HHS Public Access

Author manuscript

Stroke. Author manuscript; available in PMC 2022 August 17.

Published in final edited form as:

Stroke. 2021 August ; 52(9): 2974–2982. doi:10.1161/STROKEAHA.121.032616.

Stroke Genetics: Turning Discoveries into Clinical Applications

Martin Dichgans, MD[1,2,3], **Nathalie Beaufort, PhD**[1], **Stephanie Debette, MD, PhD**[4,5], **Christopher D. Anderson, MD, Msc**[6,7,8]

[1]Institute for Stroke and Dementia Research (ISD), University Hospital, LMU Munich, Munich, Germany

[2]Munich Cluster for Systems Neurology, Munich, Germany

[3]German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

[4]University of Bordeaux, INSERM, Bordeaux Population Health Center, UMR1219, Team VINTAGE, F-33000 Bordeaux, France

[5]Bordeaux University Hospital, Department of Neurology, Institute of Neurodegenerative Diseases, F-33000 Bordeaux, France

[6]Department of Neurology, Brigham and Women's Hospital, Boston, MA, USA

[7]Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA

[8]Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA

Background

While treatment options for stroke have greatly improved over the last 30 years, important gaps remain. Expectations towards neuroprotectant agents have not been met, in part because of challenges in selecting suitable agents with strong *a priory* evidence for efficacy in humans.[1, 2] Also, we are still missing targeted preventive treatments for small vessel disease (SVD), a major cause of ischemic stroke, haemorrhagic stroke, and vascular cognitive impairment.[3, 4] Without an improved understanding of the underlying molecular, cellular, and physiological mechanisms this situation is unlikely to change. Looking at atherosclerosis and the concept of residual inflammation,[5] the results from the recent CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study)[6] and COLCOT (Colchicine Cardiovascular Outcomes Trial)[7]trials illustrate how progress in understanding the initiating and propagating events in cardiovascular disease can further improve treatment options even in scenarios where highly effective therapies, such as lipid lowering agents, are in place. Consequently, there is great demand for a better understanding of fundamental disease mechanisms in stroke.

Address for correspondence: Prof. Martin Dichgans, Institute for Stroke and Dementia Research (ISD), Feodor-Lynen-Str. 17, 81377 München, Germany, Phone: +49-89-4400-46018, martin.dichgans@med.uni-muenchen.de.
Twitter handles: @ISD_Research, @CDAndersonMD, @BWHNeurology, @broadinstitute

Conflicts of interest

MD, NB and SD have no conflicts of interest to declare. CDA receives sponsored research support from Bayer AG, the American Heart Association, and has consulted for ApoPharma and Invitae.

Genetics has gone quite some way in identifying Mendelian causes for stroke.[8] These discoveries have highlighted the importance of individual pathways relevant to specific stroke subtypes. For instance, the discovery of genes implicated in hereditary SVD (*COLIVa1* and *COLIVa2*; *HTRA1*, and *NOTCH3* amongst others) pinpointed changes of the extracellular matrix (ECM) as a major initiating and propagating factor while also emphasizing the role of specific cellular constituents (endothelial cells, pericytes, and smooth muscle cells) in SVD pathogenesis.[8] They further enabled functional studies in animal models for SVD,[9–11] and allowed testing novel therapeutic strategies,[12] some of which may also have relevance for the larger group of patients with sporadic SVD.

More recently genome-wide association studies (GWAS) have identified multiple (>40) risk loci for stroke thereby pinpointing a causal role of specific genes and gene regions in stroke pathogenesis and offering additional starting points for functional studies in experimental models.[13–17] For example, the identification of *HDAC9* as a major risk locus for large artery stroke, coronary artery disease, and peripheral artery disease has enabled functional studies in atherosclerosis-prone mice and cultured cells.[18, 19] These studies revealed a mechanism, by which Hdac9 (encoding histone deacetylase 9) regulates atherosclerotic plaque vulnerability. They further showed that treatment with TMP195, a class-IIa specific HDAC inhibitor, attenuates plaque formation in mice thus offering a novel therapeutic strategy for atheroprotection.[18]

Aside from *HDAC9*, recent stroke GWAS clearly demonstrate the potential of large-scale genetic studies for drug discovery: risk loci in MEGASTROKE were substantially enriched in drug-target genes for antithrombotic therapy. This included *FGA* (encoding fibrinogen alpha chain), a target for alteplase and other thrombolytic agents, and PDE3A (encoding phosphodiesterase 3A), a target for cilostazol,[20] raising confidence, that stroke risk loci harbour meaningful targets for drug development.

Beyond its potential for discovery and molecular genetic testing for monogenic diseases, genetics offers unanticipated opportunities for clinical applications. Examples include: (1) risk prediction based on common genetic variants using polygenic risk scores (PRS); (2) the exploration of potential therapeutic targets by mendelian randomization; (3) drug discovery; (4) and pharmacogenomics. Given the rapid accumulation of genetic information and continued progress in analytical protocols these applications are becoming increasingly powerful. The current issue highlights two areas, where substantial progress has been made over the last years: Mendelian randomization (see Georgakis et al. page XXXX)[21] and risk prediction using PRS (see Abraham et al., page XXXX).[22] This issue further contains a guide on diagnostic testing and clinical management of monogenic stroke,(see Guey et al. page XXXX)[23] a topic that regularly comes up in clinical practice. Much has happened in the field of genetics of intracranial aneurysms, which is why we included an update on this topic (see Bakker et al. page XXXX).[24] The last article gives an overview on the genetics of stroke outcome, a field that is still at its infancy but gaining importance for the development of neuroprotective agents (see Lee et al. page XXXX).[25] Below, we summarize some of the highlights while also touching on other topics relevant to clinical applications.

Polygenic risk scores – ready for implementation?

By combining the effects of multiple genetic variants with individually small effects in a polygenic score (PRS; or genomic risk score, GRS) it is possible to quantify genetic predisposition to traits and conditions like blood pressure (BP), low density lipoprotein (LDL) levels, coronary artery disease (CAD), or stroke.[26] This is typically achieved through a weighted sum of allele counts. The inclusion of variants beyond those meeting stringent GWAS significance levels has been shown to boost predictive performance.[27, 28] Performance can be further enhanced by combining PRS from multiple related traits such as BP, LDL levels, and stroke in a so-called metaGRS (for discussion see Abraham et al XXXX).[22] Indeed, a meta GRS for CAD consisting of 1.7 million single nucleotide variants (SNPs) achieved a hazard ratio (HR) of 1.71 (95% CI 1.68–1.73) per standard deviation of the score.[29] The HR obtained with the latest metaGRS for stroke is considerably lower (HR=1.26; 1.22–1.31; c-index=0.585)[30] but likely to increase as information from additional GWAS will be integrated. PRS for cardiovascular disease (CVD) typically perform better than individual traditional risk factors (e.g. BP),[29, 30] and can be added into risk models as an essentially independent factor ([31] and Abraham et al. XXX-XXX).[22] In fact, PRS for CVD consistently increase predictive power when added to established clinical risk scores ([32] and Abraham et al. XXX-XXX).[22]

PRS offer distinct advantages over traditional risk factors: genetic risk is present from birth and essentially stable over time, whereas traditional risk factors may vary over time requiring multiple measurements. PRS can be ascertained by a single genotyping effort long before traditional risk factors manifest enabling early decisions on lifestyle interventions and targeted monitoring.[30, 33] In fact, there is evidence that addition of a PRS for CVD to conventional risk prediction models in the general population could help reducing cardiovascular events[34] while also being economically meaningful.[35] However, important questions remain that need be addressed before implementing these scores in clinical practice including validation across different ancestries, sex-specific aspects, and issues related to the deployment and communication of PRS results to individuals (https://doi.org/10.1101/2020.09.18.20197137; Abraham et al. XXX-XXX).[22]

Mendelian randomization – exploring causal relationships

As well-powered GWAS have accumulated for a variety of traits of interest in stroke and cerebrovascular disease, a specific use case for PRS has emerged via Mendelian Randomization (MR).[36] MR is an epidemiologic approach that uses genetic variants known to be associated with an exposure of interest to test for a causal effect of those variants on disease risk or other outcomes.[37] As a form of instrumental variable analysis, a PRS that explains a portion of the observed variance in a trait, such as plasma HDL levels, can be used to examine the causal role of a similar amount of variance in that trait in disease risk, such as myocardial infarction (MI), testing the causality of HDL levels on MI risk in a manner not possible using observational biomarker data alone.[38] Because an individual's germ-line genotypes cannot be modified by behaviour or disease, associations made in this methodological framework can be consistent with a directional or causal process, but only if a series of important assumptions are met.[39] Genetic variants must only act on the outcome

through their effect on a risk factor (independence), they must explain a reasonable degree of the variance in the risk factor (strong/weak instruments), and the variants cannot have effects on other risk factors that are themselves associated with the outcome (exclusion restriction). This last assumption can be particularly challenging, as genetic variants often impact multiple biological processes (termed horizontal pleiotropy) which may themselves be known or unknown.[4039] If a set of genetic variants were associated with alcohol intake, but also cryptically associated with tobacco intake, an observed association between genetic predisposition to alcohol intake and heart disease could be invalidated by pleiotropic associations with smoking. Fortunately, statistical tools and best practices continue to evolve that can aid in the validation of the underlying assumptions of MR and facilitate its use in exploring causal inferences between risk factors and disease in observational studies.[41] As GWAS samples sizes for human traits increase, the proportion of variance explained by PRS for these traits likewise increases, maximizing statistical power for novel causal inference and even providing opportunities to perform mediation analyses within the framework of MR for variants and traits with known confounding relationships with other risk factors. Two-sample MR utilizes genetic variants associated with a risk factor of interest from a GWAS in one population and uses them to test for association with outcomes in an independent population, allowing the genetic variants to "stand in" for biological variance in an unmeasured exposure.[42] As an example of the latent power of this approach, a recent MR analysis of serum lipid levels on both chronic and acute forms of cerebral SVD found that genetic predisposition to higher levels of HDL-C were associated with reduced risk of small vessel ischaemic stroke and lower white matter hyperintensity volumes, even after adjustment for LDL-C and triglycerides.[43] While the contributions of specific HDL subcomponents remain to be clarified, these results support a potential independent causal role of HDL in protection from cerebral SVD, and provide at least some evidence to support further investigation in formal randomized controlled trials (RCTs).

Intracranial aneurysms – sample size remains key

Of particular interest to the development of clinical applications of genomic medicine are conditions with a high heritability. Aneurysmal subarachnoid haemorrhage, due to rupture of an intracranial aneurysm (IA), is highly heritable[44] with common variants explaining about 25% of the disease.[45] The most recent GWAS meta-analysis on IA included individuals from multiple ancestries integrating the majority of previous GWAS studies.[45] Overall, this study found 17 risk loci, 11 of which were new. Further analyses of putative causative genes pointed to a prominent role of endothelial cells (ECs) in disease pathogenesis (see article by Bakker et al. on p.XXXXX [24] and [45]). Several of these genes are involved in cell signalling including the sensing of mechanical stress, which might contribute to aneurysm formation or rupture through vascular pressure sensing. An important insight from this study is a high degree of genetic correlation between ruptured and unruptured IA implying that the genetic architecture of ruptured and unruptured aneurysms is very similar. The study further found considerable genetic overlap of IA with ischemic stroke, ICH, and abdominal aortic aneurysm, which was largely accounted for by genes implicated in BP regulation and smoking.[45] As such, the available genetic evidence complements epidemiological findings, which point to a causal role of smoking and hypertension in IA

and aneurysmal subarachnoid haemorrhage. Notably, this risk can be modified by lifestyle interventions and medical therapy. Given the high heritability of IA, genetic risk prediction by means of PRS seems a logical development. The development of a powerful PRS will depend on the availability of larger genetic datasets, which can be expected over the next years. PRS could then be integrated into screening strategies for IA in selected populations although this would need to be tested in prospective studies.

Genetics of stroke outcome – the next big thing

As outlined above, there is great demand for the development of neuroprotective agents. Recent GWAS in mostly Caucasian populations have identified an initial set of genes and risk loci that were significantly associated with clinical or radiological outcomes after stroke thus demonstrating the feasibility of such studies.[46–49] In some instances these findings could be related to biological processes that show strong candidacy for influencing stroke outcome (reviewed in Lee et al. Stroke 2021).[25] By design, stroke outcome studies are conducted within cases, i.e. without a healthy control group. Key variables to consider include time from stroke onset and status at baseline. Accordingly, dynamic measures such as the change in NIHSS from 6 hours to 24 hours after stroke (ΔNIHSS24h) have been proposed as key readouts and there is good reasoning for using the ΔNIHSS24h as a primary outcome measure in genetic studies (reviewed in Lee et al. Stroke 2021).[25] Still, other measures such as hemorrhagic transformation[47] or long-term outcomes (clinical and imaging-defined) remain important alternatives as they capture complementary aspects of early injury, secondary injury (including secondary neurodegeneration) and recovery. Another readout that has repeatedly been proposed is serum neurofilament light (sNfL), a marker for neuroaxonal injury that was shown to reflect primary and secondary neuronal injury after ischemic stroke and can be quantified with high accuracy.[50] Quantitative trait GWAS have distinct advantages over binary case-control GWAS by providing more statistical power. Yet, sample sizes for stroke outcome studies have been at the lower end and there is a requirement for further harmonization across studies as highlighted by the Global Alliance in Acute and Long-term Outcome (https://genestroke.wixsite.com/alliesinstroke) initiative. While still at the beginning, the field of stroke outcome genetics is already receiving considerable interest by pharmaceutical industry highlighting its potential for drug discovery.

Leveraging genetics for drug development

Less than 10% of drugs move from phase I to marketing authorization[51, 52] and this rate is even lower for CVD.[52] Human genetics has become a crucial source of evidence for guiding the selection of targets for drug development and prioritizing their use in clinical trials. In fact, compounds targeting candidates with evidence from human genetics have a several-fold probability to reach approval than those without such evidence.[53, 54] In this issue, Georgakis and colleagues discuss the potential of Mendelian randomization for drug discovery (see Georgakis et al. p. XXXXX).[21] MR continues to have great potential for candidate selection as illustrated by a recent MR study of ATP citrate lyase and [CVD55] that was published in parallel with the results of the CLEAR (Cholesterol Lowering via Bempedoic Acid, an

ACL-Inhibiting Regimen) Harmony trial and that essentially predicted and explained the results of the trial.[51,56, 57]

Yet, there are alternative approaches with a successful track record in CVD. One of them involves naturally occurring human genetic variants that are predicted to inactivate protein-coding genes.[58] Perhaps the most compelling example is PCSK9. Discovered as a gene implicated in familial hypercholesterolemia in 2003,[59] it was subsequently shown that loss-of- function (LoF) variants in PCSK9 are associated with a reduced risk for CAD.[60] Within less than 10 years two monoclonal antibodies targeting the gene product were approved for clinical use with large prospective RCTs pointing to improved cardiovascular outcomes only shortly thereafter.[61, 62] The identification of individuals with two loss-of-function PCSK9 alleles who suffered from no apparent adverse health consequences[63–65] provided strong assurance for a beneficial safety profile early in the drug development process. Additional examples of complete loss-of-function ("knock-out") with implications for drug development include nonsense mutations in ANGPTL3[65–68] and APOC3.[69] As sequencing efforts in large-scale biobanks continue, additional human knock-outs for genes relevant to CAD and stroke drug development can be expected to emerge. Such datasets are currently generated through governmental (e.g. the UK Biobank), commercial (e.g. DeCode genetics) and institutional (e.g. Kaiser Permanente Research Bank) funding,[8] as well as through collaborative efforts across large deeply phenotyped cohort studies, such as the Trans-Omics for Precision Medicine (TOPMed) programme.[70]

Large-scale genetic data from prospective biobank cohorts further allow predicting unanticipated effects of drugs through a study design termed Phenome-wide association study (PheWAS). In such studies, one or multiple genetic variants are tested for their associations with hundreds of phenotypes spanning multiple organ systems.[65, 71] This allows exploring desirable and undesirable (adverse) clinical outcomes linked to loss-of-function of a given gene.

For instance, a PheWAS in more than 100.000 individuals from the UK Biobank showed that beyond their effects on CAD, genetically lowered Lp(a) levels are associated with a lower risk of stroke, peripheral vascular disease, heart failure, and aortic stenosis.[72] Another PheWAS identified a previously unknown effect of nondihydropyridine calcium channel blockers on diverticulosis risk that was subsequently confirmed in observational cohorts.[73] The field of genetics and its use for drug discovery is rapidly developing as highlighted by recent topical reviews.[51, 58, 65]

Pharmacogenomics

The study of how an individual's genome influences the activity, safety, or efficacy of a drug is termed pharmacogenomics. Pharmacogenomics is a broadening of the prior field of pharmacogenetics, which technically refers to the way in which variants in a single gene affect response to a drug. Both pharmacogenetics and pharmacogenomics appeal to the concept that genetic information can "personalize" medical decision-making, helping clinicians choose the correct drug, at the correct dose, to maximize therapeutic benefit and minimize side-effects or adverse outcomes. While somatic cancer mutations are

frequently utilized to select chemotherapy regimens, such as the targeting of HER2-positive breast cancer with trastuzumab.[74] there are relatively fewer examples of using germ-line variation to guide medication selection in standard clinical practice. The United States Food and Drug Administration currently requires that 12 medications include boxed warnings regarding pharmacogenomic biomarkers, out of a total of 457 that include pharmacogenomic information in the product labels. One prominent example is the FDA recommendation for *HLA-B*1502* genotyping in individuals of Asian ancestry being considered for treatment with carbamazepine.[75] Individuals harboring this variant, which can approach 10% of Asian ancestry populations, are at an increased risk of Stevens-Johnson Syndrome and toxic epidermal necrolysis when exposed to carbamazepine and FDA guidance suggests that carriers of *HLA- B*1502* not be treated with this drug. Other implementations of pharmacogenomics include clinical trial-driven support for the use of genomics to guide medication dosing and even therapeutic decision-making.

Several genes involved in warfarin metabolism, including *VKORC1*, *CPY2C9*, *CYP4F2* have been shown to impact dosing regimens required to maintain adequate anticoagulation via the International Normalized Ratio (INR).[76] The GIFT (Genetic Informatics Trial) RCT examined an aggregate genetic score of variants in these genes as a means to guide warfarin dosing, demonstrating a significant improvement in a composite outcome of INR ≥ 4, major bleeding, venous thromboembolism, and death in the genotype-informed dosing arm.[77] Further implementation studies have shown this genotype-guided approach to warfarin dosing to be feasible and even cost-effective, although these results are perhaps of diminishing significance with the interim rise of direct oral anticoagulant options.[78]

In stroke and CVD, a prominent example of pharmacogenomics has arisen from the observation of reduced platelet inhibitory effect of clopidogrel in individuals harboring *CYP2C19*2* and **3* gene mutations.[79] These loss-of-function mutations result in reduced metabolism of clopidogrel into its active metabolite, and carriers of these variants have been shown to exhibit increased incidence of cardiovascular events and stroke while on clopidogrel.[80] The recent TAILOR-PCI RCT was designed to prospectively test whether *CYP2C19* genotype-guided antiplatelet use would lead to improved outcomes after percutaneous coronary intervention compared with a genotype-agnostic approach.[81] While the primary composite ischemic endpoint did not reveal a significant difference in outcomes between groups at 12 months, there was an 80% reduction in ischemic events in the genotype-guided group in the first 3-months that was subsequently lost over the remainder of follow-up. The results of TAILOR-PCI may be considered disappointing by those advocating for increasing uptake in genotype-directed therapy in stroke and other vascular diseases, but this observed early benefit in ischemic events suggests that there may be more specific use-cases where pharmacogenomic patient benefit could be demonstrated.

Conclusions

As can be seen from these brief highlights and in detail in the accompanying articles, the field of medical and population genetics in stroke is moving at a rapid pace with multiple current and near-term opportunities to influence and improve the care and treatment of stroke and related vascular diseases. These advancements have in large

part been triggered by substantial investments in recruitment and biobanking of genetic information on increasingly large and well-characterized populations in both health and disease and facilitated by collaboration in consortia such as the International Stroke Genetics Consortium (ISGC, www.strokegenetics.org). It is both the ascertainment of data and its broad sharing that has been so critical to innovation. Summary genotype data from MEGASTROKE, currently the largest available GWAS of stroke and its subtypes, is available for download (www.megastroke.org) and this and 18 other stroke genetic datasets are also shared at the Cerebrovascular Disease Knowledge Portal (http://cd.hugeamp.org). The UK Biobank contains genetic data and a plethora of stroke-relevant phenotypes for analysis available by completing a web-based application. But there remains work to be done in this regard. While national biobanks like China-Kadoorie (https://www.ckbiobank.org), Biobank Japan (https://biobankjp.org/), or international consortia such as H3Africa (https://h3africa.org/) are providing precious information on genetic risk for human disease in East Asian and African populations, the vast majority of available genome-wide datasets continue to be of predominantly white European ancestry. Diversification of genetic research holds great promise not only for improvement in understanding of disease risk in understudied populations, but in all populations through approaches such as trans-ethnic fine-mapping and meta-analysis.[82]

Substantial progress has also been made in deciphering the genetic determinants of MRI-markers of covert cerebral SVD, such as white matter hyperintensity volume, through collaborative efforts in population-based brain imaging studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium,[83] the UK Biobank,[84]and the ISGC. So far, these studies have been more powerful than genetic studies of small vessel stroke and have revealed that SVD genes already have a significant impact on white matter microstructure in young adults. Identifying early predictors of stroke and vascular cognitive impairment could have major implications for our understanding of disease mechanisms across the lifespan and for devising effective and timely prevention strategies.

As work proceeds to map genotypes to traits, the next great frontier must be to transform these associations into biological understanding. The International Common Disease Alliance (ICDA, www.icda.bio) has established the “Maps to Mechanisms to Medicine” initiative to define barriers, identify opportunities, and coordinate partnerships to guide the field of medical genetics through the transition from variant to function. A substantial component of this transition will require the incorporation of additional high-throughput molecular data from transcriptomics, epigenomics, proteomics, and metabolomics, to capture a greater spectrum of effects induced by genome variation. Understanding the biological mechanisms through which variants impact disease risk and outcome will greatly enhance not only our conception of the basis of human disease, but also the efficiency with which we can translate these discoveries into novel therapeutics. Moreover, identifying circulating biomarkers of stroke capturing simultaneously inherited and environmental characteristics may lead to rapid applications for routine research or clinical practice given ease of access and low cost.

Finally, disease risk is imparted by both genetic and non-genetic exposures, and advances in genetics are being met by advances in understanding and quantification of the environmental exposures that often factor heavily in disease risk, both directly and through gene-environment interactions.[85] Social determinants of health (SDOH) have emerged as a critical component of potentially modifiable disease risk that are inequitably distributed across our populations. Hypertension, a critical risk factor for stroke, is a condition particularly impacted by SDOH,[86] and better understanding of the genetic and environmental factors contributing to blood pressure could improve risk modeling as well as stratification of vulnerable populations for targeted intervention to reduce the risk of stroke and related vascular and cognitive diseases.

Acknowledgments

Sources of Funding

MD received funding from the European Union's Horizon 2020 research and innovation programme No 666881, SVDs@target and 667375, CoSTREAM; the Deutsche Forschungsgemeinschaft (DFG) as part of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198), the CRC 1123 (B3) and DI 722/13–1, and the Vascular Dementia Research Foundation. NB received funding from the DFG (BE 6169 1–1). SD received funding from the French National Research Agency (ANR) as part of the "Investment for the Future Programme" ANR-18-RHUS-0002, the EU Joint Programme - Neurodegenerative Disease Research (JPND) and funding from the European Union's Horizon 2020 research and innovation programme under grant agreements No 643417, 640643, 667375, and 754517. CDA received funding from the NIH of the United States (R01NS103924, U01NS069763), the American Heart Association (18SFRN34250007, 21SFRN812095), and Massachusetts General Hospital.

Non-standard Abbreviations and Acronyms

SVD	small vessel disease
GWAS	genome-wide association studies
HDAC	Histone deacetylase
PRS	polygenic risk score
GRS	genomic risk score
BP	blood pressure
LDL	low density lipoprotein
CAD	coronary artery disease
HR	hazard ratio
CVD	cardiovascular disease
MR	Mendelian randomization
MI	myocardial infarction
IA	intracranial aneurysm
ICH	intracerebral haemorrhage

NIHSS	National Institutes of Health Stroke Scale
sNfL	serum neurofilament light
LoF	loss-of-function
PheWAS	Phenome-wide association study
Lp	Lipoprotein
FDA	United States Food and Drug Administration
ISGC	International Stroke Genetics Consortium

References

1. Tymianski M. Novel approaches to neuroprotection trials in acute ischemic stroke.Stroke; a journal of cerebral circulation2013;44:2942–2950
2. Savitz SI, Baron JC, Yenari MA, Sanossian N, Fisher M. Reconsidering neuroprotection in the reperfusion era.Stroke; a journal of cerebral circulation2017;48:3413–3419
3. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: Mechanisms and clinical implications.Lancet neurology2019;18:684–696 [PubMed: 31097385]
4. Dichgans M, Leys D. Vascular cognitive impairment.Circulation research2017;120:573–591 [PubMed: 28154105]
5. Ridker PM. Anticytokine agents: Targeting interleukin signaling pathways for the treatment of atherothrombosis.Circulation research2019;124:437–450 [PubMed: 30702995]
6. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al.Antiinflammatory therapy with canakinumab for atherosclerotic disease.The New England journal of medicine2017;377:1119–1131 [PubMed: 28845751]
7. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, Pinto FJ, Ibrahim R, Gamra H, Kiwan GS, et al.Efficacy and safety of low-dose colchicine after myocardial infarction.The New England journal of medicine2019;381:2497–2505 [PubMed: 31733140]
8. Dichgans M, Pulit SL, Rosand J. Stroke genetics: Discovery, biology, and clinical applications.Lancet neurology2019;18:587–599 [PubMed: 30975520]
9. Lacombe P, Oligo C, Domenga V, Tournier-Lasserve E, Joutel A. Impaired cerebral vasoreactivity in a transgenic mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy arteriopathy.Stroke; a journal of cerebral circulation2005;36:1053–1058
10. Ratelade J, Klug NR, Lombardi D, Angelim M, Dabertrand F, Domenga-Denier V, Al-Shahi Salman R, Smith C, Gerbeau JF, Nelson MT, et al.Reducing hypermuscularization of the transitional segment between arterioles and capillaries protects against spontaneous intracerebral hemorrhage.Circulation2020;141:2078–2094 [PubMed: 32183562]
11. Beaufort N, Scharrer E, Kremmer E, Lux V, Ehrmann M, Huber R, Houlden H, Werring D, Haffner C, Dichgans M. Cerebral small vessel disease-related protease htra1 processes latent tgf-beta binding protein 1 and facilitates tgf-beta signaling.Proceedings of the National Academy of Sciences of the United States of America2014;111:16496–16501 [PubMed: 25369932]
12. Dabertrand F, Harraz OF, Koide M, Longden TA, Rosehart AC, Hill-Eubanks DC, Joutel A, Nelson MT. Pip(2) corrects cerebral blood flow deficits in small vessel disease by rescuing capillary kir2.1 activity.Proceedings of the National Academy of Sciences of the United States of America2021;118
13. Malik R, Rannikmae K, Traylor M, Georgakis MK, Sargurupremraj M, Markus HS, Hopewell JC, Debette S, Sudlow CLM, Dichgans M, et al.Genome-wide meta- analysis identifies three novel loci associated with stroke.Annals of neurology2018;84:934–939 [PubMed: 30383316]

14. Traylor M, Persyn E, Tomppo L, Klasson S, Abedi V, Bakker MK, Torres N, Li L, Bell S, Rutten-Jacobs L, et al.Genetic basis of lacunar stroke: A pooled analysis of individual patient data and genome-wide association studies.Lancet neurology2021
15. Neurology Working Group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, the Stroke Genetics Network (SIGN), and the International Stroke Genetics Consortium (ISGC). Identification of additional risk loci for stroke and small vessel disease: A meta-analysis of genome-wide association studies.Lancet neurology2016;15:695–707 [PubMed: 27068588]
16. Reyahi A, Nik AM, Ghiami M, Gritli-Linde A, Ponten F, Johansson BR, Carlsson P. Foxf2 is required for brain pericyte differentiation and development and maintenance of the blood-brain barrier.Dev Cell2015;34:19–32 [PubMed: 26120030]
17. Azghandi S, Prell C, van der Laan SW, Schneider M, Malik R, Berer K, Gerdes N, Pasterkamp G, Weber C, Haffner C, et al.Deficiency of the stroke relevant hdac9 gene attenuates atherosclerosis in accord with allele-specific effects at 7p21.1.Stroke; a journal of cerebral circulation2015;46:197–202
18. Asare Y, Campbell-James TA, Bokov Y, Yu LL, Prestel M, El Bounkari O, Roth S, Megens RTA, Straub T, Thomas K, et al.Histone deacetylase 9 activates ikk to regulate atherosclerotic plaque vulnerability.Circulation research2020;127:811–823 [PubMed: 32546048]
19. Prestel M, Prell-Schicker C, Webb T, Malik R, Lindner B, Ziesch N, Rex-Haffner M, Röh S, Viturawong T, Lehm M, et al.The atherosclerosis risk variant rs2107595 mediates allele-specific transcriptional regulation of hdac9 via e2f3 and rb1.Stroke; a journal of cerebral circulation2019;50:2651–2660
20. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, Rutten- Jacobs L, Giese AK, van der Laan SW, Gretarsdottir S, et al.Multiancestry genome- wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes.Nature genetics2018;50:524–537 [PubMed: 29531354]
21. Georgakis M, Gill D. Mendelian randomization studies in stroke: Exploration of risk factors and drug targets with human genetic data.Stroke; a journal of cerebral circulation2021
22. Abraham G, Rutten-Jacobs L, Inouye M. Risk prediction using polygenic risk scores for prevention of stroke and other cardiovascular diseases.Stroke; a journal of cerebral circulation2021
23. Guey S, Lesnik Oberstein S, Tournier-Lasserve E, Chabriat H. Hereditary cerebral small vessel disease and stroke: A guide for diagnosis and management.Stroke; a journal of cerebral circulation2021
24. Bakker M, Ruigrok Y. Genetics of intracranial aneurysms Stroke; a journal of cerebral circulation2021
25. Lee J-M, Fernandez-Cadenas I, Lindgren A. Using human genetics to understand mechanisms in ischemic stroke outcome: From early brain injury to long-term recovery.Stroke; a journal of cerebral circulation2021
26. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores.Nature reviews. Genetics2018;19:581–590
27. Chatterjee N, Shi J, García-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention.Nature reviews. Genetics2016;17:392–406
28. Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities.Nature genetics2019;51:584–591 [PubMed: 30926966]
29. Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, Lai FY, Kaptoge S, Brozynska M, Wang T, et al.Genomic risk prediction of coronary artery disease in 480,000 adults: Implications for primary prevention.Journal of the American College of Cardiology2018;72:1883–1893 [PubMed: 30309464]
30. Abraham G, Malik R, Yonova-Doing E, Salim A, Wang T, Danesh J, Butterworth AS, Howson JMM, Inouye M, Dichgans M. Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke.Nat Commun2019;10:5819 [PubMed: 31862893]
31. Marston NA, Patel PN, Kamanu FK, Nordio F, Melloni GM, Roselli C, Gurmu Y, Weng LC, Bonaca MP, Giugliano RP, et al.Clinical application of a novel genetic risk score for

ischemic stroke in patients with cardiometabolic disease.Circulation2021;143:470–478 [PubMed: 33185476]

32. Abraham G, Havulinna AS, Bhalala OG, Byars SG, De Livera AM, Yetukuri L, Tikkanen E, Perola M, Schunkert H, Sijbrands EJ, et al.Genomic prediction of coronary heart disease.European heart journal2016;37:3267–3278 [PubMed: 27655226]
33. Rutten-Jacobs LC, Larsson SC, Malik R, Rannikmäe K, Sudlow CL, Dichgans M, Markus HS, Traylor M. Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: Cohort study of 306 473 uk biobank participants.Bmj2018;363:k4168 [PubMed: 30355576]
34. Sun L, Pennells L, Kaptoge S, Nelson CP, Ritchie SC, Abraham G, Arnold M, Bell S, Bolton T, Burgess S, et al.Polygenic risk scores in cardiovascular risk prediction: A cohort study and modelling analyses.PLoS Med2021;18:e1003498 [PubMed: 33444330]
35. Hynninen Y, Linna M, Vilkkumaa E. Value of genetic testing in the prevention of coronary heart disease events.PloS one2019;14:e0210010 [PubMed: 30645616]
36. Davies NM, Holmes MV, Davey Smith G. Reading mendelian randomisation studies: A guide, glossary, and checklist for clinicians.Bmj2018;362:k601 [PubMed: 30002074]
37. Burgess S, Butterworth A, Malarstig A, Thompson SG. Use of mendelian randomisation to assess potential benefit of clinical intervention.Bmj2012;345:e7325 [PubMed: 23131671]
38. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Holm H, Ding EL, Johnson T, et al.Plasma hdl cholesterol and risk of myocardial infarction: A mendelian randomisation study.Lancet2012;380:572–580 [PubMed: 22607825]
39. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in mendelian randomization studies.Human molecular genetics2018;27:R195–r208 [PubMed: 29771313]
40. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization.JAMA : the journal of the American Medical Association2017;318:1925–1926 [PubMed: 29164242]
41. Grover S, Del Greco MF, Stein CM, Ziegler A. Mendelian randomization.Methods Mol Biol2017;1666:581–628 [PubMed: 28980266]
42. Gleason KJ, Yang F, Chen LS. A robust two-sample transcriptome-wide mendelian randomization method integrating gwas with multi-tissue eqtl summary statistics.Genetic epidemiology2021
43. Georgakis MK, Malik R, Anderson CD, Parhofer KG, Hopewell JC, Dichgans M. Genetic determinants of blood lipids and cerebral small vessel disease: Role of high- density lipoprotein cholesterol.Brain : a journal of neurology2020;143:597–610 [PubMed: 31968102]
44. Korja M, Silventoinen K, McCarron P, Zdravkovic S, Skytthe A, Haapanen A, de Faire U, Pedersen NL, Christensen K, Koskenvuo M, et al.Genetic epidemiology of spontaneous subarachnoid hemorrhage: Nordic twin study.Stroke; a journal of cerebral circulation2010;41:2458–2462
45. Bakker MK, van der Spek RAA, van Rheenen W, Morel S, Bourcier R, Hostettler IC, Alg VS, van Eijk KR, Koido M, Akiyama M, et al.Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors.Nature genetics2020;52:1303–1313 [PubMed: 33199917]
46. Mola-Caminal M, Carrera C, Soriano-Tárraga C, Giralt-Steinhauer E, Díaz-Navarro RM, Tur S, Jiménez C, Medina-Dols A, Cullell N, Torres-Aguila NP, et al.Patj low frequency variants are associated with worse ischemic stroke functional outcome.Circulation research2019;124:114–120 [PubMed: 30582445]
47. Carrera C, Cárcel-Márquez J, Cullell N, Torres-Águila N, Muiño E, Castillo J, Sobrino T, Campos F, Rodríguez-Castro E, Llucia-Carol L, et al.Single nucleotide variations in zbtb46 are associated with post-thrombolytic parenchymal haematoma.Brain : a journal of neurology2021
48. Söderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, Cole JW, Fernandez-Cadenas I, Hankey GJ, Jimenez-Conde J, et al.Genome-wide association meta-analysis of functional outcome after ischemic stroke.Neurology2019;92:e1271–e1283 [PubMed: 30796134]
49. Ibanez L, Heitsch L, Carrera C, Farias FHG, Dhar R, Budde J, Bergmann K, Bradley J, Harari O, Phuah CL, et al.Multi-ancestry genetic study in 5,876 patients identifies an association between excitotoxic genes and early outcomes after acute ischemic stroke.medRxiv2020

50. Tiedt S, Duering M, Barro C, Kaya AG, Boeck J, Bode FJ, Klein M, Dorn F, Gesierich B, Kellert L, et al.Serum neurofilament light: A biomarker of neuroaxonal injury after ischemic stroke.Neurology2018
51. Holmes MV, Richardson TG, Ference BA, Davies NM, Davey Smith G. Integrating genomics with biomarkers and therapeutic targets to invigorate cardiovascular drug development.Nat Rev Cardiol2021
52. Dowden H, Munro J. Trends in clinical success rates and therapeutic focus.Nature reviews. Drug discovery2019;18:495–496
53. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, Floratos A, Sham PC, Li MJ, Wang J, et al.The support of human genetic evidence for approved drug indications.Nature genetics2015;47:856–860 [PubMed: 26121088]
54. King EA, Davis JW, Degner JF. Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval.PLoS genetics2019;15:e1008489 [PubMed: 31830040]
55. Ference BA, Ray KK, Catapano AL, Ference TB, Burgess S, Neff DR, Oliver- Williams C, Wood AM, Butterworth AS, Di Angelantonio E, et al.Mendelian randomization study of acly and cardiovascular disease.The New England journal of medicine2019;380:1033–1042 [PubMed: 30865797]
56. Ray KK, Bays HE, Catapano AL, Lalwani ND, Bloedon LT, Sterling LR, Robinson PL, Ballantyne CM. Safety and efficacy of bempedoic acid to reduce ldl cholesterol.The New England journal of medicine2019;380:1022–1032 [PubMed: 30865796]
57. Holmes MV. Human genetics and drug development.The New England journal of medicine2019;380:1076–1079 [PubMed: 30865805]
58. Minikel EV, Karczewski KJ, Martin HC, Cummings BB, Whiffin N, Rhodes D, Alföldi J, Trembath RC, van Heel DA, Daly MJ, et al.Evaluating drug targets through human loss-of-function genetic variation.Nature2020;581:459–464 [PubMed: 32461653]
59. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, et al.Mutations in pcsk9 cause autosomal dominant hypercholesterolemia.Nature genetics2003;34:154–156 [PubMed: 12730697]
60. Cohen JC, Boerwinkle E, Mosley TH Jr., Hobbs HH. Sequence variations in pcsk9, low ldl, and protection against coronary heart disease.The New England journal of medicine2006;354:1264–1272 [PubMed: 16554528]
61. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, et al.Evolocumab and clinical outcomes in patients with cardiovascular disease.The New England journal of medicine2017;376:1713–1722 [PubMed: 28304224]
62. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Goodman SG, Hanotin C, Harrington RA, et al.Alirocumab and cardiovascular outcomes after acute coronary syndrome.The New England journal of medicine2018;379:2097–2107 [PubMed: 30403574]
63. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, Cohen JC, Hobbs HH. Molecular characterization of loss-of-function mutations in pcsk9 and identification of a compound heterozygote.American journal of human genetics2006;79:514–523 [PubMed: 16909389]
64. Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. The c679x mutation in pcsk9 is present and lowers blood cholesterol in a southern african population.Atherosclerosis2007;193:445–448 [PubMed: 16989838]
65. Musunuru K, Kathiresan S. Genetics of common, complex coronary artery disease.Cell2019;177:132–145 [PubMed: 30901535]
66. Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C, Garimella KV, Fisher S, Abreu J, Barry AJ, et al.Exome sequencing, angptl3 mutations, and familial combined hypolipidemia.The New England journal of medicine2010;363:2220–2227 [PubMed: 20942659]
67. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, McCarthy S, Van Hout CV, Bruse S, Dansky HM, et al.Genetic and pharmacologic inactivation of angptl3 and cardiovascular disease.The New England journal of medicine2017;377:211–221 [PubMed: 28538136]

68. Graham MJ, Lee RG, Brandt TA, Tai LJ, Fu W, Peralta R, Yu R, Hurh E, Paz E, McEvoy BW, et al.Cardiovascular and metabolic effects of angptl3 antisense oligonucleotides.The New England journal of medicine2017;377:222–232 [PubMed: 28538111]
69. Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, Won HH, Karczewski KJ, O'Donnell-Luria AH, Samocha KE, et al.Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity.Nature2017;544:235–239 [PubMed: 28406212]
70. Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, Taliun SAG, Corvelo A, Gogarten SM, Kang HM, et al.Sequencing of 53,831 diverse genomes from the nhlbi topmed program.Nature2021;590:290–299 [PubMed: 33568819]
71. Denny JC, Ritchie MD, Basford MA, Pulley JM, Bastarache L, Brown-Gentry K, Wang D, Masys DR, Roden DM, Crawford DC. Phewas: Demonstrating the feasibility of a phenome-wide scan to discover gene-disease associations.Bioinformatics2010;26:1205–1210 [PubMed: 20335276]
72. Emdin CA, Khera AV, Natarajan P, Klarin D, Won HH, Peloso GM, Stitziel NO, Nomura A, Zekavat SM, Bick AG, et al.Phenotypic characterization of genetically lowered human lipoprotein(a) levels.Journal of the American College of Cardiology2016;68:2761–2772 [PubMed: 28007139]
73. Gill D, Georgakis MK, Koskeridis F, Jiang L, Feng Q, Wei W-Q, Theodoratou E, Elliott P, Denny JC, Malik R, et al.Use of genetic variants related to antihypertensive drugs to inform on efficacy and side effects.Circulation2019;140:270–279 [PubMed: 31234639]
74. Bredin P, Walshe JM, Denduluri N. Systemic therapy for metastatic her2-positive breast cancer.Semin Oncol2020;47:259–269 [PubMed: 32896428]
75. Drozda K, Müller DJ, Bishop JR. Pharmacogenomic testing for neuropsychiatric drugs: Current status of drug labeling, guidelines for using genetic information, and test options.Pharmacotherapy2014;34:166–184 [PubMed: 24523097]
76. Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, Lee MT, Gage BF, Kimmel SE, Perera MA, et al.Clinical pharmacogenetics implementation consortium (cpic) guideline for pharmacogenetics-guided warfarin dosing: 2017 update.Clinical pharmacology and therapeutics2017;102:397–404 [PubMed: 28198005]
77. Gage BF, Bass AR, Lin H, Woller SC, Stevens SM, Al-Hammadi N, Li J, Rodríguez T Jr., Miller JP, McMillin GA, et al.Effect of genotype-guided warfarin dosing on clinical events and anticoagulation control among patients undergoing hip or knee arthroplasty: The gift randomized clinical trial.JAMA : the journal of the American Medical Association2017;318:1115–1124 [PubMed: 28973620]
78. Jorgensen AL, Prince C, Fitzgerald G, Hanson A, Downing J, Reynolds J, Zhang JE, Alfirevic A, Pirmohamed M. Implementation of genotype-guided dosing of warfarin with point-of-care genetic testing in three uk clinics: A matched cohort study.BMC Med2019;17:76 [PubMed: 30961588]
79. Ross S, Paré G. Pharmacogenetics of stroke.Stroke; a journal of cerebral circulation2018;49:2541–2548
80. Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, Cannon CP, Danchin N, Giusti B, Gurbel P, et al.Reduced-function cyp2c19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for pci: A meta-analysis.JAMA : the journal of the American Medical Association2010;304:1821–1830 [PubMed: 20978260]
81. Pereira NL, Farkouh ME, So D, Lennon R, Geller N, Mathew V, Bell M, Bae JH, Jeong MH, Chavez I, et al.Effect of genotype-guided oral p2y12 inhibitor selection vs conventional clopidogrel therapy on ischemic outcomes after percutaneous coronary intervention: The tailor-pci randomized clinical trial.JAMA : the journal of the American Medical Association2020;324:761–771 [PubMed: 32840598]
82. Schaid DJ, Chen W, Larson NB. From genome-wide associations to candidate causal variants by statistical fine-mapping.Nature reviews. Genetics2018;19:491–504
83. Sargurupremraj M, Suzuki H, Jian X, Sarnowski C, Evans TE, Bis JC, Eiriksdottir G, Sakaue S, Terzikhan N, Habes M, et al.Cerebral small vessel disease genomics and its implications across the lifespan.Nat Commun2020;11:6285 [PubMed: 33293549]

84. Persyn E, Hanscombe KB, Howson JMM, Lewis CM, Traylor M, Markus HS. Genome-wide association study of mri markers of cerebral small vessel disease in 42,310 participants.Nat Commun2020;11:2175 [PubMed: 32358547]

85. Juarez PD, Hood DB, Song MA, Ramesh A. Use of an exposome approach to understand the effects of exposures from the natural, built, and social environments on cardio-vascular disease onset, progression, and outcomes.Front Public Health2020;8:379 [PubMed: 32903514]

86. Krousel-Wood M, Craig LS, Peacock E, Zlotnick E, O'Connell S, Bradford D, Shi L, Petty R. Medication adherence: Expanding the conceptual framework.American journal of hypertension2021