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Precision Medicine in Patients With Resistant Hypertension and Obstructive Sleep Apnea

Blood Pressure Response to Continuous Positive Airway Pressure Treatment

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ABSTRACT

BACKGROUND In patients with resistant hypertension (RH) and obstructive sleep apnea (OSA), the blood pressure response to continuous positive airway pressure (CPAP) treatment is highly variable and could be associated with differential micro-ribonucleic acid (miRNA) profiles. Currently, no available methods exist to identify patients who will respond favorably to CPAP treatment.

OBJECTIVES The aim of this study was to identify plasma miRNA profiles that predict blood pressure responses to CPAP treatment.

METHODS Cardiovascular system–focused circulating miRNA expression was evaluated in plasma samples using an 84-miRNA array among patients with RH and OSA at baseline and after 3 months of adherent CPAP use. Pathway analysis and miRNA target gene enrichment were performed in silico. Plasma levels of peptides and hormones related to cardiovascular function were also measured.

RESULTS The OSA responder group exhibited blood pressure decreases exceeding the observed median (>4.5 mm Hg) after CPAP, which were not present in the nonresponder group (≤4.5 mm Hg) ($p < 0.01$). Three miRNAs provided a discriminatory predictive model for such a favorable blood pressure response to CPAP (area under the curve: 0.92; $p = 0.01$). Additionally, CPAP treatment significantly altered a total of 47 plasma miRNAs and decreased aldosterone-to-renin ratios in the responder group ($p = 0.016$) but not in the nonresponder group.

CONCLUSIONS A singular pre-CPAP treatment cluster of 3 plasma miRNAs predicts blood pressure responses to CPAP treatment in patients with RH and OSA. CPAP treatment is accompanied by changes in cardiovascular system–related miRNAs that may potentially influence the risk for cardiovascular disease among patients with OSA and RH. (Effect of Continuous Positive Airway Pressure [CPAP] Treatment in the Control of Refractory Hypertension; NCT00616265) (J Am Coll Cardiol 2015;66:1023–32) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

AUC = area under the curve

BP = blood pressure

CI = confidence interval

CPAP = continuous positive airway pressure

CVD = cardiovascular disease

miRNA = micro-ribonucleic acid

OSA = obstructive sleep apnea

qRT-PCR = quantitative real-time reverse transcription polymerase chain reaction

RH = resistant hypertension

Cardiovascular disease (CVD) is the leading cause of death throughout the world. Among all risk factors associated with CVD development, hypertension is likely the most important; it is also among the most treatable cardiovascular risk factors (1). Between 12% and 27% of all patients with hypertension are considered to have resistant hypertension (RH) (2), defined as blood pressure (BP) higher than therapeutic goals (i.e., average systolic BP ≥130 mm Hg, average diastolic BP ≥80 mm Hg, or both) despite concurrent use of at least 3 antihypertensive agents prescribed at doses that provide optimal benefit, with 1 of these drugs ideally being a diuretic agent (3). Patients with RH are approximately 50% more likely to experience cardiovascular events than patients with hypertension but not RH, and the incidence of RH is anticipated to increase in the coming years (4).

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Obstructive sleep apnea (OSA), a common disorder that affects approximately 10% of the middle-age population (5), is associated with increased CVD and overall mortality risks (6–9). OSA is a common cause of systemic hypertension and should be suspected in patients with hypertension (10–13), particularly those with RH (14,15). Indeed, more than 70% of patients with RH have OSA (16). Although continuous positive airway pressure (CPAP) treatment reduces BP levels in patients with OSA (17–24), its beneficial effects are related to patient adherence as well as to baseline BP levels (25,26). However, BP responses are highly variable, even when adherent use of CPAP is documented, with some patients exhibiting major reductions in BP (>10 mm Hg) and others showing either unchanged or worsening BP levels (16,25,27). In fact, 25% to 30% of patients who use CPAP treatment for >4 h/night do not experience a positive effect on BP (25,26). The underlying causes of patient variability in response to continuous adherent use of CPAP are unknown. More important, no tools are available to date that enable clinicians to identify those patients who will respond favorably to CPAP treatment (i.e., reduced BP levels).

Micro-ribonucleic acids (miRNAs) are a class of small (19- to 25-nucleotide), noncoding RNAs that regulate gene expression at the post-transcriptional level by binding to a target messenger RNA, thereby leading to either degradation or translational repression (28). Evidence suggests that miRNAs control development and are critically involved in many biological processes related to health and disease, including CVD (29–31). Consequently, miRNAs have emerged as major protagonists for managing CVD in an era of evolving precision medicine.

We hypothesized that among patients with RH and OSA, a singular cardiovascular system–focused miRNA biomarker profile might reliably discriminate those patients with favorable BP responses to CPAP. Additionally, we posited that adherence to CPAP treatment might modify the miRNA profiles and plasma levels of peptides and hormones related to cardiovascular function. Some of these study results have been previously reported in abstract form (32,33).

METHODS

Study participants were part of a larger multicenter, randomized, controlled trial (NCT00616265) conducted at 24 Spanish teaching hospitals to evaluate the effect of 3 months of CPAP treatment on the BP levels of patients with RH and OSA; the patient characteristics have been provided in detail elsewhere (26). In this study, we included male patients who used CPAP for at least 4 h/day. CPAP adherence was objectively examined on the basis of the number of hours of CPAP use per day, according to the device's internal clock. Patients with reductions in mean BP greater than the observed median of 4.5 mm Hg were classified as responders to CPAP treatment.

To identify plasma miRNA profiles that predict BP response to CPAP treatment, we screened an initial group of 8 subjects with the best and 8 subjects with the worst BP responses to adherent CPAP use who were matched for age, sex, ethnicity, body mass index, and participant center (**Figure 1**). The initial set of differentially expressed miRNAs from these experiments was then used in a subsequent complete training set (n = 24), divided equally between responders and nonresponders, enabling final selection of the minimum number of miRNAs that specifically differentiate

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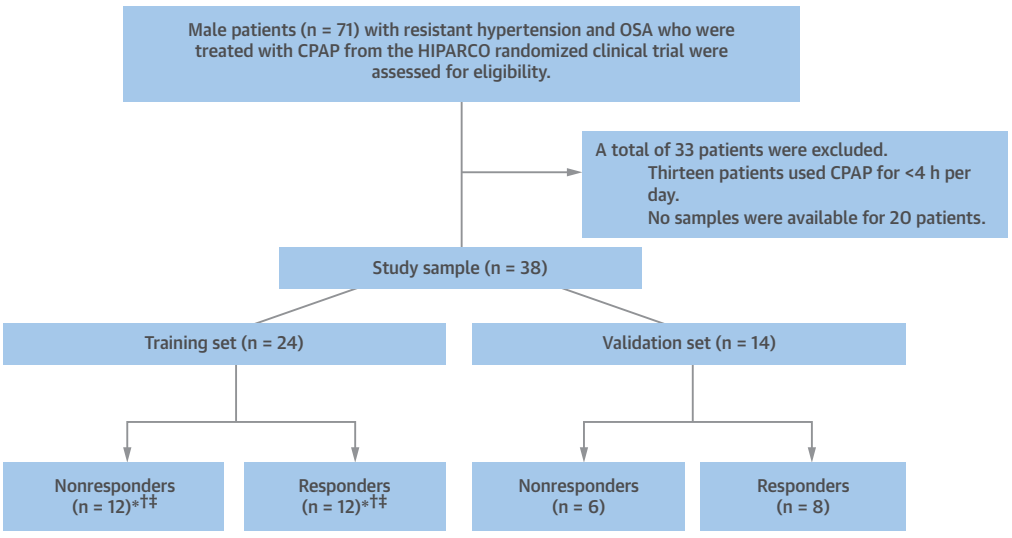
FIGURE 1 Study Flow



Patients with resistant hypertension and obstructive sleep apnea (OSA) were divided into training and validation sets and further divided for study on the basis of favorable blood pressure (BP) response (responders) and unfavorable response (nonresponders) to adherent use of continuous positive airway pressure (CPAP) treatment. *Eight patients each with the best and worst BP responses to CPAP were used to screen the initial micro-ribonucleic acid (miRNA) candidate set. †Twelve nonresponders and 12 responders were used as the training set, which included the previous 8 patients per group. ‡Five patients each with the best and worst BP responses were used to evaluate the effect of CPAP treatment on miRNA profiles.

responders and nonresponders. This latter final set of miRNAs was then further validated in an independent group of patients (n = 14) that constituted the validation set. Additionally, to assess the effect of adherent use of CPAP on cardiovascular system-focused miRNA biomarker profiling, we evaluated 5 patients with the best and 5 patients with the worst BP responses to CPAP at baseline and after 3 months of adherent CPAP use. Finally, to evaluate the impact of CPAP treatment on the plasma levels of hormones and proteins functionally related to cardiovascular functions, all patients (n = 38) were evaluated. The ethics committee of each participating center approved the study, and patients provided written informed consent.

Venous blood samples were obtained from patients between 8 and 10 AM after fasting overnight. Total RNA, including miRNA, was isolated from the plasma using the miRNeasy Mini Kit column-based system according to the manufacturer's instructions (Qiagen, Valencia, California). We used miRNA arrays (84 miRNAs) specific for the human cardiovascular system (Qiagen) using samples from patients with RH and OSA. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analyses were performed using an ABI 7500 (Applied Biosystems, Foster City, California). Complementary deoxyribonucleic acid synthesis was performed using a miScript SYBR Green PCR Kit (Qiagen). Additional details are provided in the Online Appendix. The Web-based computational tool DIANA-mirPath version 2.1 (34) was used to predict the target genes and altered Kyoto Encyclopedia of Genes and Genomes pathways of the differentially expressed miRNAs. Myeloid-related protein 8/14 and adropin have been previously identified as atherogenic markers that are related to endothelial dysfunction. Additionally, renin and aldosterone play an important role in regulating blood volume and systemic vascular resistance. Plasma concentrations of myeloid-related protein 8/14, adropin, renin, and aldosterone were measured. For additional methodological information, see the Online Appendix.

STATISTICAL ANALYSIS. Comparability between the responders and nonresponders was assessed using the Mann-Whitney *U* test for quantitative characteristics and the Fisher exact test for qualitative variables. A multivariate logistic regression model was fitted to the training set to predict the response to CPAP. Variables maximizing the discrimination ability of the model, as measured with the area under

TABLE 1 Baseline Characteristics

	Nonresponders (n = 18)	Responders (n = 20)	p Value
Change in MBP after CPAP treatment (pre- minus post-CPAP), mm Hg*	−0.25 (−5 to 2.5)	11 (7.5 to 14.1)	—
Men	18 (100)	20 (100)	1.00
Age, yrs	60 (52 to 66)	54 (50.8 to 63)	0.14
BMI, kg/m^2	32 (30.1 to 34)	32.1 (30.7 to 37.6)	0.39
Neck perimeter, cm	42.5 (42 to 43.8)	44 (42.0 to 46)	0.49
Apnea-hypopnea index, events/h	34.5 (22.2 to 47.8)	48.5 (31.5 to 59)	0.12
$TSat_{90}$	6 (1.5 to 11.2)	7 (2.00 to 27)	0.17
Epworth Sleepiness Scale score	9 (6.5 to 12)	8 (5.00 to 9)	0.26
CPAP mean use, h/day	5.5 (4.5 to 6)	5.5 (4.88 to 6.5)	0.87
24-h MBP, mm Hg	113 (107 to 114)	118 (111 to 122)	0.057
SBP, mm Hg	142 (136 to 150)	147 (138 to 152)	0.41
DBP, mm Hg	82.5 (74.2 to 85.8)	88.5 (82.5 to 94)	0.026
Nocturnal BP pattern			0.76
Dipper	4 (22.2)	7 (35)	
Nondipper	9 (50)	9 (45)	
Riser	5 (27.8)	4 (20)	
Stroke	1 (5.56)	2 (10)	1.00
Coronary heart disease	4 (22.2)	3 (15)	0.68
Peripheral arterial disease	1 (5.56)	2 (10)	1.00
Diabetes	7 (38.9)	9 (45)	0.96
Dyslipidemia	11 (61.1)	13 (65)	1.00
Tobacco use, pack-yrs	0 (0 to 28.8)	0 (0 to 29.8)	0.6
Years since diagnosis of resistant hypertension	12 (11.5 to 22)	9 (4 to 15.5)	0.077
Number of systemic hypertension drugs	3 (3 to 3.75)	3.5 (3 to 4)	0.25
Calcium-channel blockers	10 (55.6)	15 (75.0)	0.358
Angiotensin II receptor blockers	13 (72.2)	11 (55.0)	0.446
β-blockers	9 (50.0)	13 (65.0)	0.544
Angiotensin-converting enzyme inhibitors	5 (27.8)	5 (25.0)	1.00
α_1-blockers	5 (27.8)	5 (25.0)	1.00
Renin blockers	2 (11.1)	1 (5.00)	0.595
Type of diuretic agent			0.44
None	2 (11.1)	0 (0)	
Thiazides/xipamide no loop diuretic agents	7 (38)	11 (55)	
Loop diuretic agents without thiazides	8 (44.4)	7 (35)	
Potassium-sparing/antialdosterone	0 (0)	0 (0)	
Thiazides and loop diuretic agents	1 (5.56)	2 (10)	

Values are median (interquartile range) or n (%). *Descriptive values show changes in MBP in each group after CPAP treatment; p value not calculated because this was a quantitative variable used to define the groups.

BMI = body mass index; BP = blood pressure; CPAP = continuous positive airway pressure; DBP = diastolic blood pressure; IQR = interquartile range; MBP = mean blood pressure; SBP = systolic blood pressure; $TSat_{90}$ = night time spent with oxygen saturation less than 90%.

the curve (AUC), were included in the model if they exhibited statistically significant contributions according to the likelihood ratio test. Possible cutoff points for each quantitative variable were selected from those dividing the sample into 2 categories, responders and nonresponders. The Hosmer-Lemeshow test was used to test model calibration, and the continuous net reclassification index was used to determine the inclusion of those variables without statistically significant contributions to the multivariate logistic regression model but that improved its AUC. The final model was translated into an easy-to-use scoring system (the sum of the integer values), the HIPARCO-Score. Sensitivity and specificity were estimated for each possible cutoff point. The exact binomial distribution was used to estimate 95% confidence intervals (CIs) for the proportions. The Mann-Whitney *U* test was used to compare changes in miRNA expression after CPAP treatment (pre-post) between responders and nonresponders. False-discovery rate-adjusted p values were determined to adjust for the performance of multiple paired comparisons. After CPAP treatment, differences in clinical variables were assessed using a paired non-parametric Wilcoxon test for quantitative variables and a symmetry test (generalized McNemar test) for qualitative variables. The Mann-Whitney *U* test was used to compare baseline (pre) levels, post-CPAP treatment (post) levels, and changes (pre-post) of peptides and hormones related to cardiovascular function between responders and nonresponders. R software (R Project for Statistical Computing, Vienna, Austria) was used for statistical analysis, with a significance level of 0.05. Post-hoc estimation of the detectable effect size using the available sample size, statistical power of 0.80, and a significance level of 0.05 was performed using G*Power 3.1 software (35). (Additional details are provided in the Online Appendix and Online Figure 1.)

RESULTS

Patient characteristics were similar at baseline (**Table 1**), with the exception of diastolic BP, which was significantly higher in responders compared with nonresponders (p = 0.026). Online Table 1 presents the comparable characteristics between the responders and nonresponders in the training set, with the exception of the time since RH diagnosis, which was significantly shorter in responders (p = 0.041). In the validation set (Online Table 2), responders and nonresponders exhibited similar characteristics, except that the percentage of patients receiving calcium-channel blockers was significantly higher in the responder group (p = 0.015).

Eight patients with the best BP responses to CPAP and 8 patients with the worst responses were used to screen candidate miRNAs for the detection of favorable BP responses to CPAP. After miRNA PCR array-based expression analysis, a subset of 8 differentially expressed miRNAs (based on relative quantification of >1.65 or <1/1.65 when normalizing using at least 3 housekeeping miRNAs) was selected for subsequent analysis: miR-7-5p, miR-29a-3p, miR-92a-3p, miR-100-5p, miR-144-3p, miR-150-5p,

TABLE 2 Multivariate Logistic Regression Models

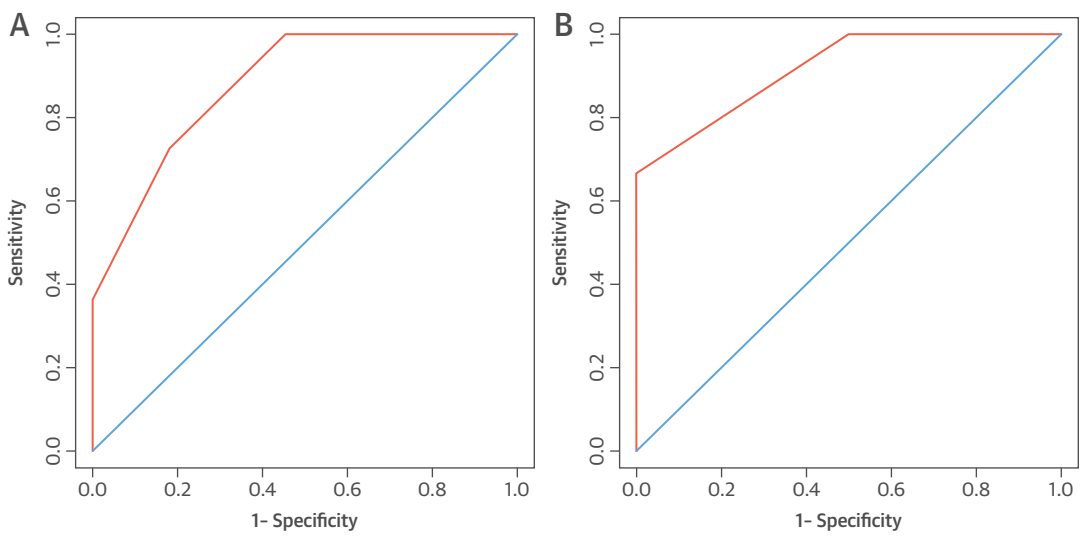
	Model M2*			Model M3†		
	Estimate	OR	p Value	Estimate	OR	p Value
dCt (miR-378a-3p) <2.6	3.01 (1.47)	20.3	0.041	2.39 (1.46)	10.9	0.102
dCt (miR-486-5p) >−7.1	2.47 (1.25)	11.8	0.048	2.67 (1.36)	14.4	0.049
dCt (miR-100-5p) ≤0.4	–	–	–	1.50 (1.32)	4.5	0.256

*Model M2 includes the 2 micro-ribonucleic acids contributing significantly to the identification of responders. †Model M3 adds miR-100-5p to model M2.
OR = odds ratio.

miR-378a-3p, and miR-486-5p. Reproducibility of these results was then confirmed in an independent assay (qRT-PCR). The microarray and qRT-PCR results were normalized using the same control (SNORD95), and all miRNA microarray data agreed with the qRT-PCR data, except for miR-29a-3p, which exhibited unreliable expression values, with no significant intraclass correlation coefficient ($p = 0.25$) (Online Table 3). The average SNORD95 Ct values were similar for the plasma collected from the responders (30.9 ± 2.5) and nonresponders (30.7 ± 1.0) ($p > 0.05$), thus confirming the applicability of this gene as a normalizer. qRT-PCR confirmed array findings in the complete training set of 24 patients.

Logistic regression models were generated using the training set of 24 patients to evaluate the discriminatory power of the combination of differentially expressed miRNAs to identify favorable BP responders and nonresponders to adherent CPAP use. Univariate logistic regression identified miR-378a-3p, miR-486-5p, and miR-100-5p as the 3 miRNAs with the highest statistical associations and a mean favorable BP response to CPAP (Online Table 4). The cutoff points maximizing the discrimination for these miRNAs were dCt (miR-378a-3p) <2.6, dCt (miR-486-5p) >−7.1, and dCt (miR-100-5p) ≤0.4. A first multivariate model (model M2 in **Table 2**) was fit to predict the response to CPAP using the expression levels of both miR-378a-3p and miR-486-5p. Both of these miRNAs contributed significantly to the model according to the likelihood ratio test. Model M2 exhibited good calibration (Hosmer-Lemeshow test $p = 1.00$) and discrimination (AUC: 0.85; 95% CI: 0.70 to 1.00). Among the subset of differentially expressed miRNAs, miR-100-5p ($p = 0.06$) (Online Table 5) improved the continuous net reclassification index

FIGURE 2 Received-Operating Characteristic Curve for the HIPARCO-Score



The discriminatory performance of the model is seen **(A)** as adjusted in the training set (Hosmer-Lemeshow test $p = 0.979$; area under the curve [AUC]: 0.88; 95% confidence interval [CI]: 0.75 to 0.99) and **(B)** when the HIPARCO-Score was applied to an independent validation set (Hosmer-Lemeshow test $p = 0.991$; AUC: 0.92; 95% CI: 0.79 to 0.99).

and was therefore added to the final model (model M3 in **Table 2**). **Figure 2A** shows the receiver-operating characteristic curve for the model M3 discrimination in the training set (AUC: 0.88; 95% CI: 0.75 to 1.00). Model M3 showed good calibration (Hosmer-Lemeshow goodness-of-fit $p = 0.979$) (**Table 2**). This model was validated in an independent group (validation set), yielding an AUC of 0.92 (95% CI: 0.79 to 1.00; Hosmer-Lemeshow $p = 0.991$) (**Figure 2B**). By translating the logistic regression model into a score by adding 1 if dCt (miR-100-5p) ≤0.4, 2 if dCt (miR-378a-3p) <2.6, and 3 if dCt (miR-486-5p) >−7.1, we were able to simplify the model's use by producing a simple numeric score (the HIPARCO-Score, ranging from 0 to 6) that predicts the probability of favorable BP response to adherent CPAP use (**Table 3**).

Among the patients with RH and OSA, adherent use of CPAP treatment for 3 months resulted in decreases in mean BP ($p < 0.001$), systolic and diastolic BP ($p = 0.008$), and Epworth Sleepiness Scale score ($p < 0.001$). No significant changes were observed in the number of antihypertensive agents taken per patient (Online Table 6). We evaluated the miRNA biomarker profiles in 5 patients with the best and worst BP responses to CPAP treatment; adherent use differentially altered the expression of 47 of the 84 cardiovascular system-focused miRNAs evaluated (with a false-discovery rate multitesting-corrected p value of <0.05) (Online Table 7). In favorable BP responders, most of the cardiovascular system-focused miRNAs were down-regulated after adherent CPAP treatment (**Figure 3**, Online Figure 2).

The enrichment analysis, including the 7 miRNAs confirmed to be differentially expressed at baseline in BP responders and nonresponders, revealed 2,868 putative target genes and 42 pathways (Online Table 8, Online Figure 3). Enrichment analysis, which included the 47 miRNAs that were found to be differentially expressed after treatment with CPAP, revealed 8,069 putative target genes and 67 pathways (Online Table 9, Online Figure 4). In both analyses, the most frequently enriched pathways were categorized under biological processes connected with cancer, CVD, and signal transduction, as well as with the endocrine and nervous systems (Online Tables 8 and 9).

Decreases in aldosterone-to-renin ratios were significantly greater in the responder group ($p = 0.016$) (Online Table 10) and were positively correlated with the change in mean BP values after adherent CPAP use ($r = 0.46$; $p = 0.001$) (Online Table 11). A complementary analysis identifying changes in mean BP that were most significantly associated with plasma levels of peptides and hormones is presented in the Online Appendix (Online Tables 12 and 13).

TABLE 3 HIPARCO-Score to Estimate Favorable BP Response

HIPARCO-Score	Observed Probability	Predicted Probability	Expected Frequency	Observed Frequency	Total
0	0%	6%	0.34	0	6
1	50%	21%	0.42	1	2
2	50%	46%	1.85	2	4
3	67%	75%	2.24	2	3
4	67%	80%	2.38	2	3
5	100%	90%	1.81	2	2
6	100%	98%	1.95	2	2

Calibration table, including observed and predicted frequencies and probabilities, showing no significant lack of fit (with a resulting Hosmer-Lemeshow test $p = 0.979$).

DISCUSSION

A singular cluster of miRNAs functionally associated with the cardiovascular system appears to specifically discriminate patients with RH and OSA who respond to CPAP treatment with favorable decreases in mean BP from those who do not (**Central Illustration**). The measurement of a specific cluster of miRNAs enabled generation of a predictive screening tool (HIPARCO-Score) to identify those responders. The present study also demonstrated that adherent CPAP treatment is associated with changes in circulating cardiovascular system-related miRNAs that could potentially influence the risk for developing CVD in patients with RH and OSA. Finally, we showed that CPAP treatment significantly decreases the aldosterone-to-renin ratio in responders (Online Figure 5).

Adherent use of CPAP treatment results in a median decrease in 24-h mean BP of 4.5 mm Hg. Studies of different antihypertensive drug regimens have demonstrated that small reductions of BP ranging from 1 to 2 mm Hg may be clinically significant on the basis of an associated reduction in the risks for cardiovascular and cerebrovascular events. Moreover, responders exhibited a median BP decrease of 11 mm Hg, a reduction associated with a dramatic reduction in the relative risk for stroke, coronary heart disease, heart failure, major cardiovascular events, cardiovascular death, and total mortality (36).

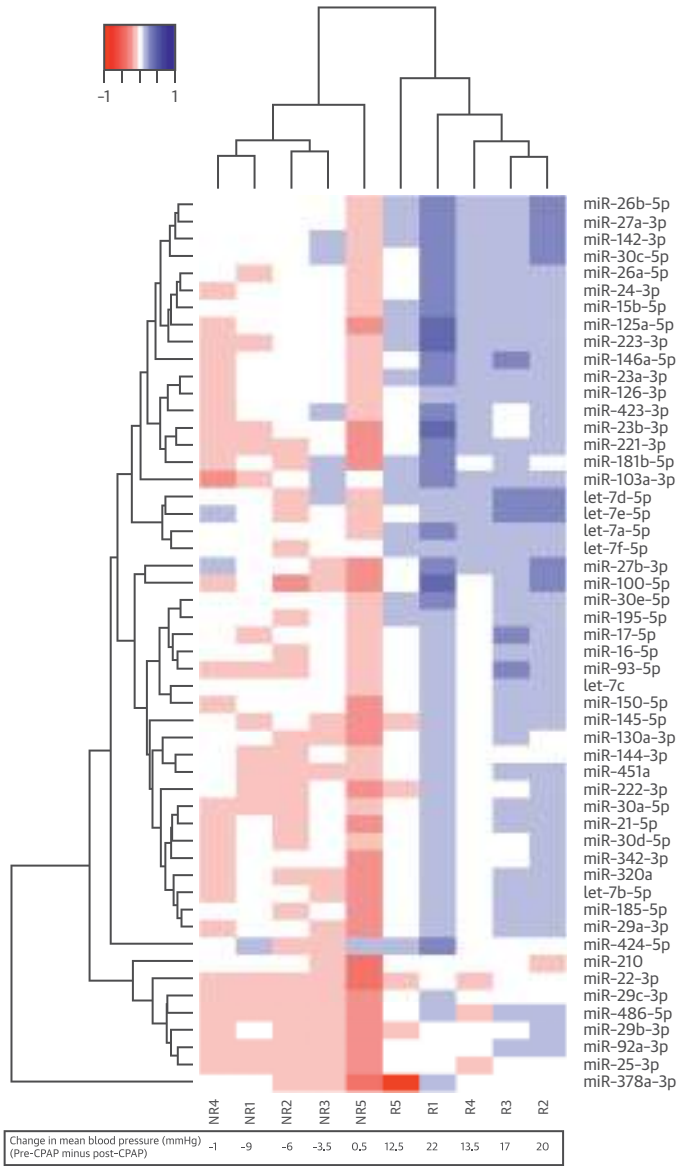
A very large degree of variability in the effect of CPAP treatment on BP levels has been observed, likely because of the multifactorial nature of systemic hypertension. This variability has led to increased interest in prospectively identifying subgroups of patients who will more likely benefit from CPAP treatment. Previous studies have reported that 25% to 30% of patients with OSA with adequate adherence

to CPAP (>4 h/night) did not exhibit BP reductions (25,26). In this study, we showed that the HIPARCO-Score enabled the correct identification of 100% of the patients who adequately responded to adherent CPAP use. Additionally, the HIPARCO-Score identified 50% of the patients who did not show any BP reduction despite adherent CPAP use and therefore will require complementary intervention for BP control. Diagnostic and personalized therapeutic decision-making tools are needed to manage sleep apnea and to effectively predict responses to adherent CPAP use. Reinforcement approaches aiming to guarantee adequate CPAP adherence may be particularly important for these patients. Determination of miRNAs can be performed in tertiary university hospitals at a low cost ($35 to $50).

Adherent CPAP use was associated with changes in the expression of 47 of the 84 miRNAs evaluated. Changes in the miRNA profile differed significantly between responders and nonresponders. In responders, CPAP treatment was associated with an overall decrease in cardiovascular system-focused circulating miRNAs; nonresponders demonstrated no change or even an increase in the expression levels of some of the circulating miRNAs post-CPAP treatment. The unsupervised hierarchical clustering of the miRNA microarray revealed a set of miRNAs with expression changes that differed significantly between the 2 response groups. Our study design allowed us to compare the circulating miRNA profile of each patient before and after OSA resolution, enabling the incorporation of interindividual variability into the analyses, which increased the statistical power to detect expression changes after adherent CPAP use. This process provided an opportunity to implement bioinformatic procedures and identify enriched putative biological pathways in response to CPAP. Most of these pathways for the group of miRNAs with altered expression levels post-CPAP are related to CVD and cancer. Each miRNA regulates a large number of distinct target genes. Selected miRNAs are emerging as pivotal modulators of mammalian cardiovascular development and diseases, such as cardiac fibrosis, arrhythmia, myocardial infarction, cardiac hypertrophy, cardiomyopathy (37), and cancer (additional information is provided in the Online Appendix). These results may indicate an association between long-term adherent CPAP use and a reduced incidence of CVD.

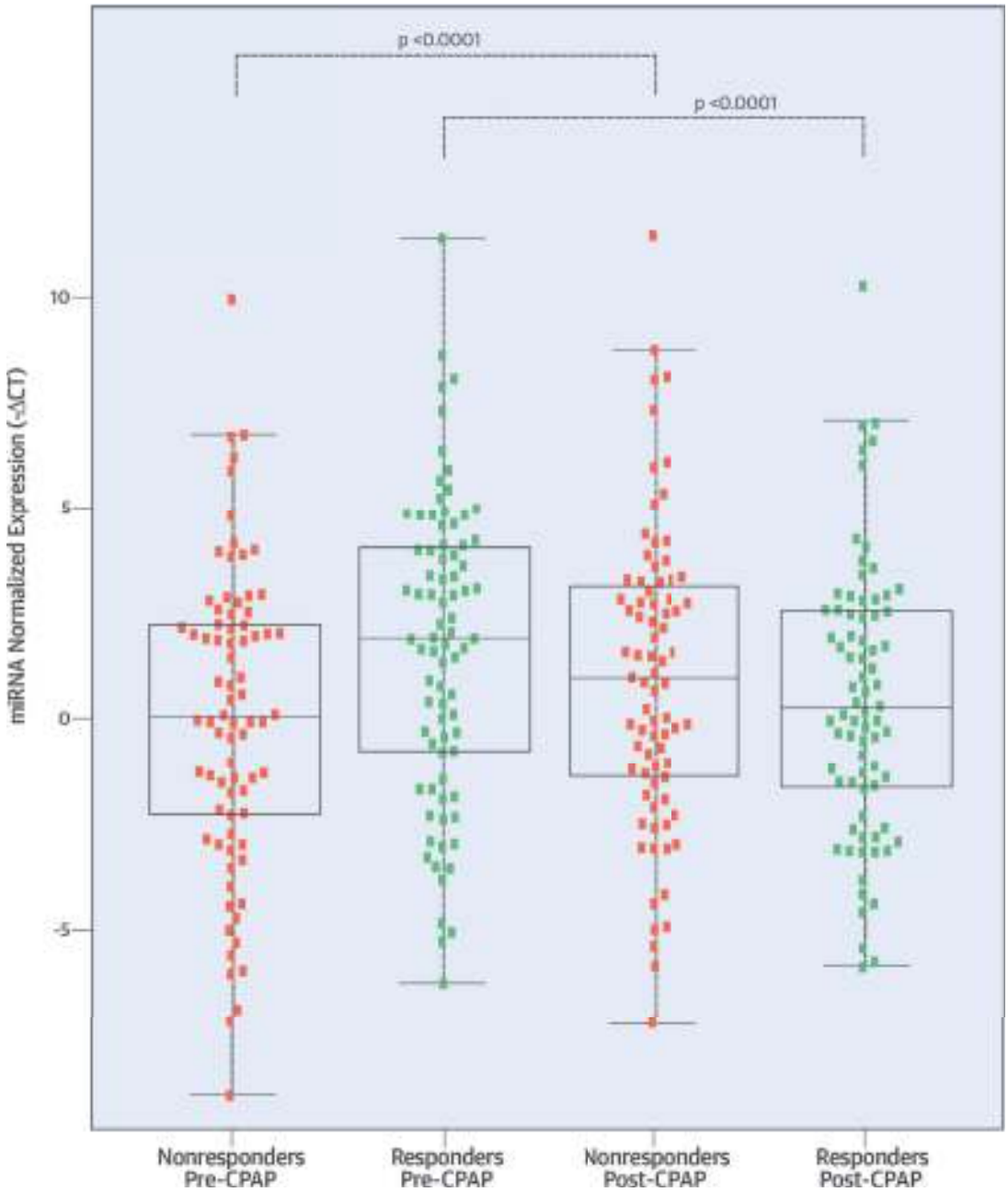
However, categorization of the enriched biological pathways also yielded somewhat unexpected results. Indeed, we observed that most of the enriched biological pathways for the miRNAs that exhibited altered expression levels after CPAP treatment fell under categories of biological processes that were closely connected to cancer. Recent studies have reported an association between OSA and cancer incidence (38) and mortality rates (39). Moreover,

FIGURE 3 Unsupervised Hierarchical Clustering of the miRNA Microarray



This heat map illustrates the differential micro-ribonucleic acid (miRNA) expression after continuous positive airway pressure (CPAP) compared with baseline for each patient. This set was constructed using a random forest plot to identify the miRNAs with maximum importance according to the Gini index. The heat map colors range from **red** (or negative ddCt, denoting increased expression after CPAP) to **blue** (or positive ddCt, denoting decreased expression after CPAP) to **white** (no change in ddCt after CPAP), without scaling by row or column. The bottom of the image presents the mean blood pressure change for each subject after 3 months of adherent CPAP use. Dendrograms show hierarchical clustering representing the similarities and dissimilarities in expression profiles among subjects and among miRNAs. NR = nonresponder; R = responder.

CENTRAL ILLUSTRATION **Predicting BP Response to CPAP Treatment: Box Plot of Responders and Nonresponders**



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Box plot of micro-ribonucleic acid (miRNA)–normalized expression in patients with obstructive sleep apnea and resistant hypertension (RH) with favorable (responders) and unfavorable (nonresponders) blood pressure (BP) responses to adherent use of continuous positive airway pressure (CPAP) at baseline and post-CPAP treatment. The data points represent the median of each miRNA-normalized expression for each group of patients. To facilitate interpretation, we present the −dCt values because high Ct values are indicative of lower expression. Consequently, positive values indicate high expression normalized to the housekeeping gene SNORD95. Data were obtained from the 5 patients with the best and 5 patients with the worst BP responses to CPAP (defined as a greater than 4.5 mm Hg change in mean BP after treatment). In patients with RH, there was a significantly different post-CPAP treatment trend in miRNA expression. Thus, whereas nonresponders showed a significant increase in miRNA expression, responders showed an inverse behavior, reducing miRNA expression.

a recent study demonstrated that effective CPAP treatment is associated with transcriptional suppression of cancer-related pathways (40). Thus, even though our selected miRNA array was a priori focused on cardiovascular functions, further in silico exploration of gene targets of differentially expressed miRNAs revealed gene candidates involved in oncological processes as well.

Finally, we observed that in patients with RH and OSA, CPAP treatment was associated with changes in plasma levels of the aldosterone-renin system. High aldosterone-to-renin ratios, indicative of hyperaldosteronism, were significantly decreased post-CPAP in responders. Aldosterone secretion is elevated in patients with systemic hypertension. Moreover, increased secretion of aldosterone and renin during repetitive apneic events may play a role in regulating BP levels and fluid balance in OSA (41). Previous studies have demonstrated that plasma aldosterone is positively correlated with OSA severity only in subjects with RH (41). The results of the present study further suggest that adherent CPAP use decreases the aldosterone-to-renin ratio only in patients with RH and OSA whose BP levels significantly decrease after CPAP treatment.

STUDY LIMITATIONS. The major strength of the present study is its multicenter prospective design. However, this study had several potential limitations. First, we examined only male patients, and our conclusions cannot be extrapolated to women. In fact, we performed a goodness-of-fit test for the HIPARCO-Score for women, which revealed poor calibration and poor discrimination (Online Appendix).

Second, the present study did not include numerous patients who refused blood sampling, and thus possible relevant relationships cannot be extrapolated to those patients. However, this study allowed us to detect high-magnitude associations with favorable BP responses to adherent CPAP use.

CONCLUSIONS

A singular cluster of cardiovascular system-related functional miRNAs was identified that specifically differentiates between patients with RH and OSA with favorable BP responses to CPAP treatment and those with unfavorable responses. The HIPARCO-Score is an easy-to-use and highly predictive clinical practice tool for identifying favorable BP responders to CPAP treatment among patients with RH and OSA; further studies using this score are therefore warranted. Additionally, adherent CPAP treatment is associated with changes in circulating cardiovascular system-related miRNAs that may affect the risk for developing CVD in patients with RH and OSA. Finally, CPAP treatment was significantly associated with decreases in aldosterone-to-renin ratios that were restricted to favorable BP responders to CPAP.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Many patients with RH have OSA, and CPAP treatment reduces BP in patients with these conditions, but responses are variable even among those who adhere to treatment. A cluster of microRNAs related to cardiovascular function reliably discriminates those patients with favorable BP responses to CPAP.

TRANSLATIONAL OUTLOOK: Further studies are needed to elucidate the mechanisms mediating the relationship between these microRNAs, OSA, RH, and the response to CPAP.

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KEY WORDS cardiovascular disease, miRNA, personalized medicine, sleep apnea

APPENDIX For additional Methods, Results, Discussion, and Reference sections as well as tables and figure, please see the online version of this article.