Check for updates

Journal of the American Heart Association

ORIGINAL RESEARCH

Circulating microRNA Biomarkers of Thiazide Response in Hypertension

Lakshmi Manasa S. Chekka, PharmD, PhD; Marwa Tantawy, PhD; Taimour Langaee, PhD; Danxin Wang, MD, PhD; Rolf Renne, PhD; Arlene B. Chapman, MD; John G. Gums, PharmD; Eric Boerwinkle, PhD; Rhonda M. Cooper-DeHoff, PharmD, MS; Julie A. Johnson, PharmD

BACKGROUND: Thiazide diuretics are the second most frequently prescribed class of antihypertensives, but up to 50% of patients with hypertension have minimal antihypertensive response to thiazides. We explored circulating microRNAs (miRNAs) in search of predictive biomarkers of thiazide response.

METHODS AND RESULTS: We profiled 754 miRNAs in baseline plasma samples of 36 hypertensive European American adults treated with hydrochlorothiazide, categorized into responders (n=18) and nonresponders (n=18) on the basis of diastolic blood pressure response to hydrochlorothiazide. miRNAs with ≥2.5-fold differential expression between responders and nonresponders were considered for validation in 3 cohorts (n=50 each): hydrochlorothiazide-treated European Americans, chlorthalidone-treated European Americans, and hydrochlorothiazide-treated Black individuals. Different blood pressure phenotypes including categorical (responder versus nonresponder) and continuous diastolic blood pressure and systolic blood pressure were tested for association with the candidate miRNA expression using multivariate regression analyses adjusting for age, sex, and baseline blood pressure. After quality control, 74 miRNAs were available for screening, 19 of which were considered for validation. In the validation cohort, miR-193b-3p and 30d-5p showed significant associations with continuous SBP or diastolic blood pressure response or both, to hydrochlorothiazide in European Americans at Benjamini-Hochberg adjusted $P<0.05$. In the combined analysis of validation cohorts, let-7g (odds ratio, 0.6 [95% CI, 0.4–0.8]), miR-142-3p (odds ratio, 1.1 [95% CI, 1.0, 1.2]), and miR-423-5p (odds ratio, 0.7 [95% CI, 0.5–0.9]) associated with categorical diastolic blood pressure response at Benjamini-Hochberg adjusted $P<0.05$. Predicted target genes of the 5 miRNAs were mapped to key hypertension pathways: lysine degradation, fatty acid biosynthesis, and metabolism.

CONCLUSIONS: The above identified circulating miRNAs may have a potential for clinical use as biomarkers for thiazide diuretic selection in hypertension.

REGISTRATION: URL: ClinicalTrials.gov. Unique identifiers: NCT00246519, NCT01203852, NCT00005520.

Key Words: antihypertensive response ■ biomarkers ■ blood pressure response ■ chlorthalidone ■ circulating microRNA ■ hydrochlorothiazide ■ thiazide diuretics

Downloaded from http://ahajournals.org by on February 25, 2024

Hypertension is defined as persistent elevation in blood pressure (BP), with systolic BP (SBP) >130 mm Hg or diastolic BP (DBP) >80 mm Hg.[1] Hypertension affects ≈1.4 billion people globally and is estimated to increase to 1.6 billion by 2025.[2] It is an independent risk factor for cardiovascular, cerebrovascular, and renal diseases[3] and a leading contributor to all-cause morbidity and death worldwide.[4] Despite the availability of a variety of effective antihypertensive drugs, BP control rate in the population is only about

Correspondence to: Julie A. Johnson, PharmD, Department of Pharmacotherapy and Translational Research, Center for Pharmacogenomics and Precision Medicine, College of Pharmacy, University of Florida, Box 100486, Gainesville, FL 32610-0486. Email: julie.johnson@ufl.edu; julie.johnson@osumc.edu

This manuscript was sent to Ajay K. Gupta, MD, MSc, PhD, FRCP, FESC, Senior Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.032433

For Sources of Funding and Disclosures, see page 11.

© 2024 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What Is New?

- MicroRNAs are important regulatory mediators in hypertension with a plausible role in antihypertensive drug response.
- Circulating microRNAs are ideal for developing noninvasive, disease/drug response–specific, and easily quantifiable biomarkers.
- We identified plasma microRNAs associated with blood pressure response to thiazide diuretics, both with hydrochlorothiazide and chlorthalidone, and in 2 ancestral groups (European Americans and Black individuals), with several having a plausible biological role in regulating blood pressure homeostasis.

What Question Should Be Addressed Next?

- Future research is essential to confirm these findings and identify their utility as independent biomarkers or complementing the existing clinical factors such as age, sex, and baseline blood pressure in predicting antihypertensive drug response in individuals.
- Our research highlights the potential of circulating microRNAs as biomarkers of drug response in hypertension and furthers the utility of additional omics beyond genomics for achieving personalized medicine in hypertension.

Nonstandard Abbreviations and Acronyms

BH	Benjamini-Hochberg
Ct	cycle threshold
EA	European American
FDR	false discovery ate
GERA	Genetic Epidemiology of Responses to Antihypertensives
PEAR	Pharmacogenomic Evaluation of Antihypertensive Responses
SBP	systolic blood pressure
TZD	thiazide diuretic

50% in high-income countries[5] and even lower in low-middle-income countries.[2] While adherence to medications and lifestyle factors can partially explain the low control rates, a major factor is suboptimal therapy selection and clinician inertia when the originally selected therapy fails to adequately control BP. Clinical predictors such as age, ancestry, and compelling indications guide the antihypertensive therapy selection to a certain extent,[6] but there is a dearth of predictive tools to guide precise therapy selection in hypertension.

MicroRNAs (miRNAs) are small noncoding RNAs involved in the posttranscriptional regulation of gene expression and are implicated in playing a vital role in a variety of biological functions. Because of their capacity to regulate and thus fine-tune the expression of multiple target genes relevant in hypertension pathophysiology, such as vascular smooth muscle cell proliferation and functioning, endothelial dysfunction, impaired vascular integrity, and angiogenesis,[7–10] miRNAs are often explored for their potential diagnostic, prognostic, and therapeutic relevance in hypertension. Circulating miRNAs have an added advantage of stability and accessibility, with the evidence of being reflective of miRNA dysregulation in a variety of disease-related tissues. Thus, several studies profiled circulatory miRNAs as prognostic markers for hypertension and related complications,[11] but very few studies explored their association with antihypertensive drug response. A previous study[12] from our lab identified circulating miRNAs associated with antihypertensive response to β blockers in uncomplicated individuals with hypertension, from a candidate list of potential miRNAs targeting known β blocker response–related genes. Another group has independently documented association between a β blocker response phenotype and one of the miRNAs we identified,[13] highlighting the potential for this line of research.

With an aim to identify potential biomarkers of antihypertensive response to thiazide diuretics (TZDs), which are important first-line agents in the management of hypertension, we chose to identify plasma miRNAs associated with TZDs' BP response. We sought to use an unbiased approach to conduct a genome-wide profile of miRNAs in participants with uncomplicated hypertension to identify those associated with BP response to TZDs and validate them in 3 large antihypertensive clinical trials testing for associations across thiazide subclass and across ancestry.

METHODS

Study Participants

PEAR, PEAR-2, and GERA Study Protocols

Biological samples and clinical data used in this study were collected as part of the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses), PEAR-2, and GERA (Genetic Epidemiology of Responses to Antihypertensives) trials (ClinicalTrials.gov Nos. NCT00246519, NCT01203852, NCT00005520). Study participants in all 3 studies were those with uncomplicated hypertension, including newly diagnosed or untreated hypertension or hypertension being treated with 1 or 2 drugs. They were excluded if they had

Downloaded from http://ahajournals.org by on February 25, 2024

any cardiovascular diseases, diabetes, or serum creatinine >1.5 mg/dL, among others. Details of designs and objectives of the 3 studies have been previously described.[14–16]

PEAR Study

PEAR was a multicenter, randomized clinical trial with the primary aim of evaluating the role of genetic variability on BP response in hydrochlorothiazide and atenolol-treated patients.[14] Study participants (n=768) with uncomplicated hypertension were randomized to receive monotherapy of either the thiazide diuretic hydrochlorothiazide (12.5 mg/day for 2–3 weeks and titrated to 25 mg/day for 6 additional weeks if BP was >120/70 mm Hg) or atenolol. Fasting blood samples were collected at baseline (untreated) and after 9 weeks of monotherapy. BP responses were assessed using office, home, and 24-hour ambulatory BP; home BP is used in the current study. Samples and data from 36 European Americans (EAs) were used in the screening/discovery step and 50 Black individuals for the cross-ancestry validation. (See below for subject selection.)

PEAR-2 Study

PEAR-2 was a multicenter sequential trial testing chlorthalidone (15 mg/day for 2 weeks and titrated to 25 mg/day for 6 additional weeks if BP was >120/70 mm Hg) and metoprolol,[15] with the same aims and protocol as that of the PEAR study. Samples and data from 50 EAs were used in the cross-drug validation.

GERA Study

GERA was a prospective study in participants with hypertension, aged 30 to 59 years.[16] After a 4-week washout period of any antihypertensive therapies, participants were treated with 25 mg/day of hydrochlorothiazide for 4 weeks. BP was measured at baseline and after 4 weeks of therapy. Samples and data from 50 EAs were used to replicate our findings from the discovery step.

Samples from all these studies have undergone genome-wide genotyping, and continental ancestry was estimated from the genome-wide association study data, as previously described.[17–19] Those who were estimated on the basis of genotype data to be of European ancestry are labeled as EAs and those of African ancestry are labeled as Black individuals.

All participants provided written informed consent, and all the studies were approved by the institutional review boards at each site. The data that support the findings of this study (miRNA data) are available from the corresponding author upon reasonable request, and the remaining data plus other omic data are available on the Database of Genotypes and Phenotype (Study Accession: phs000649.v2.p2).

Phenotype Based on BP Response

BP response in all the above cohorts is measured as posttreatment BP–pretreatment (baseline/untreated) BP. Participants in each cohort were ranked according to their DBP response to TZD and were divided into 2 groups: Those in the top quartile, with a higher response to TZD, were considered responders and those in the bottom quartile were considered nonresponders. We selected 18 responders and 18 nonresponders from the discovery cohort and 25 responders and 25 nonresponders from each of the replication/validation cohorts. In each cohort, patients were selected to have a balance of baseline characteristics such as age, sex, body mass index, and baseline SBP and DBP between the comparison groups (responders and nonresponders). miRNA expression was tested for association with categorical (responder versus nonresponder) DBP response, as this is the response phenotype on which participants were selected for inclusion, and because study participants were enrolled in all the trials on the basis of diastolic hypertension. Thus, DBP categorical response is treated as the primary outcome, given the study design features. We also evaluated the associations with categorical SBP response (the selected patients from above were recategorized into responders and nonresponders on the basis of their SBP response; those with at least 10 mm Hg SBP reduction from baseline were considered responders: GERA includes 28 responders and 22 nonresponders; PEAR-2 includes 27 responders and 23 nonresponders; the cohort of Black individuals from PEAR-1 includes 25 responders and 25 nonresponders). Data are presented for SBP and DBP categorical and continuous responses.

Study Design

Figure 1 presents an overview of the study strategy, which involves a screening/discovery step, followed by validation of selected candidates in 3 stages: (1) replication (n=50) in EAs treated with hydrochlorothiazide in the GERA trial; (2) cross drug, within ancestry validation (n=50) in EAs treated with chlorthalidone in the PEAR-2 trial; (3) cross-ancestry, within drug validation (n=50) in Black individuals treated with hydrochlorothiazide in the PEAR trial, followed by combined analysis across all 3 replication/validation cohorts of miRNAs detected in all cohorts.

miRNA Profiling

Screening/Discovery Sample Assays

We used The TaqMan OpenArray Human MicroRNA Panels, QuantStudio™ 12K Flex (Applied Biosystems,

Downloaded from http://ahajournals.org by on February 25, 2024

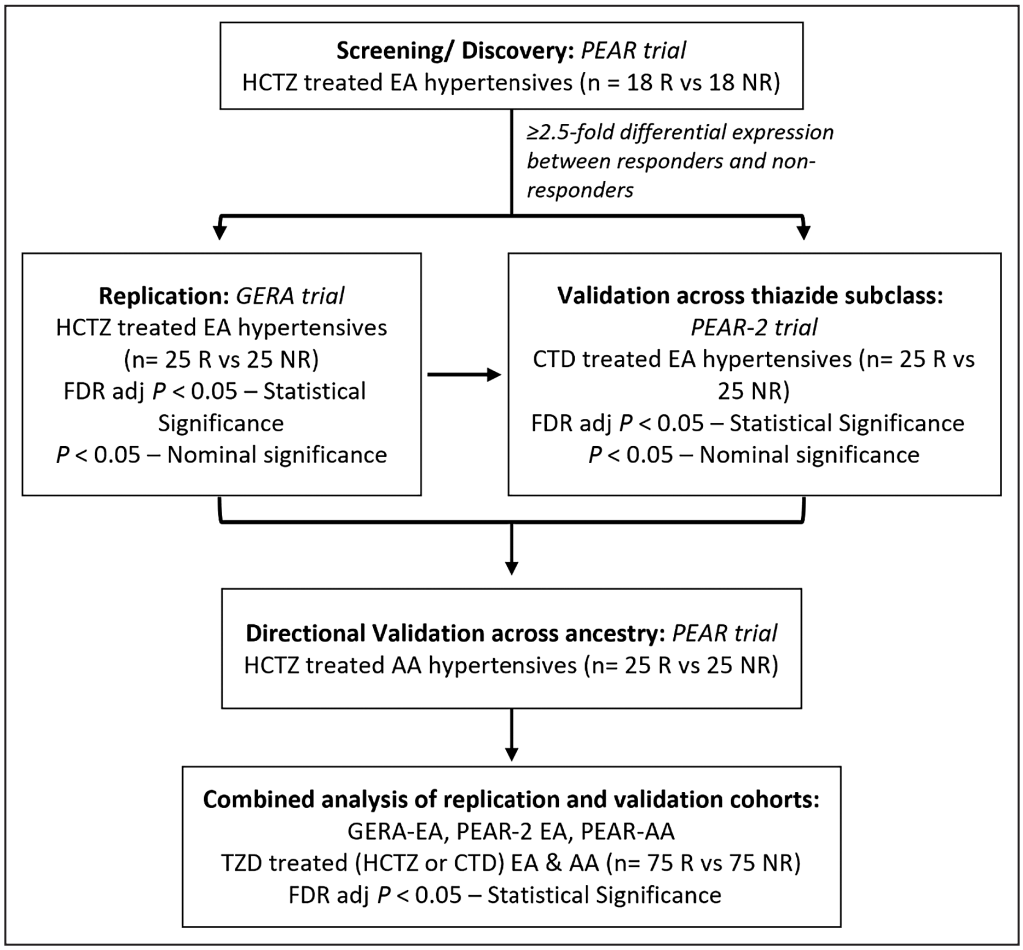

Figure 1. Overview of study strategy.
AA indicates Black individuals; CTD, chlorthalidone; FDR, false discovery rate; GERA, Genetic Epidemiology of Antihypertensive Responses; EA, European Americans; HCTZ, hydrochlorothiazide; NR, nonresponders; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and R, responders.

Waltham, MA) for the initial screening step. The TaqMan OpenArray Human MicroRNA Panel is a fixed-content panel (754 miRNAs) containing validated human TaqMan MicroRNA assays derived from Sanger miRBase release version 14.

EDTA-plasma was prepared from whole blood samples at baseline and were stored at −80°C for further processing. Total RNA extraction was done from 100-μL plasma samples using the MagMAX mirVana Total RNA Isolation Kit (Applied Biosystems). Approximately 100 ng of total RNA was split and processed in parallel with Megaplex RT primer pools A and B and TaqMan MicroRNA Reverse Transcription Kit for preparing cDNA, followed by preamplification with TaqMan preamplification master mix with Megaplex preamplification primer pools A and B. The preamplified product was diluted and mixed in 1:1 ratio with TaqMan OpenArray Real-Time PCR Master Mix and added onto the 384-well OpenArray Sample Loading Plate. TaqMan OpenArray Human MicroRNA Panels were automatically loaded by the AccuFill System and then placed in the QuantStudio 12K Flex Real-Time PCR System for polymerase chain reaction (PCR) cycling.

Validation Sample Assays

Total RNA was extracted from 100 μL of baseline plasma as described above. Samples were normalized to 10 ng/μL total RNA concentration; 2 μL of normalized samples were used for further steps. The TaqMan Advanced miRNA cDNA Synthesis Kit was used to perform polyAtailing, adapter ligation, reverse transcription reaction, and preamplification using manufacturer's protocols for Taqman Advanced miRNA single tube assays. After 1:10 dilution of the preamplified product, we performed PCR reactions in 10-μL volumes in triplicate using TaqMan Fast Advanced Master Mix and the selected TaqMan Advanced miRNA Assays.

Downloaded from http://ahajournals.org by on February 25, 2024

Quality Control and Statistical Analysis

Screening/Discovery Phase

Cycle threshold (Ct) values with amplification score <1.24 and quantification cycle confidence <0.8 were filtered out. Samples with low miRNA expression/detection and miRNAs with missing Cts in >50% of samples were excluded from analysis. Missing Cts were replaced with 40, which is the number of PCR cycles performed or maximum allowable Ct.[20] Data were normalized by global normalization, that is, by subtracting the individual Ct with the mean of the expressed miRNAs. Fold change in miRNA expression between responders and nonresponders was estimated by a $2^{-\Delta\Delta Ct}$ method,[21] calculated as $2\text{^}-(Ct_{Resp} - Ct_{mean}) - (Ct_{Non\text{-}resp} - Ct_{mean})$. miRNA prioritization was done on the basis of fold change. miRNAs with ≥2.5-fold change were taken forward for replication/validation.

Replication/Validation Phase

Ct values with amplification score <1.1 were filtered out. This lower amplification score cutoff, as compared with discovery, was considered appropriate because quantitative PCR was done in triplicate, which would provide sufficient confidence on miRNA expression and Ct values. The average of the triplicate Cts was considered for analysis. Samples with low miRNA expression/detection and miRNAs with missing Cts in >50% of samples were excluded from analysis. Missing Cts were replaced with 40.[20] After normalizing the data with mean of the commonly expressed miRNAs, a multiple logistic regression model was used to test the association between the thiazide response (responders versus nonresponders) and the relative expression of miRNA, adjusting for age, sex, and baseline DBP (or SBP in the SBP analyses). For continuous BP response, multiple linear regression was used. Tests with Benjamini-Hochberg (BH) adjusted *P* value less than false discovery rate (FDR) of 0.05 were identified as statistically significant and unadjusted $P<0.05$ as nominally significant.

The results for categorical DBP and SBP were presented as odds ratio (interpreted as odds of being a responder) and for continuous DBP and SBP as the β estimate in the regression model.

A combined analysis was conducted by pooling data from all 150 study participants who comprised the 3 quantitative PCR–based cohorts: the replication cohort, the cross-drug validation cohort, and the cross-ancestry validation cohort. Only the miRNAs that were detected across all 3 cohorts were used for analysis. We renormalized the miRNA expression in each cohort with the mean of the commonly detected miRNAs. The categorical DBP response (responders versus nonresponders) was regressed with miRNA expression, adjusting for age, sex, baseline BP, ancestry, and study.

We conducted a pathway analysis of miRNAs that showed nominal or statistical significance either in replication or validation cohorts, and directional consistency with the discovery cohort, by using Diana miRPath.[22] We looked at pathway unions using a significance clustering algorithm in Diana miRPath that uses exact significance levels calculated in the previous overrepresentation analyses.

Statistical analyses were performed on R Studio (R version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria) and SPSS version 26 (IBM, Armonk, NY).

RESULTS

Demographics and baseline characteristics of study participants in each comparison group of the 4 cohorts are presented in Table 1. As described above, the comparison groups were balanced for baseline characteristics of age, sex, body mass index, and baseline SBP and DBP.

Discovery Phase

Of the 754 tested miRNAs on the panel, after quality control filtering, 309 unique miRNAs were detected in the plasma in at least 1 sample. An average of 95 miRNAs were detected per sample, and 74 of those were detected consistently across samples with <50% missingness. Figure S1 represents the frequency of subjects with the number of miRNAs detected in plasma. Six (3 responders and 3 nonresponders) of the 36 samples from the discovery cohort were excluded from the analysis due to low sample quality and low miRNA expression/detection.

A total of 74 commonly detected miRNAs were normalized with the global mean and tested for differential expression using fold change between responders and nonresponders. Considering nonresponders as reference, 11 miRNAs were underexpressed and 8 overexpressed in the responder cohort with ≥2.5-fold difference between groups. Table S1 shows a list of the 19 miRNAs with ≥2.5-fold differences that were taken forward for replication/validation steps.

Replication Phase

After quality control filtering, 2 of the 50 samples from GERA were excluded from analysis due to low miRNA expression/detection. Of the 19 miRNAs that were tested, 12 were consistently detected across samples with <50% missingness and were used for further analysis.

None of the miRNAs tested fully replicated, based on categorical DPB response and BH adjusted *P* <0.05 (Table 2), though miR-193b-3p (fold change=2.7)

Downloaded from http://ahajournals.org by on February 25, 2024

Table 1. Demographics

	PEAR-1 EA (hydrochlorothiazide)			GERA EA (hydrochlorothiazide)			PEAR-2 (chlorthalidone)			PEAR-1 Black individuals (hydrochlorothiazide)		
	Responders (n=18)	Nonresponders (n=18)	*P* value	Responders (n=25)	Nonresponders (n=25)	*P* value	Responders (n=25)	Nonresponders (n=25)	*P* value	Responders (n=25)	Nonresponders (n=25)	*P* value
Age, y	47±12.2	48±7.29	0.73	50±6.1	47±6.2	0.066	54±7.3	50±7.5	0.078	49±8.5	45±8.3	0.097
Female, n (%)	9 (50)	7 (38.9)	0.74	15 (60)	8 (32)	0.089	13 (52)	9 (36)	0.39	16 (64)	13 (52)	0.566
Body mass index, kg/m^2	29.0±5.1	30.9±5.1	0.28	32.1±4.3	31.8±6.7	0.86	31.5±4.7	30.1±5.1	0.29	31.5±5.8	31.4±4.9	0.96
Baseline DBP, mm Hg	94.5±4.7	93.9±4.6	0.72	96±3.9	95±4.2	0.37	94.7±3.8	92.9±4.4	0.13	96.8±7.8	94.4±6.3	0.22
Baseline SBP, mm Hg	147±10.6	143±9.33	0.26	145±10.2	142±11.7	0.36	148.1±9.6	143.3±8.3	0.064	148.6±10.8	150±12.7	0.6869
Delta DBP, mm Hg	−10.2±5.3	0.9±3.8	4.21e-08	−18±1.9	7±3.9	1.1e-25	−14.4±3	0.6±1.9	7.9e-24	−16.7±2.9	2.4±2.5	1.4e-28
Delta SBP, mm Hg	−14.4±5.6	−0.2±6.5	5.04e-08	−25±10.8	2±11.9	1.05e-10	−22.8±6.7	−1.8±7.0	1.6e-14	−23.9±5.5	−11±6.9	6.4e-17
Duration of hypertension, y	3.1±4.8	5.8±6.8	0.18	4±4.7	5±7.0	0.35	9±10.0	6±8.2	0.53	8±8.7	9±8	0.68
Currently smoking, n (%)	2 (11.1)	4 (28.6)	-	0	2 (8)	-	1 (4)	5 (20)	-	1 (4)	7 (28)	-

DBP indicates diastolic blood pressure; EA, European Americans; GERA, Genetic Epidemiology of Antihypertensive Responses; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and SBP, systolic blood pressure.

nominally replicated for the categorical DBP response (nominal *P*=0.027). Of note, this miRNA was also nominally associated with the SBP categorical response and met FDR significance for both SBP and DBP continuous responses.

The other miRNA of interest in the replication cohort was miR-30d-5p, which met FDR significance for continuous SBP response and nominal significance or trends for the other BP phenotypes (Table 2).

Cross-Drug Validation (Chlorthalidone Response)

After quality control filtering, 2 of the 50 samples from the PEAR-2 cohort were excluded due to low miRNA expression/detection. Thirteen of the 19 tested miRNAs were consistently detected across samples with <50% missingness and were considered for analysis. The strongest candidate miRNA from the replication cohort (miR-193b-3p) was not significantly associated with DBP response to chlorthalidone, and the fold change between responders and nonresponders was in the opposite direction of the discovery and replication cohorts. None of the other tested miRNAs were fully validated, based on BH adjusted *P* <0.05, but 3 miRNAs (let-7g-5p, miR-142-3p, and miR-423-5p) were nominally associated with multiple BP phenotypes (Table 3).

Cross-Ancestry Validation

In the cross-ancestry analysis, which sought to determine if the associations observed in European Americans were consistent in Black individuals, after quality control filtering, 1 of the 50 samples from the PEAR Black cohort was excluded due to low miRNA expression/detection. Twelve of the tested 19 miRNAs were consistently detected across samples with <50% missingness and were considered for analysis. None of the tested miRNAs showed significant association with categorical DBP response. miR-193b-3p was not consistently detected across patients in the Black cohort and hence was not considered for analysis. Fold changes for miR-30d, let-7g, miR-142-3p, miR-423-5p in the Black cohort were directionally consistent with discovery as well as with other replication and validation cohorts (Figure 2).

Combined Analysis

In a combined analysis of the 11 miRNAs that were detected in all 3 replication/validation cohorts, miRNAs let-7g-5p, miR-142-3p, and miR-423-5p were consistently associated with categorical DBP response across all cohorts, at BH adjusted *P*<0.05. Figure 2 shows the fold changes across all 4 cohorts for the miRNAs with the most compelling data across the replication and validation cohorts and Figure 3 presents the adjusted odds ratio and CIs for those miRNAs across all tested

Downloaded from http://ahajournals.org by on February 25, 2024

Table 2. Replication of miRNAs Associated With BP Response to Hydrochlorothiazide Among European Americans in GERA: Significantly Associated miRNAs in the Logistic and Linear Models for DBP and SBP Response, Adjusted for Age, Sex, and Baseline BP

	Categorical DBP response		Categorical SBP response		Continuous DBP response		Continuous SBP response	
miRNA	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Estimate±SE	*P* Value	Estimate±SE	*P* value
miR-193b-3p	0.66 (0.42–0.89)	0.027	0.60 (0.35–0.86)	0.026	1.8±0.57	0.0028*	3.07±0.71	8.9e-05*
miR-30d	1.78 (1.05–3.75)	0.081	2.74 (1.34–7.21)	0.018	−2.11±0.99	0.04	−3.73±1.29	6.2e-03*

Adjusted=Multivariate regression model adjusted for covariates age, sex, baseline BP. *P* values presented here are raw unadjusted *P* values. Table presents raw *P* values. BP indicates blood pressure; DBP, diastolic blood pressure; FDR, false discovery rate; GERA, Genetic Epidemiology of Antihypertensive Responses; OR, odds ratio; and SBP, systolic blood pressure.

*Significant at FDR of 0.05 after Benjamini-Hochberg adjustment.

cohorts. To understand if the effects of age and sex varied by study, interaction analyses of age and sex with study were conducted (data not shown) and were not significant, justifying adjustments of models for age, sex, ancestry, and study. Figure S2 presents the fold changes for an extended list of all 11 miRNAs in the combined analysis as well as the 19 miRNAs in the discovery cohort that were taken forward for replication.

Pathway Analysis

For the pathway analysis, we focused only on the miRNAs (miR-193b-3p, miR-30d-5p, miR-let-7g, miR-142-3p, and mir-423-5p) that showed nominal or statistical significance in at least 1 quantitative PCR–based cohort and consistent direction of association with that of the discovery. Pathway analysis of the above 5 miRNAs in Diana miRPath[22] identified fatty acid biosynthesis, lysine degradation, and fatty acid metabolism as the most significant pathways involved, which may have potential relevance to hypertension and antihypertensive drug response (Table 4; Figure S3).

DISCUSSION

In this first-ever genome-wide profile of circulating miRNAs related to antihypertensive drug response, we identified plasma miRNAs associated with BP response to thiazides and tested them across thiazide subclass and ancestry. We prioritized miRNAs (n=19) associated with hydrochlorothiazide response from an array-based discovery study, and based on strict multiple-comparisons BH adjustment, none of these replicated in a separate cohort for the primary end point (categorical DBP), though miR-193b-3p nominally replicated for categorical DBP and SBP and met FDR criteria for continuous SBP and DBP responses. The modeling data (Tables S3 and S4) show that addition of miR-193b-3p to clinical models increased the adjusted R-squared values of the model and model significance (Akaike information criterion) improved from unadjusted to adjusted models, suggesting that miR-193b-3p is an important contributor to variability in BP response. miR-193b-3p had a directionally opposite, nonsignificant association with chlorthalidone response and was not consistently detected in the samples from Black individuals. Nonetheless, given the consistency in findings across the hydrochlorothiazide-treated cohorts, and given that hydrochlorothiazide is one of the most widely used drugs in the world, further study of this miRNA may be warranted.

Evaluation of the previous literature for the biological explanation of this finding provides some insights. In our study, higher levels of miR-193b-3p were associated with better response to hydrochlorothiazide, as seen from the fold change >1 in responders versus nonresponders as well as from the positive coefficient in the linear regression models (positive coefficient implies that lower Ct [translating to higher expression] is associated with greater BP reduction [represented by a larger negative value for BP change]). Though the biological basis of this association is unclear, elevated

Table 3. Cross-Drug Validation: Association of miRNAs With BP Response to Chlorthalidone in PEAR-2 European Americans: Significantly Associated miRNAs in the Logistic and Linear Models for DBP and SBP Response, Adjusted for Age, Sex, and Baseline BP

	Categorical DBP response		Categorical SBP response		Continuous DBP response		Continuous SBP response	
miRNA	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Estimate±SE	*P* value	Estimate±SE	*P* value
Let-7g	0.54 (0.28–0.91)	0.034	0.54 (0.28–0.94)	0.043	1.68±0.79	0.039	1.68±1.13	0.15
miR-142-3p	1.2 (1.04–1.47)	0.025	1.21 (1.02–1.49)	0.046	−0.49±0.23	0.035	−0.47±0.34	0.21
miR-423-5p	0.64 (0.40–0.96)	0.046	0.64 (0.39–0.95)	0.045	1.19±0.62	0.06	1.08±0.89	0.23

BP indicates blood pressure; DBP, diastolic blood pressure; OR, odds ratio; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and SBP, systolic blood pressure.

Downloaded from http://ahajournals.org by on February 25, 2024

miRNA	Discovery FC	GERA-1 FC	PEAR-2 FC	PEAR-1AA FC	Combined analysis FC	Combined analysis OR, 95% CI	Combined analysis *P* value
hsa-let-7g-5p	5.62	1.28	2.51	1.38	1.59	0.6 (0.42-0.82)	0.0022*
hsa-miR-142-3p	0.14	0.52	0.071	0.57	0.21	1.13 (1.05-1.24)	0.0032*
hsa-miR-423-5p	9.28	1.028	2.91	1.37	1.58	0.72 (0.55-0.93)	0.015*
hsa-miR-30d-5p	0.36	0.51	0.633	0.68	0.69	1.1 (0.93-1.34)	0.29
hsa-miR-193b	5.71	2.73	0.88	-	-	-	-

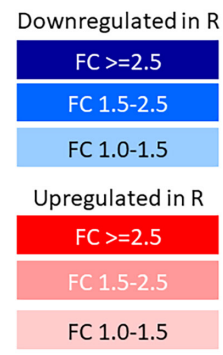

Figure 2. Combined analysis of replication and validation cohorts for association of miRNA expression with categorical DBP response to TZD therapy and directional fold change across all cohorts.
P value=raw *P* value from multivariate linear regression *Significant at FDR of 0.05 after Benjamini-Hochberg adjustment; model: response (responders versus nonresponders)=miR expression+age+sex+baseline BP+ancestry+study; OR interpreted as odds of being a responder. Combined analysis does not include discovery cohort. List presents microRNAs that showed either nominal or significant associations with BP response in at least 1 replication/validation cohort and directional consistency with discovery. BP indicates blood pressure; FC, fold change; FDR, false discovery rate; GERA, Genetic Epidemiology of Antihypertensive Responses; NR, nonresponders; OR, odds ratio; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and R, responders.

plasma miR-193b-3p levels have been previously associated with prediabetes, as it potentially downregulates *PPARGC1A*, a gene that encodes PGC-1α, a transcriptional coactivator that orchestrates the expression of genes involved in several metabolic pathways.[23] A previous study identified that a PGC-1α Gly482Ser polymorphism in this gene was associated with lower SBP and DBP.[24] Further work is required to replicate these findings and, if replicated, to understand the biological basis for the association.

Our study also identified miR-30d with nominal associations with DBP and significant association with continuous SBP in response to hydrochlorothiazide in the replication cohort. Similar to miR-193-3p, miR-30d also improved the explained variability in BP response, independent of baseline BP and other clinical variables (Tables S3 and S4). Though miR-30d was not associated with TZD response in the cross-drug or cross-ancestry validation, the direction of response was consistent across the 4 tested cohorts. Mir-30d-5p is predicted to target several hypertension-related genes: *CAT, ADRA1D, PLEKHA7, EDNRA, ADRA2A, ADRA2B, ATP2B1, SH2B3*, and *ADRB1*.[25] Plasma miR-30d was also previously shown to be downregulated in patients with hypertension.[26] Pharmaco-miR, a miRNA pharmacogenomics database identified genes associated with hydrochlorothiazide and chlorthalidone response such as *ADD1, NOS3, WNK1, STK39*, and *YEATS-4* as predicted targets of miR-30d. Our findings, coupled with the above literature, highlight the biologically plausible involvement of miR-30d-5p in TZDs' mechanism of action. But its utility as a biomarker of TZDs' response needs further confirmation in larger independent studies.

While the most interesting finding of this study from a traditional replication approach is miR-193b-3p and miR-30d to some extent, the analyses across the 4 cohorts suggest that miR-let-7g-5p, miR142-3p, and miR-423-5p also warrant further study. Each of these miRNAs had consistent associations across all 4 cohorts (2 cohorts of EAs treated with hydrochlorothiazide, 1 cohort of EAs treated with chlorthalidone, and 1 cohort of Black individuals treated with hydrochlorothiazide), and in the combined analysis of the quantitative PCR–based experiments all met FDR significance. The modeling data (Table S5) show that addition of these miRNAs to a clinical model increased the adjusted R-squared values of the model and model significance improved from unadjusted to adjusted models, suggesting that these miRNAs are important contributors to variability in BP response. In our study, higher miR-let-7g-5p levels were associated with better BP response to TZDs. A study by Huang et al[27] identified plasma let-7 levels to be positively correlated with SBP, carotid intima thickness, and C-reactive protein. It is in line with our results as higher baseline SBP corresponds to larger BP reductions, and responders in our study were shown to have higher expression of let-7g. Let-7g-5p is also predicted to target several mRNAs of hypertension-related genes such as *TBX5*, *ADRB1*, *EDN1*, and *FGF5*.[25] The Pharmaco-miR database identified genes associated with hydrochlorothiazide and chlorthalidone response such as *ACE* and *STK39* as targets of let-7g. Our study also identified miR-423-5p to be associated with TZD response, with higher plasma levels in responders. A study by Zhang et al[28] identified serum miR-423-5p levels to be positively correlated with hypertension and

Downloaded from http://ahajournals.org by on February 25, 2024

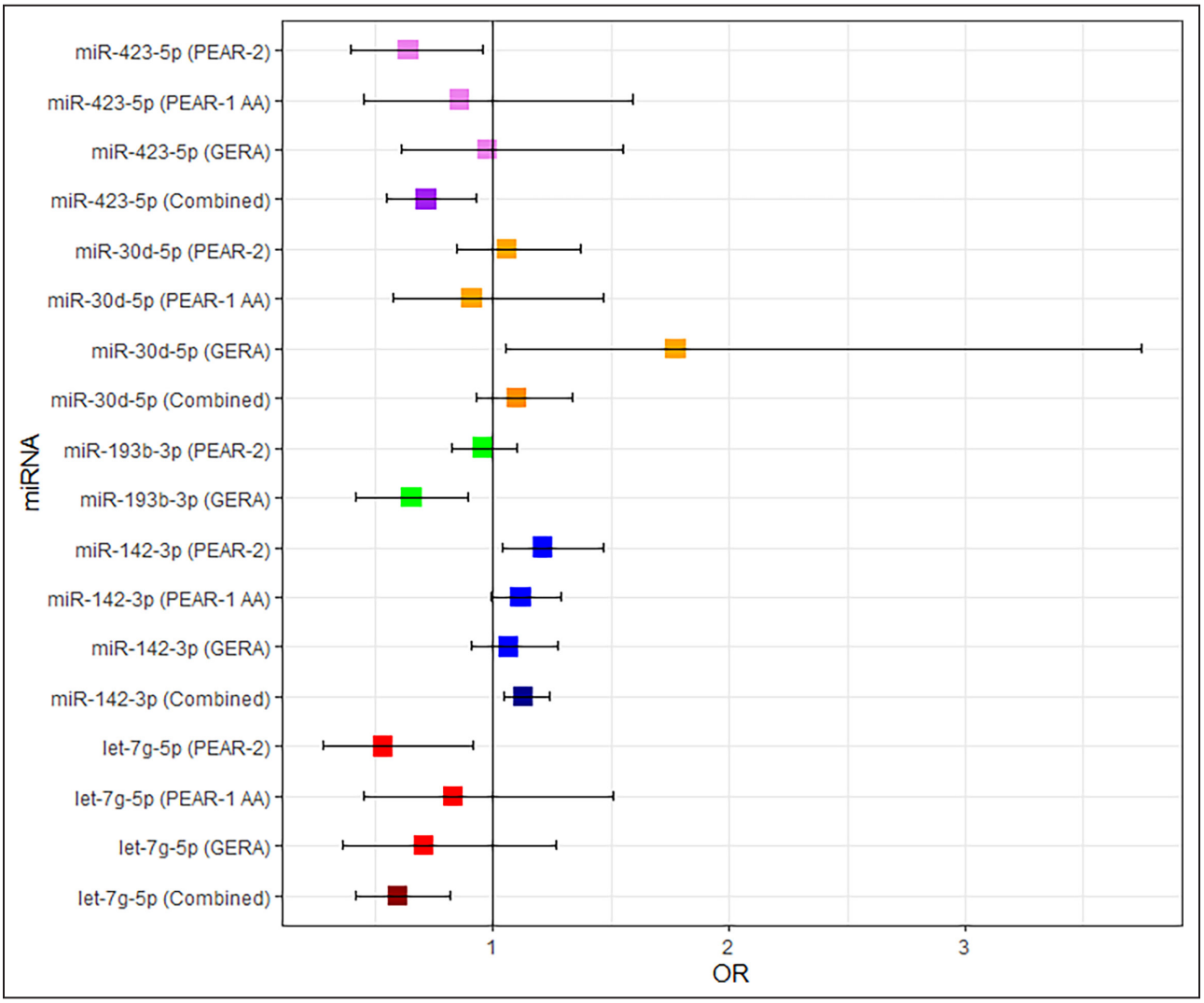

Figure 3. Adjusted ORs and 95% CIs for each miRNA regressed with categorical DBP response to thiazide diuretic therapy. Effect sizes and CIs are estimated from the logistic regression model with miRNA expression as independent variable; categorical DBP response as dependent variable; and baseline DBP, age, and sex as covariates. DBP indicates diastolic blood pressure; FC, fold change; GERA, Genetic Epidemiology of Antihypertensive Responses; NR, nonresponders; ORs, odds ratios; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and R, responders. *Missing or not applicable.

had a good predictive ability (area under the receiver operating characteristic curve=0.74) for hypertension. Given that higher BP levels are often associated with greater response to antihypertensive therapy, our results are in line with literature as higher miRNA levels, associated with higher BP, were observed in responders. There are fewer clear biological associations with hypertension or the TZD response for miR-142-3p, but miR-142-3p is predicted to target *GNAS*, which is an important pharmacogenetic region previously identified to be associated with TZD response.[29]

Our pathway analysis of the 5 miRNAs with the strongest associations in the study (Table 4) identified lysine degradation as an enriched pathway involving all the

Table 4. Most Significant Kyoto Encyclopedia of Genes and Genomes Pathways Enriched by Genes Targeted by the 5 Candidate miRNAs

Pathway	FDR-corrected *P* Value	No. of genes	No. of miRNAs
Fatty acid biosynthesis	<1e-325	4	3 (miR-423-5p, 30d-5p, miR-193b-3p)
Lysine degradation	<1e-325	26	5 (let-7g-5p, 142-3p, 423-5p, 30d-5p, miR-193b-3p)
Fatty acid metabolism	<1e-325	15	2 (miR-423-5p, 30d-5p, miR-193b-3p)

FDR indicates false discovery rate.

Downloaded from http://ahajournals.org by on February 25, 2024

identified miRNAs. Lysine metabolism has been shown previously to be associated with hypertension.[30,31] Lysine metabolism was shown to be reduced in the early stages of hypertension. Lysine administration diminished the development of salt-sensitive hypertension in Dahl salt-sensitive rat models and increased diuresis. Thus, a dysregulation of the circulating miRNAs identified in our study could potentially explain a deregulation in lysine metabolism and thereby their role in hypertension phenotype and, in turn, the response to therapy. Additionally, our pathway analysis showed that miR-423-5p and miR-30d-5p and miR-193b-3p target genes enriched in fatty acid biosynthesis and metabolism. These results are in line with the fact that patients with hypertension have several metabolic complications such as hyperlipidemia, hyperglycemia, and decreased insulin sensitivity, among others.[32]

We were unable to truly validate in Black individuals any of the signals in EAs, though many were directionally consistent. While ancestral or ethnic differences in the miRNA expression are not completely known, studies identified certain miRNA differences that could potentially drive some of the health disparities in hypertension.[33] Our study identified differences in expression of miRNAs let-7g, miR-16, miR-126, miR-30d, and miR-423-5p, between EAs and Black individuals (Table S2), which could explain the difficulty in replicating the results from EA cohorts.

Strengths and Limitations

An important strength of our study is that we had access to multiple cohorts, with nearly identical study designs, conducted by the same investigators. This allowed us to test associations across 4 cohorts, including a traditional replication cohort (same drug, same ancestral group), along with a cross-drug validation and cross-ancestry validation. This provides greater confidence in the findings and greater external validity.

Our study also has several limitations. First, our discovery sample size was relatively small, which limited our power to identify additional novel signals associated with thiazide BP response. Additionally, the age of the samples, and the fact that the samples may have been through 1 to 3 previous freeze–thaw cycles could have affected the miRNA levels during storage. Small plasma sample volumes (100 μL) also limited our ability to detect miRNAs with lower levels of expression.

While let-7g, miR-142-3p, and miR-423-5p were directionally consistent across all 4 cohorts and met FDR significance in the combined analysis, they were not significantly associated in GERA (the replication cohort). A potential explanation is the differences in measuring BP in PEAR compared with GERA. In PEAR, the BP response was based on home BP, which we have shown previously to be a more accurate measurement of BP response with a better signal-to-noise ratio,[34] whereas in GERA, the BP response was based on office BP measurements, which has more signal-to-noise ratio compared with the home BP used in PEAR, thus making biomarker associations more difficult to detect. This is consistent with findings that are directionally consistent but do not meet statistical significance.

It is also important to note that the sample selection in each cohort was intended to represent extreme response phenotypes (responders or nonresponders), which does not necessarily represent the complete range of the phenotype in the general population. It is important to test the signals identified in this study in a larger population with a continuous phenotype to determine the utility of the miRNA as a biomarker across the entire range of the BP response phenotype.

CONCLUSIONS

In this first circulating miRNA study of TZD response, and the first-ever genome-wide profile of miRNA associations with antihypertensive drug response, we identified miRNAs 193b-3p, miR-30d, let-7g, miR-142-3p, and miR-423-5p as having associations with BP response to TZDs, with potential utility as biomarkers. Considering consistent associations across all 4 cohorts, let-7g, miR-142-3p, and miR-423-5p represent the most interesting signals, and 2 of the 3 have previous associations consistent with a BP phenotype. Thus, these are likely to be the miRNAs from this study most worthy of further study. Pathway analysis of the miRNAs with interesting findings in this study point to potential involvement of lysine metabolism and fatty acid biosynthesis and metabolisms as possible pathways for divergent responses to thiazide diuretics.

ARTICLE INFORMATION

Received August 29, 2023; accepted December 21, 2023.

Affiliations

Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics and Precision Medicine (L.M.C., M.T., T.L., D.W., J.G.G., R.M.C.-D., J.A.J.) and Department of Molecular Genetics and Microbiology, College of Medicine (R.R.), University of Florida, Gainesville, FL; Division of Nephrology, University of Chicago, Chicago, IL (A.B.C.); University of Texas at Houston Center for Human Genetics, Houston, TX (E.B.); Division of Cardiovascular Medicine, Department of Medicine, University of Florida, Gainesville, FL (R.M.C.-D., J.A.J.); and now with Center for Clinical and Translational Science, The Ohio State University, Columbus, OH (J.A.J.).

Acknowledgments

Author contributions: All the authors had access to data and a role in writing or editing this manuscript.

Sources of Funding

The PEAR study 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),

Downloaded from http://ahajournals.org by on February 25, 2024

and UL1 TR000135 (Mayo Clinic). The PEAR study was also supported by funds from the Mayo Foundation. L.M.S. Chekka received the predoctoral fellowship grant 20PRE35210065 from the American Heart Association.

Disclosures

None.

Supplemental Material

Tables S1–S5
Figures S1–S3

REFERENCES

1. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/BlackPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Hypertension*. 2018;71:e13–e115. doi: 10.1161/HYP.0000000000000065
2. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, Chen J, He J. Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. *Circulation*. 2016;134:441–450. doi: 10.1161/CIRCULATIONAHA.115.018912
3. Kokubo Y, Iwashima Y. Higher blood pressure as a risk factor for diseases other than stroke and ischemic heart disease. *Hypertension*. 2015;66:254–259. doi: 10.1161/HYPERTENSIONAHA.115.03480
4. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol*. 2020;16:223–237. doi: 10.1038/s41581-019-0244-2
5. Kotchen TA, Cowley AW Jr, Liang M. Ushering hypertension into a new era of precision medicine. *JAMA*. 2016;315:343–344. doi: 10.1001/jama.2015.18359
6. Cooper-DeHoff RM, Johnson JA. Hypertension pharmacogenomics: in search of personalized treatment approaches. *Nat Rev Nephrol*. 2016;12:110–122. doi: 10.1038/nrneph.2015.176
7. Li X, Wei Y, Wang Z. microRNA-21 and hypertension. *Hypertens Res*. 2018;41:649–661. doi: 10.1038/s41440-018-0071-z
8. Sawan J, Abhishek M, Ashis M, John B, Sadanand F, Ravindra K. MicroRNAs: as a novel potential tool for diagnosis, prognosis, and therapeutic agents in hypertension. *J Hypertens Cardiol*. 2015;1:14–22. doi: 10.14302/issn.2329-9487.jhc-15-677
9. Batkai S, Thum T. MicroRNAs in hypertension: mechanisms and therapeutic targets. *Curr Hypertens Rep*. 2012;14:79–87. doi: 10.1007/s11906-011-0235-6
10. Jusic A, Devaux Y. Action EU-CC. noncoding RNAs in hypertension. *Hypertension*. 2019;74:477–492. doi: 10.1161/HYPERTENSIONAHA.119.13412
11. Romaine SP, Charchar FJ, Samani NJ, Tomaszewski M. Circulating microRNAs and hypertension—from new insights into blood pressure regulation to biomarkers of cardiovascular risk. *Curr Opin Pharmacol*. 2016;27:1–7. doi: 10.1016/j.coph.2015.12.002
12. Solayman MH, Langaee TY, Gong Y, Shahin MH, Turner ST, Chapman AB, Gums JG, Boerwinkle E, Beitelshees AL, El-Hamamsy M, et al. Effect of plasma MicroRNA on antihypertensive response to beta blockers in the pharmacogenomic evaluation of antihypertensive responses (PEAR) studies. *Eur J Pharm Sci*. 2019;131:93–98. doi: 10.1016/j.ejps.2019.02.013
13. Nevola KT, Kiel DP, Zullo AR, Weiss S, Homuth G, Foessl I, Obermayer-Pietsch B, Motyl KJ, Lary CW. miRNA mechanisms underlying the association of beta blocker use and bone mineral density. *J Bone Miner Res*. 2021;36:110–122. doi: 10.1002/jbmr.4160
14. Johnson JA, Boerwinkle E, Zineh I, Chapman AB, Bailey K, Cooper-DeHoff RM, Gums J, Curry RW, Gong Y, Beitelshees AL, et al. Pharmacogenomics of antihypertensive drugs: rationale and design of the pharmacogenomic evaluation of antihypertensive responses (PEAR) study. *Am Heart J*. 2009;157:442–449. doi: 10.1016/j.ahj.2008.11.018
15. Hamadeh IS, Langaee TY, Dwivedi R, Garcia S, Burkley BM, Skaar TC, Chapman AB, Gums JG, Turner ST, Gong Y, et al. Impact of CYP2D6 polymorphisms on clinical efficacy and tolerability of metoprolol tartrate. *Clin Pharmacol Ther*. 2014;96:175–181. doi: 10.1038/clpt.2014.62
16. Chapman AB, Schwartz GL, Boerwinkle E, Turner ST. Predictors of antihypertensive response to a standard dose of hydrochlorothiazide for essential hypertension. *Kidney Int*. 2002;61:1047–1055. doi: 10.1046/j.1523-1755.2002.00200.x
17. McDonough CW, Magvanjav O, Sa ACC, El Rouby NM, Dave C, Deitchman AN, Kawaguchi-Suzuki M, Mei W, Shen Y, Singh RSP, et al. Genetic variants influencing plasma renin activity in hypertensive patients from the PEAR study (Pharmacogenomic Evaluation of Antihypertensive Responses). *Circ Genom Precis Med*. 2018;11:e001854. doi: 10.1161/CIRCGEN.117.001854
18. Singh S, El Rouby N, McDonough CW, Gong Y, Bailey KR, Boerwinkle E, Chapman AB, Gums JG, Turner ST, Cooper-DeHoff RM, et al. Genomic association analysis reveals variants associated with blood pressure response to beta-blockers in European Americans. *Clin Transl Sci*. 2019;12:497–504. doi: 10.1111/cts.12643
19. Turner ST, Boerwinkle E, O'Connell JR, Bailey KR, Gong Y, Chapman AB, McDonough CW, Beitelshees AL, Schwartz GL, Gums JG, et al. Genomic association analysis of common variants influencing antihypertensive response to hydrochlorothiazide. *Hypertension*. 2013;62:391–397. doi: 10.1161/HYPERTENSIONAHA.111.00436
20. McCall MN, McMurray HR, Land H, Almudevar A. On non-detects in qPCR data. *Bioinformatics*. 2014;30:2310–2316. doi: 10.1093/bioinformatics/btu239
21. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. *Nat Protoc*. 2008;3:1101–1108. doi: 10.1038/nprot.2008.73
22. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. *Nucleic Acids Res*. 2015;43:W460–W466. doi: 10.1093/nar/gkv403
23. Mollet IG, Macedo MP. Pre-diabetes-linked miRNA miR-193b-3p targets PPARGC1A, disrupts metabolic gene expression profile and increases lipid accumulation in hepatocytes: relevance for MAFLD. *Int J Mol Sci*. 2023;24:3875. doi: 10.3390/ijms24043875
24. Andersen G, Wegner L, Jensen DP, Glümer C, Tarnow L, Drivsholm T, Poulsen P, Hansen SK, Nielsen E-MD, Ek J, et al. PGC-1α Gly482Ser polymorphism associates with hypertension among Danish Whites. *Hypertension*. 2005;45:565–570. doi: 10.1161/01.HYP.0000158946.53289.24
25. Kriegel AJ, Baker MA, Liu Y, Liu P, Cowley AW Jr, Liang M. Endogenous microRNAs in human microvascular endothelial cells regulate mRNAs encoded by hypertension-related genes. *Hypertension*. 2015;66:793–799. doi: 10.1161/HYPERTENSIONAHA.115.05645
26. Ye Y, Yang J, Lv W, Lu Y, Zhang L, Zhang Y, Musha Z, Fan P, Yang B, Zhou X, et al. Screening of differentially expressed microRNAs of essential hypertension in Uyghur population. *Lipids Health Dis*. 2019;18:98. doi: 10.1186/s12944-019-1028-1
27. Huang YQ, Huang C, Chen JY, Li J, Feng YQ. Plasma expression level of miRNA let-7 is positively correlated with carotid intima-media thickness in patients with essential hypertension. *J Hum Hypertens*. 2017;31:843–847. doi: 10.1038/jhh.2017.52
28. Zhang X, Wang X, Wu J, Peng J, Deng X, Shen Y, Yang C, Yuan J, Zou Y. The diagnostic values of circulating miRNAs for hypertension and bioinformatics analysis. *Biosci Rep*. 2018;38:38. doi: 10.1042/BSR20180525
29. Shahin MH, Johnson JA. Mechanisms and pharmacogenetic signals underlying thiazide diuretics blood pressure response. *Curr Opin Pharmacol*. 2016;27:31–37. doi: 10.1016/j.coph.2016.01.005
30. Rinschen MM, Palygin O, El-Meanawy A, Domingo-Almenara X, Palermo A, Dissanayake LV, Golosova D, Schafroth MA, Guijas C, Demir F, et al. Accelerated lysine metabolism conveys kidney protection in salt-sensitive hypertension. *Nat Commun*. 2022;13:4099. doi: 10.1038/s41467-022-31670-0
31. Poggiogalle E, Fontana M, Giusti AM, Pinto A, Iannucci G, Lenzi A, Donini LM. Amino acids and hypertension in adults. *Nutrients*. 2019;11:1459. doi: 10.3390/nu11071459
32. Polak-Iwaniuk A, Harasim-Symbor E, Golaszewska K, Chabowski A. How hypertension affects heart metabolism. *Front Physiol*. 2019;10:435. doi: 10.3389/fphys.2019.00435
33. Dluzen DF, Noren Hooten N, Zhang Y, Kim Y, Glover FE, Tajuddin SM, Jacob KD, Zonderman AB, Evans MK. Racial differences in microRNA and gene expression in hypertensive women. *Sci Rep*. 2016;6:35815. doi: 10.1038/srep35815
34. Turner ST, Schwartz GL, Chapman AB, Beitelshees AL, Gums JG, Cooper-Dehoff RM, Boerwinkle E, Johnson JA, Bailey KR. Power to identify a genetic predictor of antihypertensive drug response using different methods to measure blood pressure response. *J Transl Med*. 2012;10:47. doi: 10.1186/1479-5876-10-47

Downloaded from http://ahajournals.org by on February 25, 2024