Gene variants at loci related to blood pressure account for variation in response to anti-hypertensive drugs between black and white individuals: genomic precision medicine may dispense with "ethnicity"

Brief title: genomic precision medicine for hypertension

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Selection of antihypertensive treatment according to self-defined ethnicity (SDE) is recommended by some guidelines but might be better guided by individual genotype rather than “ethnicity” or “race”. We compared the extent to which variation in blood pressure (BP) response across different ethnicities may be explained by genetic factors: genetically defined ancestry (GDA) and gene variants at loci known to be associated with BP. We analysed data from five trials in which genotyping had been performed (n=4696) and in which treatment responses to beta-blockers (BB), angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blocker (ARB), thiazide or thiazide-like diuretic and calcium channel blocker were available. GDA for proportion of African ancestry was computed using the 1000 genomes population database as a reference. Differences in response to the thiazide diuretic hydrochlorothiazide (HCTZ), the BBs atenolol and metoprolol, the ACEi lisinopril, and the ARB candesartan were more closely associated to GDA than SDE in admixed subjects. A relatively small number of gene variants related to loci associated with drug-signalling pathways (*KCNK3, SULT1C3, AMH, PDE3A, PLCE1, PRKAG2*) with large effect size (-3.5 to + 3.5 mmHg difference in response per allele) and differing allele frequencies in black versus white individuals explained a large proportion of the difference in response to candesartan and HCTZ between these groups. These findings suggest that a genomic precision medicine approach can be used to individualise antihypertensive treatment within and across populations without recourse to surrogates of genetic structure such as self-defined ethnicity.

Key words: ancestry, antihypertensive drugs, blood pressure, ethnicity, race

Introduction

Hypertension is the single largest cause of mortality worldwide, with a prevalence increasing in lower and middle-income countries, particularly in Africa, where in some countries more than half the adult population require treatment.[1] Effective lowering of blood pressure (BP) and hypertension control is hampered by the relatively modest BP lowering effect of first-line drugs and differences in response across populations and between individuals. Drugs that inhibit the renin-angiotensin-aldosterone system (RAAS) such as angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) are among the most widely used antihypertensive agents worldwide. However, they are less effective in reducing BP in "black" African compared to "white" European Americans,[2] whereas the response to calcium channel blockers (CCB) that act independent of the RAAS may be similar in black and white individuals.[3] For this reason some guidelines suggest selecting drugs according to self-defined ethnicity (SDE).[4-6] However, if the drug response is genetically determined, SDE will not predict the genotype and drug response of an individual and its use will limit the degree to which treatment can be accurately individualised.

The objective of the present study, therefore, was to determine if the inter-ethnic difference in response to antihypertensive drugs is genetically determined rather than being due to environmental/lifestyle factors associated with ethnicity and to seek individual gene variants that might account for this difference and hence to be responsible for inter-individual variation in drug response. We first examined the relation of antihypertensive drug response to a genetically defined ancestry (GDA) as compared to SDE. This confirmed a likely genetic component to inter-ethnic and admixed variation in response and provided a means to adjust for population structure in later analysis. We exploited the greater genetic variation likely to underlie drug response among all our study individuals (compared to that within a single

ethnic group) to seek individual gene variants associated with this response. Rather than performing a GWAS, for which available sample sizes would provide inadequate power, we sought associations with gene variants at loci already robustly associated with BP, reasoning that these would include drug response pathways in which effect sizes for drug response might be expected to be larger than for BP itself. To confirm an effect of gene variants on drug response (rather than acting as a marker for population structure) we examined their association with drug response within ethnic groups and also when adjusting for GDA across groups. Finally, to determine the degree to which such variants might account for inter-ethnic differences in response we computed the combined effect size from the effect size per allele and allele frequencies in individual ethnic groups.

Methods

Data and analytical methods for use by other investigators may be available subject to data sharing agreements being established between relevant institutions (as was the case in the present study). Please contact the author for correspondence for further details.

Clinical trial cohorts

We analysed data from five clinical trials of hypertension treatment performed in multi-ethnic cohorts in the US for which genotyping had been performed: the Pharmacogenomic Evaluation of Antihypertensive Responses studies (PEAR and PEAR-2),[7, 8] the Genetic Epidemiology of Responses to Antihypertensives studies 1 and 2 (GERA 1 and 2)[9, 10] and the Genetics of Hypertension Associated Treatments (GenHAT) study[11] in which treatment

responses to drug classes including the BB atenolol and metoprolol, ACEi lisinopril, ARB candesartan, the thiazide and thiazide-like diuretics hydrochlorthiazide and chlorthalidone and CCB amlodipine were available. Clinical characteristics and details of drug treatment for these cohorts are summarised in the supplement. Genome-wide genotyping was performed on participants in PEAR and PEAR-2 (Illumina Human Omni1-Quad BeadChip and Human Omni2.5S Beadchip) and GERA 1 and 2 (Affymetrix GeneChip Human Mapping 500,000 and 6.0 Array Sets). Exome chip data for selected variants in hypertension and cardiovascular disease related genes were available for GenHAT (Illumina Human Exome array).

Genetically defined ancestry (GDA)

Genetic data were used to determine genetically-defined ancestry (GDA) scores for each participant. Each GDA score provides the percentages of ancestry, inferred from an individual's genetic data in comparison to samples from discrete indigenous populations. We used ADMIXTURE software[12] to estimate allele frequencies for ancestral populations using the Pritchard-Stephens-Donnelly population genetics model[13] and reference data from the 1000 Genomes dataset (phase 3). The LEAPFROG R package[14] was then used to estimate individual-level GDA scores from these allele frequencies. For each individual we computed the proportion of total African ancestry as the sum of the proportions of Kenyan and Gambian ancestry. Further details are provided in supplementary material.

Blood pressure (BP) response

The primary outcome used in each study was the change in systolic BP (ΔSBP) defined as baseline SBP (before treatment) minus the final on treatment SBP, thus a greater ΔSBP represented a greater antihypertensive response (fall in BP). The on treatment response was measured after approximately 4 weeks of treatment in GERA 1 and 2, 6 weeks in PEAR and

PEAR-2 and 6 months in GenHAT. BP measurements in PEAR and PEAR-2 were home BP obtained in triplicate with the accuracy of patient's measurement technique verified by trained study coordinators. Measurements in GERA 1 and 2 were office measurements taken in triplicate by study coordinators, as were those in GenHAT. All measurements were performed seated with a validated oscillometric device except those in GenHAT which were obtained with mercury sphygmomanometry. Because study designs differed and the object was to assess the relation of drug response to ethnicity (rather than to establish a response relative to placebo or to another drug), each group receiving a drug were treated separately giving responses to 7 individual drugs in a total of 9 drug/study combinations: HCTZ in GERA 1 and PEAR; chlorthalidone in PEAR-2 and GenHAT; atenolol in PEAR; metoprolol in PEAR-2; candesartan in GERA 2; amlodipine and lisinopril in GenHAT). A component of the response related to regression to the mean was thus common to all these combinations and was adjusted for by incorporating baseline SBP in statistical models as described below.

Association of anti-hypertensive drug response to SDE and GDA

For each of nine drug/BP response combinations, we fitted a linear regression model (*baseline* model) with ΔSBP as a quantitative independent variable and baseline SBP, sex (coded as 0 for female, 1 for male), age, and BMI as covariates. To this *baseline* model we then added either (i) SDE coded as 0 for white and 1 for black (*baseline+SDE* model), or (ii) GDA proportion of African ancestry (*baseline+GDA* model). We used likelihood ratio tests (LRT) to assess statistical significance of the addition, and considered $P < 0.003$ to be significant, following a Bonferroni adjustment for multiple testing of nine study/drug combinations for two models.

Because of the high correlation between SDE and GDA, we used an elastic net (EN) machine learning framework (a modified regression model that integrates the correlation

structure of variables into the predictive accuracy calculation, preventing the models from overfitting) to assess individual and combined predictive value of SDE and GDA variables (together with baseline SBP, age, sex and BMI) as predictors of ΔSBP[15] in all individuals and also over the subset of individuals who were highly admixed (defined as those having a GDA score between 0.4 and 0.6). Further details of the EN model are given in supplementary material.

Association of BP response to genetic variants at known BP loci

To determine whether differing frequencies of previously reported gene variants associated with BP might account for some or all of the ethnic variation in drug response we examined associations of BP response with 163 validated SNPs previously published and identified in BP GWAS at the time of analysis.[16] Because we did not have coverage for all of the BP SNPs in GenHAT, we excluded GenHAT data from analysis. We also combined data for responses to HCTZ from the GERA 1 and PEAR studies to increase power. We first examined bivariate associations of drug response (correcting for multiple comparisons) with individual BP SNPs, to determine those that were robustly associated with drug response across ethnic groups (applying a Bonferroni correction for number of comparisons). We then examined associations within each ethnic group and used multivariate models with adjustment for both SDE and GDA (as well as baseline SBP, age sex and BMI) to determine independent effects of gene variants on BP response. Differences in allele frequencies between ethnic groups were tested by χ^2-test. Finally, we computed the difference in mean BP response in black compared to white subjects predicted by the multivariate models using allele frequencies of the gene variants observed in the self-defined black and white groups and the beta-

coefficients in the multivariable model. This allowed us to estimate the proportion of the inter-ethnic variation in drug response accounted for by the gene variants identified above.

Results

Participant characteristics and genetically defined ancestry

Characteristics of the participants in the treatment arms of the various studies are shown in Table 1. Participants were predominantly middle-aged black and white men and women (mean age for each study ranging from 48 - 52 years) with an approximately equal representation of men and women, except in GenHAT where participants were older (mean age 69 years) and there were fewer women (37%). The majority of participants were overweight/obese (mean BMI ranging from 29 - 31 $Kg.m^{-2}$ across the studies).

The most frequently detected genetic ancestries across the various studies were white European (white British and Iberian, and Finnish) and black African (Gambian and Kenyan) and a small portion of other ancestry populations were also detected (figure 1). For participants that self-identified as white, the mean proportion of genetically-defined European ancestry within each study ranged from 0.79 to 0.86. Mean African ancestry for each study was lower than 0.08 in self-identified white individuals. In participants that self-identified as black, the proportions of black African ancestry ranged from 0.67 to 0.71 across studies, with similar proportions of Kenyan and Gambian ancestries. Self-identified black participants showed higher genetic admixture than those self-identified as white, with a mean proportion of European ancestry (British and Iberian, and Finnish) ranging from 0.21 to 0.28 across studies (figure 1).

BP response to treatment

Baseline (pre-treatment) SBP and the response to drug treatment (expressed as mean baseline minus final on-treatment SBP, adjusted for age and sex) according to self-identified ethnicity are shown in Table 1. Mean baseline SBP ranged from 146±14.3 to 151.9.5±12.7 mmHg (means±SD). The mean response to drugs ranged from 0.0 mmHg (lisinopril in black

individuals) to -17.6 mmHg (HCTZ in black individuals). Relative to white participants, black participants showed a greater BP response to HCTZ and a lesser BP response to atenolol, metoprolol, lisinopril, candesartan and amlodipine (Table 1). These differences were both statistically and clinically significant with difference in the average reduction in BP in black and white groups ranging from +63% (HCTZ in GERA 1, P<0.001) to -100% (Lisinopril, P<0.001). Responses to chlorthalidone were similar in black and white subjects in both the GenHAT and PEAR-2 studies (Table 1).

Association of response to SDE and GDA: linear regression analysis

For all drugs, there was a significant association of response with baseline SBP consistent with regression to the mean and/or a greater drug response in participants with higher baseline BP. The proportion of total variance accounted for by baseline SBP ranged from 0.08 to 0.41 across treatments (Table S1). For all drugs apart from chlorthalidone there was a significant association between drug response and SDE and between drug response and GDA when either SDE or GDA were considered in a regression model with baseline SBP, age, sex and BMI incorporated as additional covariates (Table S1). The association of BP response with black compared to white SDE varied from + 4.57 mmHg (greater response for black compared to white) for HCTZ to -7.25 mmHg and -7.54 mmHg for candesartan and atenolol respectively. For GDA, the association of BP response with GDA expressed as mmHg per unit proportion of black ancestry varied from + 6.24 mmHg for HCTZ (+0.624mmHg per 10% change in black ancestry) to – 11.6 mmHg and – 13.11 mmHg (-1.16 mmHg and -1.311 mmHg per 10% change in black ancestry) for candesartan and atenolol respectively (figure 2).

Association of response to SDE and GDA: elastic net model combining SDE and GDA

As in regression models above, either SDE or GDA were selected as a predictor of response for all drugs except chlorthalidone when elastic net models were restricted to allow the entry of either SDE or GDA but not both. When both SDE and GDA were allowed to enter the elastic net, GDA was selected either in addition (hydrochlorothiazide, lisinopril, and amlodipine) or in preference to SDE (atenolol and metoprolol) except in the case of candesartan when SDE was selected in preference to GDA (Table S2). Importantly, in all cases, differences between models containing SDE and GDA were minimal. Comparison of the prediction of GDA vs. SDE in admixed subjects was limited by the small number of subjects and the use of a binary classification of SDE (rather than an estimated percentage of black or white ancestry) but in these subjects GDA was a better predictor of response than SDE (table S3). In the most admixed subjects (with a GDA between 0.4 and 0.6 who were within the top 10^{th} percentile of the most admixed individuals in every treatment group), the elastic net model significantly selected GDA as the unique "ethnicity" predictor (GenHAT/chlorthalidone, PEAR/atenolol, PEAR-2/metoprolol, GERA/candesartan, Table S3) or in addition to SDE (PEAR/HCTZ, GenHAT/lisinopril, table S3). There were no cases in which SDE was selected in preference to GDA (p value 0.036 for inclusion of GDA). Neither SDE nor GDA were selected as predictors of response to amlodipine in GenHAT. Inclusion of separate GDA scores for each ancestral population (e.g Kenyan and Gambian) did not significantly increase the variance explained by the GDA models either in the whole population or in the most admixed subjects.

Association of response to genetic variants in known BP loci

For each drug (apart from chlorthalidone) between 1 and 7 of the 163 previously identified SNPs from blood pressure GWAS were found to be significantly associated with BP response

after adjustment for multiple testing (P values ranging from 2.6 E-04 to 3.8E-08, Table S4). Responses to candesartan were related to 4 loci: *KCNK3, SULT1C3, AMH* and *SH2B3* and those to HCTZ to 7 loci: *CYP1A1-ULK3, PDE3A, ADO, PLCE1, PRKAG2, c5orf56* and *NUCB2*. Responses to the BB atenolol and metoprolol were related to *OBFC1, TXB2, RRP1B* (atenolol) and *FIGN-GRB14* (metoprolol). Allele frequencies for all SNPs associated with drug response differed significantly between black and white ethnic groups (Table 2). However, when examined within individual ethnic groups the effect was in the same direction and several loci were significantly associated with response within individual ethnic groups ($P < 0.001$ for all variants associated with response to hydrochlortiazide in white individuals, $P< 4.9E-05$ for association of PLCE1 with response to hydrochlorthiazide in both white and black individuals, $P< 0.005$ for KCNK3 association with response to candesartan in white participants, Table 2). Differing allele frequencies but similar effect size in black vs. white groups resulted in the mean effect of these SNPs differing between black vs. white groups and thus accounting for a substantial proportion of the difference in response between white and black individuals (Table 2).

When considered in multivariate models, the addition of SNPs (identified above) to a baseline model including age, sex, BMI and either SDE or GDA improved the fitting of the model as judged by the AIC criteria (Table S5) and increased the amount of variability explained for candesartan and HCTZ (by 5 and 2% respectively compared to models incorporating either SDE or GDA alone). For candesartan and HCTZ, neither SDE nor GDA remained significant when the SNPs were already included. When effect sizes per allele (taken from the beta-coefficients in the multivariable models) and allele frequencies in black and white groups were used to compute the expected BP response difference between black vs white groups, the SNPs were seen to account for the majority of this difference, accounting for 85% and 94% of the difference in response between black and white groups

for candesartan and HCTZ respectively. Loci that remained significantly associated with BP response in multivariate modes included: *KCN3, SULT1C3* and *AMH* for candesartan; *PDE3, PLCE1* and *PRKAG2* for HCTZ; and *TBX2* for atenolol (Table S5). Adjusting for study in the data for HCTZ (combined across GERA1 and PEAR studies) made minimal difference to results and accounted for < 1% of the variability in BP response.

Discussion

BP response to antihypertensive drugs in black and white Americans (as categorized using SDE) in the trials examined in this study was broadly in agreement with findings from previous meta-analyses.[3, 17] The responses to BB, ACEi and ARB were lower in black compared to white Americans and the response to HCTZ was greater in black compared to white Americans. The response to amlodipine was lower in black compared to white subjects which is a finding that differs from a previous meta-analysis. [3] It is notable that, in contrast to HCTZ, the response to chlortalidone was similar in black and white subjects in both the GENHAT and PEAR-2 studies. Thus variation in response to diuretic between ethnic groups may depend on the pharmacokinetics or mechanism of action of the drug. Whilst a relatively modest amount of the variance in response to BB, ACEi, ARB and HTCZ was explained by SDE (possibly because of relatively large contributions of physiological variation and measurement error to the variance in response), the difference in response between self-defined ethnic groups was nevertheless large, being between 39 to 160% of the average drug response across ethnic groups. This variation in response according to SDE has often been attributed to a genetic difference. However the existence of population-specific genetic factors accounting for variation of common phenotypes has been disputed since common variants are likely present in the human genome at the time of African outmigration and therefore global.[18] Furthermore, SDE encapsulates a complex interaction of psychosocial, lifestyle and environment factors that are not genetic.[18]

To our knowledge this is the first study to examine the association of the response to antihypertensive treatment with a GDA rather than SDE. For most drugs, the association of response to a GDA providing the amount of black genetic ancestry was similar to that of SDE with concordant directions of effect. Although the prediction of response by GDA vs. SDE in admixed subjects was limited by the small number of subjects and the use of a binary

classification of SDE, GDA in these subjects was a better predictor of response than SDE. For drugs acting directly on the RAAS (beta-blockers, lisinopril and candesartan), for which there is the most consistent variation of response between ethnic groups, GDA was the unique/most important predictor of response. These results are consistent with a genetic component underlying some or all of the ethnic variation in response to these drugs but do not identify individual causal gene variants.

To identify potential causal gene variants, we investigated whether drug response may be explained by variants at known genetic loci associated with blood pressure, since many of these relate to drug targets.[19] A relatively small number of these were found to be significantly associated with drug response. Although this association could arise from the variants acting as a surrogate marker of ethnicity (since for most variants, the allele frequencies differed markedly between black and white groups), this is unlikely since, for many variants, associations with drug response were also observed within individual ethnic groups. Secondly, in multivariate models, the effects of these genetic variants were significant when adjusting for either SDE or GDA provided better prediction of response than SDE or GDA. Furthermore, the sets of gene variants were *drug-specific.* That is they differed according to the individual drug classes, as would be predicted from their action on specific targets. Effect sizes for several loci were large with a change in blood pressure response > 3 mmHg per allele (in fully adjusted models) which is several times greater than the size of the association with blood pressure from a main effect blood pressure GWAS, typically < 1 mmHg per allele. Perhaps because blood pressure is a polygenic trait determined by multiple homeostatic pathways, whereas drug response may be more closely related to fewer genetic variants linked to a specific drug signalling pathway. When the mean difference in response between black and white subjects was computed from the combined effect of the different

allele frequencies of the variants found to be significantly associated to drug response, this was seen to account for the majority of the observed inter-ethnic difference in drug response for candesartan and HCTZ.

Suppression of plasma renin and renin activity is recognized to be more prevalent in black compared to white subjects and has been attributed to increased sodium retention rather than increased sodium intake,[9, 20-22] which may influence response to diuretics and drugs inhibiting the renin-angiotensin system.[23] It is notable that rare monogenic syndromes of hypertension are mainly mediated through sodium retention,[24] and one explanation of the present study is a polygenic effect on sodium retention with greater frequency of sodium retaining gene variants in black compared to white subjects. It is notable that of the loci we identified as related to drug response many are in pathways that could influence drug response through sodium retention and/or through other drug signalling pathways. KCNK3 encodes a potassium channel (TASK-1) involved in aldosterone synthesis.[25] SH2B3, a member of the SH2B adaptor protein family, is an intracellular adaptor protein that functions as a negative regulator in many signalling pathways (Janus kinase and receptor tyrosine kinases) and is thought to influence sodium retention via modulation of inflammation.[26, 27] The CYP1A1-ULK3 loci contains several genes that have been linked to sodium retention.[28] NUCB2 is a precursor protein of nesfatin-1 which may influence blood pressure probably through hypothalamic ERK signalling leading to sympathetic activation and sodium retention.[29] Studies with larger sample size in different populations together with functional studies will be required to determine the biological significance of all of the potential loci identified here.

Our study is subject to several limitations. The sample size, particularly for admixed subjects, was small. The trials we analysed were of varying design, with varying follow-up periods, we

did not account for variable adherence to drug treatment and the blood pressure response was derived mainly from office readings. These factors would have tended to mask the relation of blood pressure response with SDE, GDA and individual genetic variants. GenHAT participants were excluded from the association analysis of BP response to known genetic variants (because of incomplete coverage of these variants in GenHAT). However the association of BP response with GDA was stratified by both drug and study and was similar for chlorthalidone, the only drug studied in both in GenHAT and PEAR-2. We studied only black and white Americans and the confirmation of the predictive value of genetic ancestry and predictive and biological role of the individual gene variants identified here will require further large-scale pharmacogenetic studies performed in multi-ethnic groups in different geographical locations.

Perspectives

Selection of treatment according to SDE, as recommended by some current guidelines[6] has been criticised as potentially disadvantaging peoples in whom SDE is a misleading description of the pharmacogenomic determinants of an individual person's response.[30] Furthermore, it may perpetuate the use of an imprecise measure of genetic and environmental determinants of the blood pressure response. The present study shows that although individualising treatment through a GDA is likely to be of marginal benefit in populations that are homogenous, it may be of benefit in admixed populations including those in or originating from Latin America and the Caribbean. Furthermore, when genetic variation across ethnic groups was exploited to increase the power to detect association of response with known BP loci, a small number of gene variants with large effect sizes were identified that explain much of the inter-ethnic variation in antihypertensive drug response. The relatively small sample size and lack of replication cohorts (which to our knowledge are not

available) means that we cannot be certain that all the variants identified here are causally associated with drug response. However, the strength of the associations makes it highly likely that a pharmacogenetics approach involving relatively few variants will be able to individualise therapy irrespective of ethnicity. Furthermore, it is likely that loci can be identified that will provide insight into molecular pathways determining response to treatment both within and across populations.

Conclusion

In conclusion, exploiting genetic variation across ethnic groups and examining associations with known BP loci identifies a small number of gene variants with large effect sizes that may explain much of the inter-ethnic variation in antihypertensive drug response. These findings suggest that a genomic precision medicines approach can be used to individualise antihypertensive treatment within and across populations without recourse to surrogates of genetic structure such as self-defined ethnicity.

Sources of Funding

This work was performed as part of the AIM HY (Ancestry and biological Informative Markers in stratification of HYpertension) stratified medicines programme in hypertension funded by the Medical Research Council and The British Heart Foundation. We also acknowledge support from the Department of Health via a National Institute for Health Research (NIHR) Biomedical Research Centre and Clinical Research Facility award to Guy's & St Thomas′ NHS Foundation Trust in partnership with King's College London, and the NIHR Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King’s College London. PEAR and PEAR-2 was supported by the National Institute of Health Pharmacogenetics Research Network grant U01-GM074492 and the National Center

for Advancing Translational Sciences under the award number UL1 TR000064 (University of Florida); UL1 TR000454 (Emory University) and UL1 TR000135 (Mayo Clinic). GenHAT was supported by NIH Heart, Lung and Blood Institute grant 5 R01 HL-63082, Genetics of Hypertension Associated Treatment. The ALLHAT study was supported by a contract with the National Heart, Lung and Blood Institute. GenHAT genotyping was funded by NIH Heart, Lung and Blood Institute grant 1R01HL103612.

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Novelty and Significance

What is New

A genetically-derived ancestry (GDA) predicts ant-hypertensive drug response better than self-defined ethnicity (SDE). A small number of gene variants with large effect sizes explain much of the inter-ethnic variation in antihypertensive drug response.

What is Relevant

A pharmacogenetics approach involving relative few variants will be able to individualise therapy irrespective of ethnicity. Genetic loci may be identified that will provide insight into molecular pathways determining response to treatment both within and across populations.

Summary

Differences in the systolic BP fall in response to the thiazide diuretic hydrochlorothiazide (HCTZ), the beta-blockers atenolol and metoprolol, the angiotensin converting enzyme inhibitor lisinopril, and the angiotensin receptor blocker candesartan were more closely associated to GDA rather than than SDE in admixed subjects. A relatively small number of gene variants related to loci associated with drug-signalling pathways (*KCNK3, SULT1C3, AMH, PDE3A, PLCE1, PRKAG2*) with large effect size (-3.5 to + 3.5 mmHg difference in response per allele) and differing allele frequencies in black versus white individuals explained a large proportion of the variation in response to candesartan and HCTZ. These findings suggest that a genomic precision medicine approach can be used to individualise antihypertensive treatment within and across populations without recourse to surrogates of genetic structure such as self-defined ethnicity.

Legends

Figure 1

a) Distribution of genetic ancestries amongst participants in trials GERA1 and PEAR, (n=780) receiving hydrochlorthiazide (HTCZ). b) Proportion of black ancestry (as Gambian plus Kenyan) plotted for each individual (with lowest to highest black ancestry from left to right) and the associated decrease in systolic blood pressure (SBP) induced by HTCZ c) Association of decrease in SBP induced by HTCZ with differing allele frequencies at 3 variants previously linked to (BP). Decrease in SBP in response to HTCZ is proportional to % African ancestry but can also be largely explained by differing allele frequencies at these variants.

Figure 2

Relationship of systolic blood pressure response to self-defined ethnicity (SDE, yellow bars) and genetically defined ancestry (GDA, blue bars) in multi-ethnic drug trials examining BP response. The response variable (ΔSBP) was the change in systolic blood pressure (SBP) defined as baseline SBP minus final SBP. The plot shows the Beta coefficient relating ΔSBP to self-defined ethnicity (SDE) and genetically defined ancestry (GDA) in a linear regression model including baseline SBP, age, sex and BMI and either SDE or GDA. Units are mmHg difference in ΔSBP between black and white subjects (SDE) or per unit proportion of African ancestry (GDA) or change in response per 100% change in African ancestry. A positive Beta coefficient represents a greater SBP reduction in black compared to white subjects (SDE) or in subjects with greater African ancestry (GDA). HCTZ, hydrochlorothiazide; CTD, chlortalidone; * Likelihood Ratio Test - comparing each model to a baseline model including

SBP, age, sex and BMI - was significant after Bonferroni adjustment for multiple comparisons.

Table 1. Characteristics of subjects and systolic blood pressure response in multi-ethnic trials of anti-hypertensive drugs.

		Measures at baseline					ΔSBP (mmHg)			
Drug	Study	SBP (mmHg)	Age (years)	Sex (% Female)	BMI	Ethnicity (% Black)	All	White[1]	Black[1]	White - Black (95%CI)
HCTZ	GERA1 n=517	146±14.3	48.2±6.7	44.87	31.2±6.1	48.5	14.1±13	10.8±11.7	17.6±13.3	-6.8 (-7.4, -6.3)*
HCTZ	PEAR n=363	151.9±12.7	48.9±9.4	47.66	30.8±5.1	39.4	12.9±15.9	11±12.8	15.9±13.6	-5.0 (-5.6, -4.3)*
CTD	PEAR-2 n=226	149.6±13.2	50.4±9.2	42.92	31.1±5.2	49.1	15.6±20.1	15.5±12.8	15.7±13.8	-0.2 (-1.6, 1.2)
CTD	GenHAT n=1190	148.1±16.1	69.4±7.9	36.89	29.3±6	30.76	6.7±19.26	7.2±18.8	5.7±20.1	1.5 (-1.0, 4.0)
Atenolol	PEAR n=367	151.6±12.2	48.6±9.2	56.13	30.7±5.7	39.5	13±19	15.5±14.8	9.2±16.9	6.3 (5.9, 6.7)*
Metoprolol	PEAR-2 n=250	150.1±12.8	50.5±9.6	44.8	31.1±5.2	50	9.7±21.4	12.2±12.6	7.2±16.1	5.0 (4.2, 5.7)*
Candesartan	GERA 2 n=365	147.6±12.4	48.9±6.6	50.41	30.1±4.3	49	14.8±18.1	18.5±14.5	11±14.9	7.5 (7.0, 8.0)*
Amlodipine	GenHAT n=689	147.9±15.7	69.3±7.7	34.91	29.3±5.6	31.8	6.4±19.6	8.2±18.6	2.5±21.2	5.7 (2.4, 9.1)*
Lisinopril	GenHAT n=729	149.2±15.5	69.4±7.9	38.37	28.9±5.4	37.6	4.2±20.7	6.7±19.1	0±22.5	6.7 (3.4, 10.1)*

Values are mean±SD or % of subjects. Change in systolic blood pressure (ΔSBP) is defined as baseline SBP minus final SBP, adjusted for age and sex. See text for description of GERA, PEAR and GenHAT studies; BMI, Body Mass Index; CI, confdence interval for difference in means; CTD, chlortalidone; HCTZ, hydrochlorthiazide. *P<0.001. [1]Self-defined ethnicity.

Table 2. Associations of SNPs at known blood pressure loci with blood pressure response within individual ethnic groups† and computation of expected difference in response according to allele frequencies.

Locus/Drug		Black: effect size		White: effect size		Black: genotype frequency	White: genotype frequency	White vs Black		
Locus Name	Drug	Effect per allele	p	Effect per allele (mmHg)	p	AA ; AB ; BB	AA ; AB ; BB	p	difference in response (mmHg)	% observed difference
KCNK3	CAND	-2.85	1.8E-01	-3.95	**4.9E-03**	0.022 ; 0.257 ; 0.721	0.403 ; 0.452 ; 0.145	**1.9E-31**	-1.90	25
SULT1C3	CAND	3.57	1.7E-02	1.82	2.4E-01	0.408 ; 0.425 ; 0.168	0.081 ; 0.457 ; 0.462	**5.5E-15**	0.20	-3
AMH	CAND	3.57	3.7E-02	2.86	3.0E-01	0.078 ; 0.363 ; 0.559	0.005 ; 0.124 ; 0.871	**1.1E-10**	-0.05	1
SH2B3	CAND	-1.81	5.0E-01	-0.76	5.9E-01	0.006 ; 0.184 ; 0.810	0.285 ; 0.505 ; 0.210	**3.3E-31**	-2.56	34
CYP1A1-ULK3	HCTZ	2.58	**4.9E-05**	2.78	**1.2E-06**	0.015 ; 0.166 ; 0.819	0.392 ; 0.475 ; 0.133	**2.1E-92**	2.59	43
PDE3A	HCTZ	-1.41	1.8E-02	-2.72	**4.3E-06**	0.383 ; 0.454 ; 0.163	0.064 ; 0.283 ; 0.653	**2.2E-52**	3.23	53
ADO	HCTZ	1.99	1.3E-02	2.82	**1.7E-05**	0.010 ; 0.140 ; 0.849	0.171 ; 0.503 ; 0.325	**1.0E-53**	0.41	7
PLCE1	HCTZ	2.00	**5.1E-03**	2.29	**4.3E-04**	0.036 ; 0.286 ; 0.679	0.182 ; 0.507 ; 0.310	**7.1E-28**	0.71	12
PRKAG2	HCTZ	-2.25	**8.5E-04**	-2.10	**1.2E-03**	0.158 ; 0.452 ; 0.390	0.099 ; 0.407 ; 0.495	**2.1E-03**	0.16	3
c5orf56	HCTZ	1.98	1.0E-02	2.47	**2.0E-04**	0.003 ; 0.168 ; 0.829	0.161 ; 0.465 ; 0.375	**1.1E-42**	0.62	10
NUCB2	HCTZ	1.25	1.3E-01	2.79	**4.0E-05**	0.008 ; 0.094 ; 0.898	0.137 ; 0.439 ; 0.424	**1.5E-46**	-1.23	-20
OBFC1	ATEN	2.34	2.5E-01	1.30	4.6E-01	0.455 ; 0.448 ; 0.097	0.023 ; 0.293 ; 0.685	**5.1E-35**	-0.66	10
TBX2	ATEN	2.79	2.0E-01	1.71	2.2E-01	0.510 ; 0.428 ; 0.062	0.086 ; 0.392 ; 0.523	**3.3E-26**	-0.92	15
RRP1B	ATEN	-0.83	8.5E-01	-2.13	9.0E-02	0.000 ; 0.103 ; 0.897	0.189 ; 0.464 ; 0.347	**1.4E-24**	0.90	-14
FIGN-GRB14	METOP	-4.12	1.3E-01	-3.46	7.1E-02	0.024 ; 0.224 ; 0.752	0.280 ; 0.616 ; 0.104	**7.4E-25**	-4.27	85

CAND, candesartan, HCTZ, hydrochlorthiazide; ATEN, atenolol; METOP, metoprolol. Difference between black and white subjects attributable to each SNP is derived from effect size (in multivariate model) and allele frequencies in the respective groups. P values in bold are significant associations when making a Bonferroni correction for 15 comparisons (p values lower than 3.33E-03), for the 15 SNPs that were significantly associated with response in the whole sample. †Excluding those in GenHAT for whom genetic coverage was insufficient to identify SNPs of interest.

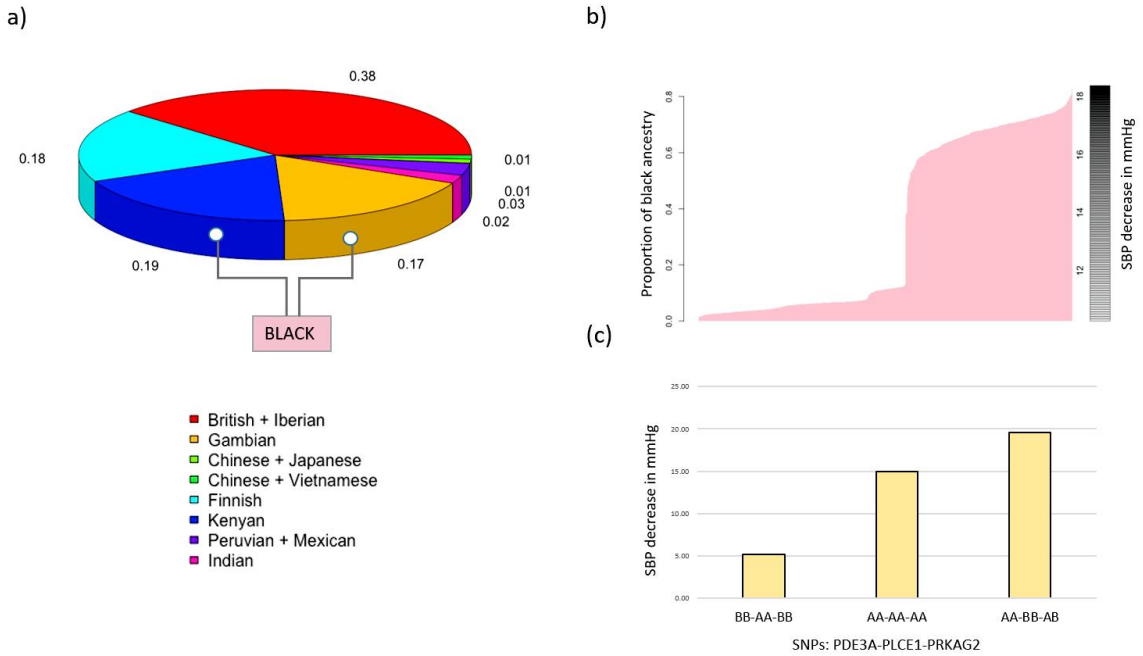

Figure 1.

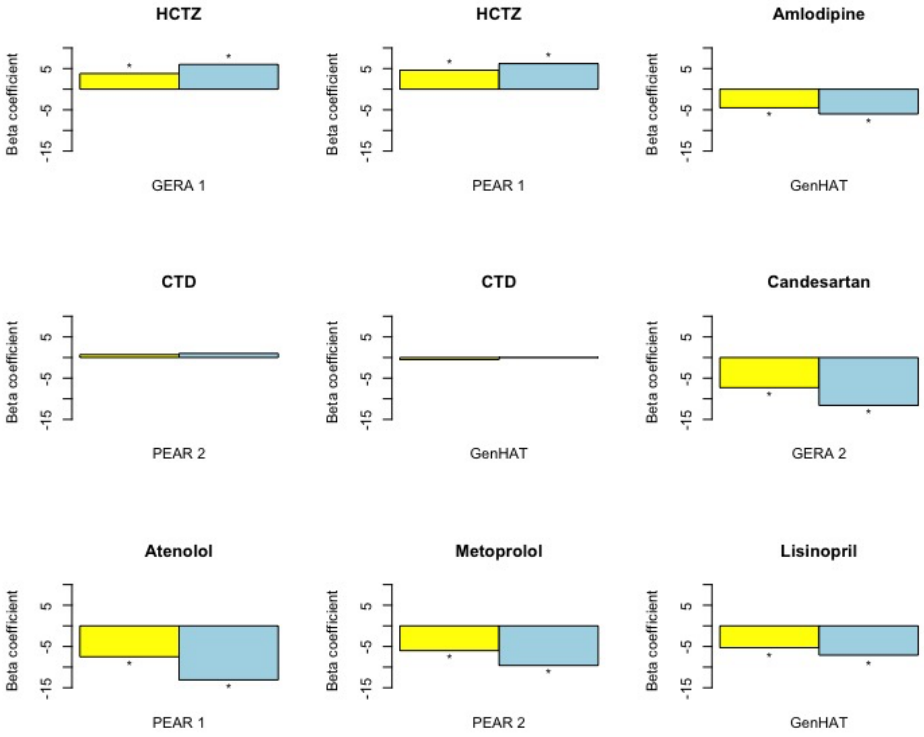

Figure 2.