ORIGINAL RESEARCH

Catechol-O-Methyltransferase and Cardiovascular Disease: MESA

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***Background*—**Genetic variation in catechol-O-methyltransferase (*COMT*), a key enzyme in estrogen and catecholamine metabolism, has plausible physiological links to cardiovascular disease (CVD) and its risk factors. In WHS (Women's Health Study), *COMT* variants rs4818 and rs4680 were associated with a lower risk of CVD among women receiving placebo but not aspirin, suggesting a possible role of *COMT* in thrombosis.

***Methods and Results*—**To evaluate potential pathways linking *COMT* with CVD, and *COMT* effect modification of aspirin in prevention, we examined *COMT* association with CVD risk and subclinical measures, coronary artery calcium, and carotid intima-media thickness in MESA (Multi-Ethnic Study of Atherosclerosis). In 65 957 person-years of follow-up, during which 498 events occurred, *COMT* rs4818 was associated with lower CVD risk (hazard ratio, 0.85; 95% CI, 0.74–0.97 [*P*=0.02]). This association remained virtually unchanged after adjusting for common CVD risk factors. Fibrinogen was the only risk factor associated with rs4818 (β, −3.65; SE, 1.35 mg/dL [*P*=0.007]). Results were directionally similar but not significant for rs4680. Adjusted hazard ratios for *COMT* rs4818 CVD association were 0.79 (95% CI, 0.65–0.95; *P*=0.02) among individuals who used aspirin <3 days per week and 0.89 (95% CI, 0.71–1.13; *P*=0.34) among more frequent users ($P_{interaction}$=0.39). Neither intima-media thickness nor coronary artery calcium was associated with *COMT*.

***Conclusions*—**In a multiethnic prospective cohort of men and women, the *COMT* rs4818G allele was associated with lower CVD risk and lower fibrinogen levels but not with radiographic measures of subclinical atherosclerosis. These results suggest a plausible role of *COMT* in the latter stages of CVD. **(*J Am Heart Assoc*. 2019;8:e014986. DOI: 10.1161/JAHA.119.014986.)**

Key Words: aspirin • cardiovascular disease risk factors • catecholamine • catecholaminergic polymorphic ventricular tachycardia • catechol-O-methyltransferase

Genetic variation in catechol-O-methyltransferase (*COMT*), a key enzyme in catecholamine metabolism, has plausible physiological links to both cardiovascular disease (CVD) and its risk factors.[1] *COMT* metabolizes catechol estrogen and the catecholamines epinephrine, norepinephrine, and dopamine by catalyzing the transfer of a methyl group from S-adenosyl methionine onto the catechol moieties.[2] *COMT* links to estrogen regulation are made more complex by the presence of estrogen receptor response elements in the *COMT* promoter region,[3] and the downregulatory effects of estradiol on *COMT*.[4] These estradiol effects are thought to contribute to sexual variation in *COMT* effects, with lower activity in women compared with men.[5,6] *COMT* is expressed in platelets,[7] and the highest levels are expressed in the liver and adrenal glands.[8] COMT's roles in reducing the toxic effects of catechol estrogen exposure and catecholamine flux are important in maintaining cardiovascular and renal function. Hence, rs4818 and rs4680, genetic single nucleotide polymorphisms (SNPs) in *COMT*, have been associated with CVD,[1,9] hypertension,[1,10–13] type 2 diabetes mellitus,[14,15] and preeclampsia.[16,17] The rs4680 variant encodes a functional G (Val) to A (Met) substitution that results in a 3- to 4-fold reduction in COMT enzyme activity. Both rs4680 and rs4818 disrupt stability of *COMT* mRNA and have effects related to *COMT* mRNA secondary structure and gene expression.[18] We previously demonstrated that both of

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Accompanying Tables S1 through S8 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014986

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Clinical Perspective

What Is New?

- A common coding variant in the catechol-O-methyltransferase locus (rs4818) was associated with lower rates of incident cardiovascular disease in MESA (Multi-Ethnic Study of Atherosclerosis) cohort.
- Two variants (rs4818 and rs4680) linked closely in white populations exhibited much less linkage in other populations, enabling separate analyses that were generally stronger for the former variant.

What Are the Clinical Implications?

- The catechol-O-methyltransferase variant was also associated with lower levels of fibrinogen, but not with radiographic measures of subclinical atherosclerosis, suggesting that the underlying mechanism of action likely impacts the latter stages of cardiovascular disease.

these *COMT* variants were associated with rates of CVD in women of European ancestry[1] in the genetics cohort of the WHS (Women's Health Study),[19] the WGHS (Women's Genome Health Study),[20] and men and women in CARDIoGRAM (Coronary Artery Disease Genome-Wide Replication and Meta-Analysis).[21] In the WGHS and large international consortia, *COMT* was also associated with several CVD risk factors including systolic blood pressure (BP),[1] triglycerides, and glycated hemoglobin (HbA_{1c}) levels.[14,15] We also observed a significant *COMT*-aspirin interaction, such that the statistically significant 33% CVD protection associated with the G alleles of rs4818 and rs4680 was attenuated with randomized assignment to aspirin ($P_{interaction}<0.001$), suggesting that benefit may result from antiplatelet activity that is superseded by aspirin. However, the WGHS comprised a white population of women aged 45 years or older, limiting the generalizability of the WGHS findings. Further, the WGHS included few physiological measures with which to evaluate potential pathophysiologic pathways of CVD. To address more fully the cardiovascular effects of the *COMT* locus on rates of clinical and subclinical CVD and the potential interaction with aspirin, we studied these factors in MESA (Multi-Ethnic Study of Atherosclerosis), an observational study of individuals of European, African, Asian, and Hispanic ancestry free of CVD at baseline.

Materials and Methods

Data for the National Heart, Lung, and Blood Institute's SHARe (SNP Health Association Resource), a substudy of the MESA cohort used in this analysis, is publically available through dbGaP (Study Accession: phs000420.v6.p3). The methods and materials used in this analysis are available to any researcher for purposes of reproducing the results or replicating the procedures.

The MESA Cohort

The MESA cohort includes 6814 participants from 6 field centers in the United States: Baltimore, Maryland; Chicago, Illinois; New York, New York; Forsyth County, North Carolina; Los Angeles, California; and St. Paul, Minnesota. Participants self-identified as white (38%), black (28%), Asian (12%), or Hispanic (22%). Specifics of the MESA design have been previously reported.[22] At baseline, participants were free from CVD (as defined by physician-diagnosed angina, stroke, myocardial infarction, transient ischemic attack, heart failure, or resuscitated cardiac arrest). Since the initiation of the study in 2000, participants have undergone 5 in-person examinations: baseline (July 17, 2000 to August 29, 2002) and 4 follow-up examinations, examination 2 (September 9, 2002, to February 7, 2004), examination 3 (March 10, 2004, to September 16, 2005), examination 4 (September 23, 2005, to May 30, 2007), and examination 5 (April 19, 2010, to February 4, 2012). MESA was conducted under institutional review board approval and oversight and with informed consent of participants. The study was performed in accord with the principles of the Declaration of Helsinki. This study was conducted under institutional review board approval and oversight from Partners HealthCare.

Outcome Measures

Our primary cardiovascular outcome was defined as myocardial infarction, stroke, resuscitated cardiac arrest, and death from stroke or coronary heart disease. We prespecified this outcome to be similar to the composite primary CVD outcome in the WGHS, which encompassed myocardial infarction, stroke, or death from CVD. CVD events in MESA were assessed at intervals of 9 to 12 months, between 2000 and 2013, by contacting participants or family members about CVD outpatient diagnoses and procedures, hospitalizations, and deaths. Self-reports were verified by review of death certificates and medical records for all hospitalizations and selected outpatient cardiovascular diagnoses and procedures. Two MESA physicians independently reviewed and classified events; disagreements were adjudicated by the MESA mortality and morbidity review committee.

Data on CVD risk factors including smoking status, medical history of diabetes mellitus, and hypertension were collected using questionnaires and laboratory evaluation at each examination. Body mass index was calculated as weight/height (kg/m^2). BP was determined as the average of the

second and third measurements taken after 5 minutes of seated rest. Laboratory measures including HbA_{1c}, fasting glucose, cholesterol, lipoprotein, and triglyceride levels were performed on blood samples following a 12-hour fast.

Carotid intima-media thickness (IMT) was assessed at examination 1 by B-mode ultrasonography of the right and left near and far walls of the internal and common carotid arteries as previously described.[23,24] Maximum IMT from examination 1 was computed as previously described by creating a composite *Z* score based on the standardized 2 carotid IMT site measurements from the common and internal carotid artery.[24] When only one of the measures was available, that one was used. Coronary artery calcium (CAC) was assessed by chest computed tomography.[24] Agatston scores of CAC were computed from phantom-adjusted coronary artery plaque density and area from replicate scans taken at the 5 examinations. CAC was stratified according to absolute cut points of Agatston scores <1, 1 to 100, 101 to 400, and >400,[25] and *COMT* association with these categories over the 5 MESA examinations was assessed using a repeated measures analysis.

The medication inventory method was used to assess aspirin use at each examination. Self-reported frequency of aspirin use at least 3 days per week was recorded at examinations 1 through 5.

COMT SNPs rs4818 and rs4680 were genotyped using the Illumina CARe iSelect (IBC) chip. Of the 6814 participants in MESA, 6316 had genotyping data available for *COMT* rs4818.

Statistical Analysis

Cox proportional hazard models were used to assess genetic associations with incident CVD, stroke, and myocardial infarction assuming a standard additive genetic model. Models were either adjusted for age, sex, and site or fully adjusted for the latter plus cardiovascular risk factors, which included medical history of hypertension and diabetes mellitus, smoking status, systolic and diastolic BP, cholesterol, triglycerides, high-density lipoprotein levels, and fibrinogen. In all cases, we performed race/ethnicity-stratified analyses adjusting for population substructure with 5 race-specific principal components. *COMT* association with CVD has not been studied in black and Hispanic populations. Based on the WGHS, we estimated that we would be adequately powered to observe a difference by *COMT* genotype if the frequency of the rs4680G allele was >0.30 (Table S1). *COMT* expression is regulated by estrogen and COMT is also a key enzyme in estrogen metabolism. In the WGHS, our initial observation of *COMT* effects on CVD were among white women, 44% of whom took exogenous estrogen hormone replacement therapy (HRT). In MESA, 68% of white women reported HRT use in examination 1. Hence, in addition to secondary analyses stratified by sex, we conducted sensitivity analyses in white women stratified by ever/never use of HRT and examined potential *COMT* effect modification of HRT ever/never use. For each model, the proportionality assumption was verified. Analyses were performed in SAS 9.4 (SAS Institute).

Linear regression was used to evaluate *COMT* cross-sectional associations with the IMT composite *Z* score and baseline risk factors: systolic BP, diastolic BP, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting glucose, HbA_{1c}, fibrinogen, and triglycerides for each race/ethnicity subgroup and then meta-analyzed for the overall associations. For right-skewed data (triglycerides), we determined SE estimates using robust SEs. Models for BP and lipids were adjusted for hypertensive and lipid-lowering medication use, respectively. For CAC, Agatston scores were categorized as previously described[26] and updated for each examination. Multinomial logistic regression was used to examine *COMT* cross-sectional association with CAC in models adjusted for age, sex, site, and the first 5 principal components for race/ethnicity.

Modification of the *COMT* association with incidence of CVD by aspirin use was tested on the Cox model coefficients with a term corresponding to the cross-product of allele number and aspirin use categorized as 0 (not currently used or used <3 times per week) or 1 (used currently at least 3 times per week). Because aspirin use changed over time, and because the proposed mechanism of action for aspirin involves irreversible platelet inhibition, we used a simple time-varying covariate for aspirin use that was updated at each examination. We also conducted sensitivity analyses stratified by HRT ever/never use specifically among white women to approximate most closely the WGHS population previously analyzed. Individual race/ethnicity *COMT* and *COMT* by aspirin use interaction estimates were meta-analyzed across races using inverse variance fixed effects models in Comprehensive Meta-Analysis, version 3.3.070 (Biostat, New Jersey).

Results

Participant Characteristics

Characteristics of the study sample at baseline stratified by race are reported in Table 1 and those stratified by rs4818 are reported in Table S2. Age and sex distributions did not differ by race. The allele frequencies of rs4818 and rs4680 (Table S3) varied by race/ethnicity. Both SNPs were in Hardy-Weinberg equilibrium within each race/ethnicity subgroup (P>0.05). In the overall population, the minor allele frequencies for rs4818 (G allele) and rs4680 (A(Met) allele), were 32% and 40%, respectively. Linkage disequilibrium and correlation

between the *COMT* SNPs was strongest for whites (0.72) and lower among others (0.12–0.24).

COMT Association With CVD Risk Factors and Subclinical Disease

In meta-analyses of CVD risk factors across races/ethnicities, only fibrinogen was significantly associated with *COMT* rs4818 (Table 2 and Table S4). In gene-dosage models of fibrinogen, the direction of the rs4818 association was consistent across the races/ethnicities (β, −3.65 mg/dL; SE, 1.35 [*P*=0.007]) and was strongest and statistically significant among Asians. In secondary analyses using sex-stratified models, the rs4818 association was negative for both women (β, −5.29 mg/dL; SE, 1.93 [*P*=0.006]) and men (β, −2.28 mg/dL; SE, 2.98 [*P*=0.44]). In contrast, the overall association of rs4680 with fibrinogen was null, with significant differences in direction between women (β, −2.98 mg/dL; SE, 1.83 [*P*=0.10]) and men (β, 4.19 mg/dL; SE, 1.75 [*P*=0.02]) ($P_{interaction}$=0.005) (Table S4).

COMT associations with other CVD risk factors HbA_{1c}, fasting glucose, high-density lipoprotein cholesterol, and intercellular adhesion molecule were directionally consistent for rs4818 and rs4680 and within 2 SDs of previously reported statistically significant associations (Table S4).[1,14] Adjustment for BP medication or lipid medication use in lipid and systolic and diastolic BP models were similarly null.

No statistically significant associations with carotid artery IMT at examination 1 were observed for *COMT* rs4818 (β, −0.004; SE, 0.014 [*P*=0.76]) and rs4680 (β, −0.019; SE, 0.014 [*P*=0.17]) (Table 3). Further, no statistically significant associations were observed for *COMT* categories of CAC over the 5 examinations for rs4818 (odds ratio, 1.00; 95% CI, 0.93–1.08 [*P*=0.93]) or rs4680 (odds ratio, 1.00; 95% CI, 0.93–1.08 [*P*=0.96]). These results did not differ substantively with stratification by sex.

COMT Association With Incident CVD

MESA documented 524 primary incident CVD events during 65 957 person-years of follow-up. Consistent with our findings in the WGHS and CARDIoGRAM, the rs4818G allele was associated with a 15% lower rate of CVD (hazard ratio [HR], 0.85; 95% CI, 0.74–0.97 [*P*=0.02]) across the 4 racial/ethnic groups (Table 4 and Figure—Panel A). The association was directionally consistent in each of the race/ethnicity populations and significant among Hispanics. This inverse association with CVD risk was not attenuated by adjustment for CVD risk factors: body mass index, triglycerides, high-density lipoprotein, cholesterol, systolic BP, history of smoking, diabetes mellitus, hypertension, or fibrinogen. The overall direction of the rs4680 Val allele association with CVD was consistent with rs4818 but was statistically nonsignificant (Table 4 and Figure—Panel B). No substantive changes were observed in models adjusted for risk factors (including fibrinogen) or stratified by sex (Table S5).

In the WGHS, our initial observation of *COMT* effects on CVD were among white women, 43% of whom took exogenous

Table 1. Baseline Characteristics of MESA Participants Genotyped for rs4818 by Race

	Race			
	White	Black	Hispanic	Asian
Participants, No. (%)	2481	1639	1428	768
Age, y	62.7 (10.3)	62.2 (10.1)	61.4 (10.3)	62.3 (10.4)
Women, %	1304 (52.5)	903 (54.0)	740 (51.7)	389 (50.7)
History of diabetes mellitus, %	150 (6.8)	285 (20.2)	255 (21.2)	101 (16.0)
History of hypertension, %	967 (38.9)	989 (59.2)	603 (42.1)	288 (37.5)
Current smoker, %	286 (11.5)	306 (18.3)	192 (13.4)	44 (5.7)
Body mass index, kg/m^2	27.7 (5.1)	30.2 (5.9)	29.5 (5.2)	24.0 (3.3)
Systolic BP, mm Hg	123.6 (20.5)	131.8 (21.8)	126.9 (22.1)	124.4 (21.7)
Diastolic BP, mm Hg	70.2 (10.0)	74.6 (10.3)	71.6 (10.2)	71.9 (10.3)
HDL cholesterol, mg/dL	52.4 (15.8)	52.3 (15.2)	47.5 (13.0)	49.3 (12.4)
Triglycerides, mg/dL	133.1 (90.5)	104.9 (69.9)	158.3 (102.2)	143.2 (85.9)
Total cholesterol, mg/dL	195.8 (35.4)	189.5 (36.4)	198.3 (37.7)	192.5 (31.5)
COMT rs4818 MAF (G)	0.41	0.21	0.27	0.33
COMT rs4680 MAF (A)	0.51	0.31	0.39	0.28

Numbers in parentheses are expressed as SD unless otherwise indicated. BP indicates blood pressure; *COMT*, catechol-O-methyltransferase; HDL, high-density lipoprotein; MAF, minor allele frequency; MESA, Multi-Ethnic Study of Atherosclerosis.

Table 2. Effect Estimates and Standard Error of *COMT* rs4818 (Per G Allele) and rs4680 (Per Val Allele) Association With Baseline Fibrinogen Levels (mg/dL) in Gene-Dosage Models

		All			White			Black			Hispanic			Asian		
SNP	Sex	β	SE	*P* Value	β	SE	*P* Value	β	SE	*P* Value	β	SE	*P* Value	β	SE	*P* Value
rs4818	All	−3.65	1.35	0.007	−0.81	2.11	0.70	−2.29	3.03	0.45	−4.94	3.02	0.10	−9.70	3.06	0.002
	Women	−5.29	1.93	0.01	−4.70	4.69	0.32	−2.92	4.40	0.51	−3.78	2.89	0.19	−11.76	4.42	0.01
	Men	−2.28	2.98	0.44	−0.42	4.35	0.92	−8.00	4.46	0.07	3.53	2.80	0.21	−6.90	4.53	0.13
rs4680	All	0.53	1.29	0.68	0.84	1.96	0.67	1.70	2.78	0.54	−1.43	2.89	0.62	0.54	3.31	0.87
	Women	−2.98	1.83	0.10	−3.05	2.81	0.28	−0.44	4.09	0.92	−7.68	4.00	0.06	−0.02	4.45	0.99
	Men	4.19	1.75	0.02	5.05	2.69	0.06	4.01	3.71	0.28	5.09	4.08	0.21	1.37	4.20	0.74

COMT indicates catechol-O-methyltransferase; SNP, single nucleotide polymorphism.

estrogen through HRT. In MESA, use of HRT varied by race, with white women using the most—64.1% used HRT at examination 1 (Table S6). In subset analyses among white women in MESA stratified by HRT use, both *COMT* rs4680 and rs4818G alleles were associated with higher rates of CVD among women who had never taken HRT. Among white women who had taken HRT, the *COMT* association was protective for rs4818 and rs4680, but only statistically significant for rs4818 (Table S6). A test for a *COMT*-HRT interaction was significant for both SNPs ($P_{interaction}$<0.05). The *COMT*-HRT interaction was not observed among women in the other race/ethnic subpopulations.

COMT and Aspirin

Aspirin use >3 days per week appeared to increase across the 5 examinations, but did not vary by *COMT* rs4818 genotype (Table S7). When stratified by time-varying aspirin use over the 5 examinations, the *COMT* rs4818G allele was statistically significantly associated with lower rates of CVD among individuals who used aspirin <3 days per week (HR, 0.79; 95% CI, 0.65–0.95 [*P*=0.02]); the corresponding HR among those who used aspirin ≥3 days per week was 0.89 (95% CI, 0.71–1.13; *P*=0.34 [$P_{interaction}$=0.39]) (Table 5). There was no difference in rates of CVD associated with *COMT* rs4680 by aspirin use.

In subset analyses of white women stratified by HRT use at examination 1 and aspirin <3 days per week, *COMT* rs4818 (HR, 0.34; 95% CI, 0.16–0.69 [*P*=0.004]) and rs4680 (HR, 0.46; 95% CI, 0.25–0.85 [*P*=0.01]) G alleles were associated with lower rates of CVD. These associations were attenuated among patients using HRT and aspirin ≥3 days per week, with a significant interaction for rs4680 but not rs4818 (Table S8). Among white women who did not use HRT, rates of CVD were higher with or without aspirin use.

Discussion

In MESA, we replicated our previous finding from the WGHS and CARDIoGRAM,[5] that the *COMT* rs4818G allele is associated with lower rates of CVD. This association was similar across race/ethnicity groups and remained significant after

Table 3. *COMT* rs4818 and rs4680 Gene-Dosage (Per Allele) Association With Carotid IMT Composite *Z* Score* and CAC Overall and by Race

	IMT		CAC	
	rs4818	rs4680	rs4818	rs4680
Race/Ethnicity	β (SE), *P* Value	β (SE), *P* Value	β (SE), *P* Value	β (SE), *P* Value
All	−0.004 (0.014), 0.76	−0.019 (0.014), 0.17	1.00 (0.93–1.08), 0.93	1.00 (0.93–1.08), 0.96
White	−0.014 (0.022), 0.52	−0.003 (0.021), 0.16	0.94 (0.85–1.05), 0.28	0.95 (0.86–1.06), 0.34
Black	0.006 (0.031), 0.85	−0.007 (0.029), 0.81	1.06 (0.90–1.25), 0.46	1.05 (0.91–1.21), 0.54
Hispanic	−0.011 (0.031), 0.73	−0.006 (0.027), 0.82	1.05 (0.90–1.24), 0.52	1.10 (0.95–1.28), 0.20
Asian	0.018 (0.037), 0.62	−0.027 (0.038), 0.48	1.09 (0.89–1.33), 0.40	0.92 (0.75–1.14), 0.45

*Intima-media thickness (IMT) composite *Z* score is based on the standardized 2 carotid IMT site measurement from the common and internal carotid artery. CAC indicates coronary artery calcium; *COMT*, catechol-O-methyltransferase.

Table 4. *COMT* rs4818 and rs4680 Gene-Dosage (Per Allele*) Association With Rates of CVD in MESA

SNP	Race/Ethnicity	Events/No.	HR (95% CI), *P* Value
rs4818	All, model 1†	498/5984	0.85 (0.74–0.97), 0.02
	All, model 2‡	497/5961	0.85 (0.74–0.98), 0.02
	White	203/2332	0.90 (0.74–1.09), 0.29
	Black	122/1518	0.85 (0.62–1.16), 0.32
	Hispanic	130/1375	0.71 (0.53–0.95), 0.02
	Asian	43/759	0.90 (0.74–1.09), 0.29
rs4680	All, model 1†	524/6157	0.95 (0.84–1.08), 0.46
	All, model 2‡	495/6066	0.96 (0.85–1.09), 0.56
	White	214/2476	1.02 (0.84–1.23), 0.86
	Black	130/1575	0.97 (0.75–1.25). 0.79
	Hispanic	137/1426	0.94 (0.73–1.19), 0.59
	Asian	43/768	0.68 (0.43–1.07), 0.10

*Allele key: rs4818 coded allele=G, reference=C; rs4680 coded allele=Val (G), reference=Met (A).
†Model 1: meta-analysis of Cox proportional models adjusted for age, sex, race, site, and the first 5 principal components specific to each of the 4 race/ethnicities.
‡Model 2: meta-analysis of Cox proportional models adjusted for time-varying cardiovascular disease (CVD) risk factors from examination 1 to 5: body mass index, triglycerides, high-density lipoprotein, low-density lipoprotein, cholesterol, systolic blood pressure and history of smoking, diabetes mellitus, and hypertension in addition to age, sex, race, and the first 5 principal components specific to each of the 4 race/ethnicities.
COMT indicates catechol-O-methyltransferase; HR, hazard ratio; MESA, Multi-Ethnic Study of Atherosclerosis; SNP, single nucleotide polymorphism.

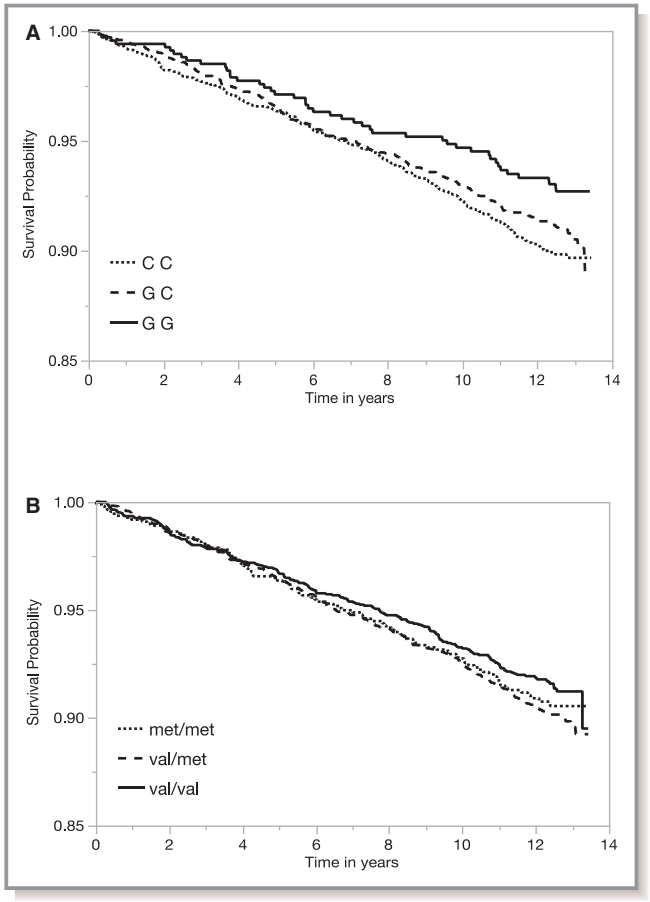

Figure. Kaplan–Meier curves of cardiovascular disease event-free survival probability over the duration of MESA (Multi-Ethnic Study of Atherosclerosis) (in years) by catechol-O-methyltransferase (*COMT*) (**A**) rs4818 and (**B**) rs4680 genotypes.

adjusting for CVD risk factors. The overall direction of *COMT* associations with CVD risk factors tended to be consistent with previous findings for systolic BP,[1,10–13] HbA_{1c},[14] and triglyceride levels,[1,27] but they were not statistically significant in the smaller and more diverse MESA sample. The only risk factor significantly associated with *COMT* in MESA was fibrinogen. The direction of this association was consistent with a nonsignificant effect observed in the WGHS.[1] We did not observe effect modification of the rs4818 association with CVD by aspirin use. With the exception of systolic BP, these findings were directionally similar, but statistically nonsignificant for rs4680. Further, neither *COMT* SNP was associated with carotid artery IMT or CAC. In contrast to the European ancestry of the WGHS participants, the MESA population is representative of multiple racial/ethnic groups and consists of both men and women. Hence, our findings of significant *COMT* rs4818 effects on CVD in MESA allow generalization to men, blacks, and Hispanics, where the findings were for the most part consistent. Among Asians, who represented the smallest subpopulation in MESA, the main associations were nonsignificant, although the *COMT* association with fibrinogen in this group was significant. Whether the modest differences across races represent the play of chance or specific differences in linkage by race will also require dedicated study in larger samples.

These results were not wholly consistent with our original observations in the WGHS where the population we examined consisted of white women, older than 45 years, of whom 43% reported taking estrogen HRT at baseline. In MESA, a majority of white women (64%) reported having used HRT at examination 1. Given the modulatory effects of estrogen on *COMT* gene expression and COMT's role in estrogen metabolism (converting catechol estrogens to 2- and 4-methoxyestradiol), we conducted stratified analyses among a subset of the MESA cohort most similar to the WGHS population. These subset analyses suggested that the discrepancy between the WGHS and MESA may indeed be attributed to differences in estrogen levels related to sex and HRT use. However, neither the WGHS or MESA were designed to look at the influence of *COMT* and HRT on the incidence of CVD. Therefore, these findings are hypothesis generating and warrant examination in other cohorts. Further, in the WGHS, the associations of rs4818 and rs4680 with CVD were similar, as the 2 are highly linked in most white populations. However, in the racially diverse MESA sample, where the linkage was much weaker among nonwhite

Table 5. *COMT* rs4818 and rs4680 Gene-Dosage (Per Allele*) Association With Rates of CVD Stratified by Aspirin Use Overall and by Race

	Aspirin <3 d/wk		Aspirin ≥3 d/wk		
Race/Ethnicity	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	$P_{interaction}$
Overall†	0.79 (0.65–0.95)	0.02	0.89 (0.71–1.13)	0.34	0.39
White	0.83 (0.61–1.11)	0.21	0.90 (0.66–1.22)	0.34	0.70
Black	0.65 (0.40–1.04)	0.07	1.27 (0.77–2.11)	0.38	0.06
Hispanic	0.70 (0.48–1.02)	0.06	0.65 (0.39–1.08)	0.10	0.81
Asian	1.09 (0.63–1.89)	0. 76	0.19 (0.01–2.40)	0.20	0.21

COMT indicates catechol-O-methyltransferase; CVD, cardiovascular disease; HR, hazard ratio.
*Allele key: rs4818 coded allele=G, reference=C.
†Meta-analysis of Cox proportional models adjusted for age, sex, race, site, and the first 5 principal components specific to each of the 4 race/ethnicities.

participants, the CVD association with rs4680 was not significant, suggesting that either rs4680 is not the causal locus or its effects on COMT activity or gene expression levels are potentially sensitive to estrogen. Still, much finer mapping, and likely whole genome sequencing, in similarly diverse samples will be needed to determine whether variation at rs4818, which appears to disrupt mRNA stability,[18] is the causal locus for the CVD effects observed here.

IMT and CAC have both been shown to be associated with incident CVD in MESA and elsewhere.[28] Our findings that *COMT* was not clearly associated with either subclinical measure suggests that this locus is likely to influence risk of CVD through mechanisms other than atherosclerosis. Notably, the effect estimates for intercellular adhesion molecule, SBP, and HbA_{1c} were directionally consistent with the WGHS and the Diabetes Prevention Program,[1,14] although, in the smaller more diverse MESA cohort, the associations were nonsignificant. The decidedly null results for subclinical disease, when combined with the suggestive effects for interaction with aspirin and the observed effect on fibrinogen, tend to suggest that *COMT* is most likely to exert its effects on the progression of subclinical to clinical CVD rather than on the development of subclinical disease per se.

COMT is expressed in platelets, where catecholamine flux influences development of thrombotic events via platelet activation.[29] Thus, genetically derived variation in COMT functionality could modify circulating and platelet levels of catecholamines, in turn shifting the threshold for vascular events. The link between COMT, platelets, and estrogen may also contribute to its effects in cancer development.[30,31] Interestingly, the fibrinogen gene has a corticosteroid response element in its regulatory region,[32] and fibrinogen levels are influenced by estrogen in women who use HRT,[33,34] pointing to a potential link between catecholamines, COMT, and fibrinogen.

It is important to acknowledge that some of our results, and especially those in smaller subgroups, had wide CIs, reflecting smaller numbers of participants and events. Results in these smaller subgroups should be treated as hypothesis-generating and, if possible, confirmed in other multiethnic cohorts that may have similar information.

Conclusions

These results build upon our previous work in the WGHS in demonstrating that common genetic variation in *COMT* is associated with risk of CVD in a multiracial population of men and women.

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Disclosures

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SUPPLEMENTAL MATERIAL

Table S1: Power estimates of hazard ratios for incident CVD based on rs4680 G(val) allele frequencies over range of GAF from 0.3-0.6.

GAF	HR	Power
0.30	0.70	0.98
	0.75	0.91
	0.80	0.74
0.40	0.70	0.99
	0.75	0.95
	0.80	0.80
0.50	0.70	0.99
	0.75	0.96
	0.80	0.83
0.60	0.70	0.99
	0.75	0.96
	0.80	0.82

Abbreviations: HR=Hazard Ratio; GAF= Gene allele frequency

Table S2: Demographics, baseline characteristics and aspirin use by *COMT* rs4818 genotype use in MESA.

	CC	GC	GG
Participants, n	2888 (47.7)	2515 (41.6)	649 (10.7)
Demographic data			
Age	62.1 (10.1)	62.3 (10.4)	63.0 (10.5)
Female	1501 (52.0)	1315 (52.3)	340 (52.4)
Race/ethnicity			
White	828 (28.7)	1129 (44.9)	379 (58.4)
Black	981 (34.0)	528 (12.9)	72 (11.1)
Hispanic	733 (25.4)	533 (21.0)	110 (17.0)
Asian	346 (12.0)	325 (21.2)	88 (13.6)
Medical History			
History of diabetes	395 (13.7)	312 (12.4)	54 (8.4)
Current smoker	373 (12.9)	338 (13.4)	66 (10.2)
Aspirin Use at Exam 1 (%)	579 (20.1)	512 (20.4)	126 (19.4)
Physical Exam			
Body mass index, kg/m^2	28.7 (5.7)	28.1 (5.4)	27.8 (5.1)
Systolic blood pressure, mmHg	127.5 (21.7)	126.0 (21.6)	124.8 (20.7)
Diastolic blood pressure, mmHg	72.3 (10.4)	71.5 (10.3)	71.2 (9.7)
Laboratory Measurements			
HDL cholesterol, mg/dL	50.6 (14.7)	50.8 (14.9)	51.0 (14.4)
Triglycerides, mg/dL	131.3 (87.7)	134.9 (96.9)	133.9 (79.2)
Total cholesterol, mg/dL	193.5 (35.8)	194.6 (36.6)	195.2 (34.3)

Table S3: Distribution of *COMT* rs4818 and rs4680 genotypes by race in MESA.

	rs4818					rs4680					
Race	**GG**	**GC**	**CC**	**MAF (G)**	**HWE**	**AA**	**AG**	**GG**	**MAF (A)**	**HWE**	**LD r^2**
Overall	677	2635	3004	0.32	<0.01	1106	2928	2316	0.40	<0.001	0.31
White	406	1206	869	0.41	>0.05	655	1236	593	0.51	>0.05	0.72
Black	74	547	1018	0.21	>0.05	178	685	806	0.31	>0.05	0.12
Hispanic	109	555	764	0.27	>0.05	211	704	514	0.39	>0.05	0.24
Asian	88	327	353	0.33	>0.05	62	303	403	0.28	>0.05	0.19

Abbreviations: MAF = minor allele frequency; HWE = Hardy-Weinberg Equilibrium; LD = Linkage disequilibrium

Table S4: Parameter estimates of COMT rs4818 and rs4680 (per G allele) association with baseline cardiometabolic risk factors.

		All			White			Black			Hispanic			Asian		
Risk Factor		**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**
Fibrinogen,	rs4818	-3.65	1.35	0.007	-0.81	2.11	0.70	-2.29	3.03	0.45	-4.94	3.02	0.10	-9.70	3.06	0.002
mg/dL	rs4680	0.53	1.29	0.68	0.84	1.96	0.67	1.70	2.78	0.54	-1.43	2.89	0.62	0.54	3.31	0.87
CRP, mg/L	rs4818	-0.06	0.10	0.58	0.32	0.16	0.05	-0.19	0.33	0.58	-0.44	0.23	0.05	-0.31	0.20	0.12
	rs4680	0.17	0.11	0.11	0.33	0.15	0.03	0.09	0.27	0.74	0.003	0.22	0.99	-0.01	0.27	0.97
ICAM, ng/mL	rs4818	-1.05	2.22	0.64	-2.86	2.93	0.33	10.52	7.85	0.18	-3.22	5.84	0.58	1.09	4.95	0.82
	rs4680	-1.12	2.11	0.59	-1.20	2.77	0.66	3.83	6.92	0.58	-0.34	5.66	0.95	-3.97	4.91	0.42
IL-6, pg/mL	rs4818	-0.02	0.02	0.44	0.03	0.04	0.37	-0.01	0.06	0.91	-0.05	0.05	0.30	-0.09	0.05	0.08
	rs4680	0.02	0.04	0.50	0.02	0.03	0.47	0.03	0.05	0.59	0.10	0.04	0.02	-0.10	0.06	0.13
SBP, mmHg	rs4818	-0.23	0.39	0.56	0.13	0.56	0.81	-0.12	0.93	0.90	-0.49	0.83	0.55	-1.28	1.08	0.24
	rs4680	0.01	0.36	0.99	0.17	0.52	0.74	0.21	0.78	0.78	-0.45	0.76	0.55	-0.16	1.07	0.88
DBP, mmHg	rs4818	0.03	0.19	0.88	0.16	0.28	0.55	0.03	0.46	0.96	-0.25	0.38	0.52	0.06	0.52	0.91
	rs4680	0.03	0.18	0.85	0.09	0.26	0.74	-0.03	0.37	0.94	-0.30	0.36	0.41	0.71	0.56	0.21
Hemoglobin	rs4818	-0.02	0.02	0.22	0.00	0.02	0.95	-0.07	0.05	0.15	-0.05	0.06	0.34	-0.07	0.04	0.12
A1c, %	rs4680	-0.02	0.01	0.20	-0.01	0.02	0.41	-0.01	0.04	0.86	-0.02	0.06	0.69	-0.08	0.05	0.12
Fasting	rs4818	-0.45	0.48	0.35	0.69	0.59	0.24	-3.36	1.34	0.01	-2.50	1.74	0.15	-2.36	1.39	0.09
glucose, mg/dl	rs4680	-1.15	0.96	0.23	0.44	0.52	0.40	-1.69	1.21	0.16	-0.86	1.64	0.60	-3.86	1.65	0.02
Triglycerides,	rs4818	0.17	1.66	0.92	-1.57	4.38	0.72	-0.12	4.71	0.98	0.60	2.56	0.81	0.51	3.00	0.86
mg/dL	rs4680	-3.42	1.83	0.06	0.06	2.43	0.98	-4.14	3.33	0.21	-5.88	3.47	0.09	-8.52	4.90	0.08
HDL-C,	rs4818	0.03	0.26	0.89	-0.05	0.41	0.90	-0.07	0.64	0.91	0.42	0.51	0.42	-0.22	0.62	0.72
mg/dL	rs4680	0.33	0.24	0.17	-0.03	0.40	0.93	0.43	0.52	0.41	0.60	0.44	0.18	0.59	0.69	0.39
LDL-C,	rs4818	0.08	0.63	0.90	0.75	0.92	0.42	-0.69	1.53	0.65	-0.79	1.37	0.56	0.10	1.55	0.95
mg/dL	rs4680	-0.14	0.58	0.81	0.13	0.86	0.88	-1.11	1.24	0.37	-0.74	1.27	0.56	1.79	1.76	0.31

Table S5: *COMT* rs4818 and rs4680 gene dosage (per allele*) association with rates of CVD stratified by race and sex.

SNP	Females			Males		
Race/Ethnicity	**Events/N**	**HR 95% CI**	**P**	**Events/N**	**HR, 95% CI**	**P**
rs4818						
Overall	226/3271	0.93 [0.75-1.14]	0.48	298/2977	0.80 [0.67-0.97]	0.02
White	97/1300	1.04 [0.77-1.41]	0.80	117/1179	0.85 [0.65-1.11]	0.24
Black	55/846	0.77 [0.45-1.32]	0.34	75/729	0.87 [0.58-1.32]	0.52
Hispanic	54/736	0.75 [0.47-1.18]	0.21	83/690	0.67 [0.46-0.98]	0.04
Asian	20/389	0.94 [0.50-1.79]	0.86	23/379	0.83 [0.42-1.64]	0.59
rs4680						
Overall	226/3271	0.95 [0.78-1.15]	0.59	298/2977	0.95 [0.79-1.14]	0.59
White	97/1300	1.14 [0.85-1.52]	0.38	117/1179	0.95 [0.73-1.22]	0.68
Black	55/846	0.88 [0.61-1.29]	0.52	75/729	1.04 [0.72-1.48]	0.84
Hispanic	54/736	0.75 [0.51-1.11]	0.15	83/690	1.04 [0.76-1.43]	0.79
Asian	20/389	0.89 [0.43-1.81]	0.74	23/379	0.55 [0.30-1.03]	0.06

Abbreviations: HR = Hazard ratio

* Allele key: rs4818 coded allele = G; rs4680 coded allele = G

Table S6: Hormone replacement therapy (HRT) use among women assessed at Exam 1 and rates of CVD by *COMT* rs4818 and rs4680 genotype stratified by HRT use and race.

		Hormone replacement therapy (HRT) use ever						
SNP	**Race/ethnicity**	**Total (%)**	**CC**	**GC**	**GG**	**HRT ever – no***	**HRT ever – yes***	**$P_{interaction}$**
rs4818	**Overall**	1540 (51.1)	708 (49.6)	647 (51.2)	185 (57.6)	1.22 [0.74-2.02], 0.44	0.64 [0.45-0.89], 0.009	0.047
	White	772 (64.1)	269 (64.7)	367 (62.2)	136 (68.3)	2.20 [1.34-3.07], 0.002	0.59 [0.39-0.89], 0.01	0.02
	Black	384 (47.4)	241 (48.5)	127 (45.0)	16 (50.0)	0.94 [0.49-1.78], 0.84	0.96 [0.41-2.30], 0.94	0.97
	Hispanic	268 (40.6)	144 (39.9)	107 (42.3)	17 (37.0)	0.83 [0.51-1.34], 0.44	0.62 [0.17-1.31], 0.46	0.39
	Asian	116 (34.2)	54 (34.8)	46 (32.9)	16 (36.4)	1.28 [0.53-3.07], 0.58	0.59 [0.22-1.58], 0.30	0.84
			met/met	**val/met**	**val/val**			
rs4680	**Overall**	1701 (55.4)	296 (57.7)	723 (51.8)	521 (47.1)	1.14 [0.66-1.95], 0.64	0.75 [0.56-1.02], 0.07	0.26
	White	772 (64.1)	205 (66.8)	381 (63.0)	186 (62.5)	2.39 [1.42-4.01], 0.001	0.72 [0.49-1.04], 0.08	2E-04
	Black	419 (50.9)	45 (50.6)	158 (47.9)	181 (46.2)	0.93 [0.57-1.53], 0.78	0.96 [0.47-1.95], 0.91	0.43
	Hispanic	302 (45.1)	39 (41.5)	138 (40.8)	91 (39.9)	0.83 [0.54-1.28], 0.40	0.59 [0.22-1.61], 0.31	0.26
	Asian	137 (39.6)	7 (30.4)	46 (37.4)	63 (32.6)	0.81 [0.30-2.19], 0.68	0.88 [0.30-2.61], 0.82	0.24

Random effects meta-analysis of HRT ever and never use. I^2 for rs4818 HRT no = 65.1; HRT yes = 0. I^2 for rs4680 HRT no = 72.7; HRT yes = 0

Table S7: Aspirin user (%) > 3 days/week by *COMT* rs4818 genotype at the five MESA exams.

	rs4818 genotype		
Exam	**CC**	**GC**	**GG**
1	577 (20.0)	511 (20.3)	125 (19.3)
2	863 (31.6)	762 (31.4)	185 (29.2)
3	886 (33.9)	799 (34.6)	195 (32.3)
4	944 (37.2)	824 (36.4)	216 (36.0)
5	905 (45.2)	825 (44.4)	215 (44.4)

Supplementary Table S8: *COMT* rs4818 and rs4680 gene dosage (per allele*) association with rates of CVD stratified by time-varying aspirin and HRT ever use among white women.

SNP	Aspirin	No HRT HR [95% CI], P	Ever HRT HR [95% CI], P
rs4818	**<3 days/week**	3.13 [1.53-6.39], 0.002	0.34 [0.16-0.69], 0.004
	≥3 days/week	1.99 [0.90-4.40], 0.09	0.80 [0.42-1.52], 0.49
	$P_{interaction}$	0.39	0.08
rs4680	**<3 days/week**	3.02 [1.38-6.58], 0.006	0.46 [0.25-0.85], 0.01
	≥3 days/week	2.25 [0.97-5.24], 0.06	1.27 [0.69-2.35], 0.45
	$P_{interaction}$	0.613	0.02

* Allele key: rs4818 coded allele = G; rs4680 coded allele = G