ELUCIDATION OF THE GENETIC BASIS of essential hypertension is a challenging endeavor. Remarkable progress has been made studying families affected by rare Mendelian forms of high or low blood pressure. This approach has been successful because it identifies mutations with large effect sizes on single genes that regulate renal sodium (Na\(^+\)) handling and Na\(^+\) balance. However, essential hypertension is not a Mendelian trait but a complex one that is incompletely penetrant and likely governed by a mixture of genetic, environmental, and stochastic factors. Nonetheless, a candidate gene approach, informed by studies of Mendelian forms of low blood pressure (8–10), has demonstrated that rare genetic variants within a large background of neutral variants can account for a large fraction of blood pressure variation (4).

In an issue of the *American Journal of Physiology-Renal Physiology*, Ray et al. (6) adopt a similar candidate gene approach with participants of the Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) study and evaluate whether epithelial Na\(^+\) channel (ENaC) variants underlie salt sensitivity. Salt sensitivity can identify individuals who are at high risk for hypertension, cardiovascular disease, and overall mortality (5, 11). Moreover, salt sensitivity may reflect more directly the mechanisms by which each individual variant changes ENaC activity. Two ENaC variants are notable from this study. \(\alpha V481M\) variant also carry the \(\beta\) subunit of ENaC (2, 7). These gain-of-function mutations enhance expression and activity of ENaC in the distal nephron, leading to a net increase in renal Na\(^+\) reabsorption and salt-sensitive hypertension. In the current study, Ray and colleagues sequenced genes encoding \(\alpha\), \(\beta\), and \(\gamma\) subunits of ENaC from two groups of GenSalt participants: 300 with the highest and 300 with the lowest blood pressure responses to Na\(^+\) loading. The GenSalt study was designed to identify genetic polymorphisms associated with salt sensitivity (1). In the original study, participants with mild hypertension (systolic blood pressure 130–160 mmHg and/or diastolic blood pressure 85–100 mmHg) and their families were recruited from six rural villages in northern China. A total of 1,906 participants were administered a low-salt diet (51.3 mmol of Na\(^+\)/day) for 7 days followed by a high-salt diet (307.8 mmol of Na\(^+\)/day) for 7 days. Blood pressures were measured at regular intervals.

Ray and colleagues identified 16 nonsynonymous gene variants of \(\alpha\), \(\beta\), and \(\gamma\) subunits of ENaC. They examined how these variants affect ENaC activity by generating human \(\alpha\beta\gamma\)-ENaC CDNAs with individual variants and measuring the activity of resultant channels in the *Xenopus laevis* oocyte expression system. Furthermore, they assiduously characterized the mechanisms by which each individual variant changes ENaC activity. Two ENaC variants are notable from this study. \(\alpha V481M\) exhibited a 2.7-fold increase in current, which was associated with an increase in ENaC cell surface expression and a decrease in Na\(^+\) self-inhibition. Conversely, \(\alpha A334T\) exhibited a decrease in current, which was associated with a decrease in ENaC cell surface expression and an increase in Na\(^+\) self-inhibition. In addition to functional characterization of ENaC variants, the investigators searched for clinical correlations by asking if gain-of-function variants associate with salt-sensitive participants and, conversely, if loss-of-function variants associate with salt-resistant participants.

Contrary to their hypotheses, the investigators found no statistically significant associations among ENaC variants and salt sensitivity. There are several possible reasons for these null results, many of which are acknowledged by the investigators. First, it is possible that a single ENaC variant may not have an effect size large enough to alter salt sensitivity in a clinically detectable way. Similar to essential hypertension, salt sensitivity is not a Mendelian trait, so the effect of an allele at one gene locus could be diluted by competing alleles at other loci. For example, in the present study, the four salt-sensitive participants carrying the gain-of-function \(\alpha V481M\) variant also carry one of the loss-of-function ENaC variants.

Second, the investigators excluded participants whose gene variants could have increased the frequency of those that might associate with salt sensitivity. The investigators selected 15% of GenSalt participants with the largest and smallest salt-sensitive changes in blood pressure for gene sequencing. If they had sequenced the entire cohort, it might have boosted the power of the study to detect a statistically significant association, an approach that was used by Ji et al. to identify gene variants associated with low blood pressure in the Framingham Heart Study offspring cohort (4).

Third, the investigators excluded participants most likely to exhibit salt sensitivity, i.e., those with systolic blood pressure...
>160 mmHg or taking antihypertensive medications, cardiovascular disease, or chronic kidney disease. What remained in the study sample were participants with normal blood pressure or mild hypertension and perhaps a lower degree of salt sensitivity. Unfortunately, Ray et al. do not provide baseline or salt-sensitive changes in blood pressure from the 600 participants selected for study. In the original GenSalt cohort, the average baseline systolic blood pressure was 119 mmHg among men and 115 mmHg among women (3). Only a small fraction of participants exhibited baseline blood pressures over 140/90 mmHg. Moreover, salt-sensitive changes in blood pressure were relatively small, ~5 mmHg in either direction.

Finally, it remains possible that ENaC variants do not have a role in adjusting salt sensitivity. Given the limitations of the present study, no firm conclusions can yet be made. Ray and colleagues should be commended for providing new insights into the variability of ENaC activity within a population and for elucidating mechanisms by which ENaC variants affect channel function. From this vantage point, their findings carry significant physiological weight. However, this study also highlights the many challenges of human genetics studies that Newton never faced in developing his theory of light. Despite thorough functional characterization of gene variants, a study can still fall short in making the critical connection between gene variants and disease. Future studies examining larger cohorts of participants with more severe salt sensitivity may eventually lead to a definitive study, and a bona fide experimentum crucis.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.C.P. drafted manuscript; A.C.P. and T.I.C. edited and revised manuscript; A.C.P. and T.I.C. approved final version of manuscript.

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