

COMMENTARY

From GWAS to the clinic: risk factors for intracranial aneurysms

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Abstract

Subarachnoid hemorrhage (SAH) from a ruptured intracranial aneurysm is a devastating subset of stroke, occurring in relatively young people (mean age around 50 years) of whom around a third die within the initial weeks after the bleed. Environmental and genetic risk factors both have a role in SAH. A recent genome-wide association study of intracranial aneurysms in Finnish, Dutch and Japanese cohorts totaling 5,891 cases and 14,181 controls identified three new loci strongly associated with intracranial aneurysms on chromosomes 18q11.2 and 10q24.32, and replicated two previously found loci on chromosomes 8q11.23-q12.1 and 9p21.3. However, these five intracranial aneurysm risk loci identified so far explain only up to 5% of the familial risk of intracranial aneurysms, which makes genetic risk prediction tests currently unfeasible for intracranial aneurysms. New approaches, including identification of causal variants, rare variants and copy number variants, such as insertions and deletions, may improve genetic risk prediction for SAH and intracranial aneurysms. This may lead to diagnostic tools for identifying individuals at increased risk for aneurysm formation and rupture of aneurysms. In this way, genetic diagnostic tools will identify the people who will benefit most from screening by imaging studies for aneurysms and those who are most likely to benefit from preventive treatment of incidentally discovered aneurysms.

Subarachnoid hemorrhage: epidemiology and socioeconomic burden

Subarachnoid hemorrhage (SAH) from a ruptured intracranial aneurysm is a devastating subset of stroke. Intracranial aneurysms are mostly situated on the larger

arteries supplying the brain. These arteries run through the so-called subarachnoid space, which is the very small space between the brain and the skull. If such an aneurysm ruptures, a bleed under arterial pressure occurs in this subarachnoid space. Clinically, patients have a sudden, unusual severe headache, which is combined with a sudden loss of consciousness in half of them. The mean age at time of SAH is around 50 years, and the incidence is around 1 per 10,000 people per year, with highest rates in Japan and Finland and higher rates in women than in men [1]. Despite improvements in patient management and improved prognosis, around a third of patients still die in the initial months after the hemorrhage [2].

Because of the relatively young age of onset and its poor prognosis, the socioeconomic burden of SAH is considerable. The number of productive life years lost in the population from SAH is as large as that lost from ischemic stroke [3] and, according to a recent study, a total of 80,356 life years and 74,807 quality-adjusted life years were lost as a result of SAH in the UK in 2005 [4]. Further improvements in prognosis in SAH patients on a population level will be difficult to achieve, because one in eight patients dies immediately, before reaching the hospital [5]. Therefore, prevention seems an attractive option to reduce the burden of SAH, and knowledge on risk factors is essential for the development of preventive measures.

Risk factors for subarachnoid hemorrhage

Risk factors for SAH can be divided into modifiable - or environmental - and non-modifiable risk factors. Established environmental risk factors for SAH are smoking, hypertension and excessive alcohol intake [6]. Non-modifiable risk factors include a familial preponderance of SAH, female gender and systemic diseases, such as polycystic kidney disease and the vascular type of Ehlers Danlos disease [7,8]. The familial preponderance suggests a genetic component in the risk for SAH. Although SAH is a rare disease, intracranial aneurysms are relatively common, with a prevalence of around 1 per 50 people [9]. It therefore seems relevant to discriminate risk factors for SAH into risk factors for the presence of an

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aneurysm and risk factors for rupture of aneurysms. For presence of aneurysms, atherosclerosis, a familial preponderance and polycystic kidney disease are the main risk factors. On the other hand, only the size and site of the aneurysm, age and gender have been consistently identified as risk factors for rupture of aneurysm. All in all, our knowledge of risk factors for both the development and rupture of intracranial aneurysms remains rather meager, and hopes are high that genetic research will further increase our understanding of such risk factors.

Genetic factors for subarachnoid hemorrhage: insights from genome-wide association studies

Because both environmental and genetic risk factors have a role in SAH and intracranial aneurysms, it is a so-called complex disease. For the identification of the genetic factors responsible for a complex disease, candidate gene studies were initially used. These studies are hypothesis-based and genes are selected on the basis of their known function and the assumption that they are involved in the development of the disease (so-called functional candidate genes). The association between the disease and a specific allele of a single nucleotide polymorphism (SNP) within the functional candidate genes is analyzed between patients and controls. In intracranial aneurysms, most of these studies included relatively small numbers of patients and controls. Therefore, results have been conflicting or have not been replicated. These studies have been reviewed elsewhere [10-12].

A disadvantage of the hypothesis-based approach of candidate gene studies is that genes involved in the pathogenesis of a disease through unknown pathways are overlooked. The hypothesis-free approach by genome-wide association studies (GWASs) allow researchers to overcome this drawback because in these studies nearly all common variation in the entire genome can be tested for association with a disease [13,14]. In the past few years, GWASs have identified hundreds of genetic loci contributing to common complex diseases [13,14]. In a GWAS the genome is analyzed for common variability associated with the risk of disease by genotyping approximately 500,000 SNPs in several thousand cases and control participants. These genetic loci include common, low-risk variants (those that are present in more than 5% of the population) that confer a small risk of disease, typically with odds ratios (ORs) of 1.2 to 1.5 [13,15]. However, the variants identified so far by GWAS in relation to complex disease explain only a small proportion of the genetic risk for those conditions [16,17]. For example, the 18 loci associated with type 2 diabetes explain only about 6% of its heritability [18], and the 32 loci associated with Crohn's disease account for 20% of its heritability [19]. Consequently, the use of

genetic risk prediction and the subsequent opportunities for personalized medicine in complex disease are not yet possible [20].

The first GWAS of intracranial aneurysms included Finnish, Dutch and Japanese cohorts making up over 2,100 cases and 8,000 controls. Common SNPs on chromosomes 2q, 8q and 9p showed a significant association with intracranial aneurysm, with odds ratios of 1.24 to 1.36 [21]. In a follow-up GWAS, additional European case and control cohorts were included and the original Japanese replication cohort was increased, resulting in a cohort of 5,891 cases and 14,181 controls [22]. This follow-up study identified three new loci strongly associated with intracranial aneurysms on chromosomes 18q11.2 (OR = 1.22, $P = 1.1 \times 10^{-12}$), 13q13.1 (OR = 1.20, $P = 2.5 \times 10^{-9}$) and 10q24.32 (OR = 1.29, $P = 1.2 \times 10^{-9}$). The previously discovered associations of 8q11.23-q12.1 (OR = 1.28, $P = 1.3 \times 10^{-12}$) and 9p21.3 (OR = 1.31, $P = 1.5 \times 10^{-22}$) were replicated [22].

The 8q locus contains a single gene, *SOX17*, which encodes a transcription factor that has a pivotal role in endothelial cell function [23]. The strongest associated SNP within the 9p locus lies close to *CDKN2A*, which encodes the cyclin-dependent kinase inhibitor p16INK4a and the alternative open reading frame ARF, a regulator of p53 activity, and *CDKN2B*, which encodes the cyclin-dependent kinase inhibitor p15INK4b. In addition, a non-protein-coding gene (*ANRIL*) lies within this locus. A recently described mutant mouse with a deletion corresponding to the human 9p21 locus showed a marked suppression of the gene expression of *CDKN2B* and *CDKN2A* [24]. Aortic smooth-muscle cells in culture from these mice showed increased proliferative activity compared with aortic smooth-muscle cells from wild-type mice [24].

The strongest associated SNP on the 10q locus is located within the *CNNM2* gene, which encodes cyclin M2. Not much is known on its function. The 13q locus includes the gene START-domain-containing 13 (*STARD13*), of which overexpression leads to suppression in cell proliferation [25], and the gene *KLOTHO* (*KL*). *KL*-deficient mice show extensive and accelerated arteriosclerosis in association with medial calcification of the aorta and both medial calcification and intimal thickening of medium-sized muscular arteries [26]. Finally, the gene product of *RBBP8*, located within the 18q locus, is one of the proteins that bind directly to retinoblastoma protein, which regulates cell proliferation [27]. An important common denominator of the gene products of the candidate genes in the five intracranial loci seems to be involvement in cell proliferation.

Assuming a fourfold increase in the risk of intracranial aneurysm among siblings of cases [28,29], the five intracranial aneurysm risk loci identified thus far only

explain up to 5% of the familial risk of intracranial aneurysms [22]. From the results of these two GWASs we can conclude that, as for other complex diseases, the possible development of genetic risk prediction tests also remains currently unfeasible for intracranial aneurysms.

Applications for diagnosis: the need for further research

So far, the current GWAS findings explain only a small proportion of the heritability of complex diseases, including the disease SAH and intracranial aneurysms. Further research, including new approaches to detect rare variants using next generation sequencing [30] and structural variants, including copy number variants such as insertions and deletions [31], may improve genetic risk prediction for SAH and intracranial aneurysms. Knowledge of the genetic determinants for intracranial aneurysms may provide diagnostic tools for identifying individuals at increased risk for aneurysm formation; the identified individuals will benefit most from screening by imaging studies. Future studies should not only look for genetic determinants of intracranial aneurysms, but also investigate genetic determinants of rupture of aneurysms. Given that only a minority of all unruptured aneurysms do rupture, these genetic determinants may provide a powerful tool for identifying patients with unruptured aneurysms who are at high risk of rupture and who are therefore most likely to benefit from preventive treatment of the aneurysm [32].

Abbreviations

GWAS, genome-wide association study; SAH, subarachnoid hemorrhage; SNP, single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors have contributed equally to this article.

Published: 10 September 2010

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doi:10.1186/gm182

Cite this article as: Ruigrok YM, Rinkel GJE: **From GWAS to the clinic: risk factors for intracranial aneurysms.** *Genome Medicine* 2010, **2**:61.