EDITORIAL

Monogenic Stroke—Can We Overcome Nature With Nurture?

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Although genetic underpinnings of several neurological disorders like epilepsy have been described as early as fourth century BCE in the Corpus Hippocraticum,[1] discoveries in stroke have been more recent. One of the first reports demonstrating the importance of heredity in stroke pathogenesis occurred in 1974 with the generation of the stroke-prone spontaneously hypertensive rat.[2] This strain was created using selective breeding and has endured as a valuable asset in contemporary stroke research. Nonetheless, half a century later, the precise genetic determinants even in this single, highly specific rodent stroke phenotype have not been completely elucidated despite immense advances in next-generation sequencing and genome mapping.[3,4] In humans, the role of genetics in stroke is exponentially more complex. Stroke is heterogeneous, as are its predisposing risk factors, which also have their own genetic contributors. Genetics may either mediate or moderate stroke via multiple mechanisms. Genome-wide association studies since 2007 have identified several common loci and variants that typically account for a small proportion of the heritable risk of stroke, stroke subtypes, or conventional stroke risk factors.[5] Monogenic stroke has classically resided on the other end of the continuum, where rare variants in a single gene are often thought to cause diseases. Here, the genetic alteration may contribute to inherited syndromes where stroke is the primary phenotype (eg, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]), or multisystem disorders that include stroke as a manifestation (eg, sickle cell anemia).[6] The causative paradigm in monogenic stroke has evolved over the past decade with increasing recognition of variability in penetrance, expressivity, and potential for modulation by risk factors and other genes.[7-10] Simply put, variants may be more frequent than formerly estimated, resultant clinical phenotypes may have broader ranges than previously appreciated, only a subset of patients may manifest disease, and the pathobiology may vary with other genetic and nongenetic/environmental factors. Ultimately, these differences may also inform which therapies are likely to be most effective in different patient subgroups. Thus, systematically dissecting these nuances in monogenic stroke has important implications for both public health and precision medicine–driven biomarker/drug development.

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In this issue of *JAMA Neurology*, Cho et al[11] evaluate key questions of penetrance, expressivity, and modulation of monogenic stroke in a methodologically rigorous study of 454 756 UK Biobank participants with whole-exome sequencing data. They focus on pathogenic variants in the 3 most common monogenic cerebral small-vessel diseases (cSVDs): *NOTCH3*, *HTRA1*, and *COL4A1/2*. *NOTCH3* variants are the predominant cause of CADASIL, the most common monogenic cSVD.[12,13] *Htra1* is a protein that colocalizes with Notch3's extracellular domain; variants in *HTRA1* cause CADASIL2 and cerebral autosomal recessive arteriopathy with subcortical infarcts. *COL4A1/2* encodes a type IV collagen α protein expressed in basement membranes of blood vessels with pathogenic variants resulting in lacunar stroke or intracerebral hemorrhage. Earlier work in smaller cohorts (predominantly focused on *NOTCH3*) suggest that pathogenic variants are more frequent than expected based on prevalence of disease phenotype.[9,14] Some studies identify differences in penetrance, expressivity, and a globally increased risk of stroke and/or vascular dementia.[7-10,14,15] Cho et al[11] reported consistent findings: pathogenic variants in all 3 genes were markedly more frequent than expected based on prevalence of clinical disease phenotypes thought to be caused by these variants. This was most notable in *NOTCH3* where 1 in 467 patients were heterozygous variant carriers vs the estimated prevalence of CADASIL (4:100 000) yielding a more than 50-fold difference. *HTRA1* variants had a frequency of 1 in 832, and *COL4A1/2* variants were detected at a rate of 1 in 1353. These participants were neither universally nor uniformly symptomatic.

The large sample size in the current study enabled a uniquely broad and detailed evaluation of these 3 key cSVD genes and their association with stroke phenotypes; it is the largest study on these genes by more than 2-fold, to my knowledge. The results are balanced between confirmatory vs novel/hypothesis generating. In all 3 genes, the study supports previous data of lower penetrance rates and overestimated variant pathogenicity.[8,10,14,15] A significant proportion of asymptomatic carriers were identified when evaluating for prevalence and incidence of cSVD (median follow-up, 12.6 years). Expressivity for each gene was also highly variable, with differences in disease severity and clinical phenotypes. *NOTCH3* variants had the most phenotypic variability with greater odds of both prevalent and incident types of cSVD including ischemic stroke, hemorrhagic stroke, all-cause dementia, vascular dementia, and higher white-matter hyperintensity volume. Most *NOTCH3* pathogenic variants result in a gain or loss of cysteine in epidermal growth factor repeats (EGFr) in the proteins' extracellular domain; pathogenic variants on EGFr position 1 to 6 have been associated with higher CADASIL disease severity[10,15] but were present in 2% of *NOTCH3* variant carriers in this study. *HTRA1* variant carriers had greater odds of migraine with aura, ischemic stroke, and larger white-matter hyperintensity volume. The reported association of *COL4A1/2* variants with greater odds of any stroke was driven by intracerebral hemorrhage. Although presence of pathogenic variants in these

genes can no longer be considered a universal harbinger of unavoidable severe monogenic cSVDs, this study suggests that they may warrant identification and close clinical attention beyond patients diagnosed with CADASIL given their strong associations with other cSVD phenotypes.

In large part, the novelty and future impact of this work lies in the authors' evaluation of whether and how genetic and cardiovascular risk factors influence monogenic ischemic stroke. They focused on *NOTCH3* and *HTRA1* since increased stroke risk with *COL4A1/2* was due to intracerebral hemorrhage. Although common genetic variants in other diseases are reported to affect penetration of monogenic conditions by acting as modifiers,[13] this was not the case for *NOTCH3* or *HTRA1*. The ischemic stroke polygenic risk score (PRS) in this cohort was associated with greater stroke risk in all participants. However, it affected only noncarriers of *NOTCH3* and *HTRA1;* there was no additive interaction between PRS and variant carrier status. Thus, genetic propensity to common ischemic stroke as measured by PRS did not further increase stroke risk in participants with monogenic variants. In both genes, stroke risk conferred by variants was equivalent to a several-fold standard deviation increase in PRS. However, PRS may mask variant-level epistasis and does not account for common/currently unidentified genetic variants or focus on candidate genes that may have a pathophysiologic basis for acting as *NOTCH3* or *HTRA1* modifiers. These limitations may be real; a previous heritability estimate in 151 patients with CADASIL suggested modifying effects of unmeasured genetic factors that were distinct from *NOTCH3*.[16]

In striking contrast, cardiovascular risk (calculated by the comprehensive Framingham Cardiovascular Risk Score) increased stroke risk in *NOTCH3* and *HTRA1* variant carriers. Like PRS, cardiovascular risk unsurprisingly was independently associated with ischemic stroke risk in all participants. However, unlike PRS, there was a clear statistical additive interaction between cardiovascular risk and variant status. Hazard ratios for ischemic stroke were markedly lower in carriers with low cardiovascular risk (range, 2.07-3.34) and noncarriers with high cardiovascular risk (range, 3.32-3.35) vs participants who were both carriers and had high cardiovascular risk (range, 6.22-7.82). It would be tempting to infer, given these associations, that treatment of cardiovascular risk could dramatically reduce disease burden in variant carriers and forms of monogenic stroke. However, a fallacy akin to post hoc ergo propter hoc applies; while logical (and perhaps even likely), it is premature to conclude either causation or treatment response. Mechanisms of how these variants cause stroke are incompletely understood. The molecular basis for interactions with cardiovascular risk profiles remains unknown but important to elucidate. Only 22 patients in this study were carriers of *NOTCH3 EGFr* 1-6 (associated with severe CADASIL) and we cannot know if/how much cardiovascular risk reduction would benefit this subpopulation. The foundation developed by Cho et al[11] creates a launchpad for future research to explore these critical questions regarding molecular mechanisms/interactions, intermediate phenotypes, causation, and treatment response.

This study advances a paradigm shift away from the traditional dogma of monogenic disease that has long suggested that little (short of developing gene-targeted therapy) can be done about the genetic hand that has been dealt. The potential to reduce disease burden in monogenic stroke by harnessing cardiovascular risk is a powerful prospect. Developing targeted therapies remains important; indeed, given the markedly higher-than-expected prevalence of these variants, it is possible that non-CADASIL stroke phenotypes may benefit from molecularly targeted treatments. However, equally exciting is the potential impact of relatively low-cost interventions that are available immediately and can be implemented early, such as smoking cessation and controlling hypertension, hyperlipidemia, and diabetes. From the patient perspective, it provides agency; instead of being sentenced to inevitable cSVD, early medication and lifestyle interventions may meaningfully reduce risk. Knowledge of genetic predisposition may create a needed sense of urgency for primary prevention and compliance and could change and harmonize current practice patterns for these monogenic diseases. On the clinician end, if cardiovascular risk factor reduction could decrease (by 2-fold or more) the risk of stroke in variant carriers, this could have important implications for early screening and vigilant treatment. Whether or not this will pan out in future studies remains to be seen. Currently, these hypotheses are unproven. But for now, even if the complex mechanisms and effects of *NOTCH3* EGFr 1-6 vs 7-34 variants are incompletely understood, it seems plausible that paying attention to basic details such as glucose, cholesterol, and blood pressure may yield unexpected and greater dividends in the monogenic stroke population than previously realized.

ARTICLE INFORMATION

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