A pathogenetic role for microRNAs in essential hypertension at last?

Riccardo Contu, BSc; Gianluigi Condorelli, MD, PhD

As a complex (or polygenic) genetic trait, essential hypertension is determined by the interplay between many genetic variants and several environmental factors. Despite an enormous and continuous effort from the clinical and research communities, much of the genetics of essential hypertension remains elusive. The classic approach for identifying disease-causing genes, that is, linkage analyses, brought to the identification of genes responsible for genetic—and thus rare—forms of hypertension; in contrast, the candidate gene approach has led to results that are subject to controversy and definitely less straightforward than those from linkage analysis. As a matter of fact, essential hypertension is a multifactorial and multigene disease, and thus the study of its etiology must be approached with complex bioinformatic and biostatistic techniques for studying gene-environment interaction and synergism among many hypertension-associated single nucleotide polymorphisms (SNPs). Given its multifactorial nature, a single SNP may be able to modify the disease phenotype only together with other SNPs and in an appropriate environmental context.

MicroRNAs (or miRs) are now considered fundamental components of the regulatory system of eukaryotic gene expression. They act posttranscriptionally through cis-regulatory sites, located typically in 3'-untranslated regions (UTRs) of mRNAs, usually significantly reducing protein expression without completely inhibiting the targeted mRNAs. Interestingly, more than 20,000 SNPs of miRNA target sites have been catalogued thus far and, over the last few years, several independent studies have claimed to associate some of them with genetic disorders ranging from cancer to Parkinson disease and hypertension. For example, it was shown that rs5186—an SNP in the 3'-UTR of the human angiotensin II type 1 receptor (AGTR1) messenger RNA, and previously associated with hypertension—mediates allele-specific targeting of miR-155 to AGTR1, thereby modulating its protein level. Other SNPs residing on the 3'-UTRs of renin-angiotensin system genes have been described, leaving room for speculation on their potential role as causal variants of essential hypertension.

Following up their previous results from a genetic linkage study, Wei and colleagues reveal in the current issue of Circulation: Cardiovascular Genetics that the ATP6V0A1 3'-UTR common variant T3246C alters gene expression through differential binding to the microRNA miR-637. The ATP6V0A1 gene, located in chromosome 17q21, encodes the α1 subunit of vacuolar H+-translocating ATPase, a heteromultimeric complex responsible for acidification of the secretory pathway compartment that includes secretory granules, such as the catecholamine storage vesicles of the adrenal medulla. A main component of chromaffin granules is chromogranin A (CHGA), the hormonal precursor of at least 4 different bioactive peptides, including the catecholamine release inhibitor catestatin. The same authors previously established that alterations in the level and/or activity of this integral membrane complex correlate with an impaired vesicular acidification process that can ultimately lead to an altered formation and an incorrect trafficking of the chromaffin granules. At the clinical level, “granins” have been shown to be important in patients with essential hypertension in which an increased plasma level of the prohormone CHGA and a reduced hematic concentration of the CHGA-derived peptide catestatin have been assessed. Interestingly, the above-mentioned allelic variation of ATP6V0A1 (rs938671) was found to be associated with plasma catestatin concentration, chromogranin A:catestatin ratio, and systemic blood pressure in the population. The ATP6V0A1 3'-UTR variant T3246C could thus influence the levels of both CHGA and catestatin subsequent to the alteration of vacuolar pH by modulating either proteolysis of the precursor or its exocytotic secretion.

To test this hypothesis, Wei et al first demonstrated that compared with the wild-type T3246 allele, the risk haplotype carrying the C allele in the ATP6V0A1 3'-UTR significantly reduced the luminescence of a luciferase reporter gene. This result was confirmed by an in vitro translation assay, in which protein expression of the blood pressure–associated variant was found to be clearly reduced. Then, using an elegant approach with fluorescent chimeric proteins, the authors demonstrated how the expression of the T3246C variant alone was responsible for granular pH perturbation (ie, alkalization) in rodent pheochromocytoma (PC12) cells treated with a selective inhibitor of the V-ATPase complex. As hypothesized, this disruption of secretory granule core acidification significantly increased CHGA processing while reducing its regulated secretion. As suggested by the same authors, this result could theoretically explain why plasma...
concentrations of CHGA and catestatin are usually lower in the population harboring the T3246C variant. Finally, to clarify the molecular mechanism whereby the presence of T3246C could be causal to the alteration of granule acidification, the authors investigated whether a microRNA binding site was located in that region. Computational analysis of the sequences flanking the T3246 locus identified a motif complementary to miR-637, with a higher match for the C variant, raising the possibility of a more efficient translational repression for messengers bearing the C allele. Both overexpression (miR mimic) and inhibition (antagomIR) approaches in PC12 cells supported the evidence of differential miR-637–mediated regulation of ATP6V0A1, with the blood pressure–associated (C allele) haplotype showing a higher affinity for the microRNA compared with the wild-type (T allele) one.

Data presented by Wey et al12 are fascinating and support the hypothesis that a “better” miR-637 binding site within the ATP6V0A1 3’ UTR contributes to the association of this region with plasma catestatin concentration and CHGA:catestatin ratio and, ultimately, with systemic blood pressure in the population. Although very interesting, more definitive and compelling evidence is needed to conclusively link T3246C to a miR-637–mediated mechanism. Surprisingly, this microRNA is highly conserved in primates but, as of date, has not been reported in other species, such as mouse, rat, or rabbit; in addition, its biological function is still unknown. Most of the in vitro data were obtained using rodent cell lines, and this leaves room for some confounding variables in the experimental setup that will need to be further addressed. Moreover, it has been recently demonstrated that disease-associated SNPs occurring in the 3’ UTR of genes could affect the RNA structure ensemble and therefore stability and/or accessibility, independent from a potential microRNA binding site.15 For these reasons, future studies might be focused on conclusively demonstrating the α1 subunit of the vacuolar H^+ translocating ATPase as a bona fide target of miR-637. Then, as acknowledged by the authors, it would be important to confirm and validate in vivo the described H^+–ATPase mechanism in human cells.

Finally, it must be considered that given the multifactorial and multigenetic nature of essential hypertension and the modulatory—not just on-off—effect of microRNAs on gene expression, the mechanism described in this report12 may be responsible for a significant albeit small effect on blood pressure, which will concur with other genetic factors in establishing the global hypertension phenotype.

Acknowledgments
We thank Michael V.G. Latronico for editorial assistance.

Sources of Funding
This work was supported by Fondation LeDucq, Fondazione CARIPLO, and the Italian Ministry of Health.

Disclosures
None.

References
ATP6V0A1 Polymorphism and MicroRNA-637: A Pathogenetic Role for MicroRNAs in Essential Hypertension at Last?
Riccardo Contu and Gianluigi Condorelli

Circ Cardiovasc Genet. 2011;4:337-338
doi: 10.1161/CIRCGENETICS.111.960591

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/4/4/337

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/