Adrenomedullin and Arterial Stiffness Integrative Approach Combining Monocyte *ADM* Expression, Plasma MR-Pro-ADM, and Genome-Wide Association Study

Farzin Beygui, MD, PhD, **Philipp S. Wild, MD, MSc**, **Tanja Zeller, PhD**, **Marine Germain, MsC**, **Raphaele Castagné, PhD**, **Karl J. Lackner, MD**, **Thomas Münzel, MD**, **Gilles Montalescot, MD, PhD**, **Gary F. Mitchell, MD**, **Germaine C. Verwoert, MsC**, **Kirill V. Tarasov, MD, PhD**, **David-Alexandre Trégouët, PhD**, **François Cambien, MD, PhD**, **Stefan Blankenberg, MD**[*], and **Laurence Tiret, PhD**[*]

UMR_S 1166, Sorbonne Universités UPMC Université Paris 06, UMR_S 1166 INSERM, UMR_S 1166 ICAN Institute for Cardiometabolism and Nutrition, Paris, France (F.B., M.G., R.C., G.M., D.-A.T., F.C., L.T.); Department of Cardiology, Caen University Hospital, Caen, France (F.B.); Department of Medicine II (P.S.W., T.M.), Center for Thrombosis and Hemostasis (P.S.W.), Institute for Clinical Chemistry and Laboratory Medicine (K.J.L.), University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany; Department of General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany (T.Z., S.B.); Institut de Cardiologie, Centre Hospitalier Universitaire Pitié-Salpêtrire (ACTION Group, AP-HP, Université Paris 6), Paris, France (G.M.); Cardiovascular Engineering, Inc, Norwood, MA (G.F.M.); Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands (G.C.V.); and Laboratory of Cardiovascular Science, National Institute on Aging, National Institutes of Health, Baltimore, MD (K.V.T.)

Abstract

Background—Adrenomedullin (ADM) is a circulating vasoactive peptide involved in vascular homeostasis and endothelial function. Single nucleotide polymorphisms of the *ADM* gene are associated with blood pressure variability, and elevated levels of plasma midregional proadrenomedullin (MR-pro-ADM) are associated with cardiovascular diseases.

Methods and Results—We investigated the sources of variability of *ADM* gene expression and plasma MR-pro-ADM concentrations in the general population, and their relationship with markers of atherosclerosis. MR-pro-ADM levels were assessed in 4155 individuals who underwent evaluation of carotid intima-media thickness and arterial rigidity (reflection index and

stiffness index). In a subsample of 1372 individuals, *ADM* gene expression was assessed as part of a transcriptomic study of circulating monocytes. Nongenetic factors explained 45.8% and 7.5% of MR-pro-ADM and *ADM* expression variability, respectively. *ADM* expression correlated with plasma C-reactive protein, interleukin-receptor 1A, and myeloperoxidase, whereas MR-pro-ADM levels correlated with C-terminal proendothelin-1, creatinine, and N-terminal pro–B-type natriuretic peptide. Genome-wide association study of *ADM* expression and MR-pro-ADM levels both identified a single locus encompassing the *ADM* gene. *ADM* expression was associated with 1 single nucleotide polymorphism rs11042717 (P=2.36×10^{-12}), whereas MR-pro-ADM was associated with 2 single nucleotide polymorphisms with additive effects, rs2957692 (P=1.54×10^{-13}) and rs2957717 (P=4.24×10^{-8}). Reflection index was independently associated with rs11042717 (P<10^{-4}) and *ADM* expression (P=0.0002) but not with MR-pro-ADM. Weaker associations were observed for stiffness index. Intima-media thickness was not related to *ADM* single nucleotide polymorphisms or expression.

Conclusions—These results support an involvement of the *ADM* gene in the modulation of peripheral vascular tone.

Keywords

adrenomedullin; gene expression; genome wide association scan; vascular stiffness

Adrenomedullin (ADM) is a potent vasodilator and angiogenic peptide involved in cardiovascular and renal protection through autocrine, paracrine, and endocrine pathways. It is produced by a large variety of cells, not only endothelial and vascular smooth muscle cells but also inflammatory cells. Its expression is inducible by shear stress, oxidative stress, and hypoxia. The synthesis and secretion of ADM are stimulated by several proatherogenic and proinflammatory factors, such as angiotensin II, endothelin-1, aldosterone, interleukin-1α (IL-1α) and interleukin-1β, and tumor necrosis factor-α and tumor necrosis factor-β.[1,2]

In humans, plasma concentrations of ADM are increased in various disease states, including acute myocardial infarction, hypertension, chronic renal failure, and congestive heart failure. [1] Because the reliable quantification of ADM in plasma is hindered by several technical difficulties, it has been proposed to use midregional pro-ADM (MR-pro-ADM), a stable peptide present in an equimolar balance with ADM, as a surrogate marker in epidemiological studies.[3] Increased MR-pro-ADM plasma levels have been related to future cardiovascular events,[4–8] left ventricular hypertrophy,[9,10] mortality in congestive heart failure,[11] and vascular dysfunction.[12–14]

The human *ADM* gene is located on chromosome 11, and genetic polymorphisms in its DNA sequence are associated with blood pressure levels and susceptibility to hypertension. [15–17] The contribution of the *ADM* locus to blood pressure variability in the general population is further supported by the results of genome-wide association studies (GWASs). [18,19] All these findings concur to support a causal role of ADM in the cause of cardiovascular diseases although the underlying molecular mechanisms are not yet fully elucidated.

Here, we report the results of an integrative study conducted in a population-based cohort combining information on ADM issued from several levels, including genome-wide genotype data, *ADM* gene expression in circulating monocytes, and MR-pro-ADM levels in plasma. The aim of this study was to investigate the genetic and nongenetic sources of variability of *ADM* gene expression and plasma MR-pro-ADM levels and to assess their relationship with markers of atherosclerosis.

Methods

Study Population

A detailed description of the study population is provided elsewhere.[20] Study participants of both sexes aged 35 to 74 years were successively enrolled into the Gutenberg Health Study (GHS), a community-based single-center cohort study in the Rhein-Main region in Western mid-Germany. Participants were of European origin. All subjects gave written informed consent. Ethical approval was given by the local ethics committee and by the local and federal data safety commissioners. Hypertension and diabetes mellitus were defined as previously.[21]

Biological Measurements

Plasma creatinine (Abbott), NT-pro–B-type natriuretic peptide (Roche), C-reactive protein (Abbott), myeloperoxidase (Prognostix), IL-18 (MBL), IL-1 receptor antagonist (R&D Systems), and C-terminal proendothelin-1 (BRAHMS) levels were measured using commercially available assays according to manufacturer's recommendations. Plasma MR-pro-ADM levels were measured using the commercially available immunolumino-metric assay (BRAHMS AG, Henningsdorf/Berlin, Germany).[3]

Carotid Arteries Ultrasound

Ultrasound exploration of carotid arteries was performed using an 11 to 3 MHz linear array transducer of the i33 system (Philips, Bove, the Netherlands).[21] Intima-media thickness (IMT) was measured as recommended 1 cm before carotid bulb, bilaterally at the far wall. The IMT phenotype used in the present analysis was the average of right and left IMT measurements.

Evaluation of Vascular Tone and Arterial Stiffness

Reflection index (RI) reflecting vascular tone of small arteries and stiffness index (SI) reflecting large artery stiffness were measured by Pulse Trace 2000 device (Micro Medical Ltd., Rochester, United Kingdom) as described elsewhere.[22]

Genotyping and Imputation

Genome-wide genotyping was performed using the Affymetrix Genome-Wide Human Single Nucleotide Polymorphism (SNP) array 6.0 (http://www.affymetrix.com). Genotyping was performed separately in cohort GHS I including 2990 individuals first enrolled in GHS and in cohort GHS II including 1165 individuals subsequently enrolled. Calling of genotypes was performed separately in each cohort.[20] Genome-wide imputation of

genotypes was performed in cohorts GHS I and GHS II separately using MACH (version 1.0.18.c) and minimac (release 2012-03-14) software with the 1000 Genomes release of February 2012 reference data set (hg19/build37). Only SNPs with good imputation quality ($r^2 > 0.3$) were kept. Imputation-based GWAS was performed using mach2qtl (version 1.1.0, 2011-05-23).

ADM Gene Expression Measurement

Expression level of the *ADM* gene was assessed from circulating monocytes in 1372 individuals of cohort GHS I as part of a genome-wide expression study using the Illumina HT-12 version 3 Beadchip (http://www.Illumina.com). Detailed protocol for separation of monocytes, RNA preparation, and microarray hybridization is reported elsewhere.[20] *ADM* gene expression was detected by a single probe located in the 3′ untranslated region sequence of the gene (ILMN_1708934) and which had a perfect quality score according to ReMOAT (http://remoat.sysbiol.cam.ac.uk/). After preprocessing and quantile normalization, data were transformed using an arcsinh-transformation.

Statistical Analysis

R software (version 2.13.0; The R Foundation for Statistical Computing) and SAS software (version 9.3; SAS Institute Inc, Cary, NC) were used for statistical analysis. Association of *ADM* expression and plasma MR-pro-ADM with cardiovascular risk factors (age, sex, body mass index [BMI], type 2 diabetes mellitus, hypertension, and current smoking) was assessed by linear regression analysis. Association of *ADM* expression and plasma MR-pro-ADM levels with circulating biomarkers and vascular phenotypes was assessed by Pearson correlation coefficients. Partial correlations were adjusted for age, sex, BMI, and smoking. Independent predictors of *ADM* expression and plasma MR-pro-ADM levels were selected by stepwise regression analysis. All biomarkers were log-transformed to remove positive skewness. In regression of vascular phenotypes on SNPs, genotypes were introduced as categorical variables (2 df). Because the 2 SNPs had a dominant effect on vascular phenotypes, their independent effect was tested by jointly introducing them as binary variables in the regression model. SNP×SNP interaction was tested by introducing a product term in the model (1 df).

The GWAS of plasma MR-pro-ADM levels was performed separately in each cohort using PLINK.[23] After quality control filters, 629 283 SNPs were common to both cohorts. Age- and sex-adjusted MR-pro-ADM levels were analyzed assuming additive allele effects for each SNP. The validity of this assumption was then formally tested for the SNPs found associated with phenotype. The results of both cohorts were combined in a meta-GWAS using METAL[24] with a fixed-effect model relying on an inverse-variance weighting. To identify additional signals after accounting for the effect of the hit SNP of the meta-GWAS, a conditional analysis adjusting for the hit SNP was performed. Conditional analyses were repeated until no more significant association was found. The GWAS of age- and sex-adjusted *ADM* expression levels was performed with PLINK in the subsample of cohort GHS I with expression measurements.

The r^2 coefficient of linkage disequilibrium (LD) between SNPs was estimated by maximum-likelihood method. Regional association plots were obtained using SNAP.[25] Power was calculated using Quanto (http://hydra.usc.edu/gxe/). A *P* value <0.05 was considered significant, except for GWAS where the commonly used genome-wide significance level was applied ($P<5\times10^{-8}$). The potential functionality of SNPs was investigated using the HaploReg software (http://www.broadinstitute.org/mammals/haploreg/haploreg.php).

Results

Plasma MR-pro-ADM levels were measured in 4155 individuals of whom 1372 also had a measurement of monocyte *ADM* gene expression. Descriptive characteristics of the 2 samples are shown in Table I in the Data Supplement, and associations of MR-pro-ADM levels and *ADM* gene expression with risk factors are shown in Table II in the Data Supplement.

Independent correlates of *ADM* gene expression were sex, BMI, age, and smoking, which all together explained 7.5% of the variability of expression. Independent correlates of plasma MR-pro-ADM were age, BMI, smoking, sex, hypertension, and diabetes mellitus, which all together explained 45.8% of the variability of plasma levels. The correlation between monocyte *ADM* gene expression and plasma MR-pro-ADM levels was relatively modest (*r*=0.13 after adjustment for risk factors).

GWAS of *ADM* Gene Expression

The GWAS of *ADM* gene expression was performed in 1372 individuals. At a genome-wide significance level, 31 SNPs were associated with *ADM* gene expression (herein referred to as eSNPs), all located on chromosome 11p15 in a 609-kb region overlapping the *SBF2* and *ADM* genes (Figure 1). The lead eSNP rs11042717 was located in an intron of *SBF2*, 23 kb upstream from the transcription start site of the *ADM* gene. The minor allele C frequency (0.45) was associated with increased *ADM* gene expression in a codominant fashion ($P=2.36\times10^{-12}$). After conditioning on rs11042717, no other SNP was significantly associated with *ADM* expression. Imputation analysis did not reveal any stronger hit than rs11042717. The rs11042717 effect was independent of cardiovascular risk factors and accounted for 3.2% of *ADM* expression variability. We looked for potential interaction of genotype with age, sex, BMI, or smoking but none reached statistical significance. None of the *ADM*-associated SNPs were associated with *SBF2* expression (*P*>0.55), and *SBF2* expression did not correlate to *ADM* expression (*P*=0.35).

The eSNP rs11042717 was associated with plasma MR-pro-ADM levels (*P*=0.0002). The minor allele C was associated with increased levels and explained 1% of variability. The effect of rs11042717 on MR-pro-ADM levels was weakened by adjustment for *ADM* expression but remained significant (*P*=0.009).

GWAS of Plasma MR-Pro-ADM Levels

The GWAS of MR-pro-ADM levels was performed in 4155 individuals. At a genome-wide significance level, 34 SNPs were associated with plasma MR-pro-ADM levels (herein

referred to as pSNPs), all located at the 11p15 locus already associated with *ADM* expression. The lead pSNP rs2957692 was located in the 3′ region of the *ADM* gene (Figure 2), and its minor allele G (minor allele frequency, 0.39) was associated with increased MR-pro-ADM levels in a codominant fashion ($P=1.54\times10^{-13}$). Conditional analysis on rs2957692 revealed 3 additional pSNPs associated with MR-pro-ADM levels, all at the 11p15 locus, the strongest signal being observed with the pSNP rs2957717 ($P=4.24\times10^{-8}$). The minor allele T of rs2957717 (minor allele frequency, 0.30) was associated with decreased MR-pro-ADM levels. After conditioning on both rs2957692 and rs2957717, no other SNP was significantly associated with MR-pro-ADM levels. The 2 pSNPs, rs2957717 and rs2957692, located 14 kb apart from each other, were not in LD ($r^2=0.0006$). They had additive effects on MR-pro-ADM levels and accounted together for 1.5% of the variability of MR-pro-ADM levels. There was no interaction of either SNP with age, sex, BMI, or smoking.

The lead pSNP rs2957692 was in strong LD with the lead eSNP rs11042717 ($r^2=0.67$). In a regression analysis, including rs11042717 and the 2 pSNPs rs2957692 and rs2957717, only the 2 pSNPs remained significantly associated with plasma MR-pro-ADM.

The imputation-based GWAS identified the same region as the original one. The strongest hit was observed with an imputed SNP rs7925720 (minor allele frequency, 0.37; $P=8.28\times10^{-15}$). The rs7925720 was in nearly complete association with the typed lead pSNP rs2957692 ($r^2=0.95$) and explained almost the same proportion of the variability of MR-pro-ADM (1.05% versus 0.96%). Because of the nearly complete association between the 2 pSNPs, it was impossible to disentangle their respective roles.

Association of *ADM* Gene Expression and Plasma MR-Pro-ADM Levels With Vascular Phenotypes

ADM gene expression negatively correlated with RI ($r=-0.13$ and -0.08 before and after adjustment for risk factors), whereas it was not associated with SI and IMT (Table 1). Plasma MR-pro-ADM levels were positively correlated with SI and IMT in raw analyses, but these correlations disappeared after adjustment for risk factors (Table 1).

Association of Vascular Phenotypes With SNPs at the *ADM* Locus

In 4155 individuals, RI was significantly associated with the 3 *ADM* SNPs identified by GWAS of plasma and expression levels (Table 2). When the 3 SNPs were introduced simultaneously in a regression model, rs2957717 and rs11042717 showed independent effects on RI, whereas rs2957692 was no longer significant. The rs11042717/TT and rs2957717/TT genotypes were both independently associated with reduced RI ($P<10^{-4}$ and $P=0.009$, respectively; $P=0.18$ for interaction between the 2 SNPs). Further adjustment on plasma MR-pro-ADM and *ADM* gene expression maintained the association of RI with rs11042717, whereas the effect of rs2957717 was no longer significant. However, it should be noted that this last analysis had a reduced statistical power because it was restricted to the 1372 individuals with *ADM* expression. In the final model, including simultaneously cardiovascular risk factors, plasma MR-pro-ADM levels, *ADM* expression, and

rs11042717/TT genotype, *ADM* expression was negatively associated with RI independently of genotype, whereas plasma MR-pro-ADM levels was not associated with RI (Table 3).

The 3 SNPs were associated with SI in a similar fashion (Table 2). When including in the same model, MR-pro-ADM levels, *ADM* expression, and rs11042717/TT genotype, only the genotype remained associated with SI (Table 4). The association, however, was weaker than with RI. Both indexes reflect vascular rigidity and they were highly correlated (r=0.52; P<10^{-4} after adjustment for risk factors). In multivariate regression, RI remained significantly associated with rs11042717 after adjustment for SI, whereas the association of SI with SNP did not persist after adjustment for RI. The IMT phenotype was not associated with any of the 3 SNPs (data not shown).

For purpose of replication, we interrogated the data of a meta-GWAS of carotid-femoral pulse wave velocity (CFPWV), a standard measure of arterial stiffness.[26] None of the *ADM* SNPs were associated with CFPWV (all P>0.20). Conversely, we examined whether the SNP rs7152623 found associated with CFPWV in the meta-GWAS[26] was related to RI and SI GHS, but neither association was significant (P=0.85 and 0.84, respectively).

Association of *ADM* Gene Expression and Plasma MR-Pro-ADM Levels With Circulating Biomarkers

All biomarkers except IL-18 were significantly associated with *ADM* gene expression (Table 5). The strongest correlations were observed with C-reactive protein, IL1-Ra, and myeloperoxidase, which were the only biomarkers associated with *ADM* expression after adjustment for cardiovascular risk factors. In a stepwise regression, including cardiovascular risk factors (age, sex, BMI, and smoking), and circulating bio-markers, the independent predictors of *ADM* gene expression were C-reactive protein, sex, IL1-Ra, myeloperoxidase, and MR-pro-ADM levels, which all together explained 15.4% of the variability of expression.

Associations of circulating biomarkers with plasma MR-pro-ADM levels were radically different from those of *ADM* expression (Table 5). After adjustment for risk factors, the strongest correlations were observed with C-terminal proendothelin-1, creatinine, and NT-pro–B-type natriuretic peptide, none being associated with *ADM* expression. By contrast, plasma MR-pro-ADM levels did not correlate with myeloperoxidase. The independent predictors of MR-pro-ADM levels selected by stepwise regression were C-terminal proendothelin-1, BMI, age, NT-pro–B-type natriuretic peptide, IL1-Ra, creatinine, *ADM* expression, smoking, and sex. These factors accounted for 71.7% of the variability of plasma MR-pro-ADM levels, the major part being attributable to C-terminal proendothelin-1.

Discussion

The present study is the first large-scale investigation of ADM integrating data from multiple sources (ie, DNA sequence variability, gene expression in monocytes, and plasma protein levels). This integrative analysis shed new light on the role of ADM in cardiovascular pathophysiology.

The GWAS of *ADM* expression and plasma MR-pro-ADM revealed a strong effect of the *ADM* locus on both phenotypes. No other locus was detected at a genome-wide level for either phenotype, which does not exclude the possibility of more modest genetic contributors that would not have passed the GWAS stringent level of significance. A recent GWAS in 3444 Europeans reported 2 loci associated with plasma MR-pro-ADM, *KLKB1* encoding prekallikrein and *F12* encoding factor XII, these 2 loci being even more strongly associated with MR-pro-ADM than the *ADM* locus itself.[27] In our study, the lead SNP rs4253238 at the *KLKB1* locus had a nominal *P* value of 0.065. The lead SNP rs2731672 at the *F12* locus was not correctly imputed in GHS, but there was no significant SNP in the region (all *P*>0.10). Yet, the power for replicating these signals in the 4155 individuals of GHS was ≈100%. Confirmation of these loci in further studies is warranted.

ADM expression was associated with a single *cis* eSNP rs11042717 located upstream from the *ADM* gene sequence in an intron of the nearby *SBF2* gene. The rs11042717 is in almost complete association (r^2=0.87) with the rs11042725, a SNP not genotyped in the present study and whose functional role on *ADM* expression has been suggested by reporter gene assays.[28] The rs11042725 SNP is in the 5′ region of the *ADM* gene and according to the HaploReg software, it is located within a strong enhancer when tested in human umbilical vein endothelial cells, which supports a role in expression regulation. However, in the imputation-based GWAS, the rs11042725 did not yield a stronger signal than rs11042717. The rs11042717 is also in almost complete association with the lead pSNP of plasma MR-pro-ADM, rs2957692. The HaploReg software did not predict any functional role for either SNP. Because of the strong LD between all these SNPs, it is impossible to disentangle their effect. Experimental studies are required to understand the underlying functional mechanisms.

The GWAS of MR-pro-ADM identified a second pSNP rs2957717 that was not in LD with the other SNPs and had independent additive effect on MR-pro-ADM. Unlike the lead pSNP, rs2957717 was not associated with monocyte *ADM* expression, emphasizing the existence of a likely different regulation mechanism in monocytes and endothelial cells. Other possible mechanisms of genetic control consistent with a location of the SNP in the 3′ untranslated region may be those involved in protein degradation, stability, or clearance. According to HaploReg, the rs2957717 SNP is located in an intron of the nearby *AMPD3* gene, which codes for an erythrocyte-specific AMP deaminase. Given the erythroid specificity of this isoform, the functional role of *AMPD3* in endothelial cells is unlikely.

The association of *ADM* SNPs with RI and SI suggests a causal involvement of ADM in the modulation of vascular tone. Because *ADM* SNPs were associated with mono-cyte *ADM* expression and plasma MR-pro-ADM levels, a hypothesis would be that the SNP effect is mediated by one or both of these proximal phenotypes. However, the association was not modified by adjustment on plasma levels and even strengthened by adjustment on monocyte gene expression, indicating that the hypothesis of mediation by these intermediate traits was not supported. A likely explanation is that genetic variants exert their effect through modulation of *ADM* expression in other sites, especially the vascular endothelium. It is also known that the colocalization of *cis* eSNPs and disease-associated variants at the same locus are not sufficient to infer a causal mechanism because there could be 2 distinct causal

variants in LD with the SNP.[29] However, circulating MR-pro-ADM was not associated with RI and its association with SI disappeared after adjustment for cardiovascular risk factors. In the Framingham Heart Study, which is comparable with GHS in terms of sample size and study population, plasma adrenomedullin was not associated with several measures of arterial stiffness, including CFPWV.[30] The only correlation was found with pulse pressure, and exclusively in men.[30] This suggests that circulating adrenomedullin, despite being predominantly of endothelial origin, does not exactly reflect the in situ vascular synthesis. Functional experiments in endothelial cells are required to understand the molecular mechanisms involved in the genetic control of vascular tonus.

At variance with the present study, the *ADM* locus was not found in a large-scale meta-GWAS of CFPWV, a standard measure of arterial stiffness.[26] However, we failed to replicate the *BCL11B* locus identified in the meta-GWAS,[26] despite a 92% power in the GHS study. These discrepancies are likely to be explained by differences in phenotypes because CFPWV measures aortic stiffness, whereas RI is a measure of peripheral vasodilation and relative wave reflection, and SI has been shown to correlate with pulse wave velocity poorly. [31]

There are limitations in our study that should be considered. *ADM* expression was assessed in monocytes and MR-pro-ADM levels were assessed in plasma, whereas circulating ADM levels mainly originate from endothelial synthesis. The circulating levels of MR-pro-ADM may not reflect the autocrine and paracrine activities of ADM that may play a major role within the vascular wall. *ADM* expression and MR-pro-ADM levels were assessed by a single measure and did not account for time-variability. SNPs included in GWAS were biased toward high allele frequency and rare variants of interest that were not correctly imputed may have been missed. The RI and SI scores are surrogate measures of arterial stiffness that may lack sensitivity/specificity.

In conclusion, we showed that genetic variants at the *ADM* locus influence *ADM* gene expression in monocytes and circulating levels of MR-pro-ADM. These variants are also associated with modulation of vascular tone, mainly of peripheral arteries. The molecular mechanisms mediating this association need to be elucidated in further experimental work. Additional studies will be required to evaluate whether these variants are related to the occurrence of cardiovascular diseases and whether ADM should be considered as a target for therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Jougasaki M, Burnett JC Jr. Adrenomedullin: potential in physiology and pathophysiology. Life Sci. 2000; 66:855–872. [PubMed: 10714887]

2. Tsuruda T, Burnett JC Jr. Adrenomedullin: an autocrine/paracrine factor for cardiorenal protection. Circ Res. 2002; 90:625–627. [PubMed: 11934826]

3. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem. 2005; 51:1823–1829. [PubMed: 16099941]

4. Brouwers FP, de Boer RA, van der Harst P, Struck J, de Jong PE, de Zeeuw D, et al. Influence of age on the prognostic value of mid-regional pro-adrenomedullin in the general population. Heart. 2012; 98:1348–1353. [PubMed: 22821276]

5. Khan SQ, O'Brien RJ, Struck J, Quinn P, Morgenthaler N, Squire I, et al. Prognostic value of midregional pro-adrenomedullin in patients with acute myocardial infarction: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. J Am Coll Cardiol. 2007; 49:1525–1532. [PubMed: 17418290]

6. Schnabel RB, Schulz A, Messow CM, Lubos E, Wild PS, Zeller T, et al. Multiple marker approach to risk stratification in patients with stable coronary artery disease. Eur Heart J. 2010; 31:3024–3031. [PubMed: 20852293]

7. Tzikas S, Keller T, Ojeda FM, Zeller T, Wild PS, Lubos E, et al. MR-pro-ANP and MR-proADM for risk stratification of patients with acute chest pain. Heart. 2013; 99:388–395. [PubMed: 23213173]

8. Wild PS, Schnabel RB, Lubos E, Zeller T, Sinning CR, Keller T, et al. Midregional proadrenomedullin for prediction of cardiovascular events in coronary artery disease: results from the AtheroGene study. Clin Chem. 2012; 58:226–236. [PubMed: 22065157]

9. Bhandari SS, Davies JE, Struck J, Ng LL. The midregional portion of pro-adrenomedullin is an independent predictor of left ventricular mass index in hypertension. Metabolism. 2010; 59:7–13. [PubMed: 19716143]

10. Coutinho T, Al-Omari M, Mosley TH Jr, Kullo IJ. Biomarkers of left ventricular hypertrophy and remodeling in blacks. Hypertension. 2011; 58:920–925. [PubMed: 21986506]

11. Adlbrecht C, Hülsmann M, Strunk G, Berger R, Mörtl D, Struck J, et al. Prognostic value of plasma midregional pro-adrenomedullin and C-terminal-pro-endothelin-1 in chronic heart failure outpatients. Eur J Heart Fail. 2009; 11:361–366. [PubMed: 19190023]

12. Coutinho T, Turner ST, Mosley TH, Kullo IJ. Biomarkers associated with pulse pressure in African-Americans and non-Hispanic whites. Am J Hypertens. 2012; 25:145–151. [PubMed: 22012208]

13. Kita T, Kitamura K, Hashida S, Morishita K, Eto T. Plasma adrenomedullin is closely correlated with pulse wave velocity in middle-aged and elderly patients. Hypertens Res. 2003; 26:887–893. [PubMed: 14714580]

14. Schnabel RB, Wild PS, Schulz A, Zeller T, Sinning CR, Wilde S, Kunde J, et al. Multiple endothelial biomarkers and noninvasive vascular function in the general population: the Gutenberg Health Study. Hypertension. 2012; 60:288–295. [PubMed: 22689741]

15. Chen S, Lu X, Zhao Q, Wang L, Li H, Huang J. Association of adrenomedullin gene polymorphisms and blood pressure in a Chinese population. Hypertens Res. 2013; 36:74–78. [PubMed: 22932875]

16. Ishimitsu T, Tsukada K, Minami J, Ono H, Matsuoka H. Variations of human adrenomedullin gene and its relation to cardiovascular diseases. Hypertens Res. 2003; 26:S129–S134. [PubMed: 12630823]

17. Li Y, Staessen JA, Li LH, Gao PJ, Thijs L, Brand E, et al. Blood pressure and urinary sodium excretion in relation to the A-1984G adrenomedullin polymorphism in a Chinese population. Kidney Int. 2006; 69:1153–1158. [PubMed: 16609682]

18. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478:103–109. [PubMed: 21909115]

19. Wain LV, Verwoert GC, O'Reilly PF, Shi G, Johnson T, Johnson AD, et al. LifeLines Cohort Study; EchoGen consortium; AortaGen Consortium; CHARGE Consortium Heart Failure Working Group; KidneyGen consortium; CKDGen consortium; Cardiogenics consortium; CardioGram. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet. 2011; 43:1005–1011. [PubMed: 21909110]

20. Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, et al. Genetics and beyond–the transcriptome of human monocytes and disease susceptibility. PLoS One. 2010; 5:e10693. [PubMed: 20502693]

21. Neumann JT, Tzikas S, Funke-Kaiser A, Wilde S, Appelbaum S, Keller T, et al. Association of MR-proadrenomedullin with cardiovascular risk factors and subclinical cardiovascular disease. Atherosclerosis. 2013; 228:451–459. [PubMed: 23562132]

22. Schnabel RB, Schulz A, Wild PS, Sinning CR, Wilde S, Eleftheriadis M, et al. Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. Circ Cardiovasc Imaging. 2011; 4:371–380. [PubMed: 21551420]

23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

24. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–2191. [PubMed: 20616382]

25. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008; 24:2938–2939. [PubMed: 18974171]

26. Mitchell GF, Verwoert GC, Tarasov KV, Isaacs A, Smith AV, Yasmin, et al. Common genetic variation in the 3′-BCL11B gene desert is associated with carotid-femoral pulse wave velocity and excess cardiovascular disease risk: the AortaGen Consortium. Circ Cardiovasc Genet. 2012; 5:81–90. [PubMed: 22068335]

27. Verweij N, Mahmud H, Mateo Leach I, de Boer RA, Brouwers FP, Yu H, et al. Genome-wide association study on plasma levels of midregional-proadrenomedullin and C-terminal-pro-endothelin-1. Hypertension. 2013; 61:602–608. [PubMed: 23381795]

28. Glubb DM, McHugh PC, Deng X, Joyce PR, Kennedy MA. Association of a functional polymorphism in the adrenomedullin gene (ADM) with response to paroxetine. Pharmacogenomics J. 2010; 10:126–133. [PubMed: 19636336]

29. Wallace C, Rotival M, Cooper JD, Rice CM, Yang JH, McNeill M, et al. Cardiogenics Consortium. Statistical colocalization of monocyte gene expression and genetic risk variants for type 1 diabetes. Hum Mol Genet. 2012; 21:2815–2824. [PubMed: 22403184]

30. Levy D, Hwang SJ, Kayalar A, Benjamin EJ, Vasan RS, Parise H, et al. Associations of plasma natriuretic peptide, adrenomedullin, and homocysteine levels with alterations in arterial stiffness: the Framingham Heart Study. Circulation. 2007; 115:3079–3085. [PubMed: 17533184]

31. Salvi P, Magnani E, Valbusa F, Agnoletti D, Alecu C, Joly L, et al. Comparative study of methodologies for pulse wave velocity estimation. J Hum Hypertens. 2008; 22:669–677. [PubMed: 18528411]

CLINICAL PERSPECTIVE

Adrenomedullin (ADM) is a potent vasodilator and angiogenic peptide involved in cardiovascular and renal protection through autocrine, paracrine, and endocrine pathways. It is produced by a large variety of cells, not only endothelial and vascular smooth muscle cells but also inflammatory cells. Elevated circulating levels of midregional proadrenomedullin, a surrogate measure of plasma ADM levels, have been consistently associated with increased cardiovascular risk in epidemiological studies. However, whether this association is causal or consequential is unclear. To gain further insight into the role of ADM in cardiovascular pathophysiology, we performed an integrative study combining genome-wide genotype data, gene expression from circulating monocytes, plasma midregional proadrenomedullin levels, and markers of arterial stiffness in a population-based cohort, including 1372 individuals. By testing association with >600 000 variants covering the whole genome, we identified a region encompassing the *ADM* gene, which was highly associated with both *ADM* gene expression and plasma midregional proadrenomedullin. The *ADM* variant showing the strongest association was also associated with reflection index, a marker of peripheral vascular tone. These results support a causal involvement of ADM in the modulation of vascular tone. The molecular mechanisms mediating this association need to be elucidated in experimental work. Additional studies will be required to evaluate whether the same variants are related to the occurrence of cardiovascular diseases and whether ADM should be considered as a target for therapy.
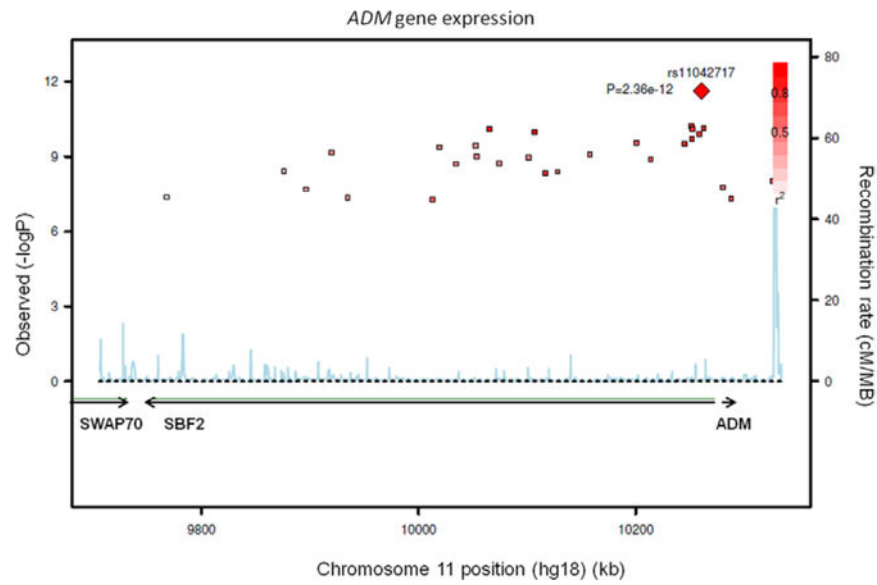
Figure 1.
Regional association plot of monocyte *ADM* gene expression with single nucleotide polymorphisms (SNPs) of the 11p15 locus encompassing the *SBF2* and *ADM* genes. The left vertical axis shows the *P* value ($-\log_{10}$) of association, the right vertical axis shows the recombination rate in the region (cM/Mb). The best SNPs associated with *ADM* gene expression (eSNPs) is shown by a red diamond. Other eSNPs significantly associated with the expression are shown by squares whose color goes from deep red to light pink according to decreasing linkage disequilibrium (r^2) with the best eSNP.
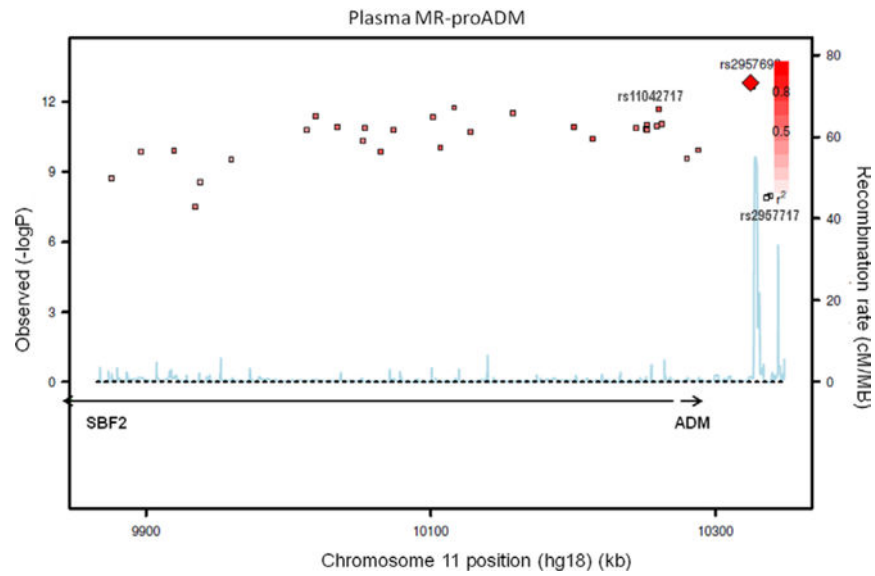
Figure 2.
Regional association plot of plasma midregional proadrenomedullin (MR-pro-ADM) with single nucleotide polymorphisms of the 11p15 locus encompassing the SBF2 and ADM genes (see legend of Figure 1).

Table 1

Correlation of ADM Gene Expression and Plasma MR-Pro-ADM Levels With *Vascular* Parameters (n=1372)

	ADM Gene Expression				Plasma MR-Pro-ADM			
	Raw Correlation	*P* Value	Adjusted Correlation[*]	*P* Value	Raw Correlation	*P* Value	Adjusted Correlation[*]	*P* Value
RI	−0.134	<0.0001	−0.082	0.003	0.023	0.412	−0.023	0.398
SI	−0.040	0.135	−0.033	0.234	0.251	<0.0001	−0.005	0.856
IMT	−0.009	0.726	−0.045	0.093	0.376	<0.0001	0.026	0.344

IMT indicates Intima-media thickness; MR-Pro-ADM, midregional proadrenomedullin; RI, reflection index; and SI, stiffness index.

[*]
Adjusted for age, sex, body mass index, and smoking. Data are Pearson correlation coefficients.

Table 2

Association of ADM SNPs With RI and SI in the Total Sample (n=4155)

SNP	Genotype	n	RI, %			SI, m/s		
			Adjusted Mean[*]	SE	P Value	Adjusted Mean[*]	SE	P Value
rs11042717	CC	874	67.4	0.47	$<10^{-4}$	9.42	0.09	0.0009
	CT	1998	67.6	0.31		9.42	0.06	
	TT	1201	65.5	0.40		9.08	0.08	
rs2957717	CC	1945	66.8	0.32	0.006	9.27	0.06	0.004
	TC	1726	67.5	0.34		9.45	0.06	
	TT	357	65.0	0.74		8.98	0.14	
rs2957692	AA	1498	66.2	0.36	0.025	9.13	0.07	0.002
	GA	1895	67.4	0.32		9.43	0.06	
	GG	645	67.5	0.55		9.45	0.10	

ADM indicates adrenomedullin; RI, reflection index; SI, stiffness index; and SNP, single nucleotide polymorphism.

[*]
Adjusted for age, sex, body mass index, and smoking

Table 3

Results of Linear Regression Analysis of RI on *ADM* Genotype, Plasma MR-Pro-ADM and *ADM* Expression (n=1372)

Model	rs11042717 (TT vs CT+CC)			MR-Pro-ADM (log)			*ADM* Expression		
	Estimate*	SE	*P* Value	Estimate*	SE	*P* Value	Estimate*	SE	*P* Value
Genotype only	−3.75	0.85	<10^{-4}	…	…	…	…	…	…
Genotype+MR-pro-ADM	−3.86	0.86	<10^{-4}	−2.88	2.13	0.18	…	…	…
Genotype+*ADM* expression	−4.33	0.86	<10^{-4}	…	…	…	−4.03	1.05	10^{-4}
Genotype+MR-pro-ADM+*ADM* expression	−4.39	0.86	<10^{-4}	−2.05	2.13	0.34	−3.95	1.06	$2×10^{-4}$

MR-Pro-ADM indicates midregional proadrenomedullin; and RI, reflection index.

*
Estimate of linear regression coefficients adjusted for age, sex, body mass index, and smoking.

Table 4

Results of Linear Regression Analysis of SI on *ADM* Genotype, Plasma MR-Pro-ADM and *ADM* Expression (n=1372)

Model	rs11042717 (TT vs CT+CC)			MR-Pro-ADM (log)			*ADM* Expression		
	Estimate[*]	SE	*P* Value	Estimate[*]	SE	*P* Value	Estimate[*]	SE	*P* Value
Genotype only	−0.36	0.16	0.02	…	…	…	…	…	…
Genotype+MR-pro-ADM	−0.38	0.16	0.02	−0.18	0.40	0.66	…	…	…
Genotype+*ADM* expression	−0.41	0.16	0.01	…	…	…	−0.32	0.20	0.12
Genotype+MR-pro ADM+*ADM* expression	−0.42	0.16	0.01	−0.11	0.41	0.78	−0.32	0.20	0.11

MR-Pro-ADM indicates midregional proadrenomedullin.

[*] Estimate of linear regression coefficients adjusted for age, sex, body mass index, and smoking.

Table 5

Correlation of ADM Gene Expression and Plasma MR-Pro-ADM Levels With Circulating Biomarkers (n=1372)

	ADM Gene Expression				Plasma MR-Pro-ADM			
	Raw Correlation	*P* Value	Adjusted Correlation*	*P* Value	Raw Correlation	*P* Value	Adjusted Correlation*	*P* Value
Creatinine	−0.103	0.0001	0.007	0.811	0.313	<0.0001	0.405	<0.0001
CRP	0.254	<0.0001	0.205	<0.0001	0.288	<0.0001	0.128	<0.0001
NT-proBNP	0.073	0.009	0.010	0.715	0.423	<0.0001	0.325	<0.0001
MPO	0.180	<0.0001	0.160	<0.0001	0.073	0.007	0.026	0.343
IL-18	0.012	0.651	0.041	0.146	0.156	<0.0001	0.077	0.006
IL1-Ra	0.260	<0.0001	0.188	<0.0001	0.359	<0.0001	0.242	<0.0001
CT-proET-1	0.065	0.016	0.042	0.137	0.675	<0.0001	0.622	<0.0001
MR-pro-ADM	0.176	<0.0001	0.128	<0.0001	…	…	…	…

CT-proET-1 indicates C-terminal proendothelin-1; CRP, C-reactive protein; IL, interleukin; MPO, myeloperoxidase; MR-pro-ADM, midregional proadrenomedullin; and NT-proBNP, NT-pro–B-type natriuretic peptide.

*
Adjusted for age, sex, body mass index, and smoking. Data are Pearson correlation coefficients.