GENETICS OF STROKE: FROM BIOLOGICAL DISCOVERIES TO CLINICAL TRANSLATION

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Abstract

Stroke genetics have been transformed from a field exploring causes of rare hereditary forms of cerebrovascular disease to an international dynamic and expanding arena offering key insights into stroke biology and exciting opportunities for clinical applications. Genome-wide association studies, which triggered this transformation, have already identified more than 40 genomic risk loci associated with stroke, which offer important windows into stroke pathogenesis and starting points for experimental explorations of pharmacological strategies. Furthermore, genome-wide explorations have enabled the development of polygenic risk scores, which represent a promising potential application for risk prediction in clinical populations. Utilization of genetic data further allows exploration of causal relationships between exposures and outcomes and the discovery of novel drug targets for stroke with the use of Mendelian randomization. In this review, I provide a brief overview of the major developments in the field and opportunities for applications.

Key words: genetics; genomics; stroke; cerebrovascular disease; polygenic risk prediction; Mendelian randomization

Stroke remains a leading cause of death and disability worldwide [1]. While major advances in the prevention and treatment of stroke have taken place, important gaps remain. For example, there is no specific preventive strategy for small vessel stroke and intracerebral hemorrhage, whereas neuroprotectant therapies have not met the initial expectations. As we learned from the past, understanding the biology underlying stroke pathogenesis is important to improve treatment options. Genetics were always considered anchors to fundamental biological mechanisms. But recent major advancements in available technologies have revolutionized the way we explore genetic information to discover disease mechanisms. Stroke genetics has grown from a small field exploring causes of rare hereditary forms of stroke to a dynamic and expanding arena offering insights about the pathogenesis of sporadic stroke. Genome-wide association studies (GWAS), which triggered this transformation, have offered not only unique windows into disease biology but also unexpected opportunities for clinical applications.

Gene discovery: from monogenic stroke to novel pathways in sporadic stroke

Early genetic studies found genes that underlie forms of Mendelian stroke [2] and pointed to pathways involved in stroke pathogenesis that were particularly relevant for stroke subtypes. Such examples include genes encoding proteins related to the extracellular matrix, which were associated with hereditary forms of cerebral small vessel disease (*COL4A1*, *COL4A2*, *HTRA1*, *NOTCH3*) [3]. However, beyond Mendelian stroke that is responsible for a very small proportion of stroke cases encountered in the clinic (around 1-2% of lacunar strokes and <5% in total) [4], studies in twins found a higher risk of stroke among monozygotic, as compared with dizygotic co-twins, thus suggesting a genetic component for sporadic stroke cases as well [5]. In further support of this, within-family studies showed that stroke is more common among individuals with a family history of stroke or vascular disease [6]. Before the mapping of the human genome and the subsequent development of GWASs, several studies explored candidate genes that might be associated with stroke risk. While such studies occasionally provided important insights into stroke biology, most of the described associations were not replicated in subsequent GWAS analyses [7]. The first GWASs in stroke estimated the heritability of stroke prevalence at around 40% for ischemic stroke[7] and 30% for intracerebral hemorrhage [8].

More recent GWASs including up to 71,147 cases have identified >40 risk loci for stroke risk [9, 10, 11]. The results of these studies highlight the role of specific genes in stroke pathogenesis and can be used as the starting point for follow-up functional experiments. One example is histone deacetylase 9

(HDAC9), which consistently came up as a major risk locus for multiple atherosclerotic phenotypes including large artery stroke, coronary artery disease, and peripheral artery disease and enabled functional studies in atherosclerosis-prone mice [12]. Other loci involved in stroke pathogenesis include genes implicated in the pathogenesis of major risk factors, such as hypertension (e.g. *FURIN-FES*) and hypercholesterolemia (e.g. *LDLR*) or genes that have been previously implicated in the development of major stroke causes, such as atherosclerosis, atrial fibrillation, and cerebral small vessel disease [3]. Finally, risk loci for stroke are enriched in target genes for approved treatments, such as *FGA*, encoding the target for thrombolytic agents, highlighting that the discovered risk loci may harbor targets for future drug development [10]. Until now, the largest GWASs have provided information about common variants, encountered in >0.5-1% of the population. As the datasets increase and low-cost sequencing technologies become more widely available, newer methods that also explore rare genetic variation are expected to be integrated into future studies. Preliminary analyses focused on rare variants in the exonic regions of specific genes, such as *HTRA1*, already provide important results about the pathogenesis of cerebrovascular disease [13].

Polygenic risk scores: a tool ready for clinical application?

Beyond new insights into disease biology, the results from GWAS analyses can be useful in stroke risk prediction. While individual genetic variants contribute to disease risk only minimally, by combining multiple variants with individually small effects in a so-called "polygenic risk score" (PRS; or genomic risk score), it is possible to additively quantify genetic predisposition to stroke risk [14]. Multiple novel methods have been developed that aim to combine information throughout the genome from GWASs in an optimal way, so as to maximize the predictive power of the tool [15]. For example, the predictive performance can be enhanced by combining PRSs for stroke with multiple PRSs from traits known to be involved in stroke pathogenesis, such as blood pressure, diabetes, and circulating lipids, in a so-called meta–genomic risk score. The hazard ratio obtained from such a score for stroke is 1.26 per standard deviation increment [16], whereas a score for coronary artery disease achieved a hazard ratio of 1.71 [17]. These scores consistently increase predictive power when added to models of established clinical risk factors [16, 17]. Because genetic information is present from birth and remains stable over time, PRSs can be assessed by a single genotyping effort long before traditional risk factors manifest, thus allowing early prognostication and decisions on targeted monitoring [18]. Already *post hoc* analyses from clinical trials suggest that PRSs can predict risk of stroke among patients with cardiometabolic risk factors [19]. Interestingly, among patients with atrial fibrillation, an ischemic stroke PRS can enhance the predictive performance of the CHA_2DS_2-VASc score for stroke prediction, thus opening a window for a potential clinical application in the decision-making algorithms for initiating anticoagulant treatment [19]. Very important topics remain however open before implementing PRSs: these include sex differences, which are not traditionally considered, the reproducibility of PRSs across different ancestries, the communication of PRS screening results to individuals, and the optimal management of individuals at high genetic risk [20].

Mendelian randomization: exploring causal associations with human genetic data

Another application of genetic results includes the exploration of causal relationships [21]. An instrumental variable analysis, called Mendelian randomization, makes use of genetic variants associated with a risk factor (*genetic instruments*) to investigate causal associations between the risk factor and a disease outcome [22, 23]. The emergence of large-scale GWASs enabled the discovery of multiple genetic variants that explain an increasing proportion of variance in risk factors of interest. Thus, Mendelian randomization studies may incorporate up to hundreds of genetic variants as instruments to explore associations between genetic predisposition to exposure traits and outcomes of interest. As genetic information is anchored to conception and is not influenced by other potential environmental confounders, Mendelian randomization is less prone to traditional biases in observational studies, such as confounding and reverse causation. However, a number of assumptions need to be fulfilled in order for the genetic variants to be valid instrumental variables: [23] the variants should (i) strongly be associated with and predict the risk factor of interest, (ii) only associate with the outcome through their relation to the risk factor and (iii) not relate to confounders of the exposure-outcome association. Importantly, genetic variants with so-called pleiotropic effects on potential confounders in the exposure-outcome association may not represent valid instruments [23]. Pleiotropy refers to the phenomenon where a gene or a genetic variant can influence more than one phenotypic traits and may represent a source of bias in Mendelian randomization analyses [24]. Developments in statistical methodology have offered analytical tools to test the validity of these assumptions and correct for deviations from the real effect estimates due to pleiotropy [25].

Mendelian randomization studies have offered unique insights into stroke etiology, and particularly into the etiological risk factors that underlie specific diagnostic subtypes [26]. For example, studies focused on blood pressure provided evidence for a strong association of higher genetically predicted blood pressure with all major ischemic stroke subtypes (large artery, cardioembolic, small vessel stroke) and deep intracerebral hemorrhage, but not with lobar intracerebral hemorrhage, which is traditionally associated with cerebral amyloid angiopathy [27]. Furthermore, on top of mean blood pressure, genetically predicted late-life pulse pressure, which is an indicator for arterial stiffness, is also a risk factor for ischemic stroke, and particularly large artery stroke [28]. Another interesting example includes lipid metabolism. Mendelian randomization studies confirmed a potentially causal association of LDL cholesterol levels only with large artery stroke [29], whereas for small vessel stroke, a protective effect of higher HDL cholesterol was more robust [30]. Interestingly, the inverse relationships were detected for intracerebral hemorrhage [30], further expanding on findings from *post hoc* analyses of randomized trials that lowering LDL cholesterol might be a risk factor for hemorrhagic stroke [31]. Other interesting insights include the role of type 2 diabetes [32], hyperglycemia [32], abdominal obesity [33], and smoking [34] on large artery and small vessel stroke and a rather linear association between alcohol consumption and risk of ischemic stroke [35]. As sample sizes further increase, unique opportunities will emerge for clarifying the role of traditional vascular risk factors in stroke pathogenesis, but also for discovering novel risk factors [26]. Expansions of more elegant analytical epidemiological tools to Mendelian randomization, such as multivariable and mediation analyses, will also enable a more accurate dissection of the pathways that lead to stroke [36, 37].

Leveraging genetic data for drug discovery

The drug discovery pipeline is costly and lengthy. Despite the increasing investment in drug development, only around 5% of cardiovascular disease drugs that enter phase I trials make it to market approval [38]. Historical retrospective analyses have shown that evidence of effect from human genetic studies for a candidate protein drug target increases the probability for a compound targeting this candidate to reach approval by 2- to 4-fold [39, 40]. Perhaps the example that most compellingly demonstrates this paradigm is *PCSK9*, which is the target of the recently developed proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [41]. *PCSK9* was first described in 2003, when it was implicated in familial hypercholesterolemia [42], soon thereafter, in 2006, loss-of-function variants in *PCSK9* were associated with lower LDL levels and a lower lifetime risk or acute coronary events [43]. Already in 2017 and 2018, two large-scale phase III trials provided robust evidence that two monoclonal antibodies against PCSK9 reduced the rates of cardiovascular events on top of statins [44, 45] .

An interesting example more focused on stroke is the evidence from human genetics on the potential atheroprotective effects of anti-inflammatory drug targets. A study exploring genetic variation in the circulating levels of 41 cytokines and growth factors showed genetic variations in the circulating levels of monocyte chemoattractant protein-1 (MCP-1 or alternatively called CC-chemokine ligand-2, CCL2) to be associated with a higher risk of ischemic stroke [46], This was particularly the case for large artery stroke, but also for other atherosclerotic phenotypes, such as coronary artery disease, and myocardial infarction [46]. These results were later confirmed in prospective cohort studies [47-49] and also agree with findings from experimental atherosclerosis models that support a role of the MCP-1/CCL2 pathway in monocyte recruitment to atherosclerotic lesions [50]. Beyond MCP-1/CCL2, genetic studies also provided evidence for a potentially causal role of interleukin-6 (IL-6) signaling in large artery stroke [51]. Specifically, genetic variants within the gene encoding IL-6 receptor (*IL6R*) show strong associations with large artery stroke, abdominal aortic aneurysm, coronary artery disease, and a more favorable cardiometabolic profile [51, 52]. These data provide evidence for a causal role of IL-6 signaling in atherosclerotic cardiovascular disease. Indeed, a monoclonal antibody against IL-6 has already been tested in phase 2 trials in patients with chronic kidney disease and a history of atherosclerotic disease [53] and is currently to be tested in a phase 3 trial. Other interesting applications include phenome-wide association studies, which can reveal previously underrecognized side-effects associated with drug targets or repurposing opportunities for available drugs targeting specific drug candidates [52].

Genetics of stroke outcome might point to mechanisms related to neuroprotection

A new generation of studies aims to explore genetic determinants of outcomes after stroke. Such studies could pinpoint pathways that might serve as targets for the development of neuroprotective agents, thus addressing the high demand for such treatments. However, these efforts are in their first steps and still suffer from low power due to the small sample sizes that do not suffice for genetic discoveries [54]. Stroke outcome genetic studies are by design more challenging than studies focusing

on stroke risk. They are focused only on cases, they need to model clinical variables that strongly predict stroke outcome, such as time from stroke onset, stroke severity, and stroke etiology, and they need to balance between data availability and accuracy of outcome measures. For example, dynamic outcome measures of early neurological change, such as the change in National Institutes of Health Stroke Scale (NIHSS) from 6 hours to 24 hours after stroke have been proposed as key readouts [55] and might be better fits for genetic studies than more traditional readouts used in clinical research, such as 3-month modified Rankin scale [54]. Although still at its birth, the field of stroke outcome genetics is already growing and has provided some results about pathways of potential interest for brain injury, repair, and recovery following ischemic stroke, which demonstrate the feasibility of the approach [56-58].

Future directions and conclusions

Over the last two decades, the field of medical and population genetics in cerebrovascular disease has been growing rapidly. As a result, several opportunities for applications have emerged that could improve stroke care in the near-term future. The advancements in the field have been the result of large-scale international collaborations, such as the International Stroke Genetics Consortium, and biobanking initiatives, such as the UK Biobank, Biobank Japan, and the China-Kadoorie Biobank. The broad data sharing mentality of the field has critically boosted innovation and accelerated paths to discovery. Still, important developments are underway, which are worth mentioning. Key initiatives to integrate data from ancestries other than Europeans are expected to lead to new discoveries and to boost the performance of PRSs in risk prediction. There is a major need for diversification in genetic research, as most data come from analyses in European populations. Large benefits are also to be expected by genetic analyses of endophenotypes of cerebrovascular disease, such as MRI biomarkers of cerebral small vessel disease. The integration of other large-scale data, such as transcriptomics and proteomics, into genetic research will allow us to link the associations between genetic variants and disease risk to biochemical footprints that will enhance our understanding of disease mechanisms. Finally, important follow-up functional experiments that will enhance our understanding about the mechanisms through which identified variants influence disease risk will accelerate the translation of genetic discoveries to novel therapeutics.

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