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Genetic susceptibility to ischemic stroke

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Abstract

Clinicians who treat patients with stroke need to be aware of several single-gene disorders that have ischemic stroke as a major feature, including sickle cell disease, Fabry disease, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and retinal vasculopathy with cerebral leukodystrophy. The reported genome-wide association studies of ischemic stroke and several related phenotypes (for example, ischemic white matter disease) have shown that no single common genetic variant imparts major risk. Larger studies with samples numbering in the thousands are ongoing to identify common variants with smaller effects on risk. Pharmacogenomic studies have uncovered genetic determinants of response to warfarin, statins and clopidogrel. Despite increasing knowledge of stroke genetics, incorporating this new knowledge into clinical practice remains a challenge. The goals of this article are to review common single-gene disorders relevant to ischemic stroke, summarize the status of candidate gene and genome-wide studies aimed at discovering genetic stroke risk factors, and to briefly discuss pharmacogenomics related to stroke treatment.

Introduction

Stroke is a leading cause of death and a major cause of acquired disability in adults.¹ In the USA, 795,000 strokes occur every year, of which 610,000 are first-ever (incident) strokes. The incidence of stroke has steadily increased despite proven strategies to reduce the risk of this disease. Ischemic stroke represents at least 80% of all strokes.

Traditional factors that increase the risk of ischemic stroke—such as hypertension, atrial fibrillation, and cigarette smoking—are well-known. Many of these traditional risk factors are modifiable or avoidable. Evidence from twin, case-control and cohort studies of familial aggregation of stroke risk indicate that stroke may be the result of shared genetic and environmental factors.² Genetic risk factors are often considered not to be modifiable; however, knowledge of genetic risk factors can provide insights into pathophysiological pathways and targets for drug therapy. The classic example of risk genes leading to therapeutic targets is familial hypercholesterolemia.³ Thus, a public health impetus exists for defining genetic stroke risk (as exemplified by several initiatives to understand the underlying genetics of stroke; Boxes 1 and 2). Furthermore, the modern human in the industrialized world does not live in a state of nature, but rather in an ecosystem that

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Competing interests

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Author contributions

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includes routine exposure to medications (for example, statins, antihypertensive agents, and platelet antiaggregants). These exposures may modify genetic risk differently from traditional (known) risk factors. Means of defining inherited risk of stroke for this population include the study of pharmacogenomics.

Box 1

Prospects in gene discovery in ischemic stroke

Once a region of the genome has been linked with stroke, using either genome-wide linkage or genome-wide association approaches, the size of the interval is typically several hundred thousand bp. At this stage, dozens of genes may emerge (obvious and non-obvious candidates) that would be targets of interrogation. The approaches to enhancing the resolution of the genomic region include both dense single nucleotide polymorphism (SNP) mapping (using available SNP resources found in dbSNP,⁸⁶ HapMap⁸⁷ and 1000 Genomes⁸⁸ databases) and targeted resequencing of either coding regions (exons) or the entire interval (introns, exons, and intergenic regions). Massively parallel sequencing is becoming feasible on a genome-wide scale and has been used with some success in novel gene discovery for both autosomal and recessive rare disorders, clinical diagnosis of conditions such as primary ciliary dyskinesia, and molecular diagnosis of clinically recognizable conditions like Charcot–Marie–Tooth disease.⁸⁹ Massively parallel sequencing is now being applied to ischemic stroke as part of the Exome Sequencing Project (funded by the US National Heart, Lung and Blood Institute). The challenge will be to decipher which gene variants are benign and which are pathogenic in stroke. Strategies include filtering on function and frequency, ranking on conservation across species, and predicting the degree to which variants would alter protein structure and function.

Box 2

Initiatives in stroke genetics research

The US National Institute of Neurological Disorders and Stroke is funding an ongoing ischemic stroke genetics consortium known as the Stroke Genetics Network (SiGN).⁹⁰ This consortium is structured as a series of Genetic Research Centers (GRC) organized around a Data Coordinating Center. The GRCs contribute DNA samples for centralized genome-wide genotyping or data when such genotypic information is already available. The original Institute goal is to aggregate around 6,000 ischemic stroke cases. Most samples were collected under previous protocols, including hospital-based case series, population-based cross-sectional studies, and longitudinal cohort studies. A key goal is to have all stroke cases gathered under diverse protocols phenotyped uniformly. SiGN uses the Causative Classification of Stroke (CCS) system for subtyping ischemic stroke.⁹¹ Physician data abstractors enter pertinent clinical and diagnostic testing information into web-based case report forms, and subtyping is then automatically generated using a computerized algorithm. The CCS has web-based training and certification modules. As of 23rd March 2011, a total of 5,287 cases have been subtyped using this system. A random sample of charts is readjudicated by a central committee of vascular neurologists to assess agreement rates. SiGN will create a permanent global scientific resource by contributing to the Database of Genotype and Phenotype (dbGAP).⁹²

The aims of this review are to summarize the clinical genetics of single-gene disorders that can lead to ischemic stroke, to review the progress in and prospects for solving the complex

genetics of sporadic ischemic stroke, and to briefly discuss pharmacogenomics in the context of care of patients with stroke.

Single-gene disorders

In general, single-gene disorders are individually rare; however, collectively they represent important clinical conditions. Some single-gene disorders have specific treatments and, in many instances, conventional treatments may be ineffective or harmful. Understanding the pathophysiology of these single-gene disorders that can lead to stroke may provide insights into the mechanisms that underlie the more-common ischemic stroke.

CADASIL

The phenotypic manifestations of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) typically begin with migraine headache followed by ischemic stroke in the deep gray structures and subcortical white matter, and cognitive decline, ending in frank dementia. Apathy is evident in approximately two-fifths of patients with CADASIL, and further increases their disability.⁴

CADASIL is associated with mutations in the *NOTCH3* gene. Classically, disease-associated mutations in *NOTCH3* lead to an odd number of cysteine residues in the protein. NOTCH 3 forms a membrane-spanning heterodimer with an extracellular domain that has 34 epidermal growth factor (EGF)-like repeats and is noncovalently attached to the transmembrane portion. The *NOTCH3* gene has 33 exons, and most of the pathogenic mutations occur in exons 2–24 and do not affect NOTCH 3 receptor function. Exceptions to this rule are mutations in the Delta/Serrate/LAG-2 (DSL) ligand-binding domain, EGF 10–11, which can disrupt Notch 3 receptor function.⁵ Individuals with mutations in the DSL-binding domain demonstrate a clinical–radiological dissociation with greater preservation of cognitive performance (despite a greater burden of white matter involvement on imaging) than patients with other pathogenic mutations.

Genetic testing for common *NOTCH3* mutations that lead to CADASIL is available clinically, although skin biopsy remains a practical alternative for securing the diagnosis. A retrospective study of European patients with CADASIL demonstrated broad genetic heterogeneity with 34 different pathogenic mutations among 131 patients, but every case had diagnostic granular osmiophilic material on skin biopsy.⁶ Biopsy of the skin for CADASIL diagnosis should include the border zone between the deep dermis and upper subcutis. An immunostaining technique directed at EGF repeats 17–21 in *NOTCH3* has been developed that may improve the yield of a diagnostic skin biopsy.⁷

In addition to skin biopsy, other nonmolecular tests can assist in the screening and diagnosis of this genetic disorder. MRI is essential to screen for both overt disease and presymptomatic carriers. Patients have patchy and, ultimately, confluent hyperintensities in the white matter on fluid-attenuated inversion recovery imaging. Anterior temporal pole involvement is considered characteristic (O’Sullivan sign), but is absent in one-third of patients between the ages of 20 years and 29 years.⁸ The arteriopathy manifests by lacunes characterized as small subcortical hypointense lesions on T1-weighted imaging and by microbleeds characterized by signal dropout on T2-weighted gradient-echo sequences. Increases in small-vessel strokes, microbleeds and ventricular size correlate with cognitive decline.⁹

In summary, CADASIL is a rare but devastating cerebrovascular disorder that provides an opportunity to understand the mechanisms underlying other types of small-vessel arteriopathies, vascular dementia, and the relationship between migraine and stroke. The

most common causal mutations for CADASIL occur in a gene coding for a cell-surface receptor (*NOTCH3*) that is widely expressed on vascular smooth-muscle cells. Investigators using animal models of CADASIL are characterizing the mechanisms in CADASIL and, just as importantly, in related phenotypes.

CARASIL

Individuals with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) typically have alopecia beginning in the second decade, spondylosis in their second and third decades, ischemic cerebrovascular disease in their third decade, and dementia in their third through fifth decades. Mutations in the HtrA serine peptidase 1 (*HTRA1*) gene were identified as the cause of CARASIL through linkage analysis.¹⁰ Patients with *HTRA1* mutations have protein products that tend to be incapable of repressing signaling by the transforming growth factor β protein family. CARASIL was initially described in individuals of Asian descent, but a European pedigree was described in 2010.¹¹

Fabry disease

Fabry disease is caused by mutations in the gene that codes for the α -galactosidase A (*GLA*) enzyme. Phenotypically, cerebrovascular manifestations of Fabry disease include premature stroke, dolichoectasia, and white matter hyper-intensities.¹² In more than half of male and one-third of female cases of Fabry disease, stroke occurs before the condition is diagnosed.¹³ Stroke remains the leading cause of death in Fabry disease.

5-year data from the Fabry Outcome Survey showed that GLA enzyme replacement led to decreased left ventricular mass and increase in medial fractional shortening, among other cardiac parameters.¹⁴ Enzyme replacement also significantly reduced pain and improved quality of life. The influence of GLA replacement on stroke and other cerebrovascular manifestations of Fabry disease are not yet known.

The incidence of Fabry disease in patients with stroke varies across different study populations. A German study found Fabry disease in 4.9% of young men with crypto-genic ischemic stroke.¹⁵ An epidemiological study in the Baltimore–Washington area in the USA observed Fabry disease in only 0.18% of all ischemic and 0.65% of crypto-genic strokes.¹⁶ A Portuguese study found missense *GLA* mutations in 2.4% (95% CI 1.3–4.1%) of all consecutive patients aged 18–55 years with first-ever stroke of any type.¹⁷ In a Belgian registry of patients aged 18–60 years presenting to neurologists with stroke, unexplained white matter lesions or vertebrobasilar dolichoectasia, Fabry disease probably had a role in 1% of cases.¹⁸ More studies are needed to better understand which cerebrovascular patient populations should be screened for Fabry disease.

Retinal vasculopathy with cerebral leukodystrophy

Retinal vasculopathy with cerebral leukodystrophy (RVCL) is caused by mutations in the *TREX1* (3' repair exonuclease 1) gene, typically in the c-terminal domain, which leads to loss of perinuclear localization of the catalytically active TREX1 protein.¹⁹ This condition has had several alternative disease designations, including cerebroretinal vasculopathy syndrome, hereditary vascular retinopathy, and HERNs (hereditary endotheliopathy, retinopathy, nephropathy, and stroke). Patients typically present in their 4th decade with vision and memory loss, seizures, hemiparesis, and dysarthria. Death ensues within 5–10 years of presentation of initial symptoms. Brain imaging can reveal a tumefactive contrast-enhancing white matter lesion that can regress in size over several months.²⁰ Lesions on MRI scans can be mistaken for multiple sclerosis. Retinal findings can include neo-vascularization of the optic disc, retinal hemorrhages, and macular edema. Anecdotally,

intravitreal bevacizumab seems to be an effective treatment for the proliferative retinopathy associated with this disease.²¹

Sickle cell disease

Sickle cell disease, historically the first molecular disease to be described, is a condition that results from the substitution of valine for glutamic acid at position 6 of the β chain of hemoglobin (encoded by *HBB*). The mutation leads to aggregation of abnormal hemoglobin and changes in the shape and rheology of red blood cells. In addition to cerebrovascular complications, patients with sickle cell disease have hemolytic anemia, mild jaundice, and vaso-occlusive crises causing attacks of pain involving the chest, back and extremities.

The earliest description of symptoms consistent with sickle cell disease dates to 1670, with more-definitive descriptions emerging in the early 20th century.²² Genetic analysis supports multiple spontaneous mutations rather than a single founder effect. Haplotype analyses have identified at least four separate mutation events arising in Africa and Asia Minor or India.²³ In areas where malaria is endemic, enhanced survival in malaria outbreaks may have provided selective pressure favoring the presence of a single variant of the *HBB* gene.²² Epidemiological data suggest that the spread of the disease across the Mediterranean basin, into Southern Europe and to the Americas is inextricably linked to slavery and the large-scale forced translocation of populations from Africa and the Arabian peninsula.^{24,25}

Sickle cell disease increases the risk of stroke 200-fold to 400-fold. Longitudinal cohort data from the late 1970s through the 1980s show a peak in the incidence of stroke between ages 2 years and 5 years.²⁶ Approximately one-quarter of patients had stroke by age 45 years. Before age 20 years, most strokes in patients with sickle cell disease are ischemic, but beyond this age most strokes are hemorrhagic. Sickle cell disease can cause a cerebral vasculopathy characterized by proximal intracranial arterial stenoses, often leading to a moyamoya pattern.

Although sickle cell disease is a paradigmatic single-gene recessive disorder, it is phenotypically complex. The clinical course of sickle cell disease ranges from early childhood mortality to a virtually asymptomatic condition. Evidence suggests that stroke risk differs among individuals with this condition. Overt stroke affects 25% of individuals with sickle cell disease by middle age.²⁶ The Stroke Prevention Trial in Sickle Cell Anemia (STOP) screened around 2,000 patients with sickle cell disease, and found that around 9% had a high-risk profile, as defined by transcranial Doppler ultrasonography (time-averaged maximal mean blood flow velocities >200 cm/s in either the internal carotid or middle cerebral arteries on either side). Long-term follow-up showed that persistently elevated transcranial Doppler ultrasonography velocities reflected continually elevated risk of stroke.²⁷

Modifier genes and environmental factors may interact to determine the susceptibility to stroke among individuals with sickle cell disease; however, these modifiers remain incompletely characterized. Using Bayesian networks, Sebastiani *et al.*²⁸ analyzed single nucleotide polymorphisms (SNPs) from 80 candidate genes in 1,398 individuals with sickle cell disease. 25 SNPs from 11 genes and four clinical factors related to hemoglobin status (such as total hemoglobin concentration and percentage fetal hemoglobin) interacted to modulate the risk of stroke.²⁸ In a validation cohort, the model predicted the stroke status with 100% sensitivity and 98% specificity, for an overall predictive accuracy of 98%. Other research suggests that various genetic factors might differentially influence large-vessel and small-vessel cerebrovascular manifestations.²⁹ Parsing of genetic modifiers of stroke risk in monogenic diseases, such as sickle cell disease, may provide important insights into genetic determinants of common ischemic stroke and stroke subtypes.

Complex genetics of ischemic stroke

Evidence for heritability in ischemic stroke extends beyond the rare single-gene disorders discussed above. Stroke tends to run in families, suggesting that genetic factors influence risk. The Framingham study found that when a parent had a history of stroke before age 65 years, the risk of stroke in the offspring was increased 2.79-fold (95% CI 1.68–4.66).³⁰ A positive parental history for ischemic stroke before the age of 65 years translated to an increased risk of ischemic stroke among the children (odds ratio [OR] 3.15; 95% CI 1.69–5.88; $P < 0.001$). Other investigators have found that early-onset stroke (stroke in patients aged <50 years) seems to be more heritable than late-onset stroke.^{31–33}

Candidate gene studies

Initial studies of stroke risk focused on selective variants in candidate genes that were thought to be involved in biological pathways related to stroke. Studied candidate genes have included those associated with diverse pathologies including atherosclerosis, thrombosis, homocysteine metabolism, and hypertension. Early studies were plagued by methodological limitations, such as small sample size, potential for population stratification, and lack of replication in the study design. Heterogeneity of the ischemic stroke phenotype was commonly ignored and, when considered, was not approached in a standardized or reliable manner.³⁴ As a historical marker of the state of the field, Dichgans and Markus³⁵ in 2005 proposed standard criteria for ischemic stroke genetics to facilitate study replication and meta-analysis. The criteria called for rigorous phenotyping, including subclassification by presumed etiology, realistic sample sizes, prespecification of hypotheses to be tested, and independent replication of the findings.

With recognition of the complexity of the genetic basis of ischemic stroke and the probable modest effect of any one variant, these small single-stage studies evolved into large studies³⁶ and multistage studies.³⁷ Eventually, meta-analytical techniques were applied to published candidate gene studies³⁸ and to coordinated genotyping efforts involving teams of investigators.³⁹ Results of these efforts have been mixed. In individuals of European descent (the most studied population), associations with modest effect (OR <1.5) have been observed for common variants in the genes for coagulation factor V, methylenetetrahydrofolate reductase (*MTFHR*), prothrombin, and angiotensin-converting enzyme.³⁸ A meta-analysis of people of non-European descent (mainly Chinese, Japanese and Korean individuals) produced similar results.⁴⁰ Though considerably smaller in size than studies of older adults, a meta-analysis of early-onset, adult ischemic stroke (aged 18–50 years) replicated associations observed with variants in *MTFHR* and apolipoprotein E (*APOE*).⁴¹

One of the great limitations of candidate gene studies is that any given genetic variant has a low pre-test probability of being truly associated with a phenotype. Candidate gene studies also presuppose more understanding about pathophysiology than is usually the case. Having not found any single genetic variant or set of variants in a single gene with major effect, investigators migrated to hypothesis-neutral (agnostic) approaches, such as conducting genome-wide linkage and genome-wide association studies (GWASs).

Genome-wide studies

Genome-wide linkage studies utilize family structure and large numbers of tagging SNPs to track the inheritance of stroke risk with the transmission of the SNP alleles. Although genome-wide linkage studies have the ability to detect single risk loci with relatively large effect, success has been limited. Early findings of a linkage between stroke and SNPs in *PDE4D* (encoding cAMP-specific 3',5'-cyclic phosphodiesterase 4D)⁴² and *ALOX5AP*

(encoding arachidonate 5-lipoxygenase-activating protein)⁴³ could not be replicated consistently.^{44,45} This inconsistent replication could be due, in part, to a limited number and low frequency in the population of genes of large effect for stroke, as well as difficulty in procuring stroke family materials that would be required for replication of the previous findings.

GWAS approaches have been used in the past few years to characterize large samples of stroke cases and controls on the basis of screening for massive numbers of SNPs distributed throughout the genome to detect common variants with modest effects on stroke risk. Many groups have confirmed an association between SNPs in the chromosome 9p21 region and coronary atherosclerotic heart disease (Figure 1).^{46–49} On the basis of consistent evidence for shared vascular risk factors between ischemic stroke and coronary atherosclerotic heart disease, the International Stroke Genetics Consortium (ISGC) investigated potential associations between ischemic stroke and variants in the 9p21 locus. A pooled analysis demonstrated that six SNPs were associated with atherosclerotic stroke independently of demographic variables, coronary atherosclerotic heart disease, myocardial infarction, and other vascular risk factors.⁵⁰ The SNP that showed the greatest statistical significance in its association with ischemic stroke (rs1537378-C) had a pooled OR of 1.21. This risk locus seems to be acting directly on stroke risk and not through risk of myocardial infarction, which could lead to cardioembolism. A locus on chromosome 4q25 adjacent to the transcription factor gene *PITX2* (pituitary homeobox 2) was initially shown to be associated with atrial fibrillation, and was subsequently found to be associated with cardioembolic stroke in an analysis of 1,661 ischemic stroke cases and 10,815 controls. Two SNPs (rs2200733 and rs10033464) were associated with cardioembolic stroke in the discovery phase.⁵¹ Somewhat unexpectedly, these SNPs were also associated with noncardioembolic stroke, leading to speculation that the noncardioembolic stroke group harbored a cryptic cardioembolic source, such as unrecognized intermittent atrial fibrillation. A separate analysis of over 4,000 cases of ischemic stroke did not confirm this reported association between noncardioembolic subtypes of ischemic stroke and SNPs in the 4q25 locus. Thus, the utility of genetic profiling to help classify individuals with cryptogenic stroke remains unresolved.⁵² Another example of shared genetic risk of atrial fibrillation and ischemic stroke was observed for variation in the zinc finger homeobox protein 3 (*ZFHX3*) gene on chromosome 16q22. *ZFHX3* encodes a protein that is involved in myogenic differentiation. A GWAS identified the rs7193343-T allele to be associated with atrial fibrillation (OR 1.21; $P = 1.4 \times 10^{-10}$) and cardioembolic stroke (OR 1.22; $P = 0.0002$), with weaker evidence for an association with all ischemic stroke (OR 1.11; $P = 5.4 \times 10^{-4}$).⁵³

The CHARGE (CoHorts for Aging Research in Genomic Epidemiology) consortium used a meta-analytical approach to perform a GWAS in four large cohorts involving 19,602 white individuals (in whom 1,544 incident strokes were recorded), with replication in a cohort of 2,430 black individuals (in whom 215 incident strokes occurred).⁵⁴ Independent replication of the association between a genetic locus on chromosome 12p13 and risk of stroke was demonstrated in the separate cohorts of black and white individuals. The finding was not, however, replicated in an analysis of nearly 9,000 individuals with stroke (primarily drawn from case-control studies).⁵⁵ Survival bias is unlikely to explain the difference in findings.⁵⁶ In contrast to the original positive report, the failed replication study exhibited absence of heterogeneity among study participants. An additional possible explanation for the difference in results for locus 12p13 could be confounding by population stratification—which can arise when individuals with disease and controls have differences in allele frequencies primarily as a result of differences in ancestral origins—within populations of European descent.

A multistage study that used a GWAS for the first stage identified SNPs in the *CELSRI* (cadherin EGF LAG seven-pass G-type receptor 1) gene as susceptibility factors for ischemic stroke in Japanese individuals.⁵⁷ *CELSRI* has not, however, been confirmed as a stroke risk gene in non-Japanese populations.

The regions of the human genome with the greatest evidence supporting association with ischemic stroke have largely been discovered in other conditions, such as coronary artery disease (chromosome 9p21) and atrial fibrillation (chromosome 4q25), with ischemic stroke confirmed to be secondarily associated. Much larger collections of stroke cases will be required to tease out true findings from false-positive findings of association and to augment identification of false negatives.⁵⁸ The Wellcome Trust Case Control Consortium-2 is a collaborative venture to report on a larger than previously published GWAS that includes about 4,000 patients with ischemic stroke in the discovery phase. However, even this study is unlikely to be sizeable enough to identify small to moderate risk alleles in stroke.

Mitochondrial studies

The syndrome of mitochondrial encephalopathy, lactic acidosis and stroke-like symptoms (MELAS) is a well-established, rare, maternally-inherited mitochondrial disorder. Classically, patients present with lesions that tend to cross vascular territories. Lesions are generally so-called metabolic strokes owing to cellular energy failure, but in rare instances strokes can be the result of cardioembolism from an associated cardiomyopathy.

Common variants in the mitochondrial genome may affect the risk of sporadic ischemic stroke (Table 1).^{59–63} A multicenter mitochondrial GWAS of ischemic stroke characterized 144 SNPs that were either directly genotyped or imputed.⁵⁹ A genetic risk score incorporating information from all the SNPs showed a statistically significant association with ischemic stroke with minimal heterogeneity across study cohorts. However, no individual variant reached statistical significance. The study, which involved 2,284 ischemic stroke cases, did not confirm a previously reported association with haplogroups H1 and K.^{60,61}

Familial aggregation of ischemic stroke

Estimates of familial aggregation of ischemic stroke have varied by population and presumed etiology. Different assumptions of risk to relatives of stroke probands and population prevalence suggest that the overall risk in siblings of a stroke proband is several-fold higher than that in the general population. Currently, the effects of known genetic loci contributing to stroke risk are substantially less than this expectation. Several factors could account for this discrepancy, including additional (undiscovered) genes, rare variants that are private to individuals and families, gene–gene and gene–environment interactions, regulatory effects through DNA splicing and/or DNA methylation, structural variation (rather than single nucleotide variation), and underlying heterogeneity owing to presumed stroke etiology (subtype). Emerging technologies that have yet to be fully applied to ischemic stroke relate to gene discovery, interactions, and regulatory or structural effects. On the other hand, much effort has been applied to better understand the underlying phenotypic heterogeneity in ischemic stroke (see below).

Several studies give insight into the relationship between phenotype and risk imparted by a positive family history of stroke. A study of 600 consecutive cases of ischemic stroke showed that family history was a risk factor for large-vessel stroke (OR 1.88), small-vessel stroke (OR 1.79) and crypto-genic ischemic stroke (OR 1.70).⁶⁴ A review of two population-based and three hospital-based family history studies found that a family history of stroke was least frequent for cardioembolic stroke and more frequent for stroke of

noncardioembolic etiologies.³³ Importantly, comparison of findings from the population-based and hospital-based studies showed little evidence for inclusion bias.

Heritability (or heritability of stroke liability) seems to have a major role in stroke in younger patients. A study of 1,000 consecutive individuals with ischemic stroke recruited at St George's Hospital, London, UK found that individuals 65 years old or younger with a positive stroke family history had a greater risk of stroke than those without such a family history.³² Two population-based studies from Oxfordshire, UK, found that family history of stroke was associated with younger stroke, the highest rates being observed in patients under 60 years (OR 1.73; 95% CI 1.02–2.91).³³ A trend towards increased risk of younger stroke was seen for large-vessel, small-vessel, cardioembolic and unknown subtypes of ischemic stroke. A population-based case-control study of young women with stroke in the Baltimore–Washington region found that the magnitude of aggregation of stroke increased with decreasing proband age.³¹ Family history of stroke and risk of stroke in probands was greatest for women aged 15–24 years (OR 2.5; 95% CI 0.4–15) and declined with increasing proband age (OR 1.6; 95% CI 0.8–3.3 for ages 25–34 years and OR 1.5; 95% CI 1.1–1.9 for ages 35–49, $P < 0.0001$).

Pharmacogenomics and ischemic stroke

Several proven medical therapies exist for preventing first or recurrent ischemic stroke, including antiplatelet agents, anticoagulants, and antihypertensive agents. Pharmacogenomics, the study of how genetic variation can influence drug response, offers the potential to individualize medical therapy for stroke and enable the use of specific drugs depending on an individual's genetic make-up.

Clopidogrel pharmacogenetics

The FDA has issued a warning that some people may respond poorly to the antiplatelet agent clopidogrel, and that these poor responders might be identified by genetic testing.⁶⁵ Clopidogrel is a prodrug that requires metabolism by the cytochrome P450 complex to act on the P2Y₁₂ platelet receptor. *CYP2C19* (cytochrome P450 family 2 subfamily C polypeptide 19) variants are associated with variable rates of metabolism of clopidogrel. Moreover, variations in *ABCB1*, which codes for a transmembrane transporter called multidrug resistance protein 1 (also known as P-glycoprotein 1), can lead to reduced concentrations of active drug.

Since the FDA warning in May 2010, substantially more information has become available that calls into question the clinical utility of gene testing for clopidogrel resistance. The TRITON-TIMI 38 Trial compared clopidogrel with another antiplatelet agent, prasugrel, in patients with acute coronary syndrome in whom percutaneous coronary intervention was planned. In the main study, prasugrel prevented more ischemic events, but caused more hemorrhages, than clopidogrel. In the pharmacogenetic analysis, patients with the *ABCB1* 3435TT genotype and patients with reduced function alleles of *CYP2C19* were at increased risk of recurrent ischemic events on clopidogrel, but this was not the case for prasugrel.⁶⁶ In the PLATO (PLATlet inhibition and patient Outcomes) trial, which compared clopidogrel versus ticagrelor in patients with acute coronary syndromes, the pharmacogenetic study found ticagrelor to be more efficacious than clopidogrel, irrespective of polymorphisms in *ABCB1* and *CYP2C19*.⁶⁷

An analysis of the CURE (Clopidogrel in Unstable angina to prevent Recurrent Events) and ACTIVE (Atrial fibrillation Clopidogrel Trial with Ibuprofen for Prevention of Vascular Events) trials showed that among patients with acute coronary syndrome or atrial fibrillation, the effect of clopidogrel as compared with placebo was consistent irrespective of

ABCB1 and *CYP2C19* genotypes.⁶⁸ In an analysis of the clopidogrel arm of the ACTIVE A trial, however, carriers of loss-of-function *CYP2C19* alleles had a significant increase in risk of bleeding ($P = 0.01$), but when both treatment arms were analyzed and an interaction with genotypes was analyzed, loss-of-function carrier status was not significantly associated with an interaction ($P = 0.16$). The analysis of the ACTIVE A trial provides an object lesson of the importance of pharmacogenetic studies within a randomized clinical trial.

Warfarin pharmacogenetics

Warfarin at therapeutic doses reduces the risk of ischemic stroke by 60% in patients with atrial fibrillation. The dosage of warfarin needed to achieve therapeutic levels of anticoagulation varies considerably, and genomics may provide a way to tailor initiation of therapy, thereby minimizing underdosing and overdosing. The International Warfarin Pharmacogenetics Consortium created and validated a dosing algorithm using genetic testing of variants of *CYP2C9* (cytochrome P450 2C9) and *VKORC1* (vitamin K epoxide reductase complex subunit 1) in over 5,000 individuals (4,043 for construction of the dose algorithm and 1,009 for validation) of various ethnic groups.⁶⁹ The pharmacogenetic algorithm outperformed a clinical dosing algorithm and a fixed-dose algorithm in identifying individuals requiring 21 mg per week and those requiring 49 mg per week. However, the study did not address whether the pharmacogenetic algorithm reduced the time to a stable international normalized ratio (INR), the number of INR measurements required or, more importantly, the number of thrombotic or hemorrhagic events. Although commercial testing is available for *CYP2C9* and *VKORC1*, the absence of strong clinical evidence of utility has kept testing from being recommended for routine warfarin initiation. Diet, concomitant medications, and comorbidities contribute to the variability in warfarin responsiveness. Several multicenter randomized trials are underway to clarify the clinical significance of pharmacogenetic dosing of warfarin.⁷⁰

Statin pharmacogenetics

Statins reduce the risk of ischemic stroke beyond what would be anticipated by their cholesterol-lowering effects; however, many patients cannot tolerate such therapy because of myalgias with or without myopathy. A GWAS of definite or incipient myopathy identified variants in the *SLCO1B1* gene, which encodes the hepatic drug transporter, solute carrier organic anion transporter family member 1B1.⁷¹ Definite myopathy was defined as myalgias with creatine kinase level greater than 10 times the upper limit of normal. Patients treated with simvastatin had a 4.5-fold increase in the odds of myopathy per copy of the rs4149056-C (also known as *SLCO1B1**5) allele; this allele results in a coding change of valine to alanine at amino acid 174 (Val174Ala, commonly known as T521C). A subsequent study of over 500 adults treated with various statin drugs found that the same allele was associated with a less severe phenotype (myalgias without substantial elevations in creatine kinase).⁷² The risk of myopathy seemed greatest for carriers treated with simvastatin and negligible in those treated with pravastatin.

Summary

The era of cerebrovascular pharmacogenomics is now upon us. Despite heavy overlap with cardiovascular pharmacogenomics, the next few years will bring insights into specific genetic determinants of treatment effects analogous to the clopidogrel data, pharmacodynamic effects analogous to the warfarin data, or adverse effects analogous to the statin data. Nearly 70 drugs include pharmacogenomic tests or biomarkers on their FDA labeling, and >10% of these drugs are used to treat or prevent cerebro-vascular disease (Box 3).⁷³ The clinical application of these data is likely to precede that of genetic risk factor data.

Box 3**The Genomics and Randomized Trials Network**

The Genomics and Randomized Trials Network (GARNET)⁹³ is funded by the US National Human Genome Research Institute to investigate genetic determinants of treatment response using data from randomized, prospective clinical trials across multiple diseases of interest, including ischemic stroke. This pharmacogenomics consortium is organized around a Coordinating Center with three research centers leveraging previously funding clinical trials, and two genotyping centers including the Center for Inherited Disease Research at John Hopkins University, Baltimore, MD, USA and the Broad Institute, Cambridge, MA, USA. All genotypic and primary phenotypic data from GARNET will be submitted to the Database of Genotype and Phenotype (dbGAP)⁹² to create a research resource broadly available to the scientific community. The stated aims of GARNET are to identify genetic variants that influence an individual's response to treatment; determine whether specific treatments are more or less effective defined by genotype; and develop and disseminate innovative methods for adding genome-wide technologies to randomized clinical trials and interpreting the results in the context of a randomized treatment assignment. Specific attention is paid to identification of cross-study phenotypes for investigation, which have been organized around two main themes—women's health and vascular disease—given the focus of the three individual studies. GARNET provides genome-wide data on 2,667 individuals with ischemic stroke.

Endophenotypes

Between risk factors and overt disease, intermediate states may serve as important indicators of cerebro-vascular disease. These intermediate phenotypes, or endophenotypes, are associated with clinical cerebrovascular disease in the population, are independently and jointly heritable with stroke, and are present in individuals with and without stroke. The endophenotype concept originally arose in psychiatric genetics,⁷⁴ but is now broadly used in clinical genetics research. The myriad manifestations and underlying mechanisms of the ischemic stroke phenotype have driven investigators to look for tangible markers of cerebrovascular disease.

Carotid disease

Large-artery atherosclerosis is an important endophenotype for ischemic stroke. Etiologically, large-artery disease accounts for 20–30% of ischemic stroke in different populations. As mentioned above, rs1537378-C on chromosome 9p21 was associated with large-artery atherosclerosis–ischemic stroke independent of coronary disease in a large international meta-analysis.

Carotid disease represents an especially appealing intermediate phenotype because it can be measured and quantified noninvasively and inexpensively. Estimates of heritability for carotid atheroma vary. Both linkage and GWAS data from the Northern Manhattan Study and the Northern Manhattan Family Study supported genetic determinants of carotid atheroma (adjusted heritability 0.50; $P < 0.0001$) and identified multiple linkage peaks in a study of individuals of Caribbean Hispanic origin.⁷⁵ One of the identified loci, on chromosome 14q32, has been previously implicated in other atherosclerotic disease. Genetic factors involved in the inflammatory milieu are also candidates that could influence the transition from asymptomatic to symptomatic state.⁷⁶

Carotid intima–media wall thickness (IMT) has been used as an intermediate phenotype for both ischemic stroke and coronary disease, although the optimal protocol and vessel for

measuring carotid IMT is debated. Estimates for the heritability of carotid IMT range from 0.30 to 0.65 depending on the study population and the site of the carotid examined (common or internal).⁷⁷ IMT correlates with atherosclerosis, but may have both shared and distinct genetic determinants. A linkage study of IMT from the Northern Manhattan Study also found linkage on chromosome 14q very near the peak for carotid plaque. A GWAS analysis of subclinical atherosclerosis in the Framingham Offspring cohort included several measures of carotid IMT.⁷⁸ Although no SNP reached the threshold for GWAS-level significance, the top hit in a generalized estimating equation analysis was associated with internal carotid IMT with an adjusted *P*-value of 3.8×10^{-7} .

Small-vessel disease

Leukoaraiosis refers to thinning of the white matter, best seen on MRI, and predominantly affects the peri-ventricular and subcortical zones. Related terms include small-vessel disease and white matter disease. A meta-analysis of 46 longitudinal studies showed that leukoaraiosis is associated with an increased risk of stroke (hazard ratio [HR] 3.3; 95% CI 2.6–4.4), dementia (HR 1.9; 95% CI 1.3–2.8) and death (HR 2.0; 95% CI 1.6–2.7).⁷⁹ Variation in leukoaraiosis is estimated to be 55–80% heritable.^{80–82} A meta-analysis of 46 studies of polymorphisms in 19 genes in 19,000 individuals found no association between a genetic polymorphism and leukoaraiosis.⁸³ Gene polymorphisms in *APOE*, angiotensinogen (*AGT*) and *MTHFR* were also investigated in 2,000 people, but no association with white matter hyperintensities was observed. Genome-wide linkage studies have also failed to identify genes accounting for the high heritability of this phenotype.⁸⁴

MRI-defined brain infarcts

The CHARGE consortium performed a meta-analysis to identify loci associated with MRI-defined brain infarcts in 9,401 participants with no history of stroke or transient ischemic attack.⁸⁵ An infarct on MRI scanning was defined as an area of abnormal signal intensity in a vascular distribution that lacked mass effect and that was at least 3 mm in size. No association reached the preset threshold for genome-wide significance ($P < 5 \times 10^{-8}$).

Conclusions and future prospects

Ischemic stroke is a major public health problem with a heritable component to risk of the disease. Ischemic stroke figures prominently in the phenotypic expression of several single-gene disorders. Although these disorders are uncommon individually, they are essential to recognize because some, such as Fabry disease and sickle cell disease, have specific treatments. As in the case of sporadic stroke, the current wave of GWASs has yielded inconsistent results. Collaborative efforts to scale up these studies to overcome statistical-power failure are ongoing. Eventually, when reliable risk loci are identified, follow-up dense mapping and sequencing will need to be done. New discovery tools like exome sequencing (that is, sequencing of the entire collection of exons in an individual) and whole-genome sequencing are beginning to be applied to ischemic stroke.

Pharmacogenomics has yielded discovery of genetic variants that influence the responses to drugs commonly used in stroke prevention, including clopidogrel, warfarin, and statins. Incorporating these findings into clinical practice has been challenging. Warfarin pharmacogenomics illustrates an interesting case study in the utility of pharmacogenomics. As trials continue to clarify the clinical utility of pharmacogenomics for predicting initial dosing of warfarin, direct thrombin inhibitors are emerging as reasonable alternative therapies. The sequence of drug approval followed by pharmacogenomic discovery will probably need to be reversed, or the two processes need to occur in parallel (so-called

theragnostics, which combines therapeutics with diagnostics) if pharmacogenomics is to become a practical clinical tool.

Next-generation sequencing of patient samples provides a detailed characterization of every individual's DNA sequence. Currently, the sequencing of coding regions (exons) provides the most likely correlation between DNA variation and functional outcome. Sequencing all coding regions of all genes in the human genome (the 'exome') can provide a comprehensive view of potential causal variants in genes contributing to disease risk, as well as a promising alternative method for characterization of response to pharmacological therapy. Currently, these next-generation sequencing methods are used primarily as discovery tools, rather than directly for personalized genomic medicine. Exome sequencing of a large panel of individuals can generate accurate allele frequency information for many known disease risk and pharmacogenetic variants, leading to a more accurate picture of the distribution of these alleles in the population at large. The accumulated exome data will reveal novel variants, both rare and common, that may have a role in disease risk or therapeutic response. In the future, as genes and gene pathways are identified that contribute to stroke risk and response to treatment, a catalog of variants will be available to permit construction of genomic risk profiles (similar in context to a Framingham Risk Score). The cost of an exome sequence will be in the same range as for a current imaging diagnostic test (under US\$1,000), but an exome sequence needs only to be generated once (although data may be accessed and utilized multiple times), whereas diagnostic tests may need to be ordered repeatedly. Thus, exome sequencing could provide information on DNA variation in the set of genes important for stroke diagnosis and treatment.

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Key points

- Several single-gene disorders, including sickle cell disease, have ischemic stroke as a major feature
- Single-gene disorders such as Fabry disease are vital to recognize because they have specific treatments beyond standard stroke preventive therapies that reduce morbidity from stroke and other complications of the underlying disease
- Genome-wide association and linkage studies have revealed no single locus of major effect applying to ischemic stroke
- A region on chromosome 9p21.3, containing genetic variants known to associate with coronary artery disease, seems to increase the risk of large-vessel atherosclerotic ischemic stroke, independent of its association with myocardial infarction
- A region on chromosome 4q25 and the zinc finger homeobox 3 (*ZFHX3*) gene on chromosome 16q22 are associated with risk of both atrial fibrillation and cardioembolic stroke

Review criteria

PubMed was searched to February 2011 using the terms “ischemic stroke”, “genetics”, “genomics”, “sequencing”, “cerebral infarction”, “CADASIL”, “CARASIL”, “RVCL”, “Fabry disease”, “sickle cell disease”, and “genome-wide association studies”. The search was limited to full-length articles and short reports in the peer-reviewed medical literature in English. Reference lists of identified papers were also searched for further relevant articles.

Figure 1.

Chromosome 9p21.3 and cardiovascular outcomes. The chromosome 9p21.3 locus comprises several genes, including *CDKN2A*, *CDKN2B* and *ANRIL*. This locus has been associated with a range of diverse vascular phenotypes, including symptomatic atherosclerotic conditions such as coronary artery disease (myocardial infarction), ischemic stroke (cerebral infarction) and peripheral artery disease, and arterial aneurysms including abdominal aortic aneurysm and intracranial cerebral aneurysm.

Table 1

Mitochondrial studies of human symptomatic cerebral infarction

Study	Number of participants	Population	Genotyping	Findings
Nishigaki <i>et al.</i> (2007) ⁶²	1,081 total	Japan	26 SNPs genotyped, covering 11 haplogroups	Haplogroup A confers increased risk in women
Rosa <i>et al.</i> (2008) ⁶¹	1,033 total	Portugal	19 SNPs	Haplogroup H1 confers reduced risk of ischemic stroke
Benn <i>et al.</i> (2008) ⁶³	Cohort study of 9,254 individuals	Denmark	Major haplogroups	No association with any haplogroup
Chinnery <i>et al.</i> (2010) ⁶⁰	950	UK	Major European haplogroups	Subhaplogroup K associated with reduced risk of stroke and transient ischemic attack
Anderson <i>et al.</i> (2010) ⁵⁹	2,284 cases of ischemic stroke; 1,728 controls	International	64 SNPs genotyped (144 SNPs total after imputation)	Genetic risk score associated with stroke

Abbreviation: SNP, single nucleotide polymorphism.