Polymorphisms in Catechol-\(\text{O}\)-Methyltransferase Modify Treatment Effects of Aspirin on Risk of Cardiovascular Disease


Objective—Catechol-\(\text{O}\)-methyltransferase (COMT), a key enzyme in catecholamine metabolism, is implicated in cardiovascular, sympathetic, and endocrine pathways. This study aimed to confirm preliminary association of COMT genetic variation with incident cardiovascular disease (CVD). It further aimed to evaluate whether aspirin, a commonly used CVD prevention agent, modified the potential association of COMT with incident CVD.

Approach and Results—We examined COMT polymorphism rs4680 (MAF [minor allele frequency], 0.47), encoding a nonsynonymous methionine-to-valine substitution, in the Women’s Genome Health Study (WGHS), a large population-based cohort of women with randomized allocation to aspirin or vitamin E when compared with placebo and 10-year follow-up. Rs4680 effects were confirmed with COMT polymorphism rs4818 and also examined in Coronary Artery Disease Genome-wide Replication and Meta-analysis/The Coronary Artery Disease Genetics Consortium, consortia for genome-wide association studies of coronary artery disease. Among WGHS women allocated to placebo (135 events/45811), the rs4680 valine allele was protective against incident CVD relative to the methionine (hazard ratio [HR]; 95% confidence interval [CI]), 0.66 [0.51–0.84]; \(P=0.007\); an association also observed in Coronary Artery Disease Genome-wide Replication and Meta-analysis and The Coronary Artery Disease Genetics Consortium (combined \(P=2.4\times10^{-8}\)). In the WGHS, the rs4680 association was abolished by randomized allocation to aspirin, such that valine/valine women experienced higher CVD rates with aspirin allocation when compared with placebo (HR [95% CI], 1.85 [1.05–3.25]; \(P=0.033\)), whereas methionine/methionine women experienced lower rates (HR [95% CI], 0.60 [0.39–0.93]; \(P=0.023\)). Allocation to vitamin E also conferred higher but nonsignificant CVD rates on valine/valine (HR [95% CI], 1.50 [0.83–2.70]; \(P=0.180\)) when compared with significantly lower rates on methionine/methionine (HR [95% CI], 0.53 [0.34–0.84]; \(P=0.006\)) women. Rs4818 results were similar.

Conclusions—Common COMT polymorphisms were associated with incident CVD, and this association was modified by randomized allocation to aspirin or vitamin E. Replication of these findings is required. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: aspirin  catecholamines  vitamin E

The catecholamines, epinephrine, norepinephrine, dopamine, and catechol estrogen play a critical role in cardiovascular, sympathetic, and endocrine pathways. Variation in the levels of these signaling molecules is implicated in a broad spectrum of disorders, including cardiovascular conditions (ie, acute coronary syndrome,\(^1\) stress cardiomyopathy,\(^2\) hyperhomocysteinemia,\(^3\) and preeclampsia).\(^4\) The enzyme catechol-\(\text{O}\)-methyltransferase (COMT) modulates the function of catecholamines. We wanted to confirm preliminary evidence that genetic variation in COMT might affect susceptibility to cardiovascular disease (CVD).\(^5,6\) Aspirin is commonly prescribed for CVD prevention. It is not known whether any potential association of genetic variation in COMT with incident CVD might be modified by aspirin treatment.

COMT degrades catecholamines by catalyzing the transfer of a methyl group donated by S-adenosyl methionine onto catechol moieties, resulting in their deactivation. The COMT genetic variant rs4680 (val158met) is an extensively studied...
single nucleotide polymorphism (SNP) that encodes a valine (G)-to-methionine (A) substitution at amino acid 158 in the membrane form and 108 in the secreted form of the enzyme. This functional polymorphism results in the methionine variant having a 3- to 4-fold lower enzymatic activity than the valine variant, and is, therefore, inversely correlated with ant having a 3- to 4-fold lower enzymatic activity than the 3- to 4-fold lower enzymatic activity.17

In addition, several small population-based studies have found genetic variation in COMT to be associated with coronary heart disease and hypertension in men and women, as well as a series of clinical outcomes some of which are shared with rs4680.13

Aspirin is the gold standard for antiplatelet therapy and is widely prescribed because it is considered a safe treatment for CVD prevention. Despite demonstrated benefit of aspirin in primary and secondary CVD prevention, particularly among men, the Women’s Health Study (WHS), a large placebo-controlled trial (n=39876) of aspirin in primary prevention among initially healthy, middle-aged women found only a 9% nonsignificant reduction of major CVD events when compared with placebo 10 years of follow-up.17 Given that aspirin like catecholamines interacts with multiple pathways to affect CVD (eg, platelet activation), we hypothesized that genetic variation in COMT might also affect response to aspirin treatment for prevention of major CVD. In the Women’s Genome Health Study (WGHS), a subset of the WHS for genome-wide genetic analysis, we therefore, performed a candidate association study of COMT SNPs rs4680 and rs4818 for association with incident CVD and potential interaction with randomized allocation to placebo or aspirin. The 2x2 factorial design of the WHS also allowed exploration of the association of COMT and incident CVD in women randomly allocated to vitamin E.

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results
The primary population for the study is the WGHS, a large prospective cohort for genetics of CVD derived from the WHS, a randomized trial of aspirin and vitamin E when compared with placebo in a balanced 2x2 factorial design. Random allocation to aspirin or vitamin E in the WHS allowed exploration of the association of genetic variation in COMT with incident CVD in the 4 treatment arms: placebo plus placebo (n=5811), aspirin plus placebo (n=5810), vitamin E plus placebo (n=5856), and aspirin plus vitamin E (n=5796; Methods in the online-only Data Supplement).

Demographics and baseline characteristics in this study did not differ by treatment arm as expected by randomization (Table I in the online-only Data Supplement). COMT SNPs rs4680 (val158met) and rs4818 were found to be in Hardy Weinberg equilibrium, and minor allele frequencies of rs4680 (G or valine) and rs4818 (G) were 0.47 and 0.39, respectively. In the WGHS, the linkage disequilibrium between these SNPs was moderate (r2=0.70; D’=1.00).

Among WGHS participants allocated exclusively to placebo (n=5811), there was a 34% lower age-adjusted incidence rate of the primary outcome, major CVD, associated with each additional valine allele in the COMT rs4680 (val158met) polymorphism (hazard ratio [HR; 95% confidence interval (CI)], 0.66 [0.51–0.84]; P=0.0007; Table 1; Figure 1). The valine allele was also associated with a decreased rate of age-adjusted secondary outcomes of total CVD (HR [95% CI], 0.77 [0.63–0.93]; P=0.0075) and myocardial infarction (HR [95% CI], 0.60 [0.41–0.90]; P=0.0130) but not of stroke (HR [95% CI], 0.76 [0.53–1.09]; P=0.14) or coronary heart disease (HR [95% CI], 0.81 [0.63–1.04]; P=0.103; Table 1; Figure 1).

In Coronary Artery Disease Genetics Consortium (C4D), a large-scale meta-analysis of 13 studies consisting of 22333 cases of coronary artery disease (CAD), a composite including myocardial infarction, revascularization, angina, and angiographic stenosis cases, and 64762 controls, the rs4680 valine allele, was associated with a decreased rate of CAD (odds ratio [95% CI], 0.960 [0.935–0.988]; P=0.0047; Table 2). Among the additional and independent 34 CAD sample collections of The Coronary Artery Disease Genetics Consortium (C4D), comprising 41513 cases and 65919 controls of European or South Asian ancestry, the association was also significant, with the valine allele again conferring protection (odds ratio [95% CI], 0.964 [0.943–0.986]; P=0.0017). Overlapping CIs of the effect estimates in the sex stratified cohort suggested that there was no significant difference between men and women (Table II in the online-only Data Supplement). In a meta-analysis of CARDIoGRAM plus C4D, the significance of the association was P=5.2×10−6. Despite the consistency of the risk allele across all 3 studies, the evident heterogeneity in the COMT SNP effect (ie, HR, 0.66 in WGHS [Table 1]; odds ratio, 0.96...
CARDiogram; odds ratio, 0.96 CVD [Table 2]) precluded the possibility of meta-analysis.

In the whole WGHS cohort, univariate analyses of rs4680 and 14 cardiovascular biomarkers measured at baseline revealed significant associations after correction for multiple hypothesis testing (P<0.05/14 risk factors). At baseline, (log) triglycerides (β [SE], −0.018 [0.005]; P=0.0004) and systolic blood pressure (β [SE], −0.367 [0.127] mm Hg; P=0.004) were significantly associated with the valine allele, consistent with less cardiovascular risk for both risk factors (Table III in the online-only Data Supplement). Similarly, the minor allele of rs4818 was significant for (log) triglycerides (β [SE], −0.018 [0.005]; P=0.0004) and nominally significant for systolic blood pressure (β [SE], −0.335 [0.130] mm Hg; P=0.01), as well as apolipoprotein B (β [SE], −0.631 [0.282] μmol/L; P=0.025) and soluble intracellular adhesion molecule 1 (β [SE], −1.707 [0.837] μmol/L; P=0.041). Despite these associations, rs4680 and rs4818 associations with incident major CVD were essentially unaffected in Cox models further adjusted by these risk factors (Table 1, fully adjusted models and data not shown). COMT rs4680 associations with triglycerides (P=0.028) and systolic blood pressure (P=0.0059) were confirmed in published results from the Global Lipids Genetics Consortium and the International Consortium for Blood Pressure Genome-Wide Association Studies, respectively.

The 2×2 design of the WGHS allowed us to evaluate how random allocation to aspirin or vitamin E might influence the association of COMT with incident CVD. Among WGHS participants randomly allocated exclusively to aspirin, the protective rs4680 valine allele association was not observed (HR [95% CI], 1.13 [0.88–1.45]; P=0.34; Table 3; Figure 2A). Comparison of these aspirin-allocated participants with those randomly allocated exclusively to placebo revealed a significant interaction between rs4680 and aspirin (P=0.0022). Similarly, random allocation exclusively to vitamin E also abolished the association of rs4680 with incident CVD (HR [95% CI], 1.08 [0.84–1.38]; P=0.52), revealing a significant interaction of rs4680 with vitamin E allocation when compared with placebo-only allocation (P=0.004; Table 3; Figure 2B). Results for rs4818 were similar (Table 3; Figure II in the online-only Data Supplement).

The consequences of the aspirin and vitamin E allocation on risk differed by rs4680 genotype. For COMT rs4680 methionine allele homozygotes, exclusive allocation to aspirin or vitamin E compared with placebo resulted in age-adjusted lower rates of incident CVD of 40% (95% CI, −7% to −61%; P=0.023) and 47% (95% CI, −16% to −68%; P=0.006; Table 4), respectively. In contrast, valine allele homozygotes had higher CVD rates of 85% (95% CI, 5% to 325%; P=0.033) and 50% (95% CI, −17% to 170%; P=0.18), respectively, although only the aspirin allocation increase was significant. Among heterozygotes, allocation to either aspirin or vitamin E did not affect the incidence rate. For methionine allele homozygotes allocated to both aspirin and vitamin E, the difference in rates compared with those allocated to placebo was not significant, implying a further interaction for allocation to both agents compared with either alone. This further interaction was...
significant ($P=0.006$; Table 4). Fully adjusted models revealed similar effects except for diminished significance of the effect of aspirin on methionine allele homozygotes ($P=0.1$, data not shown). Results for rs4818 were similar (Table 4; Figure II in the online-only Data Supplement).

**Discussion**

This is the first study to show a significant association of the COMT rs4680 (val158met) polymorphism with incident CVD in a population-based sample of women. The COMT valine allele conferred a lower rate of events in the prospective setting of the WGHS when compared with the methionine allele. The association was significantly replicated in 2 additional studies, CARDIoGRAM$^{21}$ and C4D,$^{26}$ and was also demonstrated for the first time in any study with the COMT rs4818, a SNP in partial linkage disequilibrium with rs4680. Furthermore, we found that randomized allocation to aspirin eliminated the COMT valine allele protective association with CVD, resulting in an 85% increase in the rate of incident CVD for rs4680 valine allele homozygotes allocated to aspirin when compared with placebo. Conversely, a 40% decrease in the rate of incident CVD was observed for the methionine allele homozygotes allocated to aspirin when compared with that allocated

![Figure 1. Kaplan–Meier estimates of the cumulative incidence of Women’s Genome Health Study women in the placebo arm ($n=5811$) according to COMT rs4680 genotype with a first ever A, major cardiovascular disease (CVD), B, total CVD, C, myocardial infarction, D, ischemic stroke, E, and coronary heart disease (CHD) event. Legends indicate genotype strata and number of cases/total number in each stratum. met indicates methionine; and val, valine.](http://atvb.ahajournals.org/
to placebo. Randomized allocation to vitamin E also modified the COMT CVD association as a nonsignificant increase in the rate of incident CVD in valine allele homozygotes when compared with placebo and a significant 47% rate reduction for the methionine allele homozygotes. Similar COMT effect modification by both aspirin and vitamin E was observed for another COMT SNP, rs4818.

The treatment effects described here resulting in a reduction in the rate of incident CVD for the 28% of the WGHS population that was homozygous for the methionine allele are modest. Yet, over the ≈10 years of follow-up in the WGHS, they translated into number-needed-to-treat estimates of 91 for aspirin or 74 for vitamin E as compared with 582 or 647, respectively, for the population as a whole. Conversely, for the 23% of the WGHS population that was homozygous for the valine allele, where the drug effects were deleterious, the number-needed-to-harm estimates were 91 for aspirin and 189 for vitamin E, although the effect of vitamin E was not significant. Our findings of differential CVD risk, thus, may be interpreted in the context of personalized medicine in which a subpopulation defined by COMT genotype would be identified for potential benefit or harm by either of these treatments.

Several plausible catecholamine-mediated cardiovascular functions could account for the cardiovascular protection we observed in association with the high activity COMT valine allele. COMT is present in platelets and in endothelial and vascular smooth muscle cells, where the attenuated COMT activity of methionine allele homozygotes could increase catecholamine flux and oxidant stress, thus lowering the threshold for platelet activation and endothelial dysfunction. At the same time, our findings that baseline biomarkers of cardiovascular risk, including triglycerides, systolic blood pressure, soluble intracellular adhesion molecule 1, and apolipoprotein B, were associated with COMT SNPs suggest a potential pleiotropy for cardiovascular risk.

Table 2. Meta-Analysis of COMT-Associated CAD Protection in CARDIoGRAM, C4D, and CARDIoGRAM+C4D

<table>
<thead>
<tr>
<th>SNP*</th>
<th>Stage 1†, CARDIoGRAM</th>
<th>Stage 2‡, C4D</th>
<th>Stage 3§, CARDIoGRAM+C4D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR [SE]; PValue</td>
<td>n</td>
</tr>
<tr>
<td>rs4680</td>
<td>13</td>
<td>0.96 [0.014]; 0.0047</td>
<td>34</td>
</tr>
<tr>
<td>rs4818</td>
<td>9</td>
<td>0.96 [0.016]; 0.0081</td>
<td>NA</td>
</tr>
</tbody>
</table>

C4D indicates The Coronary Artery Disease Genetics Consortium; CAD, cardiovascular disease; CARDIoGRAM, Coronary Artery Disease Genome-wide Replication and Meta-analysis; NA, not applicable; OR, odds ratio; and SNP, single nucleotide polymorphism.

*rs4680 coded allele=G(val), reference allele=A(met); rs4818 coded allele=G, reference allele=C; rs4818 was not available in Stage 2.
†Stage 1: CARDIoGRAM data set consisted of 22,233 CAD cases and 64,762 controls. C4D is a composite of myocardial infarction, revascularization, angina, and angiographic stenosis.
‡Stage 2: Analysis of 34 additional CAD sample collections of European or South Asian descent (41,513 cases and 65,919 controls).
§Stage 3: Meta-analysis of CARDIoGRAM+C4D database.

Table 3. Age-Adjusted Cox Models Relating COMT rs4680 val Allele (met Allele as Reference) and rs4818 G Allele Associations (C Allele as Reference) to Incident Major CVD and total CVD, Stratified by Randomized Treatment Assignment

<table>
<thead>
<tr>
<th>SNP*</th>
<th>Outcome†</th>
<th>Treatment Arm</th>
<th>HR [95% CI]; PValue‡</th>
<th>Gene–drug Int. PValue§</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>Major CVD</td>
<td>Placebo</td>
<td>0.66 [0.51–0.84]; 0.0007</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirin only</td>
<td>1.13 [0.88–1.45]; 0.34</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E only</td>
<td>1.08 [0.84–1.38]; 0.52</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Total CVD</td>
<td>Placebo</td>
<td>0.77 [0.63–0.93]; 0.0075</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirin only</td>
<td>1.10 [0.90–1.40]; 0.23</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E only</td>
<td>1.09 [0.90–1.32]; 0.38</td>
<td>0.011</td>
</tr>
<tr>
<td>rs4818</td>
<td>Major CVD</td>
<td>Placebo</td>
<td>0.67 [0.51–0.86]; 0.0022</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirin only</td>
<td>1.22 [0.95–1.56]; 0.11</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E only</td>
<td>1.03 [0.80–1.32]; 0.80</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Total CVD</td>
<td>Placebo</td>
<td>0.70 [0.57–0.87]; 0.001</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirin only</td>
<td>1.14 [0.93–1.40]; 0.20</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E only</td>
<td>1.06 [0.87–1.29]; 0.57</td>
<td>0.005</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; CVD, cardiovascular disease; HR, hazard ratio; and SNP, single nucleotide polymorphism.

*rs4680 coded allele=G(val), reference allele=A(met); rs4818 coded allele=G, reference allele=C.
†Major CVD, the primary Women’s Health Study outcome is a composite of myocardial infarction, stroke, or death from cardiovascular causes. Total CVD is a composite of revascularization procedures (percutaneous transluminal coronary angioplasty and coronary bypass graft), in addition to events in the primary outcome.
‡HRs refer to SNP associations with incident CVD in designated treatment arms.
§Gene–drug interaction PValue refers to the significance of the difference between the SNP association among placebo allocated Women’s Genome Health Study participants and participants allocated to the designated treatment arm.

Plac clinics also reported in Table 1.
of COMT effects in the pathophysiology underlying its association with incident CVD. In addition to these associated mechanisms, COMT activity may be modified by plasma homocysteine concentrations, thereby potentiating the adverse effects of hyperhomocysteinemia by decreasing catecholamine O-methylation and inactivation: elevated levels of homocysteine lead to an increase in S-adenosylhomocysteine, which is a noncompetitive inhibitor of COMT.\(^3\) Evidence for the effects of this theoretical interaction between COMT activity and homocysteine levels was demonstrated in the Kuopio Ischemic Heart Disease Risk Factor Study,\(^1\) as well as studies of venous thrombosis risk\(^2\) and preeclampsia.\(^5\) Interestingly, a recent trial that examined the effect of aspirin on hyperhomocysteinemia also reported effect modification of aspirin inhibition of platelet aggregation by homocysteine level.\(^3\)

How aspirin modifies CVD protection associated with COMT is not known, but candidate mechanisms include effects on platelet function or homocysteine levels and may support the hypothesis that differential response to aspirin therapy in a variety of settings is a heritable trait.\(^3\) Such genetic effects on aspirin response have precedent in our previous finding that carriers of an apolipoprotein(a) gene variant had a doubling of incident CVD rate and seemed to benefit more from aspirin therapy than noncarriers.\(^3\)

In principle, the lack of an overall effect of vitamin E on CVD risk in the original WHS report\(^19\) does not preclude the possibility that subgroups defined on the basis of genetic strata experience benefit or harm, such as implied by the novel associations reported here. The underlying mechanisms of the COMT–drug interactions are yet to be elucidated. Recent data showing platelet COMT-mediated methylation-dependent inactivation of the common dietary antioxidant, quercetin,\(^3\) may be indicative of a potential mechanism by which vitamin E effects may also depend on COMT genotype and activity. The COMT interaction with vitamin E is hypothesis generating and may potentially offer some insight into the conflicting observations between animal and in vitro studies\(^3\) that support a role for vitamin E in minimizing cardiovascular risk and overall null findings in CVD trials.\(^19\) Thus, mechanistic studies that could account for this differential aspirin and vitamin E treatment effect by genotype are warranted and may include analyses of platelet and vascular cell prostanoid and eicosanoid metabolism, oxidant stress, nitric oxide signaling, and platelet and endothelial function assessment over a wider range of doses. Moreover, the results suggest that any future studies

**Table 4.** Age-Adjusted Cox Models of Major CVD* Stratified by COMT SNP Genotype Within the 3 Drug Treatment Arms Compared With the Placebo Allocated Arm (Reference)

<table>
<thead>
<tr>
<th>Genotype Stratum</th>
<th>Placebo</th>
<th>Aspirin Only</th>
<th>Vitamin E Only</th>
<th>Aspirin+Vitamin E</th>
<th>Drug Int.</th>
<th>(P) Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>met/met</td>
<td>ref.</td>
<td>0.60 [0.39–0.93], 0.02</td>
<td>0.53 [0.34–0.84], 0.006</td>
<td>0.80 [0.53–1.21], 0.30</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>val/met</td>
<td>ref.</td>
<td>0.89 [0.63–1.27], 0.52</td>
<td>1.06 [0.76–1.49], 0.73</td>
<td>0.91 [0.64–1.29], 0.58</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>val/val</td>
<td>ref.</td>
<td>1.85 [1.05–3.25], 0.03</td>
<td>1.50 [0.83–2.70], 0.18</td>
<td>1.54 [0.87–2.74], 0.14</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>rs4818</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>ref.</td>
<td>0.69 [0.47–1.00], 0.05</td>
<td>0.67 [0.46–0.98], 0.04</td>
<td>0.77 [0.53–1.12], 0.17</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>ref.</td>
<td>0.81 [0.56–1.18], 0.28</td>
<td>1.04 [0.73–1.47], 0.85</td>
<td>1.02 [0.72–1.46], 0.89</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>ref.</td>
<td>2.98 [1.45–6.09], 0.003</td>
<td>1.81 [0.84–3.93], 0.13</td>
<td>1.67 [0.77–3.63], 0.19</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval; CVD, cardiovascular disease; HR, hazard ratio; met, methionine; SNP, single nucleotide polymorphism; and val, valine.

*Major CVD, the primary Women’s Health Study outcome is a composite of myocardial infarction, stroke, or death from cardiovascular causes.
†HR [95% CI] and \(P\) value for each genotype by drug strata relative to placebo in age-adjusted models.
‡Drug interaction \(P\) value for interaction of aspirin and vitamin E across all three drug allocations within each SNP genotype stratum relative to placebo.
exploring aspirin or vitamin E treatment in disease prevention or therapy should be mindful of COMT genotype and other genetic variation in the catecholamine metabolic pathway. More broadly, the results illustrate how gene–drug interactions may influence the interpretation of a major clinical trial.

The strengths of our study are the prospective and homogeneous nature of the WGHS cohort, the validation of cardiovascular end points by physician review of medical records, the randomized allocation of WGHS participants to aspirin or vitamin E, and the replication of the COMT rs4680 association with CVD rate in CARDIoGRAM and C4D. In addition, our inclusion of another directly genotyped SNP, rs4818, provided a mutually confirmatory safeguard against artifacts caused by genotyping errors. Despite the epidemiological strengths of the WGHS in support of the association with rs4680, the effect in CARDIoGRAM was weaker than expected given its much larger number of cases, and understanding this discrepancy may be revealing for the mechanism of COMT action. In this regard, it may be relevant to note differences between the WGHS and CARDIoGRAM/C4D in CVD end point definitions and population composition. There were a variety of CVD end points in the studies contributing to the CARDIoGRAM/C4D meta-analysis that focused on cases of CAD, a composite including myocardial infarction, revascularization, angina, and angiographic stenosis, but variously recruited with or without additional criteria related to age and family history. The studies in CAD similarly included cases recruited for a variety of CAD definitions, including angina, and also recruited from both South Asian and European populations, a strategy that may have introduced subtle heterogeneity (eg, from environmental interactions) and that could have degraded power of already weak associations. In contrast, the primary end point in the WGHS was major CVD, a composite of nonfatal myocardial infarction or stroke, or cardiovascular death, and the population had homogeneous European ancestry. A secondary end point in the WGHS, coronary heart disease, defined as myocardial infarction or coronary revascularization but not angina, is somewhat closer to the CAD used by CARDIoGRAM/C4D and had a less strong association with rs4680 in the WGHS than the primary major CVD outcome. The differences appear not to reflect a sex-specific effect because, although CARDIoGRAM included both men and women (in contrast to the all-female composition of the WGHS), there was no evidence for rs4680 differential effects according to sex. Moreover, the association between rs4680 and CVD is consistent with results from the Kuopio Ischemic Heart Disease Study, where there was a significant association of methionine homozygotes when compared with valine carriers among 69 acute coronary events in a population of 792 men.

Another possible explanation might be related to the interactions we observed with aspirin and vitamin E. Exposures to these common drugs and others that may abrogate the COMT rs4680 association with CAD were not recorded in CARDIoGRAM/C4D but may have nevertheless contributed to the weaker association observed in this study.

Genetic analysis offers 1 route to a deeper understanding of the underlying pathophysiology of CVD. Our findings of a robust association between COMT variation and incident CVD add to a range of other clinical outcomes influenced by the catecholamine pathway. Finally, the modulation of CVD risk conferred by COMT through random allocation to aspirin and vitamin E may have implications for personalized medicine and development of strategies in attenuating CVD risk.

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References


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Materials and Methods

Women’s Health Study (WHS) and Women’s Genome Health Study (WGHS)
The WHS and its genetic component, the WGHS, have been described in detail elsewhere\(^1\)-\(^3\). Briefly, the WHS was a randomized, placebo-controlled trial of aspirin and vitamin E in primary prevention over 10 years of incident CVD among 39,876 initially healthy female health-care professionals, aged >45 years at baseline. The trial had a balanced 2x2 factorial design with equal numbers of participants allocated to aspirin (100mg every other day) or placebo in one direction, and vitamin E (600 IU \(\alpha\)-tocopherol every other day) or placebo in the other direction. The WGHS (N=23,294) derives from WHS participants who provided baseline blood samples (approximately 72%). The Institutional Review Board of Brigham and Women's Hospital, Boston, approved all analyses.

In the WHS, clinical characteristics, including age at randomization, blood pressure, history of diabetes, history of smoking and family history of myocardial infarction, were collected at baseline by self-report\(^4\). As described elsewhere, plasma from baseline blood samples was used to measure CVD risk biomarkers\(^2\). The WHS primary cardiovascular outcome was incident major cardiovascular disease, a composite of myocardial infarction, stroke or death from cardiovascular causes. Endpoints were confirmed by physician review of medical records using World Health Organization criteria, and death was confirmed by review of autopsy reports, death certificates, medical records and next of kin\(^3\). A secondary outcome, total CVD, was a composite of revascularization procedures (percutaneous transluminal coronary angioplasty and coronary bypass graft), in addition to primary outcome components. Coronary heart disease (CHD) was a composite outcome of nonfatal MI or fatal CHD plus revascularization procedures.

Replication studies
CARDIoGRAM and C4D
CARDIoGRAM is a consortium combining genome-wide association studies (GWAS) by meta-analysis from 14 studies to create a discovery sample of men and women including 22,233 cases of early coronary artery disease (CAD) a composite including myocardial infarction, revascularization, angina, and/or angiographic stenosis, and 64,762 controls, all of European ancestry\(^5\). C4D is a second consortium for meta-analysis combining GWAS from 34 additional CAD sample collections including early onset CAD in men and women of European or South Asian descent totaling 41,513 cases and 65,919 controls\(^6\). Associations of rs4680 and rs4818 with CAD were examined in these pre-existing datasets (Stages 1 and 2) and the combined dataset (Stage 3).

Global Lipids Genetics Consortium and International Consortium for Blood Pressure Genome-Wide Association Studies
COMT rs4680 associations with triglycerides and systolic blood pressure were confirmed in published results from the Global Lipids Genetics Consortium\(^7\) and The International Consortium for Blood Pressure Genome-Wide Association Studies\(^8\) respectively.

Genotyping
In the WGHS, genotyping was performed using the Human-Hap300 Duo “+” (Illumina, San Diego, CA) with the Infinium II protocol as described\(^9\). The final WGHS data included 23,294 participants of self-reported European ancestry confirmed by a multi-dimensional scaling procedure in PLINK\(^10\). Genotyping was successful for rs4680 and rs4818 in 99.9% and 99.6% respectively of the WGHS participants with European ancestry.
**Statistical Analysis**
In the WGHS, linear regression was used to evaluate genetic associations with continuously-valued clinical characteristics, assuming a standard additive genetic model by encoding \(\text{COMT}\) SNPs according to dose (0, 1, or 2) of inherited minor alleles. Cox proportional-hazard models were used to assess the genetic association effect on incident CVD, again assuming a standard additive (on the log scale) genetic model. For each model, the proportionality assumption was verified. The interaction of SNPs with drug allocation was tested by hypothesis testing on the Cox model coefficients of a term corresponding to the product of SNP genotype (0, 1, or 2) and indicator variable(s) for drug allocation (0=placebo, 1=drug). In CARDIoGRAM and C4D, study level statistics for association were determined by logistic regression and combined by inverse variance-weighted meta-analysis or weighted \(p\)-value method as described previously\(^1\). The primary analysis in this study pertained to the association of rs4680 with the primary study endpoint, incident major CVD, in each of the four drug allocation arms of the trial and the interaction of this SNP with randomized allocation to aspirin, vitamin E, or both. Thus, there were seven primary tests requiring a significance threshold \(p < 0.05/7 (=0.007)\) to address multiple hypothesis testing. The additional tests described were secondary and highly correlated with the primary tests, and were therefore not included in the multiple hypothesis penalty for establishing significance. Results were confirmed by repeating the analysis for another \(\text{COMT}\), SNP rs4818. Hardy–Weinberg equilibrium was assessed by an exact test\(^2\).
References


2. Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, Buring JE. Rationale, design, and methodology of the women's genome health study: A genome-wide association study of more than 25,000 initially healthy american women. *Clinical chemistry.* 2008;54:249-255


10. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559-575


Table I. Demographics and baseline characteristics by treatment arm for women in the WGHS who were successfully genotyped for rs4860 (N=22,273).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment subgroup</th>
<th>Placebo plus Placebo</th>
<th>Aspirin plus Placebo</th>
<th>Vitamin E plus Placebo</th>
<th>Aspirin plus Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>5,811</td>
<td>5,810</td>
<td>5,856</td>
<td>5,796</td>
</tr>
<tr>
<td>Age, mean (yr)</td>
<td></td>
<td>52.9 ± 5.0</td>
<td>52.9 ± 5.1</td>
<td>52.8 ± 5.1</td>
<td>53.0 ± 5.0</td>
</tr>
<tr>
<td>BMI, mean (kg/m²)</td>
<td></td>
<td>24.8 ± 3.0</td>
<td>24.9 ± 2.9</td>
<td>25.0 ± 2.9</td>
<td>24.9 ± 2.9</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td></td>
<td>121.3 ± 21.9</td>
<td>121.3 ± 21.7</td>
<td>120.6 ± 21.9</td>
<td>122.3 ± 22.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td></td>
<td>52.2 ± 9.6</td>
<td>51.5 ± 9.6</td>
<td>51.6 ± 9.6</td>
<td>51.9 ± 9.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td>119.0 ± 45.0</td>
<td>120.0 ± 45.0</td>
<td>119.0 ± 47.5</td>
<td>119.0 ± 45.5</td>
</tr>
<tr>
<td>Family history of MI</td>
<td></td>
<td>699 (13.3%)</td>
<td>663 (12.7%)</td>
<td>670 (12.7%)</td>
<td>682 (13.1%)</td>
</tr>
<tr>
<td>History of diabetes</td>
<td></td>
<td>135 (2.3%)</td>
<td>170 (2.9%)</td>
<td>144 (2.5%)</td>
<td>137 (2.4%)</td>
</tr>
<tr>
<td>Hormone therapy user</td>
<td></td>
<td>2587 (44.6%)</td>
<td>2555 (44.1%)</td>
<td>2497 (42.7%)</td>
<td>2547 (44.0%)</td>
</tr>
<tr>
<td>History of HT</td>
<td></td>
<td>1439 (24.8%)</td>
<td>1467 (25.3%)</td>
<td>1401 (23.9%)</td>
<td>1421 (24.5%)</td>
</tr>
<tr>
<td>Smoking ever</td>
<td></td>
<td>2812 (48.4%)</td>
<td>2860 (49.3%)</td>
<td>2918 (49.9%)</td>
<td>2826 (48.8%)</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td></td>
<td>2679 (46.2%)</td>
<td>2686 (46.0%)</td>
<td>2642 (45.6%)</td>
<td>2576 (44.5%)</td>
</tr>
</tbody>
</table>

*BMI, body-mass index, (weight in kilograms/square of the height in meters); MI, myocardial infarction; HT, hypertension.

Plus–minus values are mean ±SD.
Table II. Gender stratified meta-analyses of COMT rs4680 associated CVD Protection in CARDioGRAM, C4D and CARDioGRAM+C4D.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Stage 1*: CARDioGRAM</th>
<th>Stage 2†: C4D</th>
<th>Stage 3‡: CARDioGRAM+C4D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>EAF</td>
<td>OR [95% CI]</td>
<td>EAF</td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td>0.96 [0.92-0.99]</td>
<td>0.49</td>
</tr>
<tr>
<td>Female</td>
<td>0.44</td>
<td>0.93 [0.87-0.98]</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* Stage 1: CARDioGRAM data set consisted of 22,233 coronary artery disease (CAD) cases and 64,762 controls. CAD is a composite of myocardial infarction, revascularization, angina, and/or angiographic stenosis.
† Stage 2: Analysis of 34 additional CAD sample collections of European or South Asian descent (41,513 cases and 65,919 controls).
‡ Stage 3: Meta-analysis of CARDioGRAM+C4D database.
§ EAF = effect allele frequency for G (val); reference allele = A (met)
∥ Inverse variance p-value.
Table III. *COMT* rs4680 (val158met) and rs4818 minor allele association with cardiovascular risk biomarkers from the WGHS measured at baseline.

<table>
<thead>
<tr>
<th>Biomarker (units)</th>
<th>WGHS Effect as beta [SE], P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs4680*</td>
</tr>
<tr>
<td>total cholesterol (mg/dL)</td>
<td>-0.205 [0.385], 0.595</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>-0.055 [0.325], 0.865</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>0.260 [0.144], 0.071</td>
</tr>
<tr>
<td>(log) triglycerides (mg/dL)</td>
<td>-0.018 [0.005], 0.0004</td>
</tr>
<tr>
<td>apolipoprotein A1 (mg/dL)</td>
<td>0.096 [0.274], 0.726</td>
</tr>
<tr>
<td>apolipoprotein B (mg/dL)</td>
<td>-0.535 [0.274], 0.051</td>
</tr>
<tr>
<td>lipoprotein(a) (mg/dL)</td>
<td>0.007 [0.012], 0.549</td>
</tr>
<tr>
<td>(log) C-reactive protein (mg/L)</td>
<td>-0.009 [0.011], 0.408</td>
</tr>
<tr>
<td>sICAM1 (mmol/L)</td>
<td>-1.348 [0.814], 0.098</td>
</tr>
<tr>
<td>fibrinogen (mg/dL)</td>
<td>-0.681 [0.789], 0.388</td>
</tr>
<tr>
<td>homocysteine (mmol/L)</td>
<td>-0.080 [0.047], 0.088</td>
</tr>
<tr>
<td>creatinine (mg/dL)</td>
<td>-0.007 [0.006], 0.255</td>
</tr>
<tr>
<td>systolic BP (mM Hg)</td>
<td>-0.367 [0.127], 0.004</td>
</tr>
<tr>
<td>diastolic BP (mM Hg)</td>
<td>-0.069 [0.084], 0.409</td>
</tr>
</tbody>
</table>

*rs4680 coded allele = G (val), reference allele = A (met); rs4818 coded allele = G, reference allele = C.*
Figure I. Kaplan–Meier estimates of cumulative incidence of WGHS women in placebo arm (N=5,795) according to COMT rs4818 genotype with a first ever (A) major CVD, (B) total CVD, (C) myocardial infarction (D) ischemic stroke or (E) coronary heart disease (CHD) event. Legends indicate genotype strata and (number of cases/total number) in each stratum.
Figure II. Kaplan–Meier estimates of the Cumulative Incidence of WGHS Women According to COMT rs4818 Genotype with a First Ever Major CVD event in the (A) Aspirin vs. Placebo arms, and (B) Vitamin E vs. Placebo arms. P-values are for the whole model and interaction P-value is for the drug by genotype interaction terms.