The Human Angiotensin II Type 1 Receptor +1166 A/C Polymorphism Attenuates MicroRNA-155 Binding*

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The adverse effects of angiotensin II (Ang II) are primarily mediated through the Ang II type 1 receptor (AT₁R). A silent polymorphism (+1166 A/C) in the human AT₁R gene has been associated with cardiovascular disease, possibly as a result of enhanced AT₁R activity. Because this polymorphism occurs in the 3′-untranslated region of the human AT₁R gene, the biological importance of this mutation has always been questionable. Computer alignment demonstrated that the +1166 A/C polymorphism occurred in a cis-regulatory site, which is recognized by a specific microRNA (miRNA), miR-155. miRNAs are non-coding RNAs that silence gene expression by base-pairing with complementary sequences in the 3′-untranslated region of target RNAs. When the +1166 C-allele is present, base-pairing complementarity is interrupted, and the ability of miR-155 to interact with the cis-regulatory site is decreased. As a result, miR-155 no longer attenuates translation as demonstrated by luciferase reporter and RNA binding assays. In situ hybridization confirmed that the mature miR-155 is absent in the AT₁R (e.g. endogenous) when the +1166 C-allele is present. Finally, when human primary vascular smooth muscle cells transfected with an antisense miR-155 inhibitor, endogenous human AT₁R expression and Ang II-induced ERK1/2 activation were significantly increased. These observations demonstrate that the AT₁R and miR-155 are co-expressed and that the +1166 A/C polymorphism occurs in a cis-regulatory site recognized by miR-155 in vivo. Therefore, our study provides a mechanism by which the +1166 A/C polymorphism is associated with cardiovascular disease.

Angiotensin II (Ang II), a biologically active component of the renin-angiotensin system, has been known to regulate a variety of hemodynamic physiological responses. These include fluid homeostasis, aldosterone production, renal function, and contraction of vascular smooth muscle. Apart from these hemodynamic actions, Ang II is also capable of inducing a multitude of nonhemodynamic effects, such as the induction of reactive oxygen species, cytokines, stimulation of collagen synthesis, apoptosis, and hypertrophy. The adverse effects of angiotensin II are primarily mediated through the Ang II type 1 receptor (AT₁R). A silent polymorphism (+1166 A/C) in the human AT₁R gene has been associated with cardiovascular disease, possibly as a result of enhanced AT₁R activity. Because this polymorphism occurs in the 3′-untranslated region of the human AT₁R gene, the biological importance of this mutation has always been questionable. Computer alignment demonstrated that the +1166 A/C polymorphism occurred in a cis-regulatory site, which is recognized by a specific microRNA (miRNA), miR-155. miRNAs are non-coding RNAs that silence gene expression by base-pairing with complementary sequences in the 3′-untranslated region of target RNAs. When the +1166 C-allele is present, base-pairing complementarity is interrupted, and the ability of miR-155 to interact with the cis-regulatory site is decreased. As a result, miR-155 no longer attenuates translation as demonstrated by luciferase reporter and RNA binding assays. In situ hybridization confirmed that the mature miR-155 is absent in the AT₁R (e.g. endogenous) when the +1166 C-allele is present. Finally, when human primary vascular smooth muscle cells transfected with an antisense miR-155 inhibitor, endogenous human AT₁R expression and Ang II-induced ERK1/2 activation were significantly increased. These observations demonstrate that the AT₁R and miR-155 are co-expressed and that the +1166 A/C polymorphism occurs in a cis-regulatory site recognized by miR-155 in vivo. Therefore, our study provides a mechanism by which the +1166 A/C polymorphism is associated with cardiovascular disease.

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† The abbreviations used are: Ang II, angiotensin II; AT₁R, Ang II type 1 receptor; UTR, untranslated region; miRNA, microRNA; hAT₁R, human AT₁R; VSMC, vascular smooth muscle cell; LNA, locked nucleic acid; ERK, extracellular signal-regulated kinase; CHO, Chinese hamster ovary; SNP, single nucleotide polymorphism; CMV, cytomegalovirus.

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levels in human heart failure (29). However, the physiological significance of this polymorphism is uncertain because of its location within the noncoding region of the hAT1R gene. Because we previously demonstrated that miR-155 interacted with a specific cis-response element localized in the hAT1R 3’-UTR (10), we investigated whether there was a correlation between the +1166 A/C SNP and the miR-155-binding site. Computer alignment revealed that the +1166 A/C SNP occurs in the cis-response element where miR-155 was shown to interact. Therefore, the aim of the following study was to investigate whether the presence of the +1166 C-allele decreased the ability of miR-155 to translationally regulate the expression of the hAT1R. Importantly, we demonstrate that the A→C bp change results in augmented hAT1R expression because of the inability of miR-155 to inhibit translation of hAT1R mRNAs as efficiently.

EXPERIMENTAL PROCEDURES

Cell Culture—CHO cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 80 units/ml penicillin, 80 μg/ml of streptomycin, and 0.0175 mg/ml L-proline (Sigma). Primary dermal arteriolar vascular smooth muscle cells (VSMCs) were cultured from punch biopsies as reported previously (30). These cells were grown in Ham’s F-12/Dulbecco’s modified Eagle’s medium (1:1) supplemented with 10% fetal bovine serum (Invitrogen). The human VSMCs were genotyped as described previously (24) and shown to be maintained in culture for 48 h at 37 °C.

Constructs—An 883-bp fragment encompassing the entire hAT1R 3’-UTR was PCR-amplified utilizing the sense primer (5’-CATGTTGAAAACCT–3’) and the antisense primer (5’-ATGATTATTTATTATTTATTTATTTA-3’), using standard procedures as described by the manufacturer (Platinum Pfu, Invitrogen). A forward mutagenic primer (5’-GCTCATT–CTGAAAAGTAGCTAA-3’) and a complementary reverse mutagenic primer (5’-CTGAAAGTAGCTAAAGCTACTT-3’) were synthesized and utilized in PCR experiments as described by the manufacturer. The site of the “C” mutation is shown in boldface. The amplification reaction was treated with DpnI restriction enzyme to eliminate the parental template, and the remaining DNA was used for transformation. The presence of the A→C mutation (i.e. equivalent to the +1166 A/C SNP) was confirmed by dideoxy chain termination sequencing. A full-length hAT1R cDNA which encompassed the entire open reading frame and the 3’-UTR was PCR-amplified (31), and the product was subcloned into the eukaryotic expression vector, pCR®3.1 (Invitrogen), and sequenced to ensure authenticity and proper orientation with respect to the cytomegalovirus (CMV) immediate-early promoter. This expression construct was designated pCR/hAT1R-A. The A→C SNP was generated in this construct as described above. The mutant construct was designated pCR/hAT1R-C. The A→C SNP corresponds to the +1166 A/C SNP described previously (24). Transformed bacterial cultures were grown, and each reporter construct was purified using the PureLink™ Hipure Plasmid Maxiprep kit (Invitrogen) as described previously (30).

Hybridization Prediction—The minimum free energy hybridization of miR-155 and the hAT1R 3’-UTR cis-response element harboring either the A-allele or C-allele was predicted using the miRanda software (Ambion). The human VSMCs were genotyped as described previously (24) and shown to be maintained in culture for 48 h at 37 °C.

miR-155 transfection and reporter assay—Negative Control 1 and miRNA Negative Control 2, which are “customer-defined” miRNA precursor molecules were synthesized (Ambion) that harbored a mutation in the miR-155 sequence and was designated mut-miR-155. Anti-miR-155, designed to inhibit endogenous miR-155 function, and anti-miR-155 negative control were also obtained from Ambion. Alternatively, small RNA was transfected into CHO cells with small RNAs was optimized utilizing Lipofectamine 2000 (Invitrogen) and a fluorescein-labeled double-stranded RNA oligomer designated BLOCKTM oligonucleotide (Invitrogen). Alternatively, transfection was optimized using the same fluorescent small RNA; however, magnet-assisted transfection was utilized as described by the manufacturer (IBA BioTAGnology, Göttingen, Germany; magnets were purchased separately from Engineered Concepts, Birmingham, AL). Once transfection conditions were optimized, CHO and or VSMCs (approaching 100% transfection efficiency in both cell types) were transfected with the reporter or expression constructs described above and the appropriate miRNA precursor as indicated. After 48 h, cells were washed and lysed with Passive Lysis Buffer (Promega), and firefly luciferase and Renilla luciferase activities were determined using the dual-luciferase reporter assay system (Promega) and a luminometer. The relative reporter activity was obtained by normalization to the Renilla luciferase activity. In some experiments, transfected cells were utilized for real time PCR, radioreceptor binding assays, and/or immunoassays.

AT1 receptor binding assays—Whole cell AT1 receptor binding was measured as described previously (32). Briefly, 48 h after transfection, the cell medium was aspirated and replaced with moniodinated [32P]-Sar[2],Ile[8]Ang II (2–3 × 105 cpm; Peptide Radioiodination Service, Oxford, MS) in

HAT1R POLYMORPHISMS AND MICRORNA-155
**hAT1R Polymorphisms and MicroRNA-155**

Hanks’ balanced salt solution, 20 mM Hepes, 0.1% bovine serum albumin. After incubation at room temperature for 60 min, unbound ligand was removed by washing each well twice with 1 ml of ice-cold phosphate-buffered saline. Bound ligand was recovered by dissolving the protein in each well with 1 ml of 0.5 M NaOH, 0.01% SDS. Nonspecific binding was determined by performing the binding assay in the presence of 1 μM unlabeled Ang II. The quantity of [125I-Sar1,Ile8]Ang II present in each sample was determined using a Cobra γ-spectrophotometer (Packard Bell, Palo Alto, CA). Protein content in wells was assessed using the Bio-Rad protein assay dye reagent (Bio-Rad). Values presented represent specific (total minus nonspecific) binding.

Real Time PCR—Total RNA was isolated from VSMCs and CHO cells using TRIzol (Invitrogen). The RNA was subsequently treated with RNase-free DNase I, and mature miR-155 was quantified utilizing the TaqMan microRNA assay kit for hsa-miR-155 (Applied Biosystems, Foster City, CA) as described previously (33). Briefly, 100 ng of total RNA was heated for 5 min at 80 °C with 10 μM of the 18 S rRNA antisense primer followed by cooling to room temperature. Three microliters of the 5X RT primer was then added, and the cDNA was synthesized as described (34). Real time PCR (10 μl total reaction) was performed as described using 1 μl of a 1:50 dilution of cDNA. Gene expression was calculated relative to 18 S rRNA described above and multiplied by 106 to simplify data presentation. Alternatively, total RNA was treated as described above; however, cDNA was synthesized using oligo-dT primers and of hAT1R mRNA relative to 18 S rRNA was calculated as described above. The hAT1R-specific primers were as follows: sense primer, 5′-CACCATGTTTTGAGGTTGACTGAC-3′, and antisense primer, 5′-TTCACAATGCGTTATCGGATGT-3′.

Immunoassay for ERK—Twenty-four hours after transfection, the VSMCs were washed twice with cold PBS and incubated for 30 min in 2 mg/ml of pepsin, washed in sterile water, and subsequently washed with 100% ethanol and air-dried. Hybridization was performed at 37 °C overnight followed by a short stringency wash in 0.2× SSC and 2% bovine serum albumin at 4 °C for 10 min. The probe-target complex was visualized utilizing a digoxigenin antibody conjugated to alkaline phosphatase acting on the chromogen nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. Nuclear fast red served as the counterstain.

Statistical Analysis—All data are reported as means ± S.E. When comparisons were made between two different groups, statistical significance was determined using Student’s t test. When multiple comparisons were made, statistical significance was determined using one-way analysis of variance followed by Tukey’s post-test. All statistical analysis was performed using the software package Prism 4.0b (GraphPad Software, San Diego).

RESULTS

The hAT1R 1166 A/C SNP Occurs in the miR-155-binding Site—The hAT1R 1166 A/C SNP has been associated with cardiovascular disease (23–29). However, the association of this SNP with cardiovascular disease is difficult to interpret because it is not known how a genetic variation in the coding region results in differences in hAT1R expression. To test this hypothesis, the hAT1R 3′-UTR was investigated for the presence of miR-155 to test whether miR-155 can interact with the hAT1R 3′-UTR. We have investigated the effects of the 1166 A/C SNP on the thermodynamics of binding of miR-155 to the hAT1R 3′-UTR. The hAT1R 3′-UTR cis-response element was subcloned immediately downstream from the firefly luciferase open reading frame, and the resulting construct was designated p3′-UTR-A (Fig. 2A). This construct harbors the hAT1R 3′-UTR-A. In contrast, the mutant p3′-UTR-C construct expresses the hAT1R 3′-UTR-C (Fig. 2A). To test the potency of miR-155 in regulating luciferase expression, either p3′-UTR-A or p3′-UTR-C was transfected into CHO cells with increasing concentrations of miR-155 or control nontargeting miRNAs (i.e. Con 1 or Con 2), and luciferase activities were determined.
CHO cells were utilized because they do not express miR-155, and therefore, endogenous expression of this miRNA did not mask the effects of the transfected miR-155. Dose-response experiments demonstrated that relative luciferase activity was decreased for cells transfected with the p3'-UTR-A construct. maximal decrease was obtained in p3'-UTR-A transfected cells with a concentration of 30–50 nM miR-155. In contrast, the potency of miR-155 was dramatically reduced in CHO cells transfected with the p3'-UTR-C construct (Fig. 2B). For example, firefly luciferase activity of cells transfected with the p3'-UTR-C construct was not significantly reduced until co-transfected with 30 nM miR-155. Additionaly, transfection of 50 nM miR-155 resulted in a maximal decrease of luciferase activity by only ~20% (Fig. 2B).

To investigate the importance of the SNP occurring in the middle of the miRNA and the hAT1R mRNA target seed complementarity sequence, a mutant miR-155 precursor was synthesized which compensated for the hAT1R +1166 C-allele by placing a complementary “G” residue in the miRNA 

FIGURE 1. The hAT1R +1166 A/C SNP occurs in the miR-155-binding site. A, complementarity between miR-155 and the hAT1R 3'-UTR site (70–90 bp downstream from the hAT1R stop codon). The 5' end of miR-155 corresponds to the nucleotide 86 bp downstream from the hAT1R stop codon (in boldface). The binding of miR-155 to the hAT1R 3'-UTR site requires a 7-bp seed sequence of complementarity at the miRNA 5' end (21, 22) when the 1166 A-allele is expressed. B, computational modeling of the interaction between miR-155 and the hAT1R 3'-UTR response element (cis-orientated with respect to the firefly luciferase open reading frame). The +1166 A-allele is expressed when the +1166 A-allele is expressed then the requirement of a 7-bp seed sequence of complementarity at the miRNA 5'-end is not met. C, computational modeling of the interaction between miR-155 and the hAT1R 3'-UTR site targeted utilizing the RNAHYBRID software (35). Hybridization of miR-155 and hAT1R mRNA harboring the A-allele. D, minimal free energy, is shown.

To validate the above results, expression constructs, which result in the synthesis of hAT1R mRNAs that harbor either the +1166 A-allele (i.e. phAT1R-A) or the +1166 C-allele (i.e. phAT1R-C) (Fig. 4A), were co-transfected with 50 nM miR-Con, miR-155, or mutant miR-155, and hAT1R levels were quantitated by performing radioreceptor binding assays. Importantly, only miR-155 significantly inhibited the expression of the hAT1R synthesized in CHO cells transfected with the
hAT\textsubscript{1}R Polymorphisms and MicroRNA-155

A.

| mut-miR-155 | 3′ GGGAUAUGUCUAACGGAUU 5′ |
| hAT\textsubscript{1}R mRNA (A-allele) | 5′. UUCACUCCA-AUUAGCCUUAG...3′ |

| mut-miR-155 | 3′ GGGAUAUGUCUAACGGAUU 5′ |
| hAT\textsubscript{1}R mRNA (C-allele) | 5′. UUCACUCCA-AUUAGCCUUAG...3′ |

B.

![Graph showing luciferase activity](image)

**Figure 3.** A mutation in miR-155 can restore its ability to inhibit the hAT\textsubscript{1}R +1166 C-allele. A, schematic representation of the location of the mutation introduced in miR-155 when it was chemically synthesized. A mutation eliminates a complementary bp in the miR-155 seed when the hAT\textsubscript{1}R +1166 A-allele is present. The mutation restores the complementary bp in the miR-155 seed when the hAT\textsubscript{1}R +1166 C-allele is present. B, CHO cells were transfected with p3′-UTR-A or p3′-UTR-C, pRL-CMV, and either mutant miR-155 or control miR-155 at each concentration indicated. Forty eight hours after transfection, luciferase activities were measured. Firefly luciferase expression, and mutant miR-155, experiments are shown (Data pooled from three independent experiments).

phAT\textsubscript{1}R-A plasmid (Fig. 4, B). These experiments demonstrated that transfection of anti-miR-155 not only augmented the hAT\textsubscript{1}R density and, therefore, decrease the biological efficacy of Ang II.

\textbf{Mir-155 Regulates hAT\textsubscript{1}R Expression in Primary Human VSMCs}—It is well accepted that AT\textsubscript{1}R\textsubscript{s} are expressed on endothelial cells, VSMCs, fibroblasts, and monocytes/macrophages (1, 3). To investigate whether mature miR-155 was also expressed in these same cell types, \textit{in situ} hybridization experiments were performed utilizing several paraffin-embedded, formalin-fixed human tissues from the lung, placenta, and cervix. These tissues were used for analysis because the former two tissues were known to contain many Ang II-sensitive cells, whereas few such cells should be present in the cervix. \textit{In situ} hybridization experiments demonstrated that the smooth muscle layer of the bronchial wall as well as the epithelium that lines the lumen and smooth muscle and endothelium of the arterioles in the lung express miR-155 (Fig. 5, A, C and D). Additionally, miR-155 expression was also evident diffusely in the placental endothelium (Fig. 5F). The mature miR-155 was localized in the cytoplasm and tended to concentrate around the nuclear membrane (Fig. 5C). MiR-155 expression was not evident in the cervical tissue (data not shown). Finally, the hybridization signal was not evident in tissue samples hybridized with a scrambled control probe (Fig. 5, B and E).

Because the AT\textsubscript{1}R and miR-155 are co-expressed in VSMCs, we next investigated whether the endogenously expressed miR-155 regulated hAT\textsubscript{1}R expression. Therefore, primary human VSMCs were transfected with an antisense RNA oligonucleotide complementary to miR-155 (i.e. anti-miR-155 at 20 nM final concentration) or a scrambled antisense control (i.e. anti-miR-Control at 20 nM final concentration), and the transfected cells were then subjected to radioreceptor binding assays. This concentration of miRNA was utilized because this value was within the physiological range of mature miR-155 expressed in primary human VSMCs (data not shown). Importantly, VSMCs transfected with anti-miR-155 demonstrated increased hAT\textsubscript{1}R levels (Fig. 4D). These experiments demonstrated that miR-155 expression can decrease hAT\textsubscript{1}R density and, in so doing, decrease the biological efficacy of Ang II.

We have analyzed the potential role of the AT\textsubscript{1}R and miR-155 in modulating the expression of the hAT\textsubscript{1}R gene. The biological ramifications of this mutation have always been questionable. However, with the recent discovery of miRNAs that post-transcriptionally repress mRNA through cis-regulatory sites in 3′-UTRs (15–20), polymorphisms in this region, which occur in predicted miRNA-binding sites, may be deleterious (36). In support of this conclusion, naturally occurring polymorphisms in miRNA-binding sites have been implicated in Tourette syndrome (37), papillary thyroid cancer (38), and muscularity in sheep (39). In the three examples cited, these polymorphisms either created a new target site for a miRNA or led to improved thermodynamics of binding between the miRNA and the mRNA target (i.e. increase in free energy), which in turn resulted in decreased protein expression levels because of the decreased translatability of each mRNA target described.

Systematic target site mutation experiments demonstrated that at least two classes of miRNA targets exist (20–22). One class of target sites shows perfect Watson-Crick complementarity for at least 7 bp (i.e. seed site) starting at the 1st or 2nd bp of the 5′ end of the miRNA. Furthermore, this class of miRNA does not require significant base pairing to the miRNA 3′ end to...
hAT\textsubscript{1}R Polymorphisms and MicroRNA-155

be functional. Two independent experimental approaches have suggested that $\sim$30% of all human 3'-UTRs may be regulated via this class of miRNAs (40, 41). In contrast, the second class of miRNAs has imperfect 5' matches but compensates via substantial base pairings in the 3' end of the miRNA (22).

Computer alignment of the hAT\textsubscript{1}R 3'-UTR sequence with the miR-155 sequence demonstrated that the +1166 A/C SNP occurs in the miR-155 cis-regulatory binding site (Fig. 1, A and B). The interaction between miR-155 and the hAT\textsubscript{1}R 3'-UTR harboring the A-allele would fit the first miRNA classification because there is a 7-bp region of complementarity between the 5' end of miR-155 and the hAT\textsubscript{1}R mRNA target site. In contrast, if an hAT\textsubscript{1}R mRNA that harbors the +1166 C-allele is expressed, the complementary seed site is interrupted, and the thermodynamics of the miRNA:mRNA duplex would be significantly altered (i.e., a decrease in free energy) (Fig. 1, C and D). Thus, unlike the three examples described above (36–38), the presence of the +1166 C-allele would decrease the ability of miR-155 to interact with the cis-regulatory site located in the hAT\textsubscript{1}R 3'-UTR. As a consequence, it would be expected that aberrantly high levels of the hAT\textsubscript{1}R would be synthesized.

In support of this hypothesis we demonstrated that when the hAT\textsubscript{1}R cis-response element harboring the +1166 A-allele was present in luciferase expression, the ability of miR-155 to decrease luciferase activity was significantly attenuated (Fig. 2B). When identical experiments were performed using the C-allele (i.e., the opposite orientation with respect to the CMV promoter) luciferase activity was comparable with that observed utilizing the hAT\textsubscript{1}R cis-response element harboring the A- or C-allele (Fig. 2C). To further demonstrate that the presence of the +1166 C-allele can influence hAT\textsubscript{1}R density, expression constructs that produced hAT\textsubscript{1}R mRNAs containing either the A- or C-allele were co-transfected with miR-155 or mutant miR-155. These experiments again demonstrated that when seed sequence complementarity was not fulfilled, regardless of whether miR-155 or mutant miR-155 was utilized, hAT\textsubscript{1}R levels were always higher than the levels obtained when perfect complementarity was present between the miRNA and the hAT\textsubscript{1}R cis-response element (Fig. 4, B and C). Taken together, these results suggest that the presence of the +1166 C-allele would lead to higher levels of hAT\textsubscript{1}R if miR-155 were co-expressed with hAT\textsubscript{1}R mRNAs.
To validate that the levels of miR-155 were physiologically relevant in VSMCs, we demonstrated that knocking down Ang II-induced signaling (C). In situ hybridization analysis with an LNA miR-155-specific probe in section of unremarkable human lung. No signal was evident in the serial section when the scrambled probe was utilized (A). B, Ang II-(1–38) (−, 0.01), Ang II-induced phosphorylation was determined by densitometry. Values are expressed as percentage of maximal phosphorylation of ERK1/2 in response to Ang II in mock-transfected cells. The data have been normalized for protein content and represent the mean ± S.E. from four independent transfection experiments. C, quantitation of Ang II-(1–38) (−, 0.01) and Ang II-induced ERK phosphorylation with monoclonal antibodies to phospho-ERK1/2. The data were normalized to cell numbers at harvest. ERK immunoblots are representative of four independent experiments. D, hAT1R-binding sites (Bmax) and Ang II affinity (Kd) of both miR-155 and hAT1R mRNAs and whether the genotype of a given individual is AA, AC, or CC at position 268. In VSMCs, we demonstrated that miR-155 "knockout" resulted in augmented hAT1R levels and enhanced Ang II-induced signaling (C). It has been hypothesized that the association of the C-allele with essential hypertension can be accounted for if AC and/or CC subjects have increased AT1R activity. To begin to investigate these hypotheses, several investigators (46, 47) have measured the frequency of the hAT1R SNP and physiology. However, with the low numbers of hAT1R per platelet, these investigators did not observe any differences in hAT1R number. Unfortunately, no studies have been published on the origin or generation of various nongenomic and environmental influences on the pathway between the hAT1R, SNP, and physiology. It has been hypothesized that the association of the C-allele with essential hypertension (24, 25), atrial fibrillation (26), myocardial infarction (27), cardiac hypertrophy (28), and increased oxidative stress levels in human heart failure (29) are relevant in VSMCs, we demonstrated that miR-155 translationally repressed hAT1R expression may be more pronounced contingent upon the relative expression levels of both miR-155 and hAT1R mRNAs and whether the genotype of a given individual is AA, AC, or CC at position 268.

Although others have been unable to confirm that miR-155 translated repressed hAT1R expression, the increased frequency of the hAT1R CC genotype had enhanced vascular reactivity (30). Additionally, it has been demonstrated that human heart failure patients may be attributable to differences in age, sex, ethnic origin, or various nongenomic and environmental influences on the pathway between the hAT1R, SNP, and physiology. It has been hypothesized that the association of the C-allele with essential hypertension (24, 25), atrial fibrillation (26), myocardial infarction (27), cardiac hypertrophy (28), and increased oxidative stress levels in human heart failure (29) are relevant in VSMCs, we demonstrated that miR-155 translationally repressed hAT1R expression may be more pronounced contingent upon the relative expression levels of both miR-155 and hAT1R mRNAs and whether the genotype of a given individual is AA, AC, or CC at position 268.
investigating hAT,R density on blood vessels isolated from patients with the AA, or CC genotypes. Therefore, future studies in our laboratory will examine the potential correlation between the hAT,R +1166 genotype, receptor number, and miR-155 expression levels.

In conclusion, we demonstrate that the hAT,R +1166 SNP occurs in a cis-regulatory element that is recognized by miR-155. When the +1166 C-allele is expressed, base-pairing complementarity between miR-155 and the hAT,R mRNA is reduced. As a consequence, miR-155 can no longer attenuate translation as efficiently and hAT,R density increases. Taken together, our study provides the first feasible biochemical mechanism by which the +1166 A/C polymorphism can lead to increased AT1R densities and possibly cardiovascular disease.

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