Toward an understanding of hypertension resistance

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Physiologists have long appreciated the central role of renal salt excretion in the control of blood pressure (4). Maintenance of a constant intravascular fluid volume and blood pressure depends on the ability of the kidneys to regulate urinary sodium excretion through the concerted action of several parallel sodium transport mechanisms. While abnormal function of any one transport process can usually be compensated for, there is a price. That is, a greater than normal change in arterial blood pressure must occur for natriuresis to be brought into balance with an increased Na intake. This concept has been reinforced though the study of rare Mendelian diseases of hypertension or hypotension, since all of the monogenic blood pressure syndromes characterized to date are caused by mutations in genes that influence sodium homeostasis (8). Individuals with these disorders often develop dramatic phenotypes of hypertension or salt wasting, usually through the inheritance of alleles harboring single nucleotide mutations.

In recent years, considerable effort has been directed toward understanding whether these genes or others contribute substantially to blood pressure variation in the population as a whole. The approach for a number of these analyses has involved the unbiased search for associations between common genetic polymorphisms and blood pressure through genome-wide association (GWA) studies. In some cases, GWA scans have confirmed the involvement of signaling pathways known to regulate renal salt excretion (10), or uncovered novel potential targets for antihypertensive therapy (6). In other examples, however, they have failed to identify common variants that reach genome-wide statistical significance (7, 11). More recent efforts by large-scale consortia have yielded success at identifying new associations (6). However, questions remain regarding the effect size of individual variants (3), and, in general, GWA studies have only identified a fraction of the total predicted genetic component for a variety of complex disease traits. Thus a shift in attention away from common polymorphisms, and toward the analysis of rare variants has been called for, so that their relative contributions may be assessed (3).

In a genetic tour-de-force, Ji et al. (5) recently took such an approach, testing whether rare mutations in kidney salt transport genes might contribute substantially to blood pressure variation in the general population. The authors recognized that the heterozygous carrier state for Bartter’s and Gitelman’s syndromes, two Mendelian salt-wasting disorders, should be prevalent in at least 1% of individuals worldwide. Thus they asked whether rare coding variants in genes mutated in these disorders affect blood pressure in a large study population, the Framingham Offspring Cohort. They chose to sequence the exons of three candidates that affect renal sodium transport: SLC12A1 [Na–K–Cl cotransporter (NKCC2), KCNJ1 (ROMK), mutated in type 2 Bartter’s syndrome], and SLC12A3 [Na–Cl cotransporter (NCC), mutated in Gitelman’s syndrome]. To select for relevant coding variants, Ji et al. first identified carriers for mutations in NKCC2, ROMK, and NCC already proven previously to cause hereditary salt wasting. As a second strategy, the authors applied stringent criteria to identify additional variants that could be inferred with confidence to lead to loss of function. To make the cutoff, a variant had to change an evolutionarily conserved residue and be present in the study population at a low frequency (to favor alleles under purifying selection). This approach identified 30 mutations across 49 subjects in ~3,000 individuals. The authors found that carriers for one of the proven or inferred mutations in NKCC2, NCC, or ROMK had a systolic blood pressure that averaged 6.3 mmHg lower throughout life than the mean of the cohort. Thus the study provides intriguing evidence that the carrier state for functional mutations in NKCC2, NCC, or ROMK confers protection from the development of hypertension.

While the conclusions of Ji et al. were backed with impressive statistical rigor, an important question remained, since the authors did not perform experiments to directly verify that the inferred variants actually do lead to loss of function. Now, a study in an issue of the American Journal of Physiology-Renal Physiology (9) completes a trio of papers that evaluate the inferred variants in detail. In their careful analysis, Monette et al. found that six of the nine variants in SLC12A1 decrease NKCC2 function through a variety of mechanisms. All six of the functional variants exhibited a decrease in transport activity under physiologically relevant isotonic conditions, when studied in a mammalian expression system. In at least three of the mutants, the defects were attributable to impaired biosynthetic trafficking and plasma membrane expression. One of the low-activity variants (P569H) also exhibited a decrease in sodium binding affinity, while another (N399S) had an altered regulatory response to intracellular chloride depletion. The results are broadly compatible with a recent analysis of the same NKCC2 mutations by Acuna et al. (1), although the results for at least one of the mutants (N399S) were different between studies. The reason for this discrepancy is unclear, but could be related to differences in the NKCC2 cDNAs and/or the expression systems that were used. The study by Acuna et al. also evaluated the inferred variants in SLC12A3 and found that all five of the mutations resulted in modest but statistically significant reductions in NCC activity. The third paper was an analysis of inferred variants in KCNJ1, reported recently by Fang et al. (2). In their study, all of the five identified ROMK hypertension resistance variants behaved as predicted, exhibiting measurable loss of function. Three mutations resulted in impaired biogenesis and plasma membrane expression, while
the other two introduced gating defects that rendered the channel more sensitive to inhibition in the absence of PIP2.

Together, these three studies (1, 2, 9) provide strong support for the selection criteria employed by Ji et al. (5) to identify functional variants. Even when the potential imperfections of heterologous expression are taken into consideration, it would appear that at least 90% of the identified variants have a proven functional effect. As the search for rare variants that contribute to the pathogenesis of complex disease traits continues, similar selection strategies will be of great utility.

On a final note, the three papers collectively agree that conserved sequence variants subject to purifying selection in NKCC2, NCC, and ROMK cause hypertension resistance by diverse mechanisms. Thus the mechanisms that govern the biogenesis, trafficking, and transport kinetics of these regulators of sodium homeostasis are relevant to understanding both rare Mendelian disease and a more common "hypertension-resistant" carrier state. As the molecular details of this phenotype are revealed, perhaps new targets for diuretic therapy will be identified.

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DISCLOSURES

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REFERENCES