Curr Atheroscler Rep (2013) 15:342
DOI 10.1007/s11883-013-0342-8

Genetic Profiles in Ischaemic Stroke

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Published online: 19 June 2013

Abstract Stroke represents a clinical syndrome rather than a single disease. A number of stroke subtypes can be distinguished based on careful phenotyping, with each of these having distinct and overlapping risk factor profiles. Recent evidence has suggested that genetics plays an important part in stroke risk, with at least 2 genes specific to stroke risk directly now having been identified. This review will explore our current understanding of the genetics underlying stroke risk and whether this information is currently useful in a clinical setting for patient benefit.

Keywords Stroke · Ischaemic stroke · Genetics · Genetic risk · GWAS · HDAC9

Introduction

Stroke represents a major health burden, being the third most common cause of death and the single biggest cause of adult chronic disability. Every year in the USA 795,000 people experience a new or recurrent stroke while mortality data from 2008 shows that 1 in 18 deaths are due to the condition [1]. Data from the Framingham study showed that 1 in 5 women and 1 in 6 men aged 55 to 75 years of age will experience a stroke sometime during their life [2]. It has been estimated that stroke mortality will double worldwide by 2020 owing to an aging population and an increasing incidence in developing countries. Cerebrovascular disease also causes vascular dementia, which is not only an important cause of dementia in its own right, but also appears to act synergistically with Alzheimer's disease pathology, increasing the chance of resulting clinical dementia [3]. Any treatment that could reduce the incidence or burden of this condition would therefore have significant patient and economic benefits.

Stroke represents a clinical syndrome rather than a single disease. It can be defined as a focal neurological loss of function, usually of sudden onset, resulting from disturbance in the blood supply to the brain. In 80–85 % of cases this results from occlusion of a cerebral vessel (ischaemic stroke) while 15 %–20 % are haemorrhagic in origin. A number of different and distinct pathologies cause both ischaemic and haemorrhagic stroke. Most cases of haemorrhagic stroke are due to intracerebral haemorrhage for which the most important risk factor is hypertension. A smaller number are due to intracerebral aneurysms, the genetics of which has been the subject of much research [4] but we will not cover intracerebral aneurysms in this article.

Conventional risk factors for stroke include hypertension, diabetes, smoking, and high cholesterol. Together however, these conventional risk factors account for all stroke risk [5]. Evidence suggests genetic predisposition may account for some of this unexplained risk.

Evidence for a Genetic Risk of Stroke

Considerable evidence points towards a genetic predisposition for stroke. The clearest evidence comes from monogenic forms of stroke, which display Mendelian patters of inheritance. These tend to cause specific stroke subtypes; for example CADASIL, caused by mutations in the Notch3 gene, is associated with small artery stroke and small vessel disease vascular dementia [6]. Although important to the individual patient, monogenic cases account for only a small percentage of overall stroke incidence and to date common variants in the same genes have not been associated with common polygenic stroke [7].

This article is part of the Topical Collection on *Cardiovascular Disease and Stroke*

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Twin and family history studies suggest genetic predisposition may be important for stroke risk, [8], and family history studies suggest these associations may differ for the three major ischaemic stroke subtypes, large artery stroke, cardioembolic stroke and small vessel stroke and have subtype specificity [9]. The study of intermediate phenotypes provide further evidence of genetic risks. Both carotid artery intima-media thickness (IMT), a marker for large vessel disease, and MRI white matter hyperintensities, a marker of small vessel disease, have significant heritability (the proportion of risk attributable to genetic risk factors). These estimates range from 55–70 % for IMT [10–12] and 30–68 % for WMH [13–15].

Identifying the Underlying Genetic Variants

Until recently the mainstay of investigating stroke genetics was the candidate gene method. Genetic variants (usually single nucleotide polymorphisms or SNPs) are identified in a 'candidate' gene, which is hypothesised to be involved in stroke risk. The frequency of the SNP is compared in a group of stroke patients compared with controls [16]. Many hundreds of candidate gene studies have been performed in stroke with rather disappointing results. This picture is similar to many other complex diseases, and the underlying reasons for lack of success have been explored in detail [17]. Important factors include small sample sizes, failure to replicate positive associations combined with publication bias resulting in preferential publication of positive associations and a failure to phenotype cases accurately. An additional problem with candidate gene studies is that associations can only be identified in genes already discovered and implicated in the disease. Completely novel genes cannot be identified.

Perhaps the strongest stroke candidate gene association has arisen in intracerebral haemorrhage (ICH) with APOE genotype. Presence of the ε2 or ε4 alleles of APOE have long been associated with increased risk of ICH, with a recent study demonstrating the risk is associated with lobar rather than non-lobar ICH [18].

The genome wide association study (GWAS) approach has revolutionised the genetics of many complex diseases, and is having a similar effect in stroke. GWAS allows up to 1 million or more SNPs which provide coverage of the whole genome to be genotyped in a single individual. Using a case control methodology, and rigorous statistical methods to account for the multiple comparisons made, associations between completely unexpected chromosomal loci and disease can be identified. Although first reported in 2005 examining the complement factor H gene in macular degeneration [19], it is only more recently that the technique has been fully applied to ischaemic stroke directly, while GWAS in haemorrhage is currently ongoing. Arguably greater success has been attained when examining positive findings from related cardiovascular conditions however [20]. Strong genetic risk factors for coronary artery disease (9p21) [21] and atrial fibrillation (PITX2, ZFHX3) [22, 23] have been subsequently shown to be risk factors for large vessel disease stroke [24] and cardioembolic stroke respectively [23, 25] in this manner.

GWAS and Stroke

An early study investigating the genetic basis of ischaemic stroke directly via GWAS was performed in 2007 using 249 ischaemic stroke cases and 268 controls, although we now realise this was underpowered [26]. A study in 2009 investigating incident stroke in a prospective population associated the NINJ2 gene on chromosome 12 with ischaemic stroke risk. [27], but a subsequent large replication study, in cross-sectional case control cohorts, failed to confirm the association [28].

It should be noted that while GWAS is a powerful technique, it requires very large, well phenotyped case series – typically in the thousands of samples, and even then is only powered to detect modest risks, typically with odd ratios on the region of 1.2-1.5. The collection of large, well phenotyped cases in stroke is challenging given the late age of onset of the condition. In particular detailed phenotyping, which we now realise is essential, requires in-depth and expensive investigation. As in other complex disease, collection of sufficiently large sample sizes for meaningful study requires International collaborations. To this end, the International Stroke Genetics Consortium (ISGC – www.strokegenetics.org) was established specifically to further the aim of understanding the genetic basis of stroke through large, well powered GWAS studies. This consortium reported the first full GWAS in ischaemic stroke in December 2011, identifying HDAC9 on 7p21.1 as a risk factor for large vessel disease ischaemic stroke in an initial discovery population of 3,548 cases and 5,972 controls from the UK and Germany, and replication in populations from Europe, USA and Australia resulting in a total sample size of 9,856 cases and 40,344 controls [29••]. How variants in the HDAC9 region increase large artery stroke is not yet clear. Initial studies have shown that HDAC9 is expressed in both intracranial and systemic large arteries including carotid and middle cerebral arteries. Abundant staining was found both in the endothelium and smooth muscle cells [30]. Staining was present in both nuclei and cytoplasmic locations. HDAC9 mRNA expression was upregulated in carotid atherosclerotic tissue, and the HDAC9 genetic variant was associated with both IMT and asymptomatic carotid plaque in large community populations [30]. The commonly used antiepileptic drug sodium valproate has HDAC inhibitory properties and has been shown to inhibit atherosclerosis in animal models [31]. Sodium valproate therapy in man has

been associated with lower stroke and myocardial infarction rates compared with other anti-epileptic drugs [32]. Functional studies are required to understand how the HDAC9 variant results in an increased stroke risk, but initial evidence suggest this might offer a novel stroke prevention approach.

A second GWAS study from the ISGC identified a locus on 6p21.1, also associated with large vessel disease ischaemic stroke in August 2012 [33•]. The odds ratios for these associations were within the expected GWAS range, being 1.42 and 1.62 respectively. Both of these associations were robustly replicated in larger sample cohorts than the discovery analysis.

The confirmed and replicated association of the first two genetic risk loci for ischaemic stroke directly is exciting for a number of reasons. These loci represent the first evidence that genetic risk factors can be robustly identified for common polygenic stroke, and that risks can be identified which are independent of related cardiovascular disease. These loci also confirm the subtype specificity of genetic risks in ischaemic stroke and offer the potential of new therapeutic intervention strategies in ischaemic stroke, particularly in the case of HDAC9 for which inhibitors are already known.

Validation of GWAS identified hits in ischaemic stroke and its subtypes has also been performed via meta-analysis of GWAS cohorts in the METASTROKE study in 12,389 cases and 62,004 controls [34••]. This confirmed the reported associations with HDAC9, PITX2 and ZFHX3 as well as identifying a further 12 potentially novel loci which require further efforts to replicate. As has been shown in other complex disease it is likely that as sample size increases more associations with ischaemic stroke will be identified. Yet the size of these effects are limited, being individually smaller than established risk factors such as family history, and likely represent the 'low hanging fruit' of genetic predisposition to ischaemic stroke and its subtypes. The currently identified and validated stroke gene targets from GWAS are shown in Table 1. The real challenge is how to translate the findings from genetic studies into patient benefit.

An alternative approach is to perform a GWAS to identify genetic determinants of a process believed to be important in stroke pathogenesis and then investigate the association of variants with stroke risk. Coagulation factors, and the structure/function of fibrin, have been implicated in the pathogenesis of ischaemic stroke. The EUROCLOT study used a GWAS approach to try to identify common genetic variants associated with coagulation factors and fibrin structure/function. A variant at the ABO locus (T allele of rs505922) was found to be associated with altered coagulation and also with stroke in the METASTROKE meta-analysis of ischaemic stroke GWAS data [35]. This association was present in large artery stroke and cardio-embolic stroke, but not with small vessel disease. Thromboembolism plays an important role in pathogenesis of both cardioembolic and large artery stroke. In contrast the pathogenesis of small artery stroke is unclear and the role of thrombosis uncertain. This result suggests thrombosis may be a less important disease mechanism for this stroke subtype.

Genetic Heritability in Ischaemic Stroke

Despite the initial euphoria surrounding GWAS in complex disease, it has become increasing clear that the contribution of common high penetrance high risk single alleles to overall stroke risk is small. Rather we have been able to identify a small number of loci with moderate risk of disease. Use of larger sample sizes and meta-analysis are revealing additional risks, but these are in general of decreasing effect size. The 'common disease common variant' hypothesis has been proved to be less powerful than initially envisaged, with individual variants contributing only modestly to heritability.

This has led to the concept of "missing heritability" [36]. It may be therefore that rather than single variants contributing large genetic risks, multiple risk alleles confer considerably smaller risks in a cumulative fashion. GWAS studies provide a large amount of genetic information, which can be used to test this hypothesis. By examining global variance in genotype data between cases and controls, rather than that associated with individual genotypes, an estimate of genetic

Table 1 Genetic loci and underlying genes associated with ischemic stroke through GWAS

Locus	Underlying Gene	Phenotype First Identified In	Stroke Subtype Specificity	Also Associated With
9p21	CDKN2A/CDKN2B/ANRIL	Coronary Artery Disease	LVD	Aortic and intracranial Aneurysms
7p21	HDAC9	Stroke	LVD	MI (weakly)
6p21.1	Unknown	Stroke	LVD	
4p25	PITX2	Atrial Fibrillation	CE	
16q22	ZFHX3	Atrial Fibrillation	CE	
9q34	ABO blood locus	Thrombosis	LVD and CE	

LVD—large vessel disease ischemic stroke; *CE*—cardioembolic stroke; *MI*—myocardial infarction

heritability may be arrived at that incorporates all available genetic information which has been typed on the GWAS array. Genome wide complex trait analysis (GCTA) provides such a tool to investigate the genetic heritability of complex phenotypes [37] and has been applied to ischaemic stroke [38]. This revealed that the heritability of ischemic stroke was 37 %. Intriguingly, the heritability of ischaemic stroke subtypes showed considerable variability, being as high as 40 % for large vessel disease and as low as 16 % for small vessel disease. Cardioembolic stroke showed an intermediate genetic heritability of 32 %.

These estimates are based only on those variants typed as part of the GWAS experiment, not the entirety of genetic variation within the genome. Of greater interest is the variability in heritability estimates between subtypes. This could be argued to indicate a greater genetic basis to the large vessel disease and cardioembolic stroke subtypes, or it could be related to the accuracy of phenotyping between the subtypes themselves. The tighter the phenotype the greater the underlying genetic contribution is likely to be when comparing cases and controls. One explanation for the lower heritability for small vessel disease, which is at variance with the epidemiological family history data [9] , is that this subtype is heterogeneous and has more than one underlying pathology, and these different pathologies have different genetic risk factors. Whichever is the true cause, these heritability estimates add weight to the suggestion that individual ischaemic stroke subtypes have different underlying risk profiles.

Rare Variants and Disease Risk

An alternative to the 'common variant common disease' hypothesis is that rare variants are important in common disease risk. This has gained popularity following the inability of GWAS to fully explain the genetic basis of common diseases. This hypothesis suggests that as well as common low risk allele carried by a population at high frequency, high risk alleles present at very low frequency in a population could also confer risk of disease. Such alleles could be private or restricted to individual families, but multiple risk alleles would combine to produce the final phenotype. This would prevent such alleles being identified by traditional familial linkage studies since they are not monogenic, while the low frequency of such high risk alleles would prevent their identification by GWAS. Any one affected individual would therefore be expected to carry many GWAS detectable variants of low risk and one or a few high risk alleles which would serve to dominate the phenotype. Identification of these rare variants requires a sequencing approach. While this is technically feasible through whole genome or whole exome sequencing – so called next generation sequencing – the technique is still expensive and best suited to smaller cohorts such as the search for modifiers of disease presentation in familial groups or known mutation carriers [39]. As the cost decreases however, such rare risk alleles may become more readily identifiable and therefore useful for constructing genetic risk scores. However due to their low population frequency they will require very large sample sizes to identify, unless they have very large effect sizes. The importance of rare variants to risk of stroke and other complex diseases remains to be determined.

What Have we Learnt from Candidate Gene Studies in Ischemic Stroke?

The availability of GWAS data offers the opportunity to look back at previous candidate gene studies and assess their efficacy; associations with individual SNPs can be simply performed by computer analysis of the GWAS data is nonsensical. With the main criticism of previous candidate gene studies relating to their size and poor phenotyping [17], GWAS overcomes both of these limitations by virtue of its design in a way that meta-analysis cannot. In particular, meta-analysis can be subject to publication bias [40]. A recent analysis of previous candidate gene associations in ischaemic stroke using GWAS data examined 32 gene based associations identified through candidate gene studies [38]. With stringent statistical correction for the increased number of variants available in GWAS data, none of these 32 candidate genes remained significant. This suggests that most previous candidate gene association with stroke were false positive results. This finding highlights an important aspect of genetic studies, in that the more we examine, the more we have to apply statistical correction for the number of tests performed. Availability of increasingly large amounts of data means that it is no longer acceptable to pick specific variants of choice in isolation, and account must be taken of previous testing of associations in the same dataset.

Pharmacogenetics and Stroke Risk

The clinical utility of identified genetic risks in incident stroke are currently limited, and studies of stroke recovery and recurrence are ongoing. One area where genetic variation may have more immediate clinical application is in the field of drug metabolism and drug associated adverse events – the field of pharmacogenetics [41]. While genetic background has been known to alter the efficacy of drugs such as aspirin for many years [42], the effects of this have been small or overcome by alternative antiplatelet therapies [43]. Two of the most commonly used anti-platelet treatments however, clopidogrel and warfarin, both have significant pharmacogenetic risks which alter drug efficacy.

Clopidogrel is widely used for the secondary prevention in stroke care, being more effective that aspirin alone [44] and equivalent to aspirin and dypridamole combined [45]. However a significant percentage of the population, between 5 and 30 %, show 'clopidogrel resistance' due to genetic polymorphisms in the CYP2C19 gene [46]. The effect of this genetic variation on clinical outcome is mixed, with a number of studies showing evidence for [47] and against [48] testing prior to treatment. The majority of these studies have been performed in coronary artery disease cohorts rather than stroke patients however, and testing for CYP2C19 variants prior to treatment in ischaemic stroke is not currently performed clinically.

Warfarin, used as a blood thinning agent to prevent blood clotting, is widely used in patients at high risk of cardioembolism, including ischaemic stroke patients. Response to warfarin is highly variable on an individual basis however, leading to a risk of thrombosis with under-anti-coagulation or haemorrhage with over-anti-coagulation – warfarin related mortality remains the most significant drug related cause of death [49]. Genetic variants in CYP2C9 and VKORC1 have been associated with variability in warfarin metabolism [50] with genotyping for these variants recommended to guide dosage, although this approach has not been widely adopted in clinical practice [51]. As new drugs are developed, and our understanding of genetics increases, drug related adverse events are more likely to be identified and screened for prior to individually tailored treatment.

Conclusions

There is considerable epidemiological evidence that genetics are important in stroke risk. Candidate gene studies have failed to identify much of this underlying heritability partly due to small sample sizes, lack or replication and inappropriate or poor phenotyping. GWAS is beginning to transform our understanding of stroke genetics with the identification of robustly associated novel genetic risk variants. Its success relates not only to its ability to discover completely novel and unexpected associations but also to the much larger samples sizes and replication, which is now routine, and depends upon large multicentre collaborations. As GWAS sample sizes increase further it is likely we will identify more genetic associations, but other diseases have suggested common variants identified by GWAS account for only a moderate amount of total genetic heritability. The role of other sources of genetic variance in stroke risk, including rare variants and gene-environment interactions remains to be determined. To date associations identified by GWAS have been specific to individual subtypes of ischaemic stroke emphasising that different subtypes have different pathogenesis; this may have implication for treatment approaches. It also emphasises the need for careful clinical subtyping in studies of stroke genetics as the field moves forwards.

Conflict of Interest Steve Bevan and Hugh S Markus declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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