

# Imputing Observed Blood Pressure for Antihypertensive Treatment: Impact on Population and Genetic Analyses

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## BACKGROUND

Elevated blood pressure (BP), a heritable risk factor for many age-related disorders, is commonly investigated in population and genetic studies, but antihypertensive use can confound study results. Routine methods to adjust for antihypertensives may not sufficiently account for newer treatment protocols (i.e., combination or multiple drug therapy) found in contemporary cohorts.

## METHODS

We refined an existing method to impute unmedicated BP in individuals on antihypertensives by incorporating new treatment trends. We assessed BP and antihypertensive use in male twins ( $n = 1,237$ ) from the Vietnam Era Twin Study of Aging: 36% reported antihypertensive use; 52% of those treated were on multiple drugs.

## RESULTS

Estimated heritability was 0.43 (95% confidence interval (CI) = 0.20–0.50) and 0.44 (95% CI = 0.22–0.61) for measured systolic BP (SBP) and diastolic BP (DBP), respectively. We imputed BP for antihypertensives by

3 approaches: (i) addition of a fixed value of 10/5 mm Hg to measured SBP/DBP; (ii) incremented addition of mm Hg to BP based on number of medications; and (iii) a refined approach adding mm Hg based on antihypertensive drug class and ethnicity. The imputations did not significantly affect estimated heritability of BP. However, use of our most refined imputation method and other methods resulted in significantly increased phenotypic correlations between BP and body mass index, a trait known to be correlated with BP.

## CONCLUSIONS

This study highlights the potential usefulness of applying a representative adjustment for medication use, such as by considering drug class, ethnicity, and the combination of drugs when assessing the relationship between BP and risk factors.

*Keywords:* antihypertensive; blood pressure; body mass index; heritability; hypertension; twins; VETSA.

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Population and family studies are commonly used to investigate the relationship of blood pressure (BP) with traits or disease outcomes, and twin studies are a powerful approach for investigating the genetic and environmental determinants of BP. To the extent that individuals are treated for hypertension in older populations and unmedicated BP data are inaccessible, these studies are often confounded by antihypertensive treatment. There is little consensus on how to account for BP medication use. Some studies exclude medicated individuals,<sup>1,2</sup> use BP measurements without adjusting

for medication use,<sup>3</sup> or adjust analyses by including medication use as a covariable.<sup>4</sup> Such methods may alter the estimated effect of genetic or environmental determinants of BP, obscure the relationship of BP to other factors, or reduce statistical power.<sup>5–7</sup> Further, when BP is the outcome variable, such as in genetic studies (e.g., genetic association, multivariable twin analyses) or studies identifying risk factors for BP, including an antihypertensive use variable as a predictor in a regression model is problematic because of the relationship between BP and antihypertensive use. In such

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cases, imputing unmedicated BP becomes relevant. Tobin *et al.* demonstrated by simulation and real sample data that predicting unmedicated BP based on clinical evidence is an effective strategy of imputing BP for genetic studies.<sup>6</sup>

Recent genetic studies have adopted an approach (“fixed addition”) in which a fixed value of 10/5 mm Hg is added to the observed systolic (SBP)/diastolic (DBP) BP,<sup>8</sup> but most studies do not report whether the adjustment altered the results.<sup>9–14</sup> In a family study that also included twins, Cui *et al.* investigated the genetic and environmental components of BP variation in relation to body mass index (BMI) using fixed addition<sup>8</sup> and, in a follow-up study, compared the results of genetic analysis using measured BP, fixed addition, as well as other approaches.<sup>15</sup> They introduced a “stepped addition” approach in which increments of 8/4 mm Hg, 14/10 mm Hg, and 20/16 mm Hg were added to measured SBP/DBP of treated subjects taking 1, 2, or 3 drug classes, respectively. They concluded that information was lost if treated individuals were excluded or if BP was not adjusted using imputation.

To reach optimal BP, a combination of drugs from different pharmaceutical classes is often required.<sup>16–18</sup> Treatment response varies with drug class and by ethnicity.<sup>19–21</sup> Recognizing that a refined approach integrating drug class and ethnicity information may better predict unmedicated BP, Wu *et al.* devised an algorithm to impute unmedicated BP based on drug class and ethnicity.<sup>22</sup> Using published data from 137 clinical trials of monodrug therapies, Wu *et al.* reported the weighted average effect lowering of SBP and DBP in blacks and nonblacks for 6 major classes of antihypertensives. Using data from 28 clinical trials of combination drug therapies, they estimated the effects of the second medication.<sup>22</sup> They suggested that accounting for these BP-lowering effects could be used to impute pretreatment BP levels to improve the power of studies confounded by BP medication. Adjustment based on drug class effects has been less applied than fixed addition, but its use in a large population study facilitated the identification of genetic risk factors for heart disease.<sup>23</sup>

Current therapy includes multiple ( $\geq 3$ ) drugs,<sup>24–28</sup> and fixed-dose combinations of antihypertensives in a single pill are now routinely prescribed to manage hypertension.<sup>27</sup> Thus, to infer BP more closely reflecting pretreatment values, there is a need for approaches that (i) impute effects beyond 2 drug classes; (ii) incorporate information on newer drug classes; and (iii) account for combination drugs.

We extended the approach of Wu *et al.*<sup>22</sup> to account for multiple drug therapies that reflect treatment scenarios found in recently ascertained cohorts. We applied this approach in an age-homogeneous sample comprised of middle-aged male twin pairs from the Vietnam Era Twin Study of Aging (VETSA) (age range = 51–60 years) in which hypertension is prevalent.<sup>29</sup> We reasoned that our refined imputation approach based on the best available clinical trial evidence would yield a BP that is closer to a person’s “true” BP if they were unmedicated and represents the best estimate of true BP. To the extent that there is error contained in the imputations, they will deviate from the true BP. We examined the effect of this imputation approach on the results of genetic and population studies. We first compared

the correlations between the imputed BP and BMI (a known correlate of BP)<sup>30</sup> with correlations obtained with measured BP or BP imputed with a fixed or stepped addition. Because they reduce BP, antihypertensives also restrict the range of BP. Therefore, we predicted that correlations between drug class-adjusted BP and BMI would be higher than correlations with unadjusted measured BP because of the concomitant increased range of BP. Consistent with this idea, previous studies have shown that imputing BP by adding a clinically reasonable value to medicated BP increases the power to detect determinants of BP.<sup>6</sup> We also investigated how the different imputation approaches affect estimation of heritability (i.e. the proportion of total variance in an observed trait that is due to genes<sup>31</sup>). We hypothesized that our imputation would result in higher heritability estimates for BP. There were 2 bases for this hypothesis. First, in reducing BP, antihypertensives may suppress the natural expression of genetic influences. By offsetting that suppression effect, the imputation might result in increased heritability estimates for BP. Second, medication adds an external environmental factor. Adding more environmental variance would reduce heritability, but the imputation would then offset that reduction. Heritabilities increased slightly, albeit nonsignificantly, after imputation based on drug class effects in a younger sample in which only 14.5% had hypertension.<sup>5</sup> With our further refinement of the imputation and the higher prevalence of hypertension in our older sample, we hypothesized that the imputation might increase heritability.

## METHODS

### Study participants

Participants were from Wave 1 of VETSA, a longitudinal study of cognition and aging beginning in midlife,<sup>29</sup> recruited from the Vietnam Era Twin Registry of male twin pairs who served in the US military sometime between 1965 and 1975.<sup>32,33</sup> VETSA twins resemble the larger registry sample and are representative in demographic and health characteristics of the US population of similarly aged adult men based on US Census and Centers for Disease Control and Prevention data.<sup>34</sup> Our sample (mean age = 55.4 years; age range = 51–60 years) consisted of 347 monozygotic (MZ) twin pairs, 267 dizygotic (DZ) twins pairs, and 9 unpaired twins. The study was approved by local institutional review boards at the University of California–San Diego and Boston University. Ethnicity was based on self-identification: black ( $n = 51$ ), white ( $n = 1,110$ ), and other ( $n = 76$ ; including American Indian or Alaskan Native, Native Hawaiian or Pacific Islander, mixed ethnicity). Age, education, employment status, and ethnicity were similar between the MZ and DZ groups.<sup>35</sup>

### BP and BMI

BP was measured in seated subjects using aneroid sphygmomanometry a total of 4 times, twice in the morning 5 minutes apart and twice in the afternoon 5 minutes apart. Participants rested for 5 minutes before the first BP reading, waited 1 minute, and had a second reading. Measured BP refers to the mean

value of these 4 individual measurements. BMI was defined as weight in kilograms divided by height in meters squared. Height was measured using a wall-mounted stadiometer. Weight with light clothing was measured using a digital scale to the nearest 0.1 lb. BMI was available for 1,235 participants.

## Medication

Medication use was assessed by a self-report structured interview design, which has previously been shown to be reliable when estimating recent use of cardiovascular drugs.<sup>36</sup> Antihypertensives were classified into their pharmacologic classes (Table 1). Combination drugs were counted as 2 drug classes. Beta-blockers with BP-lowering effects prescribed for the treatment of cardiovascular diseases other than hypertension were included.

## Three imputation approaches

For the fixed addition approach, 10/5 mm Hg was added to the SBP/DBP of treated individuals.<sup>15</sup> For the stepped addition approach, 8/4, 14/10, 20/16, 26/22, 32/28, or 38/34 mm Hg was added to SBP/DBP of individuals on 1, 2, 3, 4, 5, or 6 drugs, respectively. For the drug class addition approach, addition of mm Hg was based on the weighted average effect (WAE) of drug class, number of drugs, and ethnicity, as described by Wu *et al.*<sup>22</sup> The published WAEs of monodrug therapy categorized by drug class for blacks and nonblacks based on 165 clinical trials (n = 11,739) were used. The published WAE overall reported drug classes (15.4/11.2 mm Hg for SBP/DBP in blacks and 15.7/10.5 mm Hg for SBP/DBP in nonblacks) were used for the drug classes in our study that were not reported by Wu *et al.*<sup>22</sup>

For 2 drugs or combination drugs, the medication with the higher monodrug effect was designated as the first medication. The weighted effect of the second medication was calculated by the formula:

$$\left( \frac{\text{WAE}_{\text{combination drug therapy}} - \text{WAE}_{\text{1st medication}}}{\text{WAE}_{\text{2nd medication}}} \right) \times 100\%.$$

WAEs were based on published clinical trials. Weighted effects of the second medication were

computed for combinations containing and not containing diuretics and considering ethnicity. The estimated effect on BP of the combination therapy was then calculated as:  $\text{WAE}_{\text{1st drug}} + (\text{WAE}_{\text{2nd drug}} \times \text{weighted effect}_{\text{2nd drug}})$ . Our study used this method for 2 drugs and combination drugs.

An algorithm for  $\geq 3$  drugs was not reported by Wu *et al.*<sup>22</sup> Clinical trials are lacking and studies vary on the effect of additional drugs,<sup>37</sup> although there are a few published studies evaluating the effect of adding a third drug, such as adding thiazide to an angiotensin receptor blocker/calcium channel blocker.<sup>38</sup> Our imputation algorithm assumed additional drugs to have effects lower than its monodrug or second drug effect, as observed in published studies.<sup>38–41</sup> We used the reported effect of approximately 0.5 of the effect for the second drug<sup>22</sup> and conservatively added a smaller percentage effect of the third and subsequent drugs by decreasing the effect by 0.1 for each additional drug. The monodrug effect of the third drug was multiplied by 0.4, the monodrug effect of the fourth drug was multiplied by 0.3, the monodrug effect of the fifth drug was multiplied by 0.2, and the monodrug effect of the sixth drug was multiplied by 0.1. Proportions were totaled and added to the reported effects of the first and second drug class. The range of added mm Hg was 14–38/9–27 to measured SBP/DBP and is within the therapeutic range reported in clinical trials.<sup>42</sup>

The fixed, stepped, and drug class addition approaches progressively include more information in the imputation algorithm and are therefore considered in this study to be more refined in this sequential order.

## Statistical analysis

Descriptive analysis was conducted using R v2.1.2.0. Linear mixed-effects models were used to test the significance of the differences in the mean measured BP and BMI between medicated and unmedicated individuals using the non-linear mixed effects model (NLME) package in R.<sup>43</sup> By using family identification as a random effect in the model, we were able to account for the correlated nature of the twin data.

A 2-tailed significance test of the difference between dependent correlations using Fisher *z* prime transformation<sup>44</sup> was used to assess whether the phenotypic correlation ( $r_p$ ) between BMI and imputed BP was significantly different from the phenotypic correlation of BMI and measured BP.<sup>44</sup>

**Univariable twin model.** In the classical twin design, the variance of any trait is decomposed into the proportion attributed to additive genetic (A) influences (i.e., heritability), common environmental (C) influences (i.e., environmental factors that make members of a twin pair similar to one another), and unique environmental (E) influences (i.e., environmental factors that make members of a twin pair different from one another, including measurement error). This model is referred to as the ACE model (see [Supplementary Information](#)). Models were fit to raw data using the maximum-likelihood based structural equation modeling

**Table 1.** Participants per pharmacologic drug class

Drug class	Unique drugs in drug class	Number of participants
Angiotensin-converting-enzyme inhibitors	10	187
$\alpha_1$ -Blockers	3	17
$\alpha_2$ -Blockers	2	9
$\beta$ -Blockers	7	181
Calcium channel blockers	6	71
Diuretics, nonthiazide	3	26
Thiazides	3	64
Angiotensin receptor blocker	5	57
Combination	12	71
Total	51	683

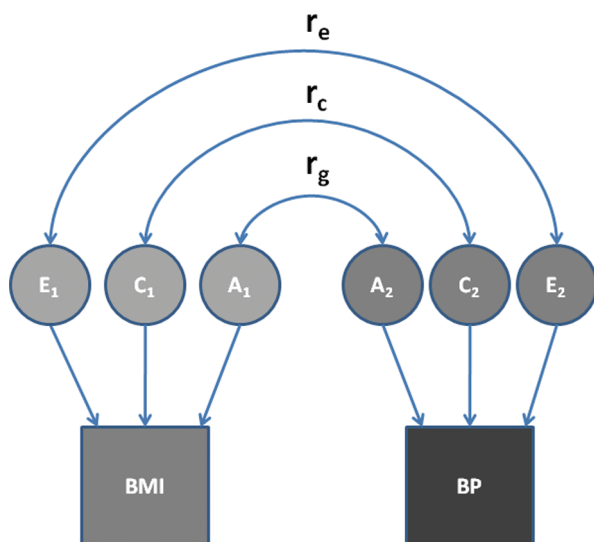
software Mx.<sup>45</sup> Analyses were conducted with both age-unadjusted and age-adjusted BP measurements because there were small correlations ( $r < 0.1$ ) between BP and age. However, results did not significantly differ after adjusting BP measurements for age, and age-unadjusted results are presented.

**Bivariable twin model.** The bivariable extension of the ACE model, in which the covariance between traits is decomposed to derive genetic and environmental covariance estimates, was used to determine the degree of covariance between the A, C, and E contributions to BP and BMI (Figure 1) and to calculate phenotypic, genetic, and environmental correlations between BP and BMI. The genetic correlation between 2 phenotypes is equal to their genetic covariance divided by the square root of the product of their separate genetic variances.<sup>46</sup> It represents the degree to which 2 traits share the same genetic influences.<sup>47</sup> Environmental correlations are defined analogously.

## RESULTS

### Descriptive analyses

There were 445 (36%) participants on BP-lowering medication (Table 2). Of the 792 unmedicated individuals, 265 (21%) had SBP >140 mm Hg or DBP >90 mm Hg. The average age of the medicated and unmedicated groups was similar, but BMI was significantly higher in the medicated group ( $F = 67.42$ ;  $P < 0.0001$ ). Measured SBP and DBP were similar between the 2 groups (Table 3), but the imputed unmedicated SBP and DBP were significantly higher in the medicated group.



**Figure 1.** Bivariable models for genetic (A), common/shared environmental (C), and unique environmental (E) influences on the phenotypic correlation between body mass index (BMI) and blood pressure (BP). Abbreviations:  $r_c$ , shared environment correlation;  $r_e$ , unique environment correlation;  $r_g$ , genetic correlation.

### Univariable twin analyses

Rates of self-reported antihypertensive use were not significantly different between MZ and DZ twins ( $\chi^2 = 2.28$ ;  $df = 1$ ;  $P = 0.13$ ); 36% of MZs and 36% of DZs were taking antihypertensive medications. The MZ correlations for BP, BMI, and antihypertensive use were greater than the DZ correlations, suggesting genetic effects (Table 4). Genetic (A) and unique environmental (E) influences, but not common environmental influences (C), for BP and BMI accounted for the majority of the total variance (Table 4). Both the MZ and DZ correlations increased with more refined imputations. Because of an increase in total variance and a parallel increase in both MZ and DZ correlations after algorithm imputations, significant changes in heritability were not observed.

### Relationship between BP and BMI

The phenotypic correlations between BMI and each imputed BP were significantly greater than the correlation between BMI and measured BP (Table 5). We observed a 61% increase in the correlation of BMI with the drug class addition imputation for SBP compared with measured SBP (from  $r = 0.18$  to  $r = 0.29$ ) and a 53% increase for DBP (from  $r = 0.21$  to  $r = 0.32$ ).

One might expect that any imputation that increases a substantial portion of BP values assigned to the medicated participants might increase the correlation between BMI and BP because it would increase the range of values. Thus, it could be that adding any value would have this effect. To address this question, we created a random addition correction that added a randomly generated integer between 9 and 27 and 14 and 38 to measured DBP and SBP, respectively, of all medicated individuals. This range of values covers the range of values in our drug class adjustment and is within the range of BP-lowering effect of antihypertensives observed in clinical trials. We created 500 sets of random addition DBPs and SBPs. We created a distribution of the 500 correlations between these sets of BPs and BMI. The mean phenotypic correlation between the randomly corrected SBP and BMI was 0.279 (95% confidence interval (CI) = 0.278–0.279) and mean phenotypic correlation between the randomly corrected DBP and BMI was 0.305 (95% CI = 0.30–0.31). The correlation of BMI with the randomly corrected BP was greater than the phenotypic correlation between BMI and measured SBP (0.18) and DBP (0.21) (Table 5) but not

**Table 2.** Participants on multiple drug classes

No. of drug classes	Number of participants
0	792
1	235
2	139
3	52
4	12
5	5
6	2
Total	1,237

**Table 3.** Blood pressure in medicated vs. unmedicated participants

	Antihypertensive medication (n = 445)	No antihypertensive medication (n = 792)			
	Mean (SD)	Mean (SD)	F (unadjusted for age)	P value (unadjusted for age)	P value (adjusted for age)
Age	55.8 (2.5)	55.3 (2.5)	0.46	NS	NA
BMI	31 (23)	28 (18)	67.42	<0.0001	<0.0001
Measured SBP	135 (16)	133 (15)	1.69	NA	NS
Measured DBP	84 (10)	83 (9)	0.26	NS	NS

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; NA, not available; NS, not significant; SBP, systolic blood pressure.

**Table 4.** Estimates of additive genetic (A), common environmental (C), and unique environmental (E) influences on blood pressure and body mass index

Trait	Variance components			MZ twins (n = 347 pairs)	DZ twins (n = 267 pairs)
	A	C	E	r <sub>MZ</sub>	r <sub>DZ</sub>
Measured SBP	0.43 (0.20–0.50)	0.00 (0.00–0.19)	0.57 (0.50–0.66)	0.43	0.21
Fixed addition SBP	0.47 (0.22–0.58)	0.04 (0.00–0.24)	0.49 (0.42–0.58)	0.50	0.28
Stepped addition SBP	0.46 (0.22–0.61)	0.08 (0.00–0.28)	0.46 (0.39–0.53)	0.52	0.33
Drug class addition SBP	0.45 (0.23–0.61)	0.13 (0.00–0.32)	0.43 (0.36–0.50)	0.56	0.37
Measured DBP	0.44 (0.22–0.51)	0.00 (0.00–0.17)	0.56 (0.49–0.65)	0.43	0.21
Fixed addition DBP	0.47 (0.21–0.56)	0.03 (0.00–0.24)	0.51 (0.44–0.59)	0.48	0.29
Stepped addition DBP	0.42 (0.18–0.59)	0.11 (0.00–0.31)	0.47 (0.40–0.55)	0.50	0.34
Drug class addition DBP	0.39 (0.16–0.60)	0.16 (0.00–0.35)	0.45 (0.38–0.52)	0.54	0.37
BMI	0.58 (0.39–0.74)	0.12 (0.00–0.30)	0.30 (0.26–0.35)	0.70	0.41
Antihypertensive medication, yes/no	0.37 (0.00–0.64)	0.15 (0.00,0.50)	0.48 (0.35–0.63)	0.52 <sup>a</sup>	0.33 <sup>a</sup>

Variance component values represent the proportion of variance in blood pressure in our sample explained by the A, C, or E variance components. Values in parenthesis are 95% confidence intervals. Analyses shown are age-unadjusted; age-adjusted BP measurements yielded similar results.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DZ, dizygotic; MZ, monozygotic; r<sub>DZ</sub>, DZ correlation; r<sub>MZ</sub>, MZ correlation; SBP, systolic blood pressure.

<sup>a</sup>Tetrachoric correlation (dichotomous variable).

greater than the correlation between the drug class addition SBP (0.29) and DBP (0.32) with BMI.

In addition we assessed the phenotypic correlation of SBP and DBP with BMI for a range of fixed addition values for the entire sample (Figure 2). The correlation peaked with the addition of 40 mm Hg to SBP and 25 mm Hg to DBP and steadily decreased with greater values. Thus, the addition of even greater values of mm Hg to medicated individuals did not significantly increase the correlation beyond that observed for the drug class adjustment. These results suggest that our imputation is meaningful and that randomly adding any value will not necessarily result in an increased BP–BMI correlation.

### Bivariable twin analyses

In parallel to our observation that heritability estimates of BP did not change significantly with the imputed values, the

genetic correlation (r<sub>g</sub>) between BP and BMI also did not significantly change (SBP: 0.17 < r<sub>g</sub> < 0.29; DBP: 0.20 < r<sub>g</sub> < 0.29), indicating that the proportion of genes shared between BP and BMI was not altered by adjustments to medicated individuals. However, the bivariable genetic analyses were useful in estimating the extent to which the phenotypic correlation between BP and BMI could be explained by genetic or environmental factors. Figure 3 shows the relative contribution of the genetic (A), common environmental (C), and unique environmental (E) influences to the phenotypic correlation between BMI and either DBP or SBP. These 2 sources of covariance combine to determine the phenotypic correlation, but it is only possible to definitively break down their relative contributions in a genetically informative study such as a twin study. Because we accounted for antihypertensives using more refined methods, the relative genetic influences on the correlation between BP and BMI tended to decrease while the environmental influences increased (Figure 3).

**Table 5.** Phenotypic correlations ( $r_p$ ) for body mass index and blood pressure in all twin participants ( $n = 1,235$ )

BP and BMI	$r_p$	$t^a$	$P$ value <sup>a</sup>
Measured SBP	0.18	--	--
Fixed addition SBP	0.24	-7.92	<0.001
Stepped addition SBP	0.26	-7.89	<0.001
Drug class addition SBP	0.29	-7.06	<0.001
Random addition of 14–38 mm Hg to SBP <sup>b</sup>	0.2785 ± 0.006 (0.278–0.279)	--	--
Random addition of 14–50 mm Hg to SBP <sup>b</sup>	0.2784 ± 0.008 (0.262–0.295)	--	--
Addition of 20 mm Hg to all medicated SBP <sup>c</sup>	0.275		
Measured DBP	0.21	--	--
Fixed addition DBP	0.26	-7.93	<0.001
Stepped addition DBP	0.29	-6.86	<0.001
Drug class addition DBP	0.32	-6.59	<0.001
Random addition of 9–27 mm Hg to DBP <sup>b</sup>	0.3046 ± 0.006 (0.304–0.305)	--	--
Random addition of 9–40 mm Hg to DBP <sup>b</sup>	0.297 ± 0.01 (0.279–0.318)	--	--
Addition of 14 mm Hg to all medicated DBP <sup>c</sup>	0.308		

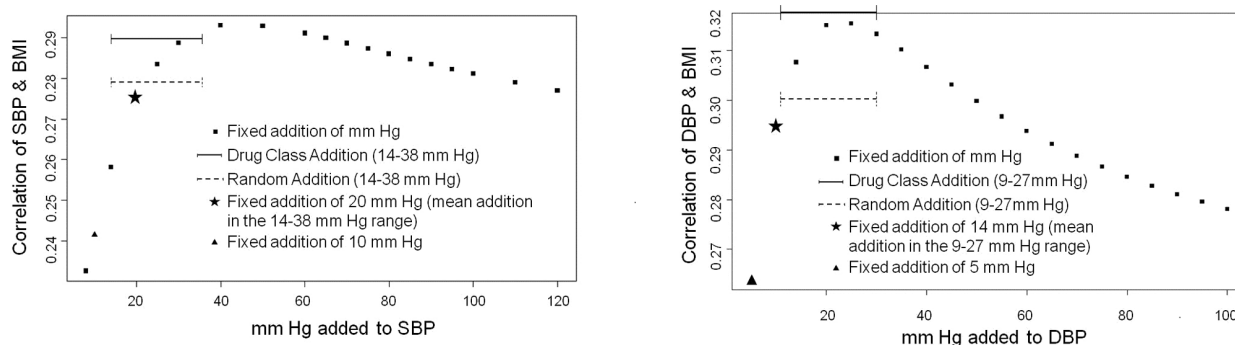
Data in parentheses are 95% confidence intervals.

Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure.

<sup>a</sup>The  $r_p$  of each imputed SBP and DBP was compared with the  $r_p$  of the measured SBP and DPB, respectively;  $t$  statistic and  $P$  value from 2-tailed significance test of the difference between dependent correlations.

<sup>b</sup>Addition of mm Hg to SBP or DBP in medicated individuals. For these, the  $r_p$  represents the mean phenotypic correlation of 500 simulations.

<sup>c</sup> The average mm Hg added to the DBP/SBP of medicated individuals in the drug class addition was 14 mm Hg/20 mm Hg.

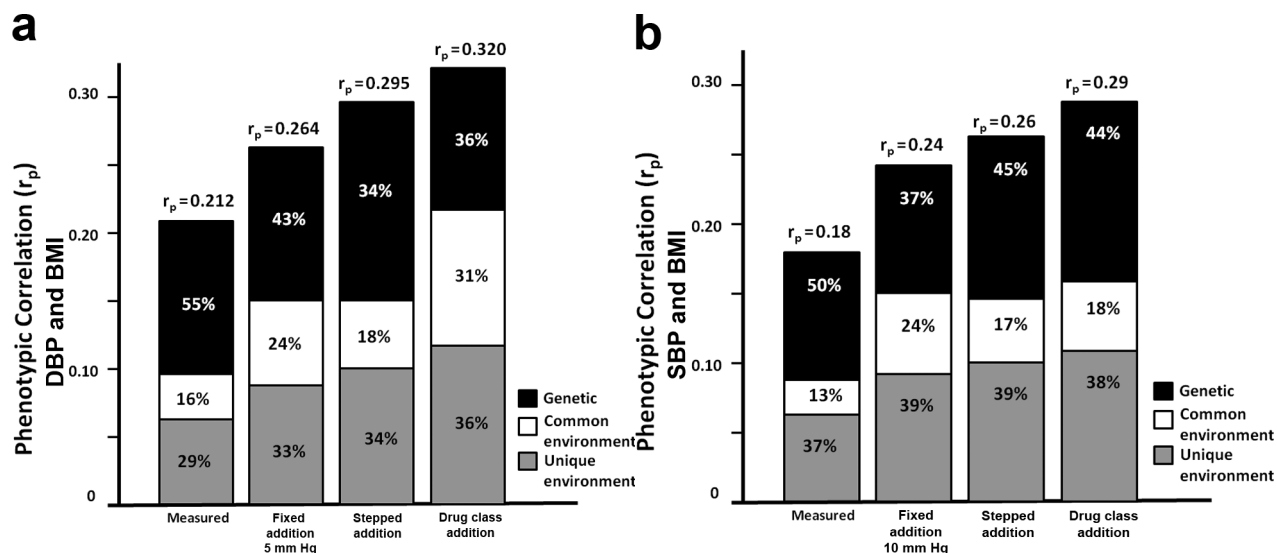


**Figure 2.** Phenotypic correlation of systolic blood pressure (SBP) and diastolic blood pressure (DBP) with body mass index (BMI) for a range of fixed addition values. Fixed addition of mm Hg represents the range of fixed additions used (8–120 for SBP; 5–100 for DBP). Fixed addition of 10 mm Hg is the fixed addition used for SBP in this study; 5 mm Hg for DBP. Solid line shows the correlation for drug class/ethnicity addition, with values ranging from 14–38 mm Hg for SBP and 9–27 mm Hg for DBP. Dashed line shows the correlation for the random addition, with values ranging from 14–38 mm Hg for SBP and 9–27 mm Hg for DBP. Fixed addition of 20 mm Hg was the mean addition when the drug class/ethnicity addition for SBP was used; 14 mm Hg was the mean addition for DBP.

## DISCUSSION

More than 30% of US adults have hypertension,<sup>48</sup> and more than 60% of hypertensive adults are treated.<sup>49</sup> Therefore, the majority of population-representative adult cohorts, particularly older cohorts, will have a large proportion of individuals on antihypertensives. Thus, the observed BP in these cohorts will be confounded by medication. Because unmedicated BP is rarely obtainable, we need to understand how imputing unmedicated BP impacts the outcome of population and genetic analyses.

We expanded an approach in which unmedicated BP is imputed based on published clinical data on drug response by ethnicity<sup>22</sup> to include additional drug classes, combination drug therapy, and use of >2 drug classes. The more refined imputations increased the range and variance of the BP in the cohort with parallel increases in MZ and DZ twin pair correlations. In contrast to our expectation, heritability estimates did not increase significantly with any imputation. However, we observed increasingly higher correlations between BP and BMI with more refined approaches (Table 5).



**Figure 3.** Contribution of genetic, common environment, and unique environment to phenotypic correlation between body mass index (BMI) and (a) diastolic blood pressure (DBP) and (b) systolic blood pressure (SBP).

The outcomes of studies investigating the impact of including BP-treated individuals in genetic studies vary depending on the age range, type of cohort, and the proportion of hypertensive and medicated individuals. Previous studies investigated BP in unrelated individuals or multigenerational families with a broad age range (typically on the order of 18–80 years).<sup>7,8,15,50</sup> A large age range may obscure results. These results are difficult to interpret because BP changes with age and hypertension penetrance and treatment occur later in midlife. In the younger extreme, even if age is adjusted for statistically, if disease has not penetrated, genetic influences cannot be detected. If the genetic architecture differs with age, heritability may change with age. In the older extreme, bias may come from isolated systolic hypertension, which is more prevalent in the elderly,<sup>51,52</sup> or loss of study participants due to the complications of elevated BP. Statistically adjusting for age as a covariable does not fully resolve the problem because this type of adjustment assumes a linear relationship of age with BP.

The lack of significantly increased heritability in our study is consistent with previous studies. Although the family/twin study of Cui *et al.*<sup>15</sup> did not include the drug class approach, our results parallel theirs in that heritabilities did not increase significantly with the imputations. In the extended twin/family study of males (mean age = 31.3 years; SD = 11.2) by Kupper *et al.*,<sup>5</sup> BP was imputed using reported drug class-specific treatment effect averages<sup>21</sup> for ambulatory BP. They showed that heritability estimates increased slightly, but not significantly, when using imputed BPs. Our hypothesis that heritability would increase was not supported because the effects of the imputation were proportional in MZ and DZ twins.

The VETSA cohort has a narrow age range in which hypertension is prevalent.<sup>48,49</sup> We observed little common environmental influences on BP. In contrast, some studies with wide age ranges tend to find that common environmental influences account for up to 20% of the BP variance. Studies using multigenerational family cohorts with a broad

age range or small twin sample sizes may not have power to differentiate between common environmental and genetic determinants. The higher common environmental component in the studies with wide age ranges may be because of the high correlation of BP and age combined with the perfectly correlated age of members within a twin pair. With our narrow age range, BP was weakly correlated with age. Ongoing longitudinal assessment will enable us to determine whether there are age-associated changes in genetic or environmental influences on BP.

Furthermore, the distribution of risk factors for BP may be different across a broad age range. In a prior report, we observed a dramatic shift in BMI over a 28-year period; 75%–80% had normal (18.5–24.9 kg/m<sup>2</sup>) BMIs at a mean age of 20, but 75%–80% had BMIs in the overweight (25–29.9 kg/m<sup>2</sup>) or obese ( $\geq 30$  kg/m<sup>2</sup>) range in midlife.<sup>53</sup> Consistent with high BMI being a known risk factor for elevated BP,<sup>54</sup> in this study we found significantly higher phenotypic correlations with BMI after imputing unmedicated BP. The increased correlations are consistent with the notion that imputed BPs are more reflective of the underlying unmedicated BP. We observed the highest correlation with our drug class imputation approach, although it was not significantly higher than the other imputation approaches. It also did not result in higher correlation with BMI compared with using a multiple linear regression approach in which BMI is the outcome variable predicted by measured BP and presence/absence of antihypertensives. However, when BP is the outcome predicted by potential risk factors (e.g., BMI, single nucleotide polymorphisms) or part of certain factor analytic analyses, including antihypertensive use as predictor is not appropriate. In such cases, an imputation approach such as our drug class imputation that predicts unmedicated BP is preferable. Although the difference in the magnitude of the correlations for the drug class approach and other less sophisticated imputation approaches were not statistically significant, it is the case that obtaining statistically significant correlations

would require larger sample sizes for BP measures that used less information to predict unmedicated BP.

Strengths of our study include the age-homogeneous sample at an age at which hypertension prevalence begins to increase and an imputation algorithm that considers contemporary BP treatment strategies. Although the age-homogeneous sample limits generalization to other age groups, ongoing assessments will provide longitudinal aging data.

A limitation of our study is that our algorithms are based on group mean data from other samples without accounting for individual variation. However, individualized data will be available only in rare cases, and the goal of this report is to assess how generalized imputation approaches affect results of studies to best predict unmedicated BP when individualized data are unavailable. As with many epidemiological studies, we relied on self-reported medication use, for which dosage and adherence to treatment were unknown; however, our method has been validated for estimating the use of cardiovascular drugs.<sup>36</sup> Dosage information would provide an even more refined method for imputation. Despite similar limitations, Tobin *et al.* demonstrated that approaches in which a sensible constant was added to the observed BP of treated individuals performed better across a range of realistic settings than using nonimputed data or including medication use as a covariable.<sup>6</sup>

If common genetic factors contribute to the underlying BP and the disease, information may be lost if one considers only the observed BPs. When elevated BP is a risk factor for a disease, it might seem that unmedicated values would be less important because the observed, treated BP may contribute to the outcome rather than the underlying unmedicated BP. However, both pieces of information are important because being treated with antihypertensives, even if BP is normalized, is still associated with increased risk for cardiovascular disease. Our study suggests the potential value of applying an adjustment for medication use. We found a small, incremental advantage with a method that was representative of the effect of the therapeutic treatment, such as by considering drug class, ethnicity, and the combination of drugs, in imputation approaches when assessing the relationship of BP with BMI. Further studies are needed to replicate this finding in other samples, for other risk factors, and for other diseases that are confounded by therapeutic treatment.

## SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (<http://ajh.oxfordjournals.org>).

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## DISCLOSURE

The authors declared no conflict of interest.

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