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The Utility of Genetic Risk Scores in Predicting the Onset of Stroke

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16. Abstract

Stroke is the fifth leading cause of death in the United States and the second leading cause of death worldwide, and is a primary cause of incapacitation. Known phenotypic risk factors for stroke include smoking, diabetes, hypertension, physical inactivity, atrial fibrillation, race, age, and sex. There has been progress in identifying genetic indicators of stroke tendency, mostly in the form of single nucleotide polymorphisms (SNPs). Certain SNPs have been statistically associated with stroke incidence in large-cohort Genome-Wide Association Studies (GWAS). Stroke-associated SNPs may be assembled into Genetic Risk Score (GRS) panels to provide improved methods of assessing individual stroke risk, either by use of the GRS panel alone or in combination with traditional phenotypic risk scores. This review surveyed the stroke GWAS and GRS literature and found 139 SNPs associated with 101 genes or chromosomal regions. Further, GRS panels composed of subsets of these SNPs slightly improve the predictive ability of traditional risk scores when combined, but are less effective than traditional risk scores when GRS panels are used as the sole means of determining stroke propensity.

Currently available GRS SNP panels, when used in combination with traditional risk scores, provide a slight improvement in predicting stroke propensity. However, when judged by the cost, time, and effort involved in utilizing such methods, as well as the lack of standardized panels or methods of evaluating results, GRS are not recommended for use as primary tools of stroke risk assessment. However, as precision medicine and personalized genomics become more common, GRS panels may ultimately prove useful in guiding stroke prevention efforts.

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Introduction

Stroke is the fifth leading cause of death in the United States (146,383 deaths in 2017) and the second leading cause of death worldwide (5,781,641 deaths in 2016), often occurring in conjunction with high blood pressure, atrial fibrillation, and cardiovascular disease [1-3]. Stroke is also a leading cause of significant long-term disability in adults over 18 years of age [4]. In the U.S., approximately 795,000 strokes occur each year, of which 25% represent a recurrence, with a total mortality rate of approximately 18% [1]. Stroke risk increases with age and the estimated 10-year stroke risk in adults fifty-five and over differs by sex and by the increasing co-occurrence of risk factors such as hypertension, diabetes mellitus, atrial fibrillation, high blood cholesterol and lipids, cigarette smoking, physical inactivity, chronic kidney disease and family history [1]. Stroke survivors have significant additional risk of death when compared with non-stroke subjects, not only related to the initial stroke but to stroke-related sequelae as well as increased cardiac disease incidence in years following a stroke [5, 6]. Accordingly, research efforts are underway to reduce stroke incidence including strategies for prevention, treatment of risk factors, and use of new drugs and therapies [7, 8].

In aerospace medicine, stroke is, and will remain, a permanent concern due to its negative impact on aviation safety and airman health. Clearly, a stroke suffered prior to, or during, flight will impair a pilot's ability to safely operate an aircraft. Stroke sequelae can contribute to airman impairment for a period far beyond stroke occurrence. Therefore, a better understanding of the genetic indications of stroke will permit a more comprehensive assessment of an individual's stroke risk and potentially enable better-informed medical decision-making that may enhance aviation safety by allowing preventative measures aimed at mitigating the risk of stroke occurrence. The intent of this review is to provide an updated assessment of genetic risk scores (GRS) for stroke compared with traditional stroke risk factors (traditional risk scores, TRS).

Stroke Classifications and Subtypes

Stroke occurs when an arterial blockage or rupture within, or leading to, the brain vasculature results in local ischemia in the brain area supplied by the restricted vessel. Strokes, depending on cause, are divided into ischemic and hemorrhagic subtypes. Ischemic stroke encompasses strokes in which a blockage within the brain vasculature results in cessation of blood flow to areas downstream of the blockage (ischemia), whereas hemorrhagic strokes result from a vascular rupture (hemorrhage) within the brain or cranium, leading to both ischemia and to tissue destruction by increased intracranial pressure [9-14].

The American Heart Association/American Stroke Association (AHA/ASA) expert consensus defined ischemic stroke as "an episode of neurological dysfunction caused by

focal cerebral, spinal, or retinal infarction" [15]. In those terms, the definition of ischemic stroke requires some clinical findings persisting ≥24 hours or until death and/or objective evidence (imaging, pathological or other) of focal ischemic injury in a defined vascular distribution [15]. Ischemic stroke has multiple etiologies with noticeable differences between subtypes related not only to the risk factors but also to the outcome [10, 16]. Several ischemic stroke classification systems exist, but the most commonly known classification is the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification system [10, 11]. The TOAST system is based on clinical history and examination findings associated with brain, cardiac and vascular imaging, and recognizes five ischemic stroke subgroups; 1) large-artery atherosclerosis, or large artery disease, 2) cardioembolism, or cardioembolic stroke, 3) small-vessel occlusion (lacune, or small-vessel disease), 4) acute stroke of other determined etiology, and 5) stroke of undetermined etiology [11, 12].

Large-artery atherosclerosis encompasses strokes in which an atherosclerotic plaque forms within cranial arteries and grows until it causes sufficient blockage by means of stenosis or thrombotic arterial occlusion, resulting in an infarction over 1.5 cm in diameter [11]. Cardioembolisms, or embolisms with a cardiac origin, may break free and become lodged in cranial arteries or arterioles, with an effect identical to that of large-artery atherosclerotic strokes. Small-artery occlusions leading to lacunar infarctions may be due to either atherosclerotic thrombi or non-cardiac embolisms, and are typified by cerebral infarctions under 1.5 cm in diameter. Small-vessel occlusions typically have less severe effects than more wide-scale large-artery atherosclerotic of cardioembolic strokes. The remaining classifications include all strokes resulting from other determined causes (acute stroke of other determined etiology) including hypercoagulable blood, vascular abnormalities, and blood disorders, and all other strokes of undetermined etiology, which do not fit under any other classification [10-12]. In order of ischemic stroke prevalence, strokes of undetermined etiology are most common, followed by cardioembolic stroke, small vessel occlusion, large artery atherosclerosis, and finally other determined etiologies [9-14, 17]. The order of incidence varies according to sample population, regional, and socioeconomic variations [13, 18-20], and a trend exists wherein the number of small vessel occlusions increases as the number of strokes of undetermined etiology decreases [18].

Hemorrhagic strokes represent approximately 15% of all strokes but approximately 40% of stroke-related deaths, and are subclassified as subarachnoid or intracerebral hemorrhages [21-23]. Hemorrhagic stroke subtypes are distinct from ischemic subtypes in that they result from vascular rupture, rather than blockage. Intracerebral hemorrhages (ICH) most commonly result from hypertension, vascular malformations (e.g., aneurysms), and head trauma [9], and have the highest mortality rate of any stroke type, with approximately 40-50% mortality within 30 days following ICH [24-26].

Subarachnoid hemorrhages (SAH) are hemorrhages within the subarachnoid space. Subarachnoid stroke mortality is similar to that of ICH at approximately 40% mortality within 30 days of occurrence [26]. Non-traumatic subarachnoid hemorrhages are caused most often by ruptured arteriovenous malformations or aneurysms and are correlated with hypertension and arteriosclerosis [27]. The separate events leading to hemorrhagic and ischemic strokes tend to make direct comparison of the two major stroke classifications difficult, and each are typically considered separately. Both major stroke classifications share hypertension as a demonstrable risk factor [24], although smoking, diabetes, obesity, arteriosclerosis, and high-risk diets are risk factors commonly associated by medical professionals with either stroke subtype [27].

Stroke Recurrence

Individuals who have had a stroke of any type are more likely to have stroke recurrence, as the risk factors and tendency toward stroke are already established, and advancing age increases the propensity toward stroke [28]. Several studies have estimated the risk of stroke recurrence and comorbidities and found that this risk is highest in the period immediately following the index stroke [29, 30]. Feng et al., examining European American and African American primary stroke patients in South Carolina in 2002, found recurrence rates of 1.8% at 1 month, 5% at 6 months, 8% at 1 year, 12.1% at 2 years, 15.2% at 3 years and 18.1% at 4 years for all stroke types, and that the risk of all events increased with age. Those researchers observed no difference in stroke incidence between sexes, but some incidence rates differed by race, with the African American cohort having 16% higher stroke recurrence and a 12% higher risk having either of 2 composite events (recurrent stroke, myocardial infarction, or vascular death) than European Americans, and that ischemic stroke recurred more often than hemorrhagic stroke [31]. Hillen et al. studied stroke patients of the South London Stroke Register and observed that the cumulative risks of stroke recurrence were 2.6% at 3 months, 8.0% at 1 year, 14.1% at 3 years, and 16.6% at 5 years; and that 45.5% of cases of recurrent strokes consist of a different subtype than the initial stroke, suggesting of a multifactorial source of stroke recurrence [32].

Stroke Risk Factors

Disease risk factors are calculated from an experimental population (the study cohort), and indicate the propensity for a condition, such as stroke, to occur more often in one group of subjects than in another group of subjects. In attempting such comparisons, each study subject is observed for a defined period, and metadata of interest is collected, such as health history, genetic profile, current/congenital conditions, presence of risk factors, and most importantly the primary condition of interest, i.e. whether or not that person has a stroke within the study's allotted time. The health data of all subjects exhibiting that condition (stroke) is then compared with that of those not

exhibiting the condition, and data points that are significantly correlated with occurrence of the primary condition are then considered to be linked with that condition.

The Framingham stroke risk assessment tool (the Framingham Risk Score, or FRS) is the most utilized stroke risk assessment, and was developed from the Framingham Study cohort [33]. The FRS includes the patient's age, systolic blood pressure, use of antihypertensive therapy, diabetes mellitus, cigarette smoking, history of cardiovascular disease (coronary heart disease, cardiac failure, or intermittent claudication), atrial fibrillation, and left ventricular hypertrophy by electrocardiogram. These risk factors together estimate the probability of stroke in subjects aged 55 to 84 over 10 years of follow-up. [33] The FRS was subsequently modified and adjusted to evaluate the effect of antihypertensive medication in the risk of stroke, and continues to be periodically updated to make use of more recent findings [34-36]. The findings of these works originated the risk stratification tables that are still used today in the evaluation of patients in daily clinical practice [34]. However, the utility of the FRS for Stroke or other stroke risk assessment tools as a way of improving the impact in primary stroke prevention is questionable [36-38].

Several phenotypic risk factors are associated with an increase in the possibility of having a stroke. Some are characteristic of each individual, like ethnicity or age (non-modifiable risks), and others are attributed to environmental or behavioral factors such as cigarette smoking (modifiable risks). Although phenotypic risk factors alone can increase the possibility of having a stroke, they may also interact, further increasing the risk of stroke when present in combination, i.e., while age, smoking and hypertension are each present a stroke risk, the risk is increased when these factors are combined [16, 28, 38, 39].

Modifiable Stroke Risk Factors

Modifiable stroke risk factors include behaviorally based phenotypic risk factors such as smoking, along with associated conditions including hypertension and diabetes that may be successfully treated and managed. Blood pressure is the most important determinant of risk for ischemic stroke [1, 40]. It is estimated that for each 10 mm Hg in systolic blood pressure there is an 8% increase in stroke risk for Caucasians, and 24% increase for African Americans [41]. Atrial Fibrillation (AF) significantly increases the risk of stroke, and this risk increases with age. Marini et al. found a 24.6% prevalence of AF in patients with a first-ever ischemic stroke, with increased risk among women 80 years and older [42]. The overall contribution of AF to stroke mortality was significant, with an association to approximately 17% of stroke deaths [42, 43]. Left atrial enlargement in sinus rhythm is another risk factor for stroke, with stroke rates from 0.64 to 2.06 per 100 person years [44]. Diabetes Mellitus (DM) is associated with higher stroke morbidity and mortality [45]. DM and Metabolic syndrome increase the risk of recurrent stroke 1.7 times when compared to people without those conditions [46]. Further, type 2 diabetes is

associated with a 1.72x higher risk of ischemic stroke [47]. The association of cholesterol levels with specific ischemic stroke subtypes is questionable [1]. Shahar et al. found no relationship between circulating cholesterol and ischemic stroke [48]. However, non-fasting triglyceride levels have been associated with an increased risk of ischemic stroke [49].

Behaviorally based risk factors include conditions that an individual may choose to avoid, as well as environmental and occupational exposures. The most significant modifiable stroke risk factor is tobacco use; cigarette smoking roughly doubles the risk of ischemic stroke, and more than doubles the risk for subarachnoid hemorrhage [38]. Studies with cohorts from diverse ethnic origins exhibit a strong association between smoking and stroke risk; when comparing current smokers with lifelong nonsmokers or people who had quit smoking for more than 10 years, the smokers showed a 1.5X to 4X increase in stroke risk [50-52]. Different lifestyle factors have been associated with increased risk for stroke including physical inactivity, obesity, nutritional and diet factors, and acute triggers such as emotional stress [53]. In women, hormonal factors such as menopause and use of oral contraceptives increase the risk for stroke, and this stroke risk increases with the co-occurrence of smoking or of migraine with aura [1]. Recreational drug use is also linked to stroke. Cocaine is the illicit drug most commonly related to risk of stroke, with hemorrhagic stroke occurring most commonly; acute cocaine use resulted in a 5.7-fold increase in the risk of stroke when compared with those who had never used cocaine [54]. Amphetamines/amphetamine derivatives and heroin are also linked with stroke occurrence, amphetamines are more likely to cause hemorrhagic stroke due to acute hypertension among users, while heroin is linked with ischemic stroke, potentially due to adulterants in the injected solution [55, 56].

As previously noted, combinations of these risk factors further increase stroke risk. Grau et al. found that ischemic stroke is a polyetiologic disease with marked differences between subtypes regarding risk factors and outcome [16]. Large artery disease showed the highest male preponderance, high early stroke recurrence, and the highest prevalence of previous transient ischemic attack, current smoking, and daily alcohol consumption among all subtypes; meanwhile the highest prevalence of hypertension, diabetes mellitus, hypercholesterolemia, and obesity was found in small-vessel disease [16].

Non-Modifiable Stroke Risk Factors

Age, gender, race, ethnicity, and inheritance/genetics are non-modifiable factors, however, their presence aids in recognizing those at greatest risk, allowing initiation of strategies to mitigate the development and/or progression of modifiable risks [53]. Age is the most important non-modifiable risk factor; the stroke lifetime risk for adults 55 to 75 years of age is 1 in 6 or higher [8]. Sex is another important risk factor; younger women had lower incidence and mortality than men of the same age group [57, 58]. However,

compared with men of all ages, female stroke risk is approximately 20% greater, potentially due to the longer lifespan of women in comparison to men [8, 57-59]. Race is also an important risk factor; African Americans had more risk for stroke than European Americans, and a 16% higher rate of stroke recurrence [31, 60]. Family history and stroke predisposition by inheritance is another risk factor. The hereditary risk of ischemic stroke is significant, but differs by stroke subtype [61]; a study with affected sibling pairs exhibit significant clustering in stroke subtype and age at stroke [62], and another study showed a 3-fold increase in risk of offspring stroke in those who had documented parental stroke at 65 years of age [63]. Non-modifiable risk factors aside from age, such as race, sex, and family history, likely have a genetic basis, and may tend to indicate the utility of genetic risk scores.

Demographic Differences in Stroke Incidence

Differences in stroke incidence between demographic and racially defined groups are evident. The incidence of stroke is lower in younger women than in men but increases with age, and after 79 years of age there is no difference in stroke incidence according to sex, with the exception that women have a higher lifetime risk of stroke due a longer life expectancy [8, 58, 59]. Racial disparities in stroke incidence have been widely noted, particularly between European- and African-origin inhabitants of the U.S. and the U.K., with stroke occurring among African-origin study participants as much as 3.5 times more frequently than in European-origin participants among all age groups, although some studies find a trend toward stroke rate equalization among cohorts over the age of 84 [64-66].

These disparities have persisted despite an overall decrease in stroke incidence [7, 8]. Koton et al., studying a cohort of 14,357 individuals from 1987 to 2011, found that stroke rates among most racial groups declined progressively over the 24-year span of the study, although not consistently among age groups, and that overall risks actually increased among males, African Americans, those of advanced age, and in individuals with hypertension, diabetes, and cardiovascular heart disease (CHD) [7]. The GBD Stroke Collaborators found a similar worldwide decline in stroke incidence, but found that this decline was less steep among developing nations [67]. This reduction in stroke incidence rates could be attributed to the implementation of strategies for prevention and treatment of risk factors, as well as the use of new drugs and reperfusion therapy [7, 8, 67]. However, data from the Behavioral Risk Factor Surveillance System between years 2006 to 2010, show that the total self-reported stroke prevalence did not change during that time, and that older adults, African Americans, people with lower levels of education, and people living in the southeastern United States had higher stoke rates [60].

While racial disparities in stroke may superficially appear to have a genetic basis, the more likely explanation for the increased stroke incidence among minority populations is a socioeconomic one, with an accompanying increase in the incidence of traditional

risk factors and a limitation in access to health care among the affected groups [68]. In support of the role of socioeconomic factors in stroke incidence, Bray et al. found that lower economic status was correlated with increased risk of both ischemic and hemorrhagic stroke, and Vincens and Stafström observed a significant positive correlation between stroke mortality and the Gini index, a measure of income inequality [69, 70]. Howard et al. found that, between 1997-2000, the African American vs. European American stroke mortality ratio was higher for all age groups in southern US states than in northern US states, and further that stroke rates were higher for southern than for northern European Americans [71]. Olawabi et al. found that stroke incidence rates were much higher in indigenous Africans than in African Americans, and that stroke incidence in both groups was higher than in European Americans [72]. Disparities in stroke incidence are also apparent between European nations, varying as much as fourfold between western and eastern European nations [67]. Thus, socioeconomic opportunity, educational attainment, and other non-genetic factors are likely more important stroke risk factors than race.

Genetic Risk Factors for Stroke

The genetic contribution to a disease is reflected in its heritability; some conditions are strongly associated with monogenic disorders, follow straightforward Mendelian modes of inheritance (dominant transmission, recessive transmission), and can be investigated using family-based study designs [73]. However, most diseases are more complex, reflecting the interaction between contributions from multiple genes and external influences such as lifestyle and environmental factors. Most of the cardiovascular and cerebrovascular disorders including stroke are complex diseases and are considered polygenic because multiple DNA variants are associated with its incidence. Such polygenic conditions require a different study approach aimed at detecting the small effects contributed by each of the individual DNA variants [73].

While stroke susceptibility may be minimized by controlling modifiable risk factors such as hypertension, diabetes, and cardiovascular disease [33, 38, 39], heredity is a significant factor in stroke risk, and this heredity varies between stroke subtypes, particularly between ischemic and hemorrhagic subtypes [61]. A sufficient understanding of the genetic basis of stroke may serve to identify people at risk, improving stroke prevention strategies and designing better treatments [74, 75]. While it is known that a family history of stroke indicates a predisposition to stroke occurrence in successive generations [76] and in monozygotic twins [77], stroke incidence has not been traceable to specific genetic mutations until recently. Advances in genetic screening have identified genomic regions, both within and outside genes that are correlated with the tendency toward stroke.

The most effective means for large-scale discovery of genomic regions correlated with stroke occurrence is the genome-wide association study (GWAS), typically

conducted with very large patient cohorts. Such GWAS projects examine the statistical significance of known genetic mutations with stroke occurrence, and thus far the strongest genetic associations are shared with related cardiovascular phenotypes, including arteriosclerosis, hypertension, and other common risk factors [61].

Genome-Wide Association Studies

GWAS are carried out to identify the genetic risk factors of complex and multifactorial diseases, testing for a correlation between a disease and specific genetic sequence variations to identify candidate genes or genome regions that contribute to disease such as stroke. The sequence variations that are screened for most often are single-base variations called single nucleotide polymorphisms (SNPs), wherein an individual's genetic sequence varies from that of a "standard" reference genome. Also important are DNA insertions or deletions (indels), in which an individual has an addition or deletion of sequence at a particular chromosomal location, although these are less frequent than SNPs. Both SNPs and indels may occur either inside or outside a gene. Although protein-coding genes make up only approximately 5% of a person's DNA, intragenic SNPs make up 38.6% of the total SNP population, with intergenic SNPs accounting for the remaining 61.4% of total SNPs [78, 79].

Unlike family-based studies, GWAS studies use large subject populations (cohorts) to detect associations between diseases and particular SNP markers [80, 81]. A SNP marker will have two or more different alleles: the more common, called the major allele, and the less common, called minor alleles. The minor allele frequency (MAF) of a SNP varies broadly among different population groups, and is used as the criterion to judge how common a specific SNP is in a population [73]; Common (MAF >5%), low frequency (0.5%<MAF<5%), and rare (MAF <0.5%). Over 95% of SNPs present within the human genome have a MAF of less than 5% [79].

Linkage equilibrium occurs when there is no genetic linkage between SNPs, and the SNPs in question display a random association. This is the case when 2 SNPs lie on different chromosomes or when 2 SNPs are at opposite end of the same chromosome; as meiotic recombination occurs at particular recombination "hotspots" distributed across chromosomes [73]. The alternative to this is linkage disequilibrium, which occurs when two SNPs are located physically near one another, and are associated together non-randomly. Recombination hotspots define separate chromosomal regions or loci; SNPs separated by a hotspot will have a low degree of linkage, and SNPs that are separated by multiple hotspots will be in linkage equilibrium, meaning that there is no significant linkage between them. SNPs not separated by hotspots will be in linkage disequilibrium (LD) consequently they will be inherited together more often by the offspring of a parent, and have high linkage scores [73].

GWAS assays ask whether, for a specific SNP, the MAF show a statistically significant difference between cases and controls, e.g., among people with and without

stroke. The frequency with which that SNP is associated with stroke occurrence is expressed as an odds ratio (OR), in which, for example, individuals carrying a given SNP experience a stroke 23% more often than those who do not. In this scenario, the odds of stroke occurring in an individual carrying the SNP will be 23% greater than non-carriers, and the SNP will have a stroke OR of 1.23 [82]. The p-value associated with that OR determines the validity of the association and of the OR.

SNPs are useful in defining a genomic interval that contains the DNA variant that causes or contributes to the pathogenesis of a disease. When found, a SNP that has a significant statistical association with the disease may not identify the causal DNA variant itself, nevertheless, it is in some degree of LD with the causal DNA variant and could be placed somewhere within the locus. While SNPs/indels that lie within a gene locus may seem to clearly indicate the potential importance of that variation through the inferred decrement to that gene's function, SNPs that lie beyond the borders of any gene may also influence gene function (Figure 1). Intergenic SNPs may be located in DNA regulatory regions, and can influence the expression and/or regulation of one or several genes [73]. The functional consequences of most SNPs are not clear, however, and an association study is concerned mainly with the correlation of SNPs with a phenotype such as stroke. Association of SNPs or other mutations with stroke incidence most importantly identifies a risk factor within the assayed population [83]. If the assayed population is of sufficient size, the results garnered from that study may be broadly applicable to the general population.

A number of different methods are utilized to examine SNP distribution. Several sources of commercial SNP arrays (GWAS 'chips') are available, some allowing examination of over one million SNPs, as well as chips optimized for specific ethnic backgrounds [84]. SNP arrays are the basis of most GWAS studies, including the large-cohort studies surveyed in this review [80, 81, 84-90]. These chips allow coverage of most of the human genome, and the presence of SNPs not present on the chip are imputed based on known frequencies of co-occurrence [80, 81, 88, 89]. Use of these relatively inexpensive microarray-based techniques allow efficient examination of very large population cohorts, and remain the standard for large population studies.

Over 84 million SNPs are known to exist, although most are not present within any single individual [78]. This large number of potential comparisons requires the use of large study populations in order to maintain sufficient statistical power, with subject populations commonly reaching into the hundreds of thousands [80, 84]. The largest meta-analysis of stroke GWAS to date includes data from 67,162 patients with stroke and 454,450 controls [80].

The number of SNPs associated with stroke, and with each stroke subtype, continues to increase as GWAS studies are completed and analytical techniques and data availability increase (Table 1). Stroke GWAS studies to date have identified 139

SNPs associated with stroke incidence, located within or near 101 different genes (Table 1). Many of these stroke-associated SNPs are located within intergenic regions relatively near genes, but there is no well-defined distance threshold within which to either associate a SNP as genic or intergenic. For example, all of the stroke-related SNPs assigned to *PITX2* are located within a region between 100,000 and 160,000 bases upstream of the start codon (Figure 1). The location of these mutations correspond approximately to the predicted location of *PITX2* enhancer C4, located 111 kb upstream of the start codon [91], and *PITX2* suppression leads to vascular and cardiac abnormalities in humans and mice [92]. Although such upstream SNPs may influence the transcriptional regulation of downstream genes, functional analyses are typically not explored in, nor necessary for, GWAS studies.

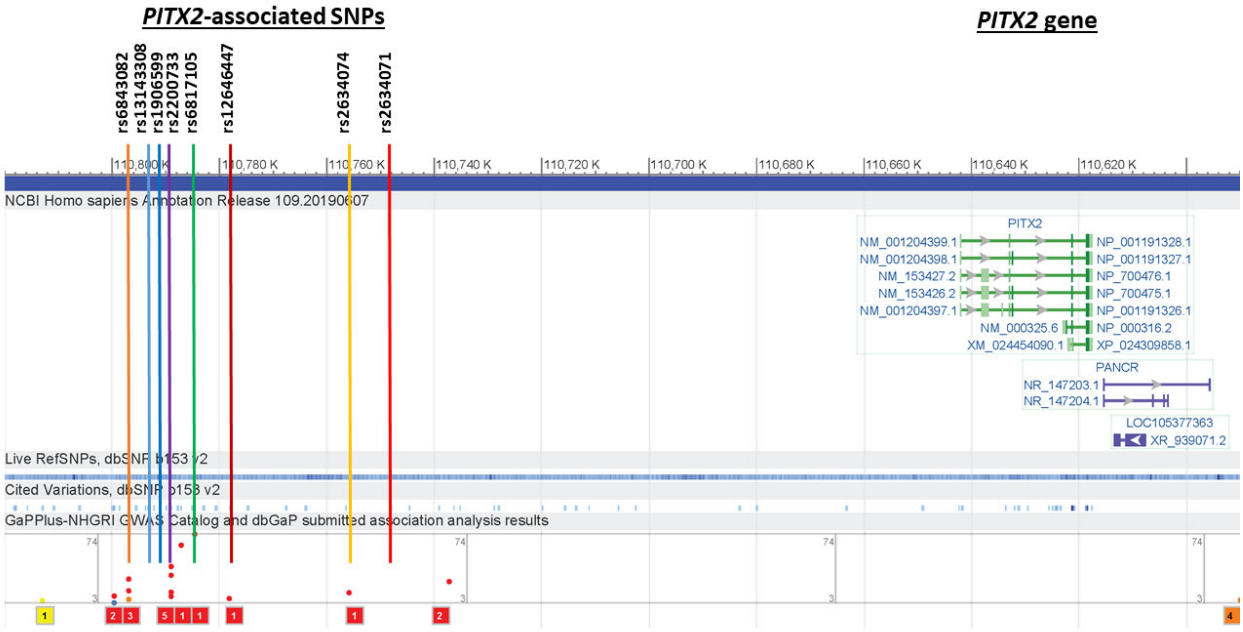

Figure 1. Single nucleotide polymorphisms with significant associations to stroke occurrence that lie in proximity to the *PITX2* gene. *PITX2* occupies 24,701 nucleotides of chromosome 4. Stroke-associated SNPs assigned to *PITX2* are located between 100,000 and 160,000 bases upstream from *PITX2*, indicated by colored vertical lines. SNP designations are indicated by vertical text at the top of each line (e.g., rs6843082). *PITX2* isoforms are indicated in green, the direction of transcription is indicated by arrows. The scale atop the figure indicates chromosome 4 nucleotide position. Red dots at the bottom of the figure indicate clinically significant SNPs registered with the National Center for Biotechnology Information.

The genes most commonly associated with stroke-related SNPs are *ABO*, *ALDH2*, *CDKN2A/B*, *FOXF2*, *HDAC9*, *PITX2*, *TSPAN2*, and *ZFHX3* (Table 1). *ABO*, the gene that determines A-B-O blood type, is variously associated with ischemic, cardioembolic, and

large-vessel disease [80, 86, 93]. *ALDH2*, an alcohol dehydrogenase, is associated with small-vessel disease [85, 90]. *CDKN2A/B*, a gene with tumor suppressor functions, is associated with large-vessel disease [75, 81, 87, 88, 93, 94]. *FOXF2*, a transcription factor, is associated with stroke in general and with small-vessel disease [80, 85, 90, 95]. *HDAC9*, a histone deacetylase that influences transcriptional efficacy, is associated with large-vessel disease [75, 81, 86-88, 93, 94]. *PITX2*, a transcription factor, is associated with cardioembolic stroke [61, 80, 81, 85, 87, 88, 90, 93, 94, 96]. *TSPAN2*, encoding a cell-surface protein with signal transduction roles, is associated with stroke in general and large-vessel disease [75, 80, 81, 86-88, 90, 93, 94]. *ZFHX3*, a transcription factor, is associated with cardioembolic stroke [61, 80, 81, 87, 90, 94, 96].

SNPs associated with cardioembolic stroke are located in or near *ABO* [75, 80, 86, 93], *DACT1* [85], *FCRL3* [85], *GLRA1* [85], *HDGFL1* [85], *MAML2* [85], *NKX2-5* [80, 85, 94], *PITX2* [87, 94], *RGS7* [85], *SLC12A2* [85], *TUSC3* [85], *ZFHX3* [61, 87, 94], *ZNF239* [85], and *ZNF608* [85]. *PITX2* and *ZFHX3* are also associated with atrial fibrillation [97]. SNPs associated with small-vessel disease are located in proximity to *ALDH2* [87, 90] and *FOXF2* [80, 85, 90, 95]. Other SNPs and genes listed as 'Large Artery Disease' or 'Any Stroke' in Table 1 are involved in large artery disease and/or in stroke in general.

Fewer SNPs are associated with intracerebral or subarachnoid Hemorrhage (ICH, SAH). SNPs proximal to *PMF1/SEMA4A/SLC25A44* were found to be strongly associated with both ischemic stroke and ICH [80, 98], and the SNP rs1052053 is the only known SNP to date with validated involvement in both ischemic stroke and intracerebral hemorrhage [80]. Three other genes/regions are associated with ICH, *APOE* [40, 98], *COL4A2* [98], the intergenic locus *17p12* [99], and *PMF1/SEMA4A/SLC25A44* [80, 98]. No SNPs have been discovered linked directly to subarachnoid hemorrhage, but several are known for intracranial aneurysm; *ALDH2* (rs671), *CDKN2BAS* (rs10757272), *EDNRA* (rs6842241), *UBR3/MYO3B* (rs4667622), *SCN11A/WDR48* (rs659901), PRDM9 (rs3932338), and *HTR1B* (rs10943471) [100, 101]. Notably, *ALDH2*, *CDKN2BAS*, and *EDNRA* are also associated with ischemic stroke (Table 1).

Most stroke GWAS have focused on European-ancestry populations, however a number of cohorts and meta-analyses include other ethnic groups, one examining African Americans identified associations for total or ischemic stroke near genes *PTPRG*, *CDC5L*, *HPS4*, *CLDN17*, *ELTD1*, *WDFY4*, *IL1F10*, *IL1RN* and those previously reported for ischemic stroke, *PITX2*, *HDAC9*, *CDKN2A/CDKN2B*, and *ZFHX3* [94]. Another large study with 13,214 patients with ischemic stroke and 26,470 controls took place in Japan and concluded that the polygenic risk score was superior to the weighted multilocus genetic risk scores for ischemic stroke, with associations nominally significant for *PITX2*, *CDKN2A/CDKN2B*, *HDAC9* and *ZFHX3* genes, and found genome-wide significance for *KCNK3* in a Japanese population [87]. A large study of 15 European-origin cohorts found statistically significant associations for cardioembolic stroke near *PITX2* and *ZFHX3*

genes, and for large-vessel stroke at a 9p21 locus (*CDKN2A/B*) and *HDAC9* genes [81]. The *PITX2* and *ZFHX3* genes have been associated with atrial fibrillation previously in GWASs [96]. Recently the same collaboration found an association of *ABO* with all ischemic stroke types [93]. Jung et al., using GWAS microarray chips created specifically for Korean populations, found that a GRS panel assembled from stroke-associated SNPs from a Korean cohort outperformed a traditional risk score assessment at stroke prediction for participants <40 years of age [84]. To discover pan-ancestry SNPs, Malik et al. combined all available stroke samples from any ancestry background to arrive at SNP associations valid across ethnic backgrounds [80].

Table 1. Genes associated with stroke-related SNPs identified by published GWAS analyses.

Gene	SNP Designations	Stroke Type	Additional Associations	References
ABCC1	rs74475935	Ischemic Stroke	NA	[90]
ABO	rs635634 rs505922 rs579459	Ischemic Stroke, Cardioembolic Stroke, Large Artery Disease	Venous Thrombo-embolism, Coronary Artery Disease	[75, 80, 86, 93]
AGBL1	rs12438353	Ischemic Stroke	NA	[94]
ADAMTS7	rs2219939 rs899997	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
AIM1	rs783396	Ischemic Stroke	NA	[95]
ALCAM	rs62262077	Ischemic Stroke	NA	[85]
ALDH1A2	rs4471613	Any Stroke	NA	[95]
ALDH2 (12q24.12)	rs10744777 rs671	Small Artery Disease, Intracranial Aneurysm	NA	[85, 90, 101]
ANK2	rs34311906	Ischemic Stroke	NA	[80]

APOE	rs429358	Intracerebral Hemorrhage	NA	[98]
C6orf155	rs9351814	Ischemic Stroke	Coronary Artery Disease	[86]
C10orf14	rs4448595	Ischemic Stroke	NA	[85]
CASZ1	rs880315	Large Artery Disease	Blood Pressure	[80]
CDC5L	rs11572061	Ischemic Stroke	NA	[94]
CDH6	rs10037362	Ischemic Stroke	NA	[85]
CDK6	rs42039	Ischemic Stroke	NA	[80]
CDKN2A/B	rs1333040 rs2383207	Large Artery Disease	NA	[75, 81, 87, 88, 93, 94]
CDKN2BAS	rs1333047 rs1333049 rs2383207 rs10757272	Ischemic Stroke, Large Artery Disease, Intracranial Aneurysm	Coronary Artery Disease	[86, 101]
CFL2	rs11627959	Any Stroke	NA	[85]
CHD3	rs9899375	Ischemic Stroke	NA	[94]
***ncRNA intron* (chr9p21)**	rs7859727	Any Stroke	Coronary Artery Disease	[80]
CLDN17	rs7283054	Ischemic Stroke	NA	[94]
COL4A2	rs9588151	Intracerebral Hemorrhage	NA	[98]
CYP17A1/CNNM2/NT5C2	rs12413409	Large Artery Disease	Coronary Artery Disease	[86]

DACT1	rs710009	Cardioembolic Stroke	NA	[85]
EDNRA	rs17612742 rs6841581 rs6842241	Large Artery Disease, Intracranial Aneurysm	Carotid Plaque, Coronary Artery Disease	[80, 86, 101]
ELTD1	rs1937787	Ischemic Stroke	NA	[94]
FCRL3	rs4284256	Cardioembolic Stroke	NA	[85]
FGA	rs6825454	Ischemic Stroke	Venous Thrombo-embolism	[80]
FLRT2	rs10400694	Ischemic Stroke	NA	[94]
FOXF2	rs4959130 rs12204590	Small Artery Disease, Any Stroke	NA	[80, 85, 90, 95]
FURIN-FES	rs4932370	Ischemic Stroke	Blood Pressure	[80]
GLRA1	rs1428155	Cardioembolic Stroke	NA	[85]
HDAC9	rs28688791 rs2107595 rs11984041	Large Artery Disease	Coronary Artery Disease	[75, 80, 81, 86-88, 90, 93, 94, 102]
HDGFL1	rs7771564	Cardioembolic Stroke	NA	[85]
HPS1	rs1804689	Ischemic Stroke	NA	[95]
HPS4	rs1804689 rs5752326	Ischemic Stroke	NA	[94]
IL1F10/IL1RN	rs11681884	Ischemic Stroke	NA	[94]

ILF3-SLC44A2	rs2229383	Ischemic Stroke	Coronary Artery Disease	[80]
ILI5	rs17007400	Large Artery Disease	NA	[89]
Intergenic (chr6p25)	rs12204590	Any Stroke	NA	[85]
Intergenic (14q31)	rs7156510 rs1564060 rs12323577	Any Stroke	NA	[89, 94]
Intergenic (10p14)	rs768606 rs17145593	Any Stroke	NA	[94]
Intergenic (17p12)	rs11655160	Intracerebral Hemorrhage	NA	[99]
KCNK3	rs12476527	Ischemic Stroke, Any Stroke	NA	[80, 87]
KRTDAP	rs8113528	Ischemic Stroke	NA	[89]
LINC01492 (ncRNA intronic)	rs10820405	Large Artery Disease	NA	[80]
LIP1/ABCC 13	rs2822388	Any Stroke	NA	[94]
LOC100505 841	rs11957829	Ischemic Stroke	White matter hyperintensity	[80]
LOC100507 163	rs768606	Any Stroke	NA	[94]
LRCH1	rs9526212	Any Stroke	NA	[80]
MAML2	rs11021485	Cardioembolic Stroke	NA	[85]
MICAL2	rs12291066	Any Stroke	NA	[94]

MGP	rs1800801	Ischemic Stroke	NA	[103]
MMP12	rs2005108	Ischemic Stroke	NA	[80]
MPDZ	rs11788316	Any Stroke	NA	[85]
NDF1P2	rs4597201	Large Artery Disease	NA	[89]
NINJ2	rs34166160 rs11833579 rs12425791	Ischemic Stroke	NA	[104]
NKX2-5	rs4867766 rs6891174	Cardioembolic Stroke, Any Stroke	NA	[80, 85, 94]
OPRM1	rs790919	Any Stroke	NA	[85]
PDE3A	rs7304841	Ischemic Stroke	NA	[80]
PITX2	rs2634071 rs2634074 rs13143308 rs6843082 rs6817105 rs2200733 rs12646447 rs1906599	Cardioembolic Stroke	atrial fibrillation	[61, 80, 81, 85, 87, 88, 90, 94, 96]
PLEKHA1	rs2281673	Ischemic Stroke	NA	[89]
PMF1/SEMA4A/SLC25A44	rs1052053 rs2984613	Ischemic Stroke, Intracerebral Hemorrhage	NA	[80, 98]
PHACTR1	rs4714955	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[61, 86]
PPAP2B	rs17114036	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]

PRPF8	rs11867415	Ischemic Stroke	NA	[80]
PTPRG	rs704341	Ischemic Stroke	NA	[94]
RAI1/PEMT/RASD1	rs12449964 rs12936587	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
RGS7	rs146390073	Cardioembolic Stroke	NA	[80]
RNU6-36	rs248812	Any Stroke	NA	[94]
SH2B3	rs3184504 rs17696736 rs11065987	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[80, 86]
SH3PXD2A	rs2295786	Any Stroke	Lipid Levels	[80]
SLC22A3/LPAL2/LPA	rs10455872	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
SLC12A2	rs72794386	Cardioembolic Stroke	NA	[85]
SLC22A7-ZNF318	rs16896398	Any Stroke	Blood Pressure	[80]
SMARCA4/LDLR	rs8103309 rs1122608	Any Stroke, Ischemic Stroke, Large Artery Disease	Lipid Levels, Coronary Artery Disease	[80, 86]
SORT1	rs599839	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
SPINK2	rs781542	Any Stroke	NA	[94]
SPRY2	rs77858481 rs77744591	Ischemic Stroke	NA	[85]

SUMO2P6/ GAPDHP71	rs7705819	Any Stroke	NA	[94]
SYNE2	rs4899120	Any Stroke	NA	[85]
TBX3	rs35436	Any Stroke	Blood Pressure	[80]
TCF21	rs12190287	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
TGFB1	rs6880837 rs13168506	Any Stroke	NA	[94]
TM4SF4	rs7610618	Large Artery Disease	NA	[80]
TRNAK27	rs2084637	Any Stroke	NA	[94]
TSG1	rs9345396	Any Stroke	NA	[94]
TSPAN2	rs12124533 rs12122341	All stroke, Large Artery Disease	NA	[75, 80, 81, 85-88, 90, 94]
TUSC3	rs1495081	Cardioembolic Stroke	NA	[85]
UBE2E3	rs6433905	All Stroke	NA	[85]
WDFY4	rs17771318	Ischemic Stroke	NA	[94]
WDR12	rs7582720	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
WNT2B	rs12037987	Any Stroke	NA	[80]
ZC3HC1	rs11556924	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]

ZCCHC14	rs12445022	Any Stroke	NA	[80]
ZFHX3	rs16971456 rs879324 rs2106261 rs7193343 rs12932445	Cardioembolic Stroke	atrial fibrillation	[61, 80, 81, 87, 90, 94, 96]
ZNF239	rs2393938	Cardioembolic Stroke	NA	[85]
ZNF259	rs964184	Ischemic Stroke, Large Artery Disease	Coronary Artery Disease	[86]
ZNF608	rs72184	Cardioembolic Stroke	NA	[85]

Genetic Risk Factor Utilization

To assess a stroke genetic risk score (GRS), which may be either monogenic or polygenic depending on the analysis method and goal, a researcher first determines the correlation of SNPs within each subject of an initial discovery population with the occurrence of stroke. The panel then screened against successive validation cohorts, and the validity of that GRS panel is assessed and following each phase. GRS panel development requires the use of a set of significantly stroke-associated SNPs to assess the stroke predisposition of individuals with one or several large cohorts. The large number of comparisons also necessitate the use of multiple comparison testing rather than judging significance by the standard p-value criteria of $p<0.05$ or <0.01. The standard p-value stringency for SNP-Stroke association in GWAS and GRS studies typically exceeds $p<1x10^{-6}$, and is often more stringent, particularly during SNP validation using additional study cohorts, with p-value criteria exceeding $p<1x10^{-8}$ or smaller [80, 85, 90, 102, 105, 106].

To develop a GRS, the ability of the single SNP or SNP panel to predict stroke among one or several study cohorts is determined by three primary methods. GRS effectiveness is assessed by comparing the OR, Hazard Ratio (HR) and/or Area Under Curve (AUC) of the receiver operating characteristic (also termed AUC-ROC or AUROC) of a SNP panel, that same SNP panel combined with additional risk factors (traditional risk factors or otherwise), and those additional risk factors without the SNP panel. HR differs from OR in that HR refers to the probability that an event (e.g., a stroke), will occur within a given period of time, and accounts for the time during which patients or subjects

participate in a study [107]. Thus, HR is utilized most often for an entire GRS panel, whereas OR is most often used for individual SNPs, although ORs are also used to assay GRS SNP panels [87, 105]. AUC is a measure similar to HR that indicates the proportion of genetic variance explained by a test, wherein an AUC of 0.5 (50%) indicates a completely random effect in which a GRS SNP panel is randomly associated with stroke, and an AUC of 1.0 (100%) indicates a perfect association of that GRS panel with stroke incidence [108, 109].

Risk scores based upon single SNPs are termed monogenic risk scores. Examples of monogenic stroke risk markers exist within the literature [86, 110], but none of those monogenic risk alleles were identified as significant within the association studies reviewed herein (Table 1). When developing a GRS SNP panel, however, it is more effective to include multiple SNPs, resulting in a polygenic risk score [87, 97, 105]. Utilization of multiple SNPs during GRS assessment improves the predictive reliability of a genetic test; Hachiya et al. noted that a selected SNP panel retained significant predictive ability in two validation cohorts, with predictive ORs of between 1.75 and 1.99, whereas single-SNP-based risk scores did not [87]. Likewise, Tada et al. pooled single stroke-associated SNPs into a panel and determined that predictive ability was improved, and that the panel slightly improved stroke prediction over TRS with an odds ratio of 1.23 [97]. Malik et al. found that combining 113 SNPs related to stroke risk factors, such as atrial fibrillation and hypertension, into a model including the covariates of subject sex and study site produced a slight predictive improvement over the covariates themselves (GRS + covariates: OR=1.806, AUC=0.6275 covariates: OR=1.0756 AUC= 0.6104 in primary and derivation samples [105]. However, screening of the combined SNP panel against validation cohorts revealed no significant improvement in stroke prediction, and the predictive ability of the combined SNP panel alone approached randomness (AUC not reported), and was significantly worse than that of the covariates [105].

Rutten-Jacobs et al. examined a 90-SNP panel among a MEGASTROKE subcohort of 306,473 subjects, and determined that while polygenic stroke GRS was useful (HR=1.35), it was not as determinative of stroke as were lifestyle factors such as smoking, diet, physical activity, and body mass index (HR=1.66) [111]. Fava et al. found that a combination of 29 stroke-associated SNPs, when combined with traditional risk factors, had a slightly improved predictive ability over the risk factors themselves (GRS OR = 1.086, AUC: TRS with GRS=0.672, TRS without GRS=0.669), and concluded that a GRS added only a marginal improvement in stroke risk assessment [112]. Ibrahim-Verbaas et al. studied adding a GRS based on 324 stroke-associated SNPs to the classical Framingham Risk Score, and found that this addition resulting in an AUC increase of 1.6% for all stroke, and 2.1% for ischemic stroke, a significant but small improvement [113]. The GRS alone, however, was significantly less predictive than the Framingham Risk Score (GRS AUC = 0.578, FRS AUC = 0.621) [113]. However, Jung et al. determined that a Korean-specific GRS panel was superior to a TRS panel

consisting of age, sex, hypertension, diabetes, dyslipidemia, and smoking status for study participant >40 years old (AUC GRS: 0.65, AUC TRS: 0.58), but that the TRS was superior for participants ≥40 years old (AUC GRS: 0.62, AUC TRS: 0.72) [84]. For comparison, the latest revision of the FRS, when screened against a multiethnic cohort of 6,712 individuals produced an AUC of 0.716, while the original FRS method screened against the same cohort produced an AUC of 0.653 [35].

The estimation of genetic risk continues to develop, and loci that are associated with stroke risk continue to be identified. The large cohorts necessary for GWAS studies, ranging from thousands to hundreds of thousands of subjects, limit the availability of such studies, although several large publicly available cohort studies exist [80, 85, 88, 90]. Further, risk alleles are often useful only for the population used as the basis of comparison, with a tendency toward specificity for the major population groups (e.g., European, Korean, etc.), and risk alleles indicated for one group may not be useful for another [38, 84, 94]. As studies examining populations of other ethnic origins and cohorts become available, it may become more possible to differentiate between risk factors by race, age, sex, or environmental factors.

Although a GRS is potentially useful information in clinical practice, enhancing the use of traditional and clinical risk factors, this usefulness remains to be proven and applied. Described genetic variants explain only a small proportion of stroke risk and even combined, their predictive value is relatively low; for this reason, the genetic screening of the general population for the prevention of a first stroke is not recommended by the Guidelines for the Primary Prevention of Stroke of AHA/ASA [38].

Stroke, Genetic Risk, and Aerospace Medicine

Stroke occurrence in flight crewmembers, especially pilots, is a major concern for aerospace medicine because of its medical and safety implications. The subtle or sudden incapacitation of a pilot in flight due to a stroke presents a high risk for the safety of the crewmembers and passengers, especially in airplanes operated by a single pilot. A flight-related stroke study found that in-flight stroke is uncommon; between 2003 and 2014 only 42 patients with flight-related stroke presented during a period in which 131 million passengers landed in Melbourne, Australia, less than one event per million [114]. Alvarez-Velasco et al. estimated that the incidence of stroke was one in 35,000 commercial flights [115]. Although those studies do not differentiate between passengers and crewmembers, they provide an indirect indication of the presentation of cases of pilots with stroke in flight, and since pilots generally belong to the group of healthy workers the incidence would be expected to be lower than in the population-at-large. However, a study of brain MRIs in 102 U-2 pilots found that occupational exposure to hypobaria, potentially in combination with hyperoxemia, induces white matter hyperintensities, probably secondary to white matter injury, even in the absence of clinical symptoms of neurologic decompression sickness. This finding is consistent with the damage pattern secondary to

microemboli in cerebral tissue, leading to thrombosis, coagulation, inflammation, and/or activation of innate immune response [116]. Commercial and general aviation pilots, however, are not exposed to conditions similar to those under which U-2 pilots operate.

There are several public reports of pilots having strokes during flight [117-120]. The Aviation Accident Database & Synopses of National Transportation Safety Board (NTSB) reported 6 accidents related to a pilot stroke in flight in the period of 1982 – 2017, 4 of these with fatal results for the airman suffering the stroke (Table 2) [120]. Data from Federal Aviation Administration (FAA) Accident and Incident Data System (AIDS) that contains incident reports from 1978 until 2017 showed a total of 8 aviation incidents related to a pilot or co-pilot stroke, with 1 injury reported (Table 3) [121]. However, these results should be interpreted with caution, since an autopsy may not reveal any evidence of stroke if the pilot does not suffer a massive stroke or if it does not last long enough to cause changes in the brain; therefore, is very difficult to diagnose stroke as an accident cause [17, 118].

Table 2. Accidents related to stroke in flight from 1982 – 2017 *

Event ID	NTSB Number	Event Date	Fatalities	Total Injuries
20001214X44299	MKC83LA208	8/30/1983	0	1
20001212X16403	ATL91LA048	2/2/1991	1	1
20001212X17374	SEA91FA121	6/2/1991	1	1
20001212X19542	IAD99LA061	8/29/1999	1	1
20020312X00332	FTW02LA090	3/6/2002	0	1
20070405X00372	NYC07FA088	3/28/2007	1	1

* Data from Aviation Accident Database & Synopses of National Transportation Safety Board [120]

Table 3. Incidents related to stroke in flight from 1978 to 2017 *

Report Number	Event Date	Fatalities	Total Injuries
19790724024099I	7/24/1979	0	0
19800714060749I	7/14/1980	0	0

19830916065869I	9/16/1983	0	0
19840904074749I	9/4/1984	0	0
19840906073039I	9/6/1984	0	0
19960712045359I	7/12/1996	0	0
19970806033309I	8/6/1997	0	0
20070617825059I	6/17/2007	0	1

* Data from FAA Accident and Incident Data System (AIDS) [120, 121]

Stroke in airmen is not only a health problem, it is an event that can induce lasting subsequent effects due to neurocognitive sequelae, use of medications, and other complications that could lead to loss of fitness to fly, and therefore of his aeromedical certificate [122]. A study conducted in the United Kingdom with commercial pilots in 2004 determined that stroke is one of the main causes of professional incapacitation, eclipsed only by cardiovascular events [123]. The FAA Airman Medical Certification System/Document Imaging and Workflow System (DIWS) shows that between June 1, 2016 and June 30, 2017, of all people who requested medical certification, 29 airmen had a history of stroke, and 22 of these were denied and did not obtain their medical certificate to fly. In a recent case on August 9, 2010, a crash resulting in five deaths (including the pilot) and four serious injuries was found by the National Transportation and Safety Board (NTSB) to most likely have resulted from pilot “temporary unresponsiveness for reasons that could not be established from the available information.” [124] However, the NTSB also found that the pilot suffered an intracerebral hemorrhage approximately four years prior to the accident, and that the pilot had a familial history of stroke. As a result of this investigation, the NTSB offered a recommendation regarding the issuance of medical certification following an airman’s ischemic stroke or intracerebral hemorrhage, as well as assessing the risk of recurrence [119]. As a result of this recommendation, the FAA held a neurological summit to review all NTSB recommendations resulting from this accident, and issued improved criteria for evaluating cerebrovascular disease in a revision of item 46 of the FAA Guide for Aviation Medical Examiners [125].

A stroke leads to a minimum 2-year disqualification from FAA medical flight certification, and certification of any airman with a history or presence of any neurological condition or disease that may incapacitate an airman during flight, such as stroke, requires a case-by-case special issuance by the FAA according to Item 46 of the Guide for Aviation Medical Examiners 2017 [125]. Similarly, other agencies like the Civil Aviation Safety Authority (CASA) in Australia or the Civil Aviation Authority (CAA) in Europe

require that pilots who have suffered a stroke meet a series of conditions to obtain a medical certificate and in some cases consider issuance with clinical and operative restrictions [126, 127].

The reliability of both traditional and genetic risk scores for ischemic stroke has continually improved since the publication of the Framingham score [33-36]. Advances in genetic testing allow researchers to examine the correlation of individual DNA profiles or polymorphisms with stroke occurrence. However, most studies comparing the predictive ability of GRS for Stroke with TRS such as the Framingham Stroke Risk Score have to date shown only very small, if any, improvement over clinical assessment, and often only when combined with clinical assessment [11, 103, 105, 113, 128-130]. Another consideration that is raised the by the potential use of genetic risk indicators is the possibility of genetic discrimination. However, the Genetic Information Nondiscrimination Act of 2008 (GINA) prevents employers from using genetic information in employment decisions such as hiring, firing, promotions, pay, and job assignments [131]. Additionally, GINA prohibits employers, employment agencies, labor organizations, joint labor-management training programs, and apprenticeship programs, from requiring or requesting genetic information and/or genetic tests as a condition of employment [131].

Conclusion and Recommendations

Although its incidence has decreased, stroke remains a major cause of morbidity and mortality worldwide [7, 67]. New technologies, analytical methods, and statistical methods have improved the ability of researchers to conduct and extract useful information from genetic studies and to discover genetic indicators of stroke susceptibility. The present trend toward personalized/precision medicine will aid in the discovery of additional genetic associations with stroke, likely identifying new genes, increasing the confidence of known associations, and refining genetic risk profiles for subpopulations. These associations will also increase comprehension of the genetic and pathophysiological mechanisms involved in the development of each stroke variety. GWAS projects have discovered a substantial number of SNPs related to stroke, and will likely continue to find additional SNPs, allowing the identification of genes and genetic regulatory profiles associated with stroke occurrence.

The true utility of genetic risk scores for stroke lies in its ability to inform individuals and health professionals of an individual's tendency toward developing the conditions that lead to stroke, and taking the proper steps to avoid or control these tendencies before they manifest [111]. With further development and validation of stroke genetic risk scores, medical professionals may be better able to use that information to identify an individual's propensity for stroke, and thereby more effectively take steps toward prevention. Stroke prevention will ultimately translate into a decrease in stroke occurrence and a concomitant decrease in stroke and stroke sequelae-related aviation accidents and incidents.

There is no current evidence of GRS panels providing a meaningful improvement in stroke prediction ability when compared with routine clinical scores like the Framingham Stroke Risk Score. Further, as no well-validated, commercially available GRS panels exist, and any GRS-based prediction of stroke risk would necessarily consist of an ad-hoc effort based upon literature review and use of reported GRS panels or other stroke-associated SNPs matching the patient's race and other relevant phenotypic indicators. The results of such ad-hoc tests would necessarily be open to interpretation and therefore useful only for advisory purposes.

In summary, a perfect tool for assessment of stroke risk does not exist. While individual genetic profiles may increase the risk of stroke, they do not predict when a stroke will occur, and are less predictive for stroke than are lifestyle factors and health indicators such as smoking, BMI, physical activity, diabetes, and others. The currently available stroke risk assessment methods have limitations, and should be used with caution because they do not include all the factors that contribute to stroke risk. Further research is needed to validate risk assessment scores through age, sex, and race/ethnic groups, to evaluate if any of the newly identified risk factors enhance the predictive accuracy of current scales, and to develop a clinically relevant and easily interpretable genetic screen for stroke propensity.

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