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A genetic variant in the catechol-O-methyl transferase (COMT) gene is related to age-dependent differences in the therapeutic effect of calcium-channel blockers

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Abstract

Hypertension is the leading risk factor for cardiovascular disease and one of the major health concerns worldwide. Genetic factors impact both the risk for hypertension and the therapeutic effect of antihypertensive drugs. Sex- and age-specific variances in the prevalence of hypertension are partly induced by estrogen. We investigated 6 single nucleotide polymorphisms in genes encoding enzymes involved in estrogen metabolism in relation to sex- and age-specific differences in the systolic and diastolic blood pressure (SBP and DBP) outcome under the treatment of diuretics, calcium-channel blockers (CCBs), angiotensin-converting-enzyme inhibitors, and angiotensin-receptor blockers (ARBs).

We included 5064 subjects (age: 40–82) from the population-based CoLaus cohort. Participants were genotyped for the catechol-O-methyltransferase gene (COMT) variants rs4680, rs737865, and rs165599; the uridine-diphospho-glucuronosyltransferase 1A gene family (UGT1A) variants rs2070959 and rs887829; and the aromatase gene (CYP19A1) variant rs10046. Binomial and linear regression analyses were performed correcting for age, sex, body mass index, smoking, diabetes, and antihypertensive therapy to test whether the variants in focus are significantly associated with BP.

All investigated COMT variants were strongly associated with the effect of diuretics, CCBs, and ARBs on SBP or DBP ($P < .05$), showing an additive effect when occurring in combination. After Bonferroni correction the polymorphism rs4680 (Val[158]Met) in COMT was significantly associated with lower SBP in participants treated with CCBs ($P = .009$) with an especially strong impact in elderly individuals (age $\geq$ 70) alone ($\Delta = -14.08$ mm Hg, $P = .0005$).

These results underline the important role of estrogens and catecholamines in hypertension and the importance of genotype dependent, age-related adjustments of calcium-channel blocker treatment.

Abbreviations: ACEI = angiotensin-converting-enzyme inhibitors, ARBs = angiotensin-receptor blockers, ATC = Anatomical Therapeutic Chemical, Beta = standardized beta coefficient, BMI = body mass index, BP = blood pressure, CCBs = calcium-channel blockers, COMT = catechol-O-methyl transferase, CVD = cardiovascular disease, CYP19A1 = aromatase, DBP = diastolic blood pressure, HWE = Hardy–Weinberg equilibrium, P_{adj} = adjusted P value obtained by using logistic regression analyses, P_{unadj} = unadjusted P value, SBP = systolic blood pressure, SNP = single nucleotide polymorphism, UGT1A = uridine-diphospho-glucuronosyltransferase 1A, UTR = untranslated region, Δ = difference of mean blood pressure levels between wildtype carriers and minor allele carriers of specific genetic variants under comparison (reference value = blood pressure level of wildtype carriers).

Keywords: age- and sex-related effect, calcium-channel blockers, catechol-O-methyltransferase, estrogen, hypertension, pharmacogenetics, single nucleotide polymorphism

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1. Introduction

Currently, hypertension affects nearly one-third of the adults worldwide.[1] It is a major risk factor for cardiovascular diseases (CVDs), which are major causes of morbidity and mortality.[2–6] The number of adults with hypertension in 2025 has been predicted to increase by about 60% to a total of 1.56 billion.[7] Hypertension is especially prevalent in the elderly population.[8,9] Furthermore, the risk for clinical complications such as coronary artery disease, congestive heart disease, stroke, chronic kidney insufficiency, and dementia is also higher in this subgroup of population.[8] The pathogenesis of high arterial blood pressure (BP) is known to be influenced by both environmental and genetic factors. It is estimated that genetic factors are responsible for 30% to 50% of BP differences seen among individuals.[3]

Various treatment strategies are available to help controlling BP and reducing the risk for associated clinical complications.[10] Especially in elderly patients, age 70 years and older, hypertension management represents a dilemma.[8,9] Treatment with antihypertensives in this patient group appears to be not as efficient with regard to BP adjustment compared with younger patients, as elderly patients taking antihypertensive medications have shown to be at much higher risk for ischemic events and poor oxygenation in brain, heart, or kidney than younger patients.[8]

First-line drugs against hypertension include diuretics, calcium-channel blockers (CCBs), angiotensin-converting-enzyme inhibitors (ACEIs), and angiotensin-receptor blockers (ARBs).[11] However, the therapeutic effect of these medications varies among individuals, which is partly induced by genetic variants.[10,12] Furthermore, a sex dimorphism has been shown to exist for BP regulation as demonstrated in several population-based studies. This effect has been attributed to different estrogen levels in men and women and associated differences in the estrogen-dependent expression of proteins involved in BP regulation.[3,4,13–16] Thus, variances in the activity of proteins involved in estrogen metabolism, as caused by, for example, genetic polymorphisms, may influence the risk for hypertension.[3]

The enzyme catechol-O-methyltransferase (COMT) catalyzes the transformation of estrogen and other catechol substrates into methylated inactive metabolites.[17] The polymorphism rs4680 (Val[158]Met) in COMT drives major individual differences in the enzymatic activity of COMT.[18] Homozygous Met (A) allele carriers showed significantly decreased COMT activity, higher BP, and hypertension prevalence in Japanese men, compared with Met/Val or Val/Val carriers.[19–21] The intronic single nucleotide polymorphism (SNP) rs737865 and the polymorphism rs165599 near the 3′ untranslated region (UTR) induce an additional variation in COMT activity.[18,22]

The enzyme family uridine-diphospho-glucuronosyltransferase 1A (UGT1A) facilitates estrogen excretion by preventing enhanced tissue exposure to the hormone, thus, weakening estrogen receptor-dependent signaling.[23] The polymorphisms rs2070959 (Thr[181]Ala) and rs887829 are located in the genes UGT1A6 and UGT1A1, respectively. Together with the variant rs1105879 (Arg[184]Ser, UGT1A6), rs2070959 causes 30% to 50% lower activity of UGT1A6.[24]

The aromatase CYP19A1 catalyzes the key step of estrogen synthesis from androgens and, thus, impacts the serum estrogen-androgen balance.[3,4] The SNP rs10046 located in the 3′ UTR of CYP19A1 has been associated with higher and lower serum estradiol levels in different studies.[3,25] Several publications have discussed the role of CYP19A1 polymorphisms as risk markers for general or sex-dependent susceptibility to hypertension.[13,26]

While several studies have addressed the impact of estrogen levels and a modulated estrogen metabolism on BP levels, no previous study has systematically investigated the impact of genetic variants in key genes involved in estrogen transformation on the therapeutic effect of specific types of antihypertensive drugs.

To close this gap of knowledge we investigated the association of 6 genetic variants within the estrogen transforming genes COMT, UGT1A, and CYP19A1 with the therapeutic effect of antihypertensive drugs (i.e., diuretics, CCBs, ACEIs, and ARBs) in a population-based cohort study CoLaus, including 5064 subjects of Swiss origin, 1337 of them treated with different types of antihypertensive drugs. Importantly, we scrutinized whether genetically induced sex- or age-specific effects are abundant with regard to treatment outcome with the mentioned therapeutics.

2. Materials and methods

2.1. Study design and subjects

We included individuals from the population-based CoLaus cohort in our analyses. The primary aim of the CoLaus study was to evaluate the prevalence and determinants of CVD in the Caucasian population living in Lausanne, Switzerland. The sampling methodology of the CoLaus study has been previously described.[27] In brief, the CoLaus study (2003–2006) enrolled 6733 participants at baseline, of whom 5064 participants were re-contacted for a follow-up visit (2009–2012). All participants attended the outpatient clinic at the University Hospital of Lausanne after an overnight fast with a minimum fasting period of 8 h. The visit included a personal interview and a physical examination conducted by trained field interviewers. At baseline, the age of participants ranged between 35 and 75 years. The average follow-up period was 5.4 years with an interquartile range of 5.3 to 5.6 years. The study was approved by the local Institutional Ethics Committee of the University of Lausanne. Written informed consent was obtained from all participants.

In the frame of our study, we included clinical and genotyping data of individuals collected during the follow-up visit. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times on the left arm in a sitting position after an initial resting period of at least 10 min. BP measurements were performed using an Omron HEM-907 automated oscillometric sphygmomanometer. The BP measurements considered in the present study are average values of the 2 last measurements. Based on smoking habits, participants were categorized into current smokers, never smokers, and former smokers. Diabetes was defined as showing fasting plasma glucose ≥7 mmol/L and/or using antidiabetic medications or insulin. Body weight and height were measured without shoes in light indoor clothes. Body mass index (BMI) was calculated as weight (kg) divided by the height squared (m^2). Menopause status was defined as the absence of menstruation. Information on perimenopause was not collected.

Antihypertensive medications were coded using the Anatomical Therapeutic Chemical (ATC) classification system. Treatment combinations were split into the medication classes they contained. For instance, the ATC code C09DA01 representing the combination of Losartan and diuretics was split into the antihypertensive medication groups of "ARBs" and "diuretics."

2.2. Genotyping

In the CoLaus study, nuclear DNA was extracted from whole blood. Genotyping was conducted using the Affymetrix 500K

SNP chip. In the present study, 6 common genetic variants were investigated. The SNPs rs737865 (A/G), rs4680 (A/G, Met[158]Val), and rs165599 (A/G) are located within the promoter, in exon 4 and within the 3′ UTR of COMT, respectively. The genetic variants rs2070959 (A/G, Thr[181]Ala) and rs887829 (C/T) are located in exon 1 of UGT1A6 and in the core promoter region of UGT1A1, respectively.[28] The genetic variant rs10046 (A/G) is located close to the 3′ UTR of CYP19A1. All variants were imputed based on linkage behavior obtaining values between 0 and 2. The applied cut-off values for inclusion into the study were 0.2, 0.8, 1.2, and 1.8, and rounded to the values 0 (wildtype), 1 (heterozygous mutated), and 2 (homozygous mutated), respectively. Hardy–Weinberg equilibrium (HWE) was tested using the package "Hardy–Weinberg" in R, which applies a Fisher exact test on allele frequencies. None of the SNPs deviated from HWE. The estimation of linkage disequilibrium between SNPs was performed using Haploview 4.1 software.

2.3. Generation of a combined genetic COMT score

Based on the effect on BP under treatment with CCB, a combined genetic COMT score was defined, taking the 3 investigated COMT variants into consideration. For each allele lowering the BP under CCB treatment 1 point was given. Thus, based on the number of alleles, individuals were reaching a COMT score between 0 and 6.

2.4. Statistical analysis

The unpaired Student t test (2-tailed) or the Mann–Whitney U test was applied to test for differences of continuous variables between 2 subgroups in unadjusted analyses.

A general linear model (univariate) was applied to investigate the additive impact of risk alleles on BP levels (mm Hg). Sitting BP levels (mm Hg) and genotypes were chosen as dependent outcome parameters in linear and logistic regression analyses, respectively. Binomial logistic and multiple-linear regression analyses were applied to correct for important covariates, such as age, sex (female = 0, male = 1), BMI (kg/m^2), current tobacco smoking, diabetes, menopause state, as well as the intake of more than one type of BP-lowering drug. Menopausal state was included as covariate for analyses investigating the group of women comprising both pre- and postmenopausal subjects. To assess whether sex or age exerted an impact on the association between genotype and the therapeutic effect of antihypertensives, different statistical models were constructed to test for potential interactions between genotypes, sex and age with sitting SBP and DBP levels as outcome variable. Specifically, the following interaction terms were included in the model: genotype*sex, genotype*age and genotype*age*sex.

A P value < .05 was considered significant in both unadjusted and adjusted analyses. In multiple comparisons, statistically significant values were evaluated based on Bonferroni corrected P value thresholds to control for type I error. Differences in SBP or DBP over 10 or 5 mm Hg were evaluated as clinically relevant.

The statistical analyses were performed using SPSS software (Version 23.0.0). The flow chart representing the steps of the analysis approach is shown in Fig. 1.

3. Results

3.1. Outcome of clinical characteristics in the investigated cohort

As shown in Table 1, sexes were equally represented in the CoLaus cohort (men = 2357, women = 2707). In the whole
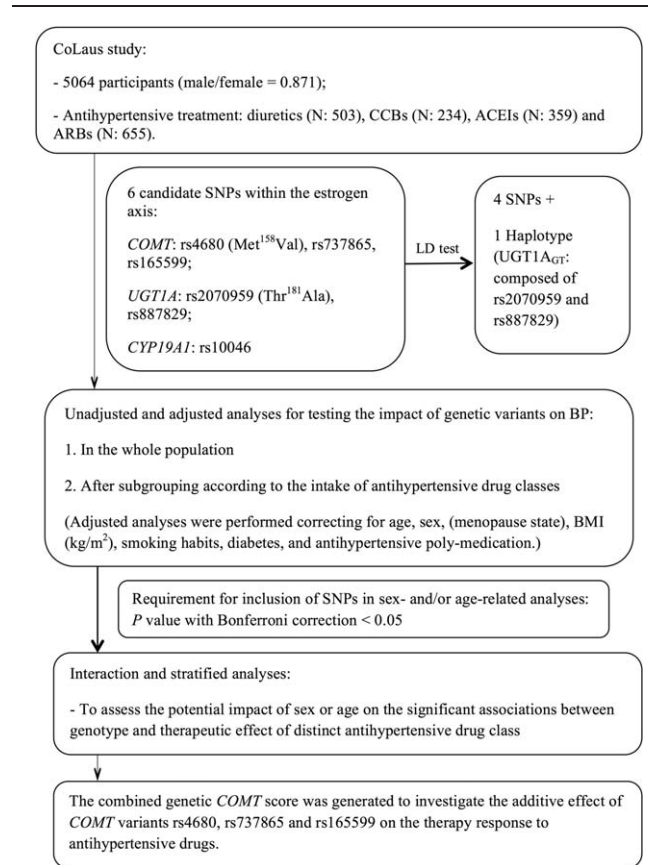
Figure 1. Flow chart illustrating study design and objectives tested. Initially, statistical analyses were performed in the whole population testing for differences in BP levels in relation to genetic variants in genes involved in estrogen metabolism. In addition, the influence of these SNPs on BP under the treatment of antihypertensive drugs was tested. Both unadjusted and adjusted analyses were performed. Adjusted analyses were conducted correcting for age, sex, BMI (kg/m^2), smoking habits, diabetes, antihypertensive polymedication, and menopausal state (included as covariate in analyses investigating specifically pre- and postmenopausal women). In multiple comparisons, statistically significant values were evaluated based on Bonferroni corrected P values. Statistical outcomes reaching Bonferroni corrected P value thresholds ($P < .01$) were further investigated in sex- and age-related interaction and stratification analyses (age cutoff: 70 years). ACEIs = angiotensin-converting-enzyme inhibitors, ARBs = angiotensin-receptor blockers, BMI = body mass index, CCBs = calcium-channel blockers, LD = linkage disequilibrium, N = number of subjects, SNP = single nucleotide polymorphism.

cohort, ARBs (men/women = 353/302) were the most often prescribed antihypertensive drug group, followed by diuretics (men/women = 262/241), ACEIs (men/women = 210/149), and CCBs (men/women = 132/102) (Table 1). Men had significantly higher sitting SBP and DBP levels than women in unadjusted analyses ($P < .001$) (Table 1). Furthermore, significant differences were detected between men and women with regard to age, smoking habits, diabetes, BMI (kg/m^2), whether or not taking antihypertensives in mono- or combination therapy ($P < .05$) (Table 1). These parameters were used as covariates in subsequently performed adjusted analyses investigating the association of genetic variants with sitting SBP and DBP levels.

3.2. Genotyping results

All 6 genetic variants investigated in the present study were in HWE (Table 2). Two SNPs rs2070959 (UGT1A6) and rs887829 (UGT1A1) were identified to be in strong linkage disequilibrium

Table 1

Clinical characteristics of the subjects from the population-based CoLaus cohort.

Clinical characteristics, N	All (5064)	Women (2707)	Men (2357)	P
Age	57.764 ± 0.148 (5064)	58.234 ± 0.202 (2707)	57.225 ± 0.217 (2357)	.001
Smoker (%)	1089/5007 (21.7%)	549/2668 (20.6%)	540/2339 (23.1%)	.033
Diabetes (%)	539/5044 (10.7%)	168/2697 (6.2%)	371/2347 (15.8%)	.000
BMI, kg/m^2	26.195 ± 0.065 (4999)	25.521 ± 0.096 (2673)	26.971 ± 0.084 (2326)	.000
Sitting systolic BP (mm Hg)	126.128 ± 0.252 (5048)	122.21 ± 0.353 (2696)	130.623 ± 0.337 (2352)	.000
Sitting diastolic BP (mm Hg)	78.059 ± 0.152 (5048)	76.403 ± 0.206 (2696)	79.958 ± 0.220 (2352)	.000
AHT (%)	1337/5064 (26.4%)	640/2707 (23.6%)	697/2357 (29.6%)	.000
Co-AHT (%)	448/5064 (8.8%)	199/2707 (7.4%)	249/2357 (10.6%)	.000
Diuretics (%)	503/5064 (9.9%)	241/2707 (8.9%)	262/2357 (11.1%)	.010
Calcium-channel blockers (%)	234/5064 (4.6%)	102/2707 (3.8%)	132/2357 (5.6%)	.002
Angiotensin-converting-enzyme inhibitors (%)	359/5064 (7.1%)	149/2707 (5.5%)	210/2357 (8.9%)	.000
Angiotensin-receptor blockers (%)	655/5064 (12.9%)	302/2707 (11.2%)	353/2357 (15.0%)	.000

The results are shown as mean ± standard error of mean. P values representing the differences of tobacco smoking, diabetes, whether or not under (distinct types of) antihypertensive treatment and whether or not taking more than one type of BP-lowering medications between men and women were obtained using chi-squared tests (2-sided); Unadjusted P values testing for differences of age, BMI, systolic and diastolic BP between men and women were obtained using the unpaired Student t test (2-sided). P < .05 was considered as significant and is shown in bold.
AHT = taking at least one type of antihypertensive drug, BMI = body mass index, BP = blood pressure, Co-AHT = taking more than one type of antihypertensive drug, N = the number of subjects.

(D′ = 0.909, r^2 = 0.804). Therefore, the haplotype UGT1A$_{GT}$ combining these 2 variants was considered in subsequent analyses; 38.6% of subjects carried the wildtype form of this haplotype while 55.2% of subjects carried the haplotype UGT1A$_{GT}$ in heterozygous and homozygous form. These 2 groups of subjects were compared in subsequent analyses; 6.2% of subjects were carrying other haplotypes composed of only 1 genetic variant.

3.3. A COMT variant is significantly associated with lower BP in patients taking CCB

Initially, we investigated to what extent the 6 SNPs in focus are associated with SBP and DBP. As shown in Table 3 (first column), none of the SNPs showed a relevant strong effect on BP before subgrouping into different treatment groups (P > .05).

After dividing participants into subgroups according to antihypertensive treatment, the COMT SNP rs4680 was associated with lower BP levels in patients taking CCBs (SBP: P_{unadj} = .009, P_{adj} = .008) or ARBs (DBP: P_{unadj} = .037) (Table 3). The other 2 COMT variants rs737865 and rs165599 were associated with higher SBP levels in participants treated with CCBs (rs737865: P_{unadj} = .041, P_{adj} = .032; rs165599: P_{unadj} = .019, P_{adj} = .035) (Table 3). Individuals treated with diuretics showed lower SBP levels compared with wildtype carriers when carrying the genetic variant rs737865 (P_{unadj} = .023, P_{adj} = .039) (Table 3). The haplotype UGT1A$_{GT}$ and rs10046 in CYP19A1 was not associated with a modulated therapeutic effect of antihypertensives (P > .05) (Table 3). Furthermore, none of the investigated genetic variants exhibited a significant effect in

individuals taking ACEIs (P > .05) (Table 3). After Bonferroni correction (P < .01 [0.05/5]), the association of COMT SNP rs4680 with SBP in individuals treated with CCBs was remained significant (Table 3).

3.4. A COMT variant is significantly associated with lower systolic BP especially in elderly individuals taking CCB

As illustrated in Table 4, multiple linear regression analyses revealed that the association of rs4680 (COMT) with SBP in participants taking CCBs (beta = −0.194, P = .007) appeared to be especially influenced by age (beta = 0.167, P = .024) and sex (beta = 0.148, P = .042). By contrast, no significant association was detected between rs4680 and DBP in participants treated with CCBs (P > .05) (Table 4).

Therefore, we investigated in the next step to what extent age or sex influence the association between rs4680 and the therapeutic effect of CCBs. As shown in Table 5, a significant interaction between rs4680 and age was observed in patients treated with CCBs (SBP: P = .004, DBP: P = .006). Interestingly, the observed significant differences in BP were limited to elderly individuals ≥70 years of age. In this subgroup, rs4680 carriers treated with CCBs showed significantly lower SBP levels compared with wildtype carriers (Δ = −14.08 mm Hg, P_{unadj} = .0005, P_{adj} = .0019) (Table 6, Fig. 2A). Although not significant, DBP levels tended to be lower in elderly rs4680 carriers taking CCBs (Δ = −4.97 mm Hg) (Table 6, Fig. 2C). In line with this observation, we detected in this subject group stepwise increasing differences in SBP levels in relation to the number of

Table 2

Genotype frequencies of SNPs investigated in the population-based CoLaus cohort.

SNP	Gene	Allele (forward)		Genotype frequency			Minor allele proportion (%)	n	P (Hardy–Weinberg equilibrium)
		Major	Minor	Wild type	Heterozygous	Homozygous			
rs4680	COMT	A (Met)	G (Val)	1013	2078	997	49.80	4088	.287
rs737865	COMT	A	G	1830	1692	367	31.19	3889	.397
rs165599	COMT	A	G	1914	1607	318	29.21	3839	.452
rs2070959	UGT1A6	A (Thr)	G (Ala)	1673	1866	514	35.70	4053	.858
rs887829	UGT1A1	C	T	1707	1877	525	35.62	4109	.799
rs10046	CYP19A1	A	G	1104	2026	977	48.45	4107	.425

SNP = single nucleotide polymorphism.

Table 3

Association of genetic variants in *COMT*, *UGT1A*, and *CYP19A1* genes with blood pressure in the whole population and in patients treated with distinct types of antihypertensive medications.

SNP	BP	All individuals $\Delta^\dagger$	$P_{unadj}^\ddagger$	$P_{adj}^\S$	Diuretics $\Delta^\dagger$	$P_{unadj}^\ddagger$	$P_{adj}^\S$	CCBs $\Delta^\dagger$	$P_{unadj}^\ddagger$	$P_{adj}^\S$	ACEIs $\Delta^\dagger$	$P_{unadj}^\ddagger$	$P_{adj}^\S$	ARBs $\Delta^\dagger$	$P_{unadj}^\ddagger$	$P_{adj}^\S$
rs4680	SBP	0.87	ns	ns	0.63	ns	ns	−6.53	**.009**[**]	**.008**[**]	−1.28	ns	ns	1.16	.525	.924
	DBP	−0.14	ns	ns	−0.92	ns	ns	−1.07	.560	.509	0.57	ns	ns	−2.37	.037[*]	.138
	N	1009/3066			104/314			55/137			71/233			134/407		
rs737865	SBP	−0.87	ns	ns	−4.09	.023[*]	.039[*]	4.76	.041[*]	.032[*]	−1.43	ns	ns	−2.59	ns	ns
	DBP	0.09	ns	ns	−1.15	.485	.574	2.77	.094	.132	1.02	ns	ns	−0.21	ns	ns
	N	1824/2052			193/213			83/102			134/155			251/279		
rs165599	SBP	−0.07	ns	ns	−1.06	ns	ns	5.76	.019[*]	.035[*]	0.48	ns	ns	−0.21	ns	ns
	DBP	0.40	ns	ns	1.36	ns	ns	2.45	.157	.222	1.34	ns	ns	1.11	ns	ns
	N	1907/1920			189/195			82/93			147/140			258/248		
UGT1A$_{GT}$	SBP	0.21	ns	ns	0.04	ns	ns	−4.32	ns	ns	0.14	ns	ns	−0.54	ns	ns
	DBP	−0.19	ns	ns	−0.25	ns	ns	−2.13	ns	ns	−0.35	ns	ns	−0.59	ns	ns
	N	1554/2229			167/223			78/100			114/160			212/292		
rs10046	SBP	−0.19	ns	ns	−0.70	ns	ns	2.53	ns	ns	−2.28	ns	ns	0.24	ns	ns
	DBP	−0.10	ns	ns	−1.17	ns	ns	0.69	ns	ns	−1.56	ns	ns	0.49	ns	ns
	N	1103/2991			106/316			48/148			80/225			156/390		

Significant associations after Bonferroni correction with $P < .01$ (0.05/5) are shown in bold.

ACEIs = angiotensin-converting enzyme inhibitors, ARBs = angiotensin-receptor blockers, BP = blood pressure, CCBs = calcium-channel blockers, DBP = diastolic BP, N = number of subjects (wildtype/variant carriers), ns = not significant, SBP = systolic BP.

† Difference of SBP or DBP mean values between wildtype and variant carriers (reference value: wildtype) (mm Hg).

‡ Unadjusted P values obtained when testing for differences of SBP or DBP between wildtype and variant carriers using the unpaired Student t test (2-sided).

§ Adjusted P values obtained using binomial logistic regression analyses correcting for age, sex, body mass index (kg/m^2), smoking, diabetes, antihypertensive therapies.

* $P < .05$.

** $P < .01$.

Table 4

Multiple-linear regression analyses investigating the association of *COMT* genetic variant rs4680 with SBP and DBP in patients treated with CCBs.

	Multiple-linear regression models					
	Outcome: SBP			Outcome: DBP		
Drug† (SNP)	Predictors	Beta	P	Predictors	Beta	P
CCBs (Rs4680)	**SNP**	**−0.194**	**.007**[**]	SNP	−0.044	.519
	Age	**0.167**	**.024**[*]	**Age**	**−0.335**	**.000**[**]
	Sex (F = 0)	**0.148**	**.042**[*]	Sex (F = 0)	−0.029	.675
	BMI	−0.039	.611	BMI	0.132	.071
	Smoking	−0.102	.159	**Smoking**	**−0.180**	**.010**[*]
	Diabetes	−0.061	.413	Diabetes	−0.133	.063
	Co-AHT	0.053	.480	Co-AHT	−0.022	.757

Models were adjusted for the specific genetic variant (whether or not carrying the minor allele), age, sex (F = 0), BMI (kg/m^2), smoking habits, diabetes, and antihypertensive polymedication. P values <.05 were considered as significant and set in bold.

Beta = standardized beta coefficient, BMI = body mass index, CCBs = calcium-channel blockers, Co-AHT = treatment with more than one type of antihypertensive medication, DBP = diastolic BP, F = female, SBP = systolic BP.

† Adjusted multiple-linear regression models were applied on Bonferroni corrected significant associations listed in Table 3.

* $P < .05$.

** $P < .01$.

Table 5

Blood pressure lowering effects of CCBs in relation to COMT rs4680 genotypes (AA vs. AG+GG) and interactions with age and sex.

Variables (N)	P Genotype*sex	Genotype*age	Genotype*age*sex	Observed power Genotype*sex	Genotype*age	Genotype*age*sex
SBP (192)	.048[*]	**.004**[**]	.077	0.589	0.858	0.514
DBP (192)	.444	**.006**[**]	.440	0.188	0.832	0.190

P value and observed power were obtained using general linear model to assess whether and to how much degree sex or age exerted a significant impact on the association between the variant rs4680 genotype and the response to CCBs. Models were tested for interactions between genotypes, sex, and age with respect to SBP and DBP levels. Specifically, the following interaction terms were included in the model: genotype*sex, genotype*age, and genotype*age*sex. P values <.05 were considered as significant. Significant associations with observed power >0.8 were shown in bold.

CCBs = calcium-channel blockers, DBP = diastolic blood pressure, N = number of subjects, SBP = systolic blood pressure.

* $P < .05$.

** $P < .01$.

Table 6

Subgroup analyses to investigate the effect of age or sex on the associations between the *COMT* SNP rs4680 and systolic or diastolic blood pressure in individuals treated with CCBs.

Stratification	Subgroups	Genotype	N	SBP				DBP			
				SBP, mm Hg	$\Delta^\dagger$, mm Hg	$P_{unadj}^\ddagger$	$P_{adj}^\S$	DBP, mm Hg	$\Delta^\dagger$, mm Hg	$P_{unadj}^\ddagger$	$P_{adj}^\S$
Age	<70	AA	35	135.03				79.53			
		AG+GG	80	132.86	−2.17	.491	.588	81.38	1.85	.415	.553
	≥70	AA	20	146.52				77.83			
		AG+GG	57	132.44	−14.08	.0005**	.0019**	72.86	−4.97	.079	.216
Sex	Male	AA	33	140.08				78.85			
		AG+GG	81	134.56	−5.52	.081	.052	77.48	−1.37	.530	.595
	Female	AA	22	137.91				79.00			
		AG+GG	56	129.96	−7.95	.051	.074	78.36	−0.64	.843	.785

P values < .05 were considered significant and shown in bold.
BP = blood pressure, CCBs = calcium-channel blockers, DBP = diastolic BP, N = number of subjects (wildtype/variant carriers), SBP = systolic BP, SNP = single nucleotide polymorphism.
† Difference of BP mean values between wildtype (reference value, AA) and variant (AG + GG) carriers.
‡ Unadjusted *P* values were obtained using unpaired Student *t* test to detect significant BP differences between wildtype (AA) and variant (AG + GG) carriers.
§ Adjusted *P* values obtained using binomial logistic regression analyses correcting for sex, age, body mass index (kg/m^2), smoking, diabetes, and antihypertensive poly-medication.
* *P* < .05.
** *P* < .01.

abundant rs4680 alleles compared with wildtype carriers (*P* = .0017) (Fig. 2B). The interaction between rs4680 genotypes and sex was also strongly associated with SBP in individuals treated with CCBs (*P* = .048). However, this result should be interpreted with caution since the observed statistical power was relatively low (Table 5). In addition, subgroups stratified by sex did not show significant differences in BP in participants taking CCBs between wildtype and rs4680 carriers in either males or females (*P* > .05) (Table 6). Investigations in the very small subgroup of individuals ≥ 70 years treated with CCBs revealed a beneficial association of SNP rs4680 with SBP and DBP in elderly male individuals [SBP/DBP: 150.62/80.04 (AA, n=31) versus 130.45/71.18 mm Hg (AG or GG, n = 13), p$_{adj}$ = 3.26×10^{-5} (SBP) and 6.07×10^{-4}(DBP)] compared to elderly women [138.93/73 mm Hg (AA, n = 7) versus 134.81/74.87 mm Hg (AG or GG, n = 26), p$_{adj}$ = not significant for SBP and DBP].

3.5. The combined occurrence of genetic variants in COMT shows an additive effect on the treatment outcome with CCBs

Interestingly, a higher COMT score, as constructed based on the individual abundance of the 3 *COMT* variants rs4680, rs737865, and rs165599, was significantly associated with lower SBP in participants taking CCBs (*P* = .019). This strong association was maintained when adjusting for sex and age (*P* = .018) (Table 7). Furthermore, after correcting for age and sex, participants treated with CCBs and reaching scores that belong to the highest quartile of the COMT score showed a significantly lower SBP than individuals belonging to the lowest COMT score quartile (*P* = .001) (Table 7).

4. Discussion

In this large population-based study, we showed that genetic variants affecting estrogen metabolism are significantly associated with a modulated effect of antihypertensives especially in elderly individuals. To our knowledge, this is the first study that identifies *COMT* as a clinically significant susceptibility gene for the therapeutic effect of CCBs.

Previous studies obtained either inconsistent results regarding the relation of here investigated estrogen-related SNPs with the prevalence of hypertension or BP regulation.[3,4,13,29,30] The association of estrogen-related polymorphisms with hypertension has only been tested for the SNPs rs4680 (*COMT*) and rs10046 (*CYP19A1*). Importantly, none of the mentioned SNPs
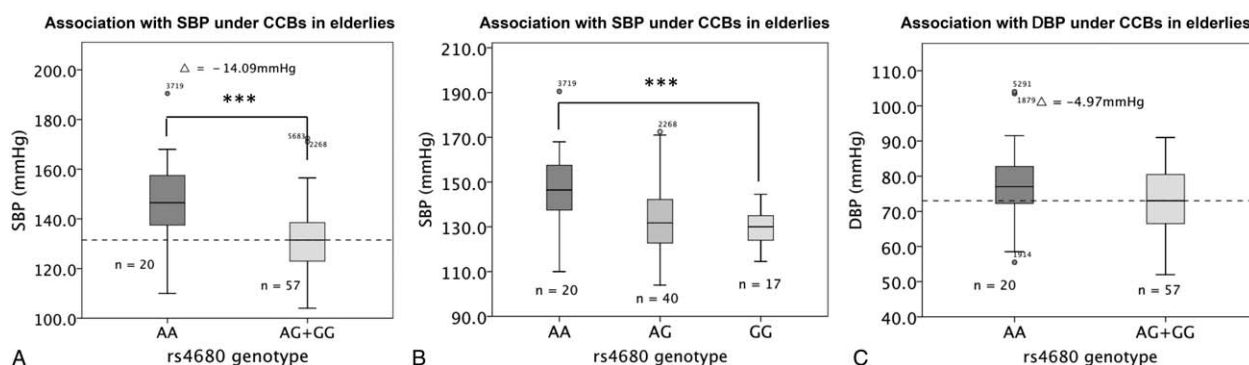
Figure 2. The association of *COMT* variant rs4680 with blood pressure under the treatment of calcium-channel blockers (CCBs) in elderly individuals (age ≥ 70). (A, B) Elderly individuals treated with CCBs and carrying the G allele of rs4680 showed significantly lower SBP levels than wildtype carriers. This observation was strongly pronounced in homozygous rs4680 variant carriers. (C) DBP was lower in elderly rs4680 carriers treated with CCBs compared with elderly wildtype carriers. * *P* < .05, ** *P* < .01, *** *P* < .005. *P* values were obtained using the unpaired Student *t* test (2-sided) or general linear model (univariate) analysis. DBP = diastolic blood pressure, SBP = systolic blood pressure.

Table 7

Associations of the genetic COMT score with blood pressure in patients treated with CCBs.

Subjects	Model	Linear regression analyses					
		Outcome: SBP			Outcome: DBP		
		Predictor	Beta	*P*	Predictor	Beta	*P*
CCBs	Model 1[†] (N=168)	**COMT**	**−0.181**	**.019**[*]	COMT	−0.075	.336
	Model 2[‡] (N=168)	**COMT**	**−0.179**	**.018**[*]	COMT	−0.073	.327
		Sex (F=0)	0.121	.108	Sex (F=0)	−0.063	.402
		Age	**0.224**	**.003**[**]	**Age**	**−0.303**	**.000**[**]
	Model 3[§] (N=123)	**qtCOMT**	**−0.283**	**.001**[**]	qtCOMT	−0.154	.075
		Sex (F=0)	0.135	.120	Sex (F=0)	−0.096	.268
		Age	0.170	.052	**Age**	**−0.304**	**.001**[**]

P values < .05 were considered as significant and shown in bold.

Beta = standardized beta coefficiency, BP = blood pressure, CCBs = calcium-channel blockers, COMT = COMT score, DBP = diastolic BP, F = female, N = number of subjects, qtCOMT = the lowest and the highest COMT score quartiles, SBP = systolic BP.

[†] Linear regression models investigating the impact of the genetic COMT score on BP.

[‡] Multiple-linear regression models adjusted for the *COMT* score, sex (F=0), and age.

[§] Multiple-linear regression models adjusted for the lowest and the highest *COMT* score quartiles, sex (F=0), and age.

[*] $P < .05$.

[**] $P < .01$.

have been investigated in relation to the therapeutic effect of distinct antihypertensive drug groups.

We demonstrate that the coding variant rs4680 (*COMT*) is significantly associated with lower SBP levels especially in elderly individuals taking CCBs. Rs4680 is commonly occurring with a minor allele frequency of 49.80% in our cohort (50% in white populations[31,32]), which allows powerful pharmacogenetic investigations even in groups comprising smaller samples sizes.

Previous studies demonstrated that rs4680 was significantly associated with increased COMT activity, lower BP levels, and lower prevalence of hypertension.[19–21,30,33,34] Interestingly, we did not per se observe a general effect of this variant on BP levels but rather in association with distinct antihypertensive treatment and after stratification according to age. This observation may be a result of a changed COMT-dependent metabolism of catecholamines and estrogens. Decreased levels of circulating catecholamines induce lower BP levels and cardiac output[35–37] (Fig. 3). The age-dependent effect of rs4680 may be a result of significantly elevated COMT mRNA expression and protein activity especially observed in higher age,[34,38] consecutively leading to a higher metabolism of norepinephrine to normetanephrine, which ultimately results in the beneficial effect on BP
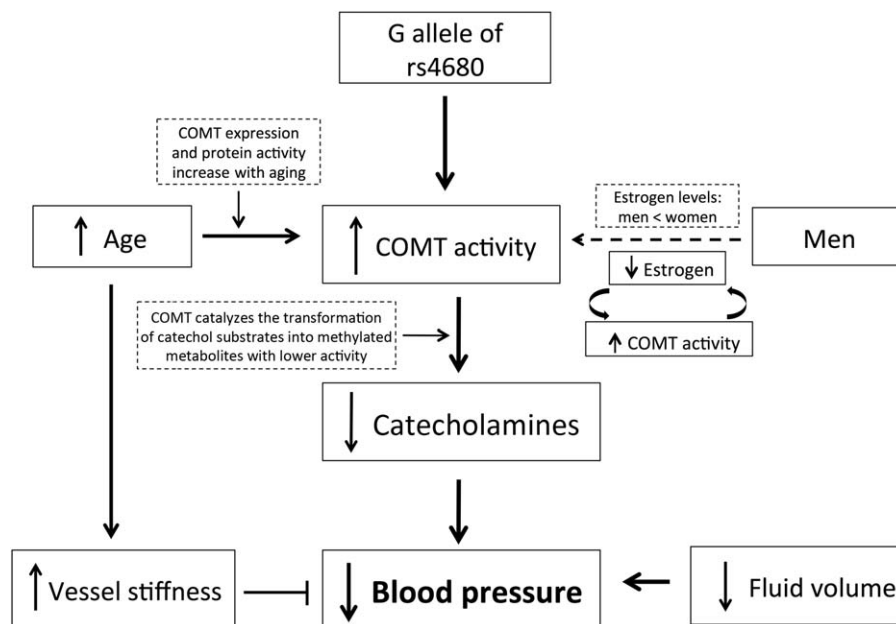
Figure 3. Hypothesized functional pathway causing the age-related impact of *COMT* SNP rs4680 on blood pressure regulation. COMT mRNA expression, COMT protein levels, and enzyme activity are influenced by age and genetic variants. The polymorphism rs4680 in *COMT* is associated with major individual differences in COMT activity. The Val (G) allele is associated with significantly higher COMT activity. Age is associated with increased COMT expression and, thus, activity across different genotypes of the coding SNP rs4680. Since COMT catalyzes the breakdown of catecholamines into methylated metabolites with lower activity, higher COMT expression and activity causes a decrease of circulating catecholamine and blood pressure levels. Furthermore, fluid volume and vessel stiffness impact blood pressure levels as well. The estrogen-dependent regulation of COMT expression induces a sexually dimorphic effect of rs4680 on COMT activity, leading to the observation that specifically elderly male variant carriers profited from a BP lowering effect of CCBs. However, this observed association was made in a very small sample and is therefore shown with a dashed arrow. BP = blood pressure, SNP = single nucleotide polymorphism.

seen in the elderly[39] (Fig. 3). These observations underline the importance of age-specific adjustments of antihypertensive treatment.

Our adjusted analyses suggest a sexually dimorphic beneficial effect of rs4680 on SBP regulation in individuals treated with CCBs. According to the results, specifically elderly male carriers of rs4680 showed lower SBP under CCBs. This observation may be a result of estrogen-dependent higher COMT activity and subsequent lower circulating catecholamine levels in males[14,40,41] (Fig. 3). This observation was, however, not confirmed in whole cohort based subgroup analyses stratifying by sex, putatively because of a too small sample size in our study.

While association of the COMT variants rs737865 and rs165599 with the therapy effect of diuretics and CCBs on BP did not remain significant after Bonferroni correction, the general importance of COMT variants for antihypertensive therapy is further supported by an additive effect of COMT SNPs on BP under CCBs that we observed in our study.

Other investigated SNPs (i.e., the UGT1A SNPs rs2070959 and rs887829, or rs10046 of CYP19A1) were not associated with changes in BP levels under antihypertensives.

Future studies should elucidate, why an age-dependent effect is specifically observed for CCBs in relation to a polymorphic expression of COMT. Those investigations should take even larger numbers of individuals into consideration specifically treated with the therapeutics in focus. Furthermore, future studies should also take applied drug dosages, estrogen replacement therapy, and the compliance to the treatment into consideration, which we were not able to correct for in our analyses.

In conclusion, we detected age-related differences in the therapeutic effect of CCBs, in association with a commonly occurring genetic variant in the COMT gene. Our results propose a relevant role of estrogen and catecholamines in the age-specific pathogenesis of hypertension and underline the need for individualized therapy approaches taking age into account.

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