Taken, the primary outcome, defined as mean number of steps per day, was monitored during 7 consecutive days with an accelerometer device. An incremental increase of 2,500 steps per day was considered clinically significant improvement.

Five validated inventories were selected *a priori* as secondary outcomes from the NorCAPITAL trial to assess the impact of clonidine versus placebo treatment on the patient's symptoms and quality of life (29). Briefly, functional disability was measured using the Functional Disability Inventory (FDI). The FDI, designed as a global measure of children's and adolescent's physical and psychosocial functioning in everyday social roles, consists of 15 items, which are scored on a 0–4 Likert scale (32). The score represents the total sum across all items, hence the total range is from 0 to 60; higher scores indicate more severe disability. Fatigue was assessed using the Chalder Fatigue Questionnaire (CFQ) (33), a validated measure for assessing CFS in adolescents (34). The 11 CFQ items are scored on a 0–3 Likert scale. The range of the sum total score is from 0 to 33; higher scores indicate more severe fatigue. Sleep disturbances were assessed using the Karolinska Sleep Questionnaire (KSQ) (35). The KSQ sleepiness subscale averages four items addressing sleep problems during the previous month and is scored on a 1–6 Likert scale. The total ranges from 1 to 24 with a lower score indicating poorer sleep. The Pediatric Quality of Life (PedsQL) Inventory combines measures of physical, emotional, social and school functioning to assess the patient's health-related quality of life (36). The Brief Pain

Inventory (BPI) (37) consists of single items addressing different aspects of pain during the past week on 0–10 Likert scales; higher scores represent more severe pain.

Laboratory Analyses

Clonidine steady-state concentration—As previously described, the plasma clonidine concentration was measured 3 and 8 weeks after the initiation of therapy (29). The trough level was estimated as the plasma concentration 12 hours after the last dosage. The mean trough value was taken as an estimate of clonidine steady-state concentration during the intervention period.

Epinephrine and norepinephrine—Urine samples were processed as previously described (30). Briefly, urine was acidified immediately after collection and samples were analyzed using high-performance liquid chromatography (HPLC) with a reversed-phase column and glassy carbon electrochemical detector (Antec, Leyden Deacade II SCC, Zoeterwoude, The Netherlands) using a Chromsystems commercial kit (Chromsystems, München, Germany).

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 SBP supine and SBP 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%.

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 BMI (38). 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 3 categories). To test interactions on the non-linear 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 (8 weeks – 0 weeks) jointly stratified by genotype and treatment allocation are presented here. Differences in clonidine levels in individuals randomized to clonidine

treatment were analyzed by ANOVA. The normal distributions of all the outcomes or the residuals of week 8 compared to week 0 were confirmed using the Shapiro-Wilk Test.

Using Spearman 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 (HWE) was calculated using the Online Encyclopedia for Genetic Epidemiology studies (39). Statistical analyses were performed using SAS 9.3 and JMP Pro 12.0.1 (SAS Institute Inc., Cary, NC).

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 3 *COMT* genotypes in the clonidine treatment arm ($p=0.5$), mean(SD) ug/L: met/met=0.25(0.09), val/met=0.23(0.11), val/val=0.23(0.08).

Outcomes

Steps Taken—In the unadjusted model of the primary outcome Steps Taken, individuals homozygous for the val allele (val/val) randomly allocated to clonidine took approximately 1,500 fewer steps than baseline, in contrast those allocated to placebo took 1,000 more steps after 8 weeks of treatment (Figure 1A and B). This finding was consistent with a significant val/val by clonidine treatment interaction ($\beta[\text{SE}] = -2026[990]$; $p_{\text{interaction}}=0.043$). In contrast, differences between clonidine and placebo in heterozygote individuals and individuals homozygous for the met allele were non-significant. In the placebo arm, individuals homozygous for the val allele had the greatest placebo response compared to those with at least one met allele (val/met and met/met), but this difference was non-significant (Figure 1A). The results were essentially equivalent when the models were adjusted for age, gender and BMI ($\beta[\text{SE}] = -2215[985]$; $p_{\text{interaction}}=0.027$).

Subjective outcomes—Table 2 shows the Spearman correlations amongst the five 8-week subjective outcomes and Steps Taken. Absolute correlations ranged from 0.11–0.74, suggesting these inventories represent complementary but incompletely overlapping domains, as expected. Consistent with the findings for Steps Taken, the unadjusted model of Karolinska Sleep Questionnaire (KSQ) also revealed a significant difference between val/val individuals treated with clonidine compared to placebo and heterozygous individuals (val/met) ($\beta[\text{SE}] = -1.25[0.42]$, $p_{\text{int}}=0.003$), 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[SE] = -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 to placebo (Figure 2B). The results were essentially equivalent when the models were adjusted for age, gender and BMI: KSQ (beta[SE] = -1.15[0.40]; $p_{\text{int}}=0.01$) and PedsQL (beta[SE] = -12.1[5.7], $p_{\text{int}}=0.04$). The direction of the *COMT* val/val by treatment interaction effects for CFQ (beta[SE]= 2.3[3.0], $p_{\text{int}}=0.43$), FDI (beta[SE] = 5.5[4.4], $p_{\text{int}}=0.22$) and BPI (beta[SE] = -0.18[1.00], $p_{\text{int}}=0.86$), (Figure 2C, D and E) were qualitatively consistent with the other three outcomes, but these effects were non-significant. The strong positive response of val/val individuals randomized to placebo treatment was consistent across all the secondary outcomes, whereas there was more variability amongst individuals with at least one met allele.

We explored the possibility of non-linear interaction of genotype with treatment allocation across the primary and secondary endpoints. 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.

Catecholamines and orthostatic tolerance

Norepinephrine: Clonidine treatment effectively reduced norepinephrine levels across all *COMT* genotypes compared to placebo (beta[SE]= -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 non-significant (beta[SE]= -0.64[47.2], $p_{\text{int}}=0.99$).

Epinephrine: Clonidine treatment reduced epinephrine levels in all three genotype subsets (beta [SE]= -6.3[2.7], $p=0.024$) relative to placebo treatment (Figure 3B). Again the val/val by treatment interaction effect was non-significant (beta[SE]= 4.2 [7.2], $p_{\text{int}}=0.6$).

Orthostatic tolerance: Individuals homozygous for the val allele in the clonidine treatment arm had a decrease in orthostatic tolerance compared to those in the placebo arm (beta[SE]= -11.5[5.0], $p=0.02$) (Figure 4). The test for a non-linear 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 to 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 1,500 fewer steps in the clonidine treatment arm versus 1,000 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, compared to 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 BMI. 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 α_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 (40). In NorCAPITAL, the expectation was that the enhanced sympathetic nervous activity reported in adolescents with CFS (18), and associated higher levels of the catecholamines norepinephrine and epinephrine compared to healthy individuals, would be restored to normal levels by the sympatholytic 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 mechanistically 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 due to 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 α_2A -adrenoreceptor (*ADRA2A*) (41), and the beta subunit of guanine nucleotide-binding protein (*GNBR*) (42) which regulates signal transduction between receptors like *ADRA2A*. Given that *COMT* is located on chromosome 22 and *ADRA2A* and *GNBR* 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 (43). 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, compared to the negative responses of those randomized to clonidine. Neuroimaging studies have demonstrated that dopamine signaling is activated in response to placebo treatment (44), and there is evidence that genetic variation

in *COMT* modifies individual response to placebo treatment (13, 14). Clonidine has been shown to inhibit dopamine activation in brain regions implicated in placebo response (27, 28); 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 amongst adolescents with CFS (8), val/val subjects may have been slightly underrepresented in this study. The number of val/val 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 α - and β -adrenergic receptors or signaling in placebo related pathways.

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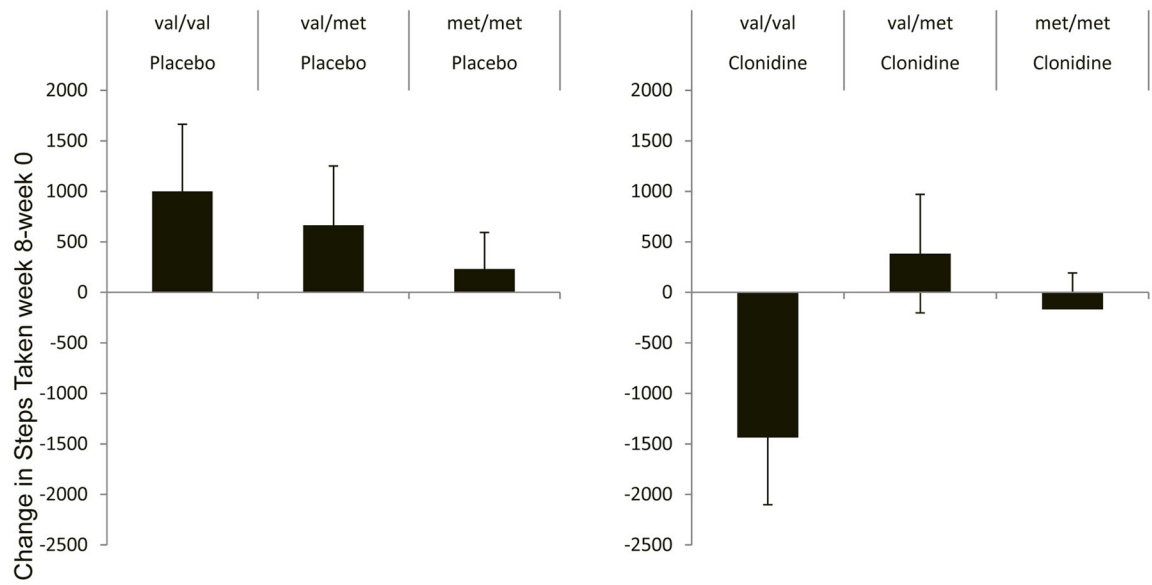


Figure 1. Differences in the primary outcome Steps Taken between 8-weeks of treatment and baseline as a function of treatment allocation and *COMT* genotype. p_{int} = p value for the effect estimate of the interaction between val/val and clonidine versus placebo allocation.

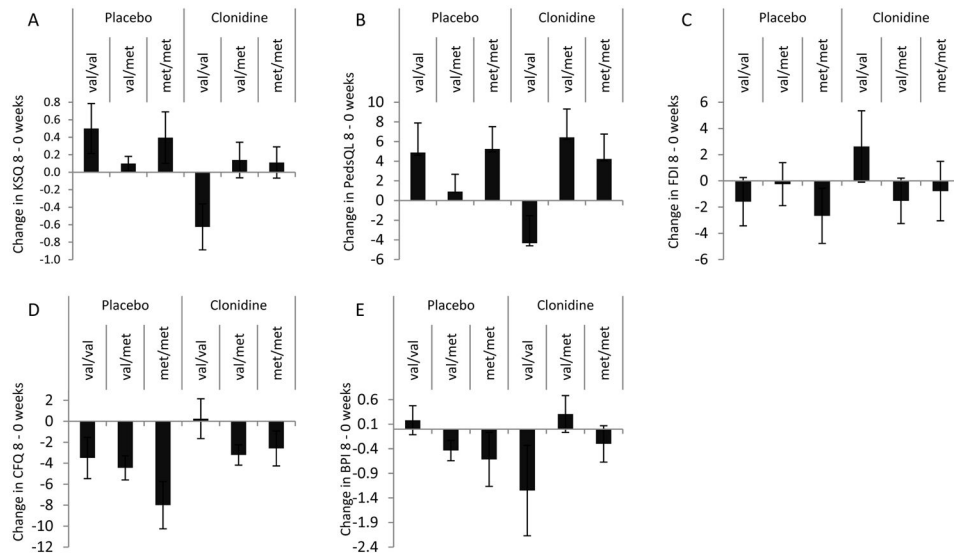


Figure 2.

Differences in subjective outcome measures between 8-weeks of treatment and baseline as a function of treatment allocation and *COMT* genotype. (A) Karolinska Sleep Questionnaire (KSQ), (B) Pediatric Quality of Life (PedsQL), (C) Functional Disability Inventory (FDI), (D) Chalder Fatigue Questionnaire (CFQ) and (E) Brief Pain Inventory (BPI). p_{int} = p value for the effect estimate of the interaction between val/val and clonidine versus placebo allocation.

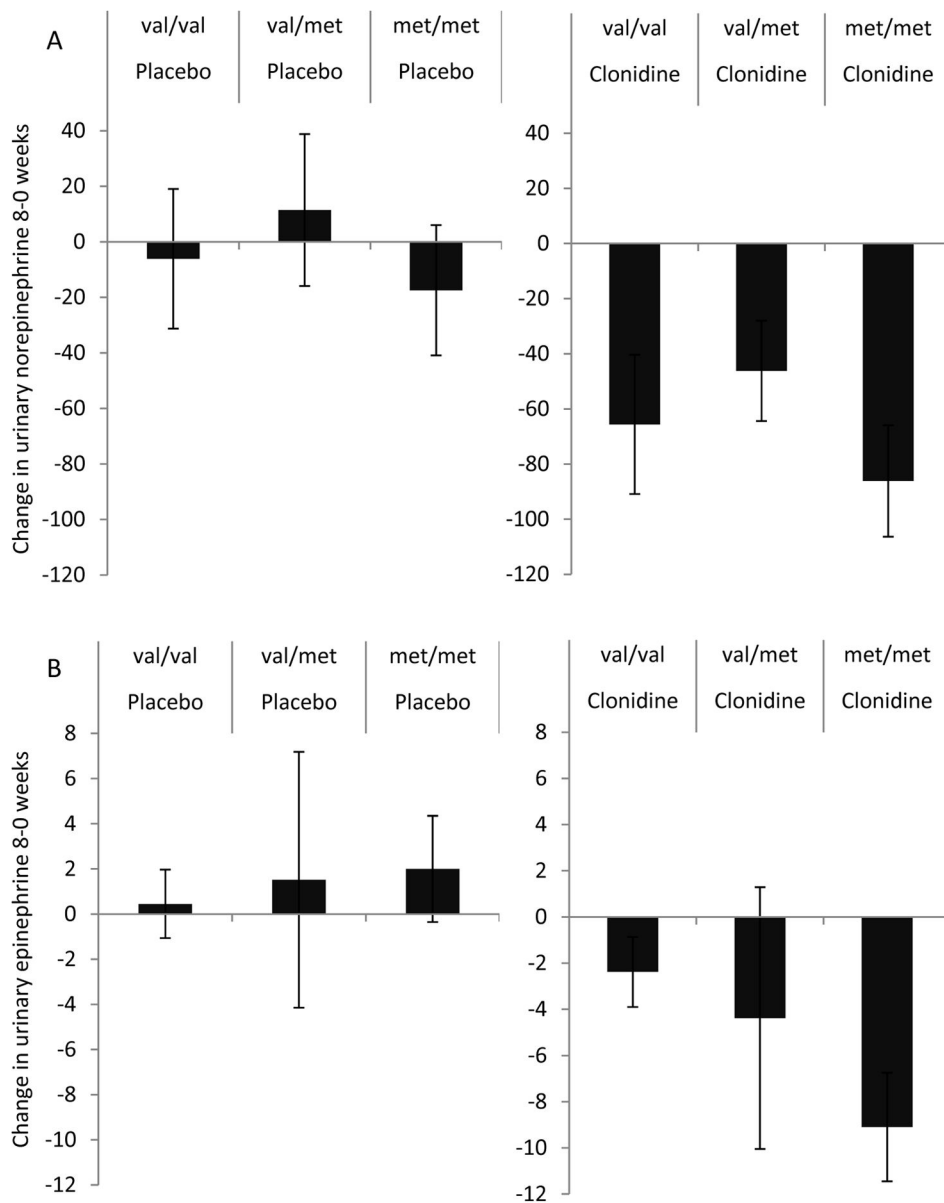


Figure 3. Change from baseline in norepinephrine (A) and epinephrine (B) levels (nM) after 8-weeks of treatment as a function of *COMT* genotype and treatment allocation. p_{int} = p value for the effect estimate of the interaction between val/val and clonidine versus placebo allocation.

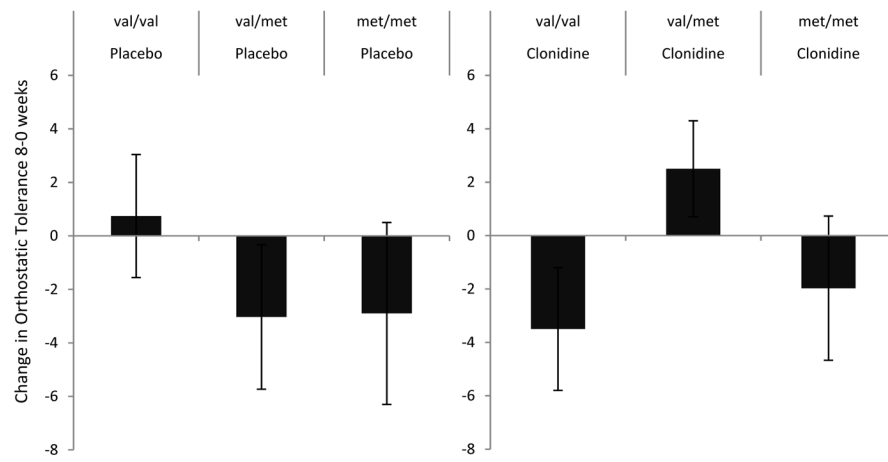


Figure 4. Change from baseline in orthostatic tolerance after 8-weeks of treatment as a function of treatment allocation and *COMT* genotype. p_{int} = p value for the effect estimate of the interaction between val/val and clonidine versus placebo allocation.

Table 1

Baseline demographics and measures of all genotyped patients (N=104) and genotyped patients by *COMT* rs4680 genotype in the NorCAPITAL trial. Numbers are mean (SD) unless otherwise indicated.

<u>Demographics</u>	All	val/val	val/met	met/met	P
N	104	20	51	33	
Age (years)	15.4 (1.6)	15 (1.7)	15.3 (1.6)	15.8 (1.4)	0.16
Female (%)	72	65	72	76	0.70
BMI (kg/m ²)	21.5 (4.1)	20.3 (4.3)	22.1 (4.4)	21.3 (3.5)	0.24
<u>Baseline Measures</u>					
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 (nM)	185 (86)	159 (80)	189 (80)	195 (99)	0.33
Epinephrine (nM)	20 (12)	17 (8)	22 (14)	21 (11)	0.31
SBP supine (mmHg)	113 (10)	111 (11)	114 (10)	115 (9)	0.35
SBP standing (mmHg)	116 (13)	115 (14)	115 (14)	119 (11)	0.35
DBP supine (mmHg)	64 (10)	64 (11)	64 (11)	63 (7)	0.93
DBP standing (mmHg)	75 (12)	74 (12)	75 (12)	76 (10)	0.82

Abbreviations in alphabetical order: BMI, body mass index; BPI, Brief Pain Inventory; CFQ, Chalder Fatigue Questionnaire; DBP, diastolic blood pressure; FDI, Functional Disability Inventory; KSQ, Karolinska Sleep Questionnaire; PedsQL, Pediatric Quality of Life Inventory; SBP, systolic blood pressure.

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				
	0.15 †					
CFQ	-0.13	-0.31	1.00			
	0.19	0.002				
FDI	-0.42	-0.31	0.34	1.00		
	<0.0001	0.001	0.001			
PedsQL	0.36	0.44	-0.38	-0.74	1.00	
	0.0002	<0.0001	<0.0001	<0.0001		
BPI	0.11	-0.34	0.19	0.36	0.42	1.00
	0.26	<0.001	0.06	<0.001	<0.0001	

Abbreviations in alphabetical order: BPI, Brief Pain Inventory; CFQ, Chalder Fatigue Questionnaire; FDI, Functional Disability Inventory; KSQ, Karolinska Sleep Questionnaire; PedsQL, Pediatric Quality of Life Inventory; Steps, Steps Taken.

* Correlation coefficients and

† p-values