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Stroke is the fourth leading cause of death in the United States and is a leading cause of long-term disability. More than 795,000 people have a stroke annually. Ischemic strokes account for 87% of all strokes, and hemorrhagic strokes account for 13% of all strokes. Although family history is an indicator for both types of stroke, ischemic strokes are more closely related to environmental risk factors, such as diet, exercise and smoking. Hemorrhagic strokes also aggregate within families but often occur at younger ages indicating a possible genetic link. The purpose of this study was to identify similarities and differences of the biological risk factors associated with hemorrhagic stroke, such as hypertension, diabetes mellitus and/or high cholesterol, and environmental risk factors, such as exercise, alcohol consumption, smoking, and perceived stress within and between families with a history of hemorrhagic stroke.

Methods: 14 individuals (8 with hemorrhagic stroke, 6 without hemorrhagic stroke) participated from 4 families with a family history of hemorrhagic stroke were recruited from stroke support groups in the southeast, social media and Casa Colina Hospital and Medical Center in Pomona, California. Participants completed medical and family history questionnaires, as well as, the Health Promoting Lifestyle Profile (HPLPII) and the Perceived Stress Scale (PSS).

Results: Nonparametric statistical analysis and visual representation were utilized to compare biological risk factors associated with hemorrhagic stroke within and between families and to measure the strength and direction of association that exists between

groups. Hypertension was the most salient biological risk factor among all study participants (87%), followed by high cholesterol (42.9%) and diabetes mellitus (14.3%). General stress and alcohol consumption was reported in all families (50% and 57% of participants, respectively). Mann-Whitney U Test indicated that PSS scores were significantly higher for participants with hemorrhagic stroke ($\bar{x}$=24.33) than for participants without hemorrhagic stroke ($\bar{x}$=15.67), U=4.0, p=.028).

Conclusion: There is not one clear biological or environmental factor identified as the cause of familial hemorrhagic stroke; however, hypertension seems aggregate within families with a history of hemorrhagic stroke suggesting that it may be a major risk factor. In addition, perceived stress was significantly higher in participants with hemorrhagic stroke compared to those without hemorrhagic stroke suggesting that it is also a risk factor for familial hemorrhagic stroke.

RISK FACTORS RELATED TO FAMILIAL HEMORRHAGIC STROKE

by

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LIST OF ACRONYMS

ACE	angiotensin-converting enzyme gene
ACTH	adrenocorticotropic
APOE	apolipoprotein E gene
ApoE	apolipoprotein E protein
ASHA	American Speech-Language-Hearing Association
AVM	arteriovenous malformation
BP	blood pressure
BMI	body mass index
CCM	cerebral cavernous malformation gene
CHD	coronary heart disease
COL4A1	collagen type IV alpha 1 chain gene
COL4A2	collagen type IV alpha 2 chain gene
CRH	corticotropic
DD	homozygous dominant
FHS	Family Health Study
FRS	Family Risk Score
HDL	high-density lipoprotein
HPA	hypothalamic-pituitary-adrenal
ICH	intracerebral hemorrhage
I/D	Insertion/Deletion variant
II	Insertion/Insertion variant
KRIT1	ankyrin repeating containing (also known as CCM1)
LDL	low-density lipoprotein
RAAS	renin-angiotensin-aldosterone system
SAH	subarachnoid hemorrhage

CHAPTER I

INTRODUCTION

Precision medicine is a term used to describe treatment and prevention of disease. It takes into account unique biological, environmental, and behavioral factors that drive disease in individuals. It recognizes variability in environment, lifestyle and genes for each person (U.S. Department of Health & Human Services, 2015). Identifying and understanding risk factor, such as family history, social history, and medical history, associated with an illness is the first step in prevention of a disease. It allows physicians and individuals to understand the pattern of disease and helps in planning a course of action which may reduce risk. One such disease that can benefit from precision medicine is stroke.

Stroke is the fourth leading cause of death in the United States when considered separately from other cardiovascular diseases, such as heart disease, coronary artery disease, and arrhythmias. It is a leading cause of long-term disability in the United States. More than 795,000 people have a stroke annually, costing an estimated $34 billion each year. According to the National Institutes of Health, National Heart, Lung, and Blood Institute (2015), 25% of people with a stroke will have another stroke within 5 years. Of these, 87% are ischemic, and 13% are hemorrhagic (Mozaffarian et al., 2015). Forty percent of hemorrhagic strokes end in death within 30 days (Woo et al., 2002).

Several long-term effects of stroke exist, including aphasia, which occurs in 21%-35% of strokes, and dysphagia, which affects more than 50% of stroke survivors (Martino et al., 2005). One way to avoid the long-term effects of stroke is to prevent stroke. The American Speech, Language and Hearing Association (ASHA) recognizes the importance of prevention of communication disorders as one of the profession's primary responsibilities. In 1973, the ASHA Legislative Council approved a policy statement entitled "Prevention of Communication Problems in Children." Since then, the statement has been modified to include all age groups. ASHA (1988) acknowledges that the traditional role of the speech-language pathologist in the identification and treatment of communication disorders has expanded to now include efforts to "eliminate the onset of communication disorders." One way to accomplish this is to determine the epidemiologies of specific communication disorders (ASHA, 1988), such as aphasia and dysphagia. Once the specific causes have been identified, preventative practices can be initiated with at risk populations, such as individuals with a family history of stroke.

Because ischemic stroke occurs more often than hemorrhagic stroke, the risk factors associated with it are more widely understood. For example, high blood pressure, high cholesterol, diabetes mellitus, heart disease, smoking, high body mass index (BMI) (Dey, Rothenberg, Sundh, Bosaeus, & Steen, 2002), race, and increasing age (Morgenstern, et al., 2004) are all known risk factors for ischemic stroke. Although several risk factors are associated with hemorrhagic stroke, they are not as clearly defined or necessarily well known. For instance, there is strong evidence to support high blood pressure (BP) (Howard et al., 2013; Matsukawa et al., 2012; Sturgeon et al., 2007) and

smoking (Kissela et al., 2002; Woo et al., 2009) as risk factors for hemorrhagic stroke but other risk factors such as BMI (Kissela et al., 2002; Matsukawa et al., 2012; Sturgeon et al., 2007), high cholesterol (Sturgeon, et al., 2007; Wieberdink et al., 2011), and diabetes mellitus (Howard et al., 2013; Kissela et al., 2002; Woo et al., 2002) yield more inconclusive evidence. Family history is a strongly associated risk factor for both ischemic stroke (Choi, Lee, Kang, Kang, & Bae, 2009; Jerrad-Dunne, Cloud, Hassan, & Markus, 2003) and hemorrhagic stroke (Kissela et al., 2002; Woo et al., 2002; Woo et al., 2009). Ischemic and hemorrhagic strokes can occur at any age; however, hemorrhagic strokes that aggregate within families occur at a younger age suggesting a genetic link (Bromber et al., 1996, Schievink, Schaid, Rogers, Piepgras, & Michels, 1994).

Two subtypes of hemorrhagic stroke exist: intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). ICH results when the bleeding occurs inside the brain and accounts for 10% of all hemorrhagic strokes. SAH occurs when the bleeding is in the subarachnoid space between the cerebrum and the arachnoid mater and accounts for 3% of all hemorrhagic strokes (Mozaffarian et al., 2015).

The causes of hemorrhagic strokes are multifactorial but appear to have a strong familial link (Kissela, et al., 2002; Woo et al., 2002; Woo et al., 2009). The low incidence and high mortality rates make it difficult to determine a specific genetic source. Each gene has its own individual purpose and plays an important role in maintaining balance within the vascular system. Two genes that have been specifically related to cell integrity and thus resulting in hemorrhagic strokes are cerebral cavernous malformation (CCM) pathway genes (Gore, Lampugnani, Dye, Dejana, & Weinstein, 2008) and

COL4A1/COL4A2 genes (Gould et al., 2006; Jeanne, Jorgensen, & Gould, 2015). Mutations in either of these gene groups significantly increase the risk of hemorrhages. CCM genes code for the malcavernin protein, which is involved in angiogenesis, defined as the process in which new blood vessels form. The malcavernin protein strengthens the interactions between cells that form blood vessels and limits leakage from the vessel (National Library of Medicine [NLM], 2016). For COL4A1/COL4A2, increased blood flow, which occurs while exercising or during the stress of the natural birth process, increases the risk even more, demonstrating that the interaction between genes and environment is an important component of disease outcome (Gould et al., 2006; Jeanne et al., 2015).

Hypertension is a primary risk factor for hemorrhagic stroke. It is the result of high blood pressure and is described in four stages based on systolic and diastolic pressure. Systolic BP 120-139 mmHg or diastolic BP 80-89 mmHg is considered prehypertension. Systolic BP 140 to 159 mm Hg or diastolic BP 90 to 99 mm Hg is Stage I. Stage II is a systolic BP 160 mm Hg to 179 mm Hg or diastolic BP 100 to 109 mm Hg. Stage III hypertension is a systolic BP ≥160 mm Hg or diastolic BP ≥110 mm Hg. Higher stages of hypertension significantly increase the risk of ICH (Chobanian et al., 2003; Howard et al., 2013; James et al., 2014). As blood pressure builds, it can cause already weakened blood vessels in the brain to rupture, resulting in a hemorrhagic stroke.

Blood pressure is affected by fluctuations in hormones. The ACE gene is part of the renin-angiotensin-aldosterone system (RAAS). RAAS is a hormone system that regulates blood pressure and bodily fluids. It functions as an endocrine axis in which the

active hormone, angiotensinogen, and angiotensin II is formed (Atlas, 2007). Long-term angiotensinogen concentration, such as during pregnancy, may be a risk factor for hypertension (Atlas, 2007). Activation of angiotensin II increases blood pressure (Atlas, 2007). The most common genetic variation related to RAAS is the insertion/deletion polymorphism of the ACE gene (ACE I/D). ACE D carriers make more ACE protein which results in a more active RAAS system than that of variant I carriers. A more active RAAS system results, such as ACE DD variant, increased sensitivity to sodium intake and produces a higher risk of hypertension as well as stroke and CHD (Dengel, Brown, Ferrell, & Supiano, 2001). It is also significantly associated with ICH, and can result in smooth muscle cell death and collagen deposition (Fleming, 2006).

The association between cholesterol levels and hemorrhagic stroke is not as clear as the relationship between hypertension and hemorrhagic stroke. ApoE (apolipoprotein E) protein is one of the key lipoproteins of lipoprotein complexes that regulate the metabolism of lipids by directing their transport, delivery, and distribution from one tissue or cell type to another through apoE receptors and proteins associated with lipid transfer and lipolysis (Mahley, 1988). Higher levels of cholesterol are associated with higher levels of apoE protein. The APOE ε4 variant is associated with faster cholesterol metabolism resulting in higher cholesterol levels, whereas the APOE ε2 variant is associated with slower cholesterol metabolism resulting in lower cholesterol level. Both APOE ε4 and ε2 have been associated with lobar ICH and play a role in the pathogenesis of amyloid angiopathy (Charidimou et al., 2015).

Some studies suggest that the type and location of hemorrhagic stroke is specifically related to a genotype or specific environmental factors. Type refers to ICH and SAH; whereas, location refers to the specific area of the brain where the hemorrhage occurred and can be divided into lobar and nonlobar (e.g. basal ganglia, thalamus, brainstem, cerebellum or periventrical white matter). Martini and colleagues (2012) found that first time patients with lobar ICH were more likely to have the APOE ε2 or ε4 genotype whereas the nonlobar ICH group were more likely to have high cholesterol and/or hypertension. Nieuwkamp and colleagues (2014) studied SAH within two groups: those with artherogenic risk factors (e.g. hypertension and smoking) and those without artherogenic risk factors and found that the middle cerebral artery was the site of aneurysm for most SAH regardless of group. These were both population-based studies and did not consider family history as a necessary risk factor. Other undetermined risk factors may not have been reported.

Individuals are more likely to make health changes based on family history when informed about the risks (Ruffin et al., 2001). In recent years, there has been a greater push to use family histories in medical settings to help inform patients of their risk for certain diseases. Healthcare professionals agree that patients' ability to recognize and understand their risk factors based on family history is important to the patients' overall health, and directly targeting and educating patients known to have a family history of a disease is desirable. However, one study found that most healthcare professionals who participated in the study did not consider it feasible (Heideman, Cleijne, Van, Snoek, & Cornel, 2013). Indeed, several barriers are recognized by healthcare professionals, such

as time-effectiveness and cost of administering and reviewing family histories (Heideman et al., 2013), as well as the concern that patients will not follow suggestions given.

Although hemorrhagic stroke has a low incidence rate, it has a high mortality rate. Identifying the biological and environmental risk factors for hemorrhagic stroke within and between families could be an important first step for decreasing the risk of hemorrhagic stroke within the general population. One study found that 10.7% of families within a Utah population had a positive family history of stroke. This group accounted for 86% of early strokes and 68% of strokes regardless of age (Hunt, Gwinn, & Adams, 2003). However, it is unclear if the stroke risk was attributed to genes, environment, or a combination of both.

It is unlikely that there is one gene that guarantees an individual will acquire a hemorrhagic stroke; instead, it is more likely that there are several genes that interact with each other and the environment that would result in hemorrhagic stroke. The first step in understanding and identifying genetic and environmental risk factors for hemorrhagic stroke is choosing families with a strong family history of hemorrhagic stroke to study. Next, administering a cost-effective questionnaire to these families to determine commonalities between and within groups may help to develop a clear picture of familial hemorrhagic stroke. Gathering an in-depth family history may help researchers identify the potential causes in order to prevent this life threatening disease.

CHAPTER II

LITERATURE REVIEW

The cause of hemorrhagic stroke is multifactorial but appear to have a strong familial link (Kissela et al., 2002; Woo et al., 2009; Woo et al., 2002). Defining and understanding family history may be an important step in the prevention of hemorrhagic stroke and thus the prevention of communication disorders associated with this disease. Several biological and environmental factors have been identified as potential risk factors for hemorrhagic stroke. The low incidence rate of hemorrhagic stroke makes the assessment of these risk factors difficult. In order to understand why some individuals will have a hemorrhagic stroke and others will not, it is important to clearly identify the genetic and environmental risk factors and how these influence the cellular underpinning that results in hemorrhagic stroke.

Family History

Family health history is one of the strongest predictors of a patient's risk for complex diseases. Family history of a disease captures the underlying complexities of gene—gene and gene—environment interactions by identifying families with combinations of risk factors that lead to disease expression (Hunt, Gwinn, & Adams, 2003). Collecting this information can dramatically improve identification of at-risk individuals (Qureshi et al., 2012; Wu et al., 2014). Discussing the importance of family history and risk factors of disease with families prior to obtaining a health assessment

significantly improves the quantity and quality of the data provided so that appropriate preventive care can be given (Wu et al., 2014). For example, a family history of hemorrhagic stroke increases the risk of hemorrhagic stroke (Woo et al., 2009). This is especially true if a first-degree relative had a hemorrhagic stroke prior to the age of 65. Familial aggregation of ICH has been observed, and the heritability of ICH risk has been estimated at 44% (Carpenter, Singh, Gandhi, & Prestigiacomo, 2016).

A strong, documented relationship exists between family history of SAH and subsequent SAH. One study found a sevenfold increase in SAH for first-degree relatives compared to second-degree relatives with SAH (Bromberg et al., 1995). In another study, Korja and colleagues (2010) conducted a large twin cohort study to determine the heritability of SAH in a Nordic population, comparing monozygotic twins to dizygotic twins, and found that 41% were heritable. In addition, researchers determined that although genetic factors in the etiology of SAH played a moderate role, environmental factors also play a significant role in SAH susceptibility at the population level (Korja et al., 2010).

Biological and environmental risk factors for hemorrhagic stroke can differ between families. Individuals with a biological predisposition for hemorrhagic stroke can be compounded by environmental factors. According to Hunt and colleagues (1986),

> determining whether a person has a positive family history of disease can be important clinically, epidemiologically and genetically because unaffected members of positive family history families tend to have increased risk of developing the disease in the future (p. 809).

The Health Family Tree Study (Hunt et al., 1986) included 122,155 family histories from students at a Utah high school to determine the risk of CHD and high blood pressure based on a positive family history. As part of the curriculum of a required health or physiology class, a specific first-degree family medical history was obtained from the parents of the students. Positive family history was determined using the number of affected first-degree relatives and whether the age of disease onset was early (< 55) or late (≥ 55) and using a quantitative family history score (FHS). The FHS was based on the overall observed number of events in the family and the expected number calculated by multiplying the age and sex specific person-years of experience (number of years with disease) of the family by the age and sex specific incidence rates of the general population.

Researchers found that using two or more affected family members as a minimum definition of a positive family history or using FHS ≥ 1 provided useful family history categorization. Comparing FHS ≥ 2 and FHS ≥1, the relative risk of high blood pressure increased from 20 – 59 years of age in males. As males aged, the relative risk leveled out. For females, there was little change in the relative risk between FHS ≥ 2 and FHS ≥1, regardless of age. However, regardless of age or sex, having two or more first-degree family relatives with hypertension increased the relative risk. Information not available from families was classified as "uninformative family history" rather than a negative family history, which decreased study bias. Coronary heart disease (CHD) was defined as having a heart attack or bypass surgery. CHD incidence rates for each family history group were calculated by following the family members who were unaffected for 8, 13,

and 18 years. Researchers reported data only for year 8. Risk of CHD significantly increased as the number of first-degree relatives with CHD increased or having an FHS ≥ 1.

The continuous nature of the FHS allowed for every family to be ranked and used in the analysis of family history. Using the FHS or two or more affected family members as a minimum definition of a positive family history resulted in similar results. This suggests that obtaining an in-depth family history can be a good predictor for disease. It also shows that the more first-degree family members affected or the higher the FHS, the greater relative risk there was for both high blood pressure and CHD.

A later study that included the Health Family Tree Study and the National Heart, Lung, and Blood Institute Family Heart Study (Williams et al., 2001) determined the cost-effectiveness of using a family health questionnaire as a tool for family-based preventive medicine and medical research. This study utilized the family risk score (FRS) (formally FHS) as it pertains to four diseases: diabetes, hypertension, CHD, and stroke. Researchers found 79% sensitivity, 91% specificity, 67% predictive value of a positive report, and 96% predictive value of a negative report for CHD when using the questionnaire. In addition, the cost for collecting and computerizing the initial family history was about $10 per participating proband (mail form, follow-up call, input form, postage, and materials). For validating information gathered, the cost was approximately $50 per proband. Furthermore, researchers found that for stroke, 10.7% of families had a positive family history. This group accounted for 86% of early strokes and 68% of strokes at any age. For the 13,106 families with a high risk for strokes, a cost of $27 per

high-risk family was identified. Interestingly, researchers found that positive family histories for all four disorders were significantly associated with each other in 2 x 2 contingency table analysis. The relatively low cost of compiling a family history is a validated tool for family-based preventative medicine and medical research.

Many variables can influence why a disease affects one family and not another. For example, environmental factors, such as dietary choices, smoking, or exercise and genetic factors, such as family history of high blood pressure, stroke, or even a specific gene variant may increase the risk of hemorrhagic stroke within certain families. Family history can help differentiate between environmental and genetic factors providing individuals a more comprehensive understanding of specific risk factors for hemorrhagic stroke. Although hemorrhagic stroke affects only a small portion of the population, it is a devastating disease with a high mortality rate. If individuals with a family history of hemorrhagic stroke were aware of the underlying pathophysiology of the risk factors, they may begin to make changes to their lifestyle or monitor their health status to decrease their risk.

Pathophysiology of Hemorrhagic Stroke

Hemorrhagic stroke occurs when a weakened blood vessel ruptures and bleeds into the brain. It causes injury to brain tissue by disrupting connecting pathways and causing localized pressure injury. Two types of weakened blood vessels typically cause hemorrhagic stroke: aneurysm and arteriovenous malformation (AVM). An aneurysm is a ballooning of a weakened region of a blood vessel. If left untreated, the aneurysm can continue to weaken until it ruptures and bleeds into the brain. An AVM is a cluster of

abnormally formed blood vessels that are comprised of snarled tangles of arteries and veins. The presence of an AVM disrupts the cyclical process of blood flow. It is estimated that approximately 300,000 Americans have AVM of the brain or spinal cord (National Institute of Neurological Disorders and Stroke [NINDS], 2015). Any one of these vessels can rupture, also causing a hemorrhage into the brain.

Hemorrhagic strokes are divided into two subtypes: intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). ICH results when the bleeding occurs inside the brain. ICH accounts for 10% of all strokes. SAH results when the bleeding is in the subarachnoid space between the cerebrum and the arachnoid mater. SAH accounts for 3% of all strokes (Mozaffarian et al., 2015).

Genetic Underpinning

Understanding the underlying structural cause of blood vessel construction/destruction is an important aspect of understanding hemorrhagic stroke. Blood vessels consist of three layers: tunica intima, tunica media, and tunica externa. The tunica intima is the innermost layer of the blood vessel. It is the only part of the vessel that is in contact with the blood. It is comprised of simple squamous epithelium called endothelium. Because of its extreme smoothness and normal chemical composition, the tunica intima prevents abnormal blood clotting by preventing the adhesion of platelets. The endothelium of blood vessels also produces chemicals, such as nitric oxide (vasodilator—stimulates relaxation of the smooth muscle) and the peptide endothelin (vasoconstrictor—contraction of the smooth muscle) that affect blood pressure. The middle layer, tunica media, is made of smooth muscle and elastic

connective tissue. These tissues are involved in the maintenance of normal blood pressure, especially diastolic blood pressure. The smooth muscle in this layer is affected by the chemicals produced by the endothelium. The outermost layer, tunica externa, is made of fibrous connective tissue, which makes it very strong (Scanlon & Sanders, 2011). An abnormal protein or an abnormal amount of certain normal proteins in the blood can cause blood vessels to become abnormally formed or weakened, thus compromising blood vessel integrity.

One gene associated with blood vessel integrity is the cerebral cavernous malformation (CCM) gene, which codes for the malcavernin protein. This protein is involved in angiogenesis, which is the process in which new blood vessels form. It strengthens the interactions between cells that form blood vessels and limits leakage from the vessel (NLM, 2016). Even minor deficits in the CCM proteins or in their effectors can trigger a hemorrhagic stroke (Gore et al., 2008). There have been three CCM genes identified in heritable disorders: CCM1/KRIT1 (Laberge, Labauge, Maréchal, Maciazek, & Tournier-Lasserve, 1999) CCM2/melcavernin (Denier et al., 2004), and CCM3/programmed cell death 10 (PDCD10) (Bergametti et al., 2005). In humans, mutations in any one of these genes cause an autosomal dominant genetic ICH disorder characterized by CCM.

Gore et al. (2008) investigated the interaction between CCM pathway genes and hemorrhagic stroke using zebrafish embryos and found that a combined minor reduction in the expression of multiple CCM pathway genes can precipitate hemorrhagic stroke. The rap1a gene is expressed normally during early development, but rap1b gene, a gene

implicated in the CCM pathway in endothelial cells, shows vascular-enriched expression. The rap1b gene encodes a RAS GTPase effector protein for CCM1/KRIT1. It helps regulate cell adhesion, growth, and differentiation. It also plays a role in regulating outside-in signaling platelets (NCBI RAP1B, 2016) and is a crucial player in the CCM pathway.

Gore and colleagues (2008) designed a morpholino oligonucleotide experiment to knockdown rap1b. During a knockdown experiment, a specific substance, such as an oligonucleotide with sequence complementary to an active gene or its mRNA transcript, is integrated with a specific gene to interfere with the expression of that gene. Researchers targeted either the ATG initiator codon or the second exon-intron boundary to interfere with rap1b translation or splicing. After injecting the zebrafish embryos with the altered gene, and within 48 hours of fertilization, more than 70% developed ICH. Most hemorrhage foci were localized in, and around, the hindbrain ventricle. There was also a left-side bias, with 62% of hindbrain hemorrhages occurring on the left side. There were no deficits in proper patterning of nervous system, endothelial specification, or arterial-venous differentiation. The specificity of the observed phenotype effects of rap1b knockout were further verified by co-injection of synthetic human Rap1b mRNA with zebrafish rap1b morpholino. Two days post fertilization, 44% of co-injected embryos manifested cranial hemorrhages compared with 74% of embryos injected with rap1b ATG morholino alone. These results indicate that rap1b morpholinos target rap1b and specifically knock out its function and suggest that human and zebrafish Rap1b proteins have a conserved function.

To determine if rap1b is specifically required in endothelial cells for maintenance of vascular integrity, researchers carried out a rescue experiment. Rescue experiments are used to determine if the results from the knockdown experiment are the result of the gene or due to other effects not identified. Researchers used zebrafish fli1 promotor to drive endothelial expression of human rap1b. They co-injected a fli1:hRap1b DNA construct together with the zebra fish rap1b morpholino. Prior to injection, cranial hemorrhage was 74%. After injection, cranial hemorrhage was decreased to 30%. These results indicate that rap1b function is required in endothelial cells for vascular integrity. Any change in the expression of rap1b increases the likelihood of ICH.

Other genes associated with the structural integrity of blood vessels are COL4A1 and COL4A2 (Gould et al., 2006; Jeanne et al., 2015). COL4A1 and COL4A1 are located on the long arm of chromosome 13 at position 34. Together, they are the most abundant and prevalent proteins in basement membranes, including those in the cerebral vasculature. Basement membranes are a composite of several large glycoproteins and form an organized scaffold to provide structural support to the tissue and also offer functional input to modulate cellular function (LeBleu, MacDonald, & Kalluri, 2007). COL4A1 and COL4A2 provide strength to the basement membrane and also participate in dynamic biological process through interactions with growth factors and cell receptors, including bridging cell-to-cell or cell-to-extracellular matrix (NLM, 2016). When mutations occur in COL4A1, small-vessel disease can result. Gould et al. (2006) identified mutations in COL4A1 in mice with porencephaly, a rare genetic disorder in which fluid-filled cysts and cavities develop on the surface of the brain that can be the

result of hemorrhages. Researchers found that all heterozygous mutant pups had cerebral hemorrhage, and approximately 50% died on the day of birth. Next, researchers repeated the experiment, but this time, half of the pups were delivered surgically instead of naturally. Of the surgically delivered mutant pups, none had severe hemorrhage. These results suggest that COL4A1 may predispose mice to vascular fragility and act with birth trauma to cause cerebral hemorrhage. For adult mice, a higher rate of death was seen among mutants compared to their control littermates with a significant number having SAH. In addition to having hemorrhagic stroke as an outcome for mutations of COL4A1, researchers also found retinal vascular tortuosity, glomerular basement membrane, and microalbuminuria in mice. These often occur in some families with small-vessel disease (Vahedi et al., 2007).

In a later study, Jeanne and colleagues (2015) examined variants in COL4A1 and COL4A2 in mice and found that intracellular accumulation of mutant collagen in vascular endothelial cells and pericytes was a key triggering factor of ICH. Researchers also determined that surgically delivered pups, as previously discussed by Gould and colleagues (2008), along with tamoxifen injections during pregnancy resulted in less ICH than non-treated, naturally born mice. Another interesting outcome of this study was that physical exertion increased ICH, and anticoagulant administration provoked fatal hemorrhages within just 7 days of use. These results demonstrate an association between hemorrhagic stroke and COL4A1 and COL4A2 in mice, as well as suggest a detrimental interaction with environment. The interaction between gene (COL4A1 and COL4A2) and environment (increased exercise) is quite interesting as it pertains to hemorrhagic

stroke. Historically, increased exercise has been associated with decreased stroke risk (Lee, Hennekens, Kerger, Buring, & Manson, 1998), but this study suggests that excessive exercise for those with mutations of the COL4A1 gene could be detrimental.

Biological Risk Factors

The risk factors for hemorrhagic stroke are not clearly defined. Several biological risk factors, such as race, ethnicity (Broderick, Brott, Tomsick, Huster, & Miller, 1992; Howard et al., 2013), and high blood pressure (Howard et al., 2013; Matsukawa et al., 2012; Sturgeon et al., 2007), are historically associated with hemorrhagic stroke. Other factors, such as diabetes mellitus (Howard et al., 2013; Kissela et al., 2002; Woo et al., 2002) and high cholesterol (Sturgeon, et al., 2007; Wieberdink et al., 2011) are not as clearly defined but may be precipitating factors.

Age, Race, Ethnicity and Sex

African-Americans have a higher incidence of hemorrhagic stroke when compared to Hispanic and non-Hispanic White populations (Broderick et al., 1992; Howard et al., 2013). Broderick and colleagues (1992) found that Blacks have a greater risk of SAH than Whites regardless of age, with the highest incidence seen in individuals 54 years and younger. Similarly, ICH was 4 times more likely in ages under 54 years for Blacks compared to Whites; however, unlike SAH, the risk at 75 years and older level off to a 25% difference between Whites and Blacks suggesting that advancing age is more important as a risk factor for ICH than SAH.

In a later study, Sturgeon et al. (2007) found that increasing age and African-American ethnicity were risk factors for ICH. In fact, an estimation of interaction of

ethnicity and age found at age 45, African-Americans had 5.8 times the ICH rate of Whites. The relative rate decreased to 1.7 by age 65 and to 0.94 by age 75.

Hispanics are another minority population with a higher risk of hemorrhagic stroke (Morgenstern et al., 2004). As part of the Brain Attack Surveillance in Corpus Christi (BASIC) project, Morgenstern and colleagues (2004) completed a study comparing the incidence of ICH and SAH in Mexican-American, Blacks, and non-Hispanic whites. Researchers found Hispanics have a higher incidence of hemorrhagic stroke than non-Hispanic Whites but lower than Blacks. In addition, investigators found an increased risk of ICH regardless of age in Mexican Americans. Young Mexican-Americans aged 45-59 years had a three-fold increased risk of ICH compared with non-Hispanic Whites. Furthermore, Mexican-Americans were 1.5 times more likely to have SAH than non-Hispanic Whites. From 1995 to 1998, age standardized mortality rates for SAH and ICH were higher among Blacks than Whites. In an earlier study, Ayala et al. (2001) found that all minority populations, including Black, Asian/Pacific, and Hispanic, had higher mortality rates than Whites. Interestingly, regardless of race and ethnicity, the risk ratio for hemorrhagic stroke was higher than the risk ratio for ischemic stroke in the 25-44 and 45-64 age groups; however, for the ≥ 65 group, numbers were higher for ischemic stroke. Researchers suggested that differences in death rates between races and ethnic groups were possibly attributed to several factors including socioeconomic status, greater severity of disease, and poor survival at younger ages. In addition, researchers suggested that variations in risk factors such as; obesity, uncontrolled high blood pressure, inactivity, poor nutrition, diabetes, and cigarette smoking attributed to higher

death rates. Other affecting factors such as a lack of access to medical care and a lack of knowledge about early warning signs of stroke can also impact a patient's death rate.

Generally, males have a higher incidence rate of hemorrhagic stroke. Howard and colleagues (2013) found that men were significantly more likely to have ICH than females. However, Appelros and colleagues (2009) completed a systematic review comparing incidence rates of hemorrhagic stroke among males and females and found that although ICH were higher among men, the rate of SAH was higher, although not statistically significant, among women.

High Blood Pressure/Hypertension

High blood pressure (BP) is the best documented risk factor for hemorrhagic stroke. According to the National Institutes of Health (2015) fact sheet, one in three adults in the United States have hypertension (systolic BP $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg). The prevalence of hypertension increases with age and is similar among men and women. 7.3% of adults aged 18 – 39, 32.2% of adults aged 40 – 59, and 64.9% of adults 60 years and older have hypertension. Men have a higher prevalence of hypertension than women among adults aged 18 – 39 (8.4% compared with 6.1%, respectively) and aged 40 – 59 (34.6% compared with 29.9%, respectively), but there is no significant difference of hypertension among men and women aged 60 and over (63.1% compared with 66.5%, respectively) (Yoon, Fryar & Carroll, 2015). Blood pressure is influenced by three biological factors—how fast the heart is beating, how open and flexible the arteries are, and how much blood is pumping through the blood vessels (Chadha et al., 2012). Blood pressure is measured by determining the systolic

and diastolic pressure. Systolic BP is the measure of the arteries when the heart beats, and the diastolic BP measures the pressure in the arteries between heartbeats.

Sturgeon and colleagues (2007) found that ICH increased most dramatically with increasing hypertension state. For example, from Stage I hypertension, systolic BP 140 to 159 mm Hg or diastolic BP 90 to 99 mm Hg, to Stage II, systolic BP 160 mm Hg to 179 mm Hg, the relative rates of ICH doubled. From Stage II to Stage III, systolic BP ≥160 mm Hg or diastolic BP ≥110 mm Hg, relative rates doubled again, indicating that higher BP rates increase the risk of ICH.

Howard and colleagues (2013) found similar results. For every 10 mm Hg higher difference in systolic BP, there was a 24% increase of ICH. In addition, the use of warfarin increased the risk of ICH more than three times. Warfarin is an anticoagulant that is often prescribed after an ischemic stroke or myocardial infarct to decrease the clotting tendency of blood. It has been shown to increase prevalence of ICH and is related to increased mortality rates (Rosand, Eckman, Knudsen, Singer & Greenberg, 2004). Surprisingly, no association with hypertension status overall was detected, indicating higher systolic BP levels are a better indicator for risk of ICH than hypertensive status alone.

An important gene in regulation blood pressure is the angiotensin converting enzyme (ACE) gene. ACE helps regulate blood pressure and balance fluids and salts in the body. It plays a vital role in the development of hypertension by cutting a protein called angiotensin I. ACE converts this protein to angiotensin II. Angiotensin II causes blood vessels to narrow, which results in increased blood pressure (NLM, 2016). Higher

ACE activity leads to higher amounts of vasoconstrictor angiotensin II, which results in smooth muscle arteriolar proliferation, death of smooth muscle cell walls, and collagen deposition (Daemen et al., 1991; Fleming, 2006).

Das and colleagues (2015) compared the ACE genotypes and allelic frequencies in an Indian based population with a first time hemorrhagic stroke (n = 200) to an age and sex matched healthy control group (n = 200). Researchers also compared other biological and environmental risk factors between groups. The frequency of the DD genotype, as well as D allele was higher among the hemorrhagic group as compared to the controls. The DD genotype and D allele associated significantly with the disease using dominant, recessive, and co-dominant genotype models. Unfortunately, this study did not report on the use of ACE inhibitors. ACE inhibitors relax blood vessels and essentially lower blood pressure. Nevertheless, this study suggests that there is a possible role of D allele in predisposing individuals to hemorrhagic stroke. Interestingly, only 5% of participants had a family history of stroke, but 34% had a family history of hypertension. This suggests that a family history of hypertension may play a more important role in hemorrhagic stroke for those individuals with the DD genotype than a history of hemorrhagic stroke alone. In addition, there was no significant difference between the group's total cholesterol level or HDL levels. LDLs were not reported in the study. Triglyceride levels and a diagnosis of diabetes mellitus were significantly higher in the hemorrhagic stroke group. Smoking and alcohol use were significantly higher in the hemorrhagic stroke group, as well. Unfortunately, these environmental factors were not clearly defined.

Sanders and colleagues (2006) studied menstrual cycles of 23 healthy females (12 with dominant insertion (II) variant of the ACE genotype and 11 with dominant deletion (DD) variant of the ACE genotype) and found that ACE activity did not vary overall regardless of genotype. Interestingly, ACE activity throughout the cycle was significantly lower in those participants with II (21.4 +/- 4.1), compared to DD (32.4 +/- 8.4). Unfortunately, the researchers did not include information about blood pressure levels, thus elevated blood pressure in the DD genotype could not be verified.

Several studies have examined the association of the DD genotype in other populations. Sun and colleagues (2013) completed a meta-analysis on 805 participants with ICH and 1,641 controls from eight case controlled studies. Asian and Caucasian populations were included in the review. Results indicate that the DD homozygote carriers among Asians are more prone to increased risk of ICH than their Caucasian counterparts. Researchers found a 58% increase in the susceptibility to ICH in the dominant genetic model of the polymorphic variant I/D. In another study of a Spanish-Mediterranean population, researchers found that the DD genotype of the ACE gene was the strongest independent predictor of hypertension in individuals with diabetes mellitus (Martínez et al., 2000).

Although hypertension can be associated with the ACE gene and has a strong familial association, its cause can also be linked to environmental factors, such as lack of exercise, poor diet, and smoking (Alderman, 2000; Ishikawa-Takata, Ohta, & Tanaka, 2003). Some studies suggest that modifying the environmental risk factors may lower blood pressure. For example, Ishikawa-Takata et al. (2003) found that exercising as little

as 30 to 60 minutes a week significantly lowered blood pressure. Alderman (2000) found that by lowering salt intake, the risk for high blood pressure can be reduced.

Cholesterol

High serum total cholesterol (greater than or equal to 240 mg/dL) is present in 12.1% of adults age 20 years and over within the general population (National Center for Health Statistics, 2016). The association between high cholesterol and hemorrhagic stroke is inconclusive. Sturgeon and colleagues (2007) studied risk factors associated with ICH. They did not find overall cholesterol to be significantly associated with ICH; interestingly, the researchers did find that low LDL was a significant risk factor for ICH. In a later study by Wieberdink and colleagues (2011), decreasing levels of total serum cholesterol were associated with an increased risk of ICH, but LDLs were not associated with the risk of ICH. On the other hand, Kim and colleagues (2004) did not find a significant association between hemorrhagic stroke and high total cholesterol, although this study consisted of females only with the majority of strokes consisting of SAH. It did not assess differences between ICH and SAH and high cholesterol, so a difference could possibly be found. Because high LDL cholesterol can leave fatty deposits within the lining of the arteries (NIH, National Institutes on Aging, 2005), it is possible that having low LDL could cause thinning areas along the artery wall, thus increasing the risk for ICH (Konishi et al., 1993).

In recent years, the apolipoprotein E (APOE) gene has also been studied to determine if it is associated with hemorrhagic stroke. The APOE gene is located on the long arm of chromosome 19. It codes for 299-amino acid protein (apoE), which is a

polymorphic glycoprotein involved in cholesterol transport and cell membrane maintenance and repair (Mahley, 1988). ApoE protein is involved in the elimination of excess cholesterol from the brain. It is regulated by the liver X receptors, which are important regulators of cholesterol, fatty acid, and glucose homeostasis (Zerbinatti, 2009). Mutations of APOE are associated with higher levels of plasma cholesterol levels and accelerated coronary artery disease. APOE has three common alleles (e.g., ε2, ε3, ε4), and each person has two alleles that together compose that person's APOE genotype (e.g. ε2/ε3, ε3/ε3, ε3/ε4) (Mahley, 1988). The APOE ε2 variant is associated with slower cholesterol metabolism, which results in lower cholesterol. In contrast, the APOE ε4 variant is associated with faster cholesterol metabolism, resulting in a higher cholesterol level (Mahley, 1988). In a meta-analysis of 11 case-control studies with 1,238 ICH cases and 3,575 control cases, Zhang et al. (2014) found that there was a significantly higher frequency of APOE ε4 allele in the ICH cases. In addition, researchers found that Asians and Caucasians had a significantly higher frequency of APOE ε4.

A mutation of the apoE protein affects the regulating mechanism. If there is a large amount of apoE protein, cholesterol level will increase. Approximately 6.2% of adults ≥ 20 years of age have undiagnosed hypercholesterolemia, defined as a total cholesterol level ≥ 240 mg/dL (Mozaffarian, et al., 2015). Zerbinatti (2009) injected rats with apoE and found significant levels of cholesterol in the cerebral spinal fluid as early as 3 days after injection. Although there appears to be a link between ICH and APOE gene and between apoE protein and cholesterol levels, the association between ICH and cholesterol levels is inconclusive.

Diabetes Mellitus and Hemorrhagic Stroke

Although diabetes mellitus is highly correlated with ischemic stroke (Jerrad-Dunne et al., 2003; Jood et al., 2005), it is not often associated with hemorrhagic stroke. Indeed, most studies do not find an association between diabetes mellitus and hemorrhagic stroke (Howard et al. 2013; Kissela et al., 2002; Matsukawa et al., 2012); however, one study (Woo et al., 2002) did find a slight association between ICH and diabetes mellitus although it was not significant. Diabetes mellitus is associated with hypertension and therefore may be a precipitating factor for hemorrhagic stroke.

Several gene variations are associated with diabetes mellitus (i.e., HLA-DRB1, HLA-DQB1, and HLA-DQA1) (NML); however, none of these gene variants have been shown to have an association with hemorrhagic stroke. Diabetes Mellitus results from defects in insulin secretion, insulin action, or both. It has been associated with blood vessel, kidney, and heart damage (American Diabetes Association, 2009). Some studies suggest that diabetes mellitus is associated with hemorrhagic stroke, but others do not. Because diabetes mellitus is associated with blood vessel damage and kidney damage, and both of these are associated with hemorrhagic stroke (Mozaffarian et al., 2015), it is possible that there may be a stronger association with hemorrhagic stroke.

Environmental Risk Factors

Smoking and Hemorrhagic Stroke

Several modifiable risk factors are associated with ICH and SAH, including cigarette smoking, alcohol consumption, and stress. Smoking has a strong association with hemorrhagic stroke and is one of the most preventable causes of a variety of

diseases (Centers for Disease Control and Prevention [CDC], 2014). When nicotine enters the bloodstream, it immediately stimulates the adrenal glands to release the hormone epinephrine. Epinephrine stimulates the central nervous system and increases blood pressure, respiration, and heart rate (CDC, 2014). In addition, cigarette smoking may have an adverse effect on lipid metabolism (beta blockers, diuretics) (Porkka & Ehnholm, 1996) due in part by free radical damage to the lipids (Miller, 1997).

Brischetto, Connor, Connor, and Matarazzo (1983) proposed a mechanism to explain the link between smoking and changes in serum lipid and lipoprotein concentrations. First, nicotine stimulates the release of adrenaline by the adrenal cortex, leading to the increased serum concentrations of free fatty acid observed in smokers. Next, free fatty acid is a well-known stimulant of hepatic secretion of very low LDL and hence higher triglyceride. Triglycerides are a type of lipid stored in fat cells in the blood. Finally, HDL concentrations vary inversely with very low LDL concentrations in serum. Complementary to this mechanism is the finding that free fatty acid also stimulates hepatic synthesis and secretion of cholesterol.

Alcohol Use and Hemorrhagic Stroke

Another environmental risk factor associated with hemorrhagic stroke is alcohol consumption. It is suspected that heavy alcohol use can stop the growth of new brain cells (NIH, 2004). Klatsky, Armstrong, Friedman, and Sidney (2002) found no increased risk of hemorrhagic stroke among light or moderate drinkers of either sex or race but did find a slight increase in hemorrhagic stroke for individuals who were heavy drinkers (≥3 drinks per day). Interestingly, the relative risk, when compared by type of beverage,

showed no independent association to hemorrhagic stroke risk (wine = 1.02, liquor = 1.06, and beer = 1.05). It is unclear exactly how biological changes due to alcohol use are associated with hemorrhagic stroke.

Psychosocial Stress

According to Centre for Studies on Human Stress (2007), when an individual interprets a situation as being stressful, it triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis whereby neurons in the hypothalamus release corticotropin-releasing hormone (CRH). The release of CRH triggers the subsequent secretion and release of adrenocorticotropin (ACTH) from the pituitary gland, which travels in the blood and reaches the adrenal glands, which triggers secretion of the stress hormones cortisol, epinephrine and norepinephrine. Several studies have shown an association between psychosocial stress and hemorrhagic stroke although the definition differs. For example, O'Donnell and colleagues (2010) found psychosocial stress, defined as a combined measure of general stress at home and in the workplace, to be significantly associated with ICH. Henderson and colleagues (2013) found distress was an analytically derived composite measure of depressive symptoms, perceived stress, neuroticism, and life dissatisfaction. For each 1-SD increase in distress, there was 1.47 increase in stroke mortality and 1.18 increase in stroke incident. O'Donnell and colleagues (2010) found psychosocial stress, defined as a combined measure of general stress at home and in the workplace; depression: feeling sad, blue or depressed for 2 or more consecutive weeks during past 12 months, significantly associated with ICH. Defining psychosocial stress is a complex concept. It considers personal and professional

aspects and varies from person to person based on perception. The same biological reaction to stress is consistent within all human beings. It is instead, the person's perception and management of that stress that is individualistic to each situation. Thus making human beings reaction to stress idiosyncratic in nature (Centre for Studies on Human Stress, 2007). Unfortunately, no known studies on cortisol levels prior to stroke exist. Without these studies, there can be only speculation that increased cortisol level as the result of stress is an increased risk factor for hemorrhagic stroke.

Risk Factors by Location in the Brain

ICH occurs more frequently than SAH and has poorer outcomes. Of patients who survive, only 28%-35% are independent at 3 months after damage (Woo et al., 2002). Some studies suggest that risk factors for ICH and SAH vary based on location and that this plays a role in prevalence (Flaherty et al., 2005; Martini et al., 2012; Nieuwkamp, Wilde, Wermer, Algra, & Rinkel, 2014).

Location of ICH Within the Brain

ICH can be divided into two primary locations, lobar and nonlobar (basal ganglia, thalamus, brainstem, cerebellum, or periventricular white matter). Martini and colleagues (2012) studied 597 Blacks and Whites with a first time history of ICH and found that regardless of location, all ICH were more likely to be associated with hypertension, current warfarin use, having a first-degree relative with ICH, having a personal history of ischemic stroke, having less than high school education, and having the APOE ε2 or ε4 genotype. When compared by different locations, risk factors for subjects with lobar damage included warfarin use, prior history of ischemic stroke, less than a high school

education, and the APOE ε2 or ε4 genotype. For subjects with nonlobar ICH, risk factors included history of hypertension, warfarin use, first-degree relative with ICH, prior history of ischemic stroke, and less than a high school education. Roughly 10% of subjects had a history of ischemic stroke and about 15% warfarin use. Had researchers excluded these two groups, the study could have been a little more compelling. Interestingly, Blacks with ICH, regardless of location, were six times more likely to have hypertension than the control group, and Whites with ICH, regardless of location, were two times more likely to have hypertension compared to the control group. Nevertheless, the major difference between risk factors when compared between the two locations was that hypertension and high cholesterol were associated with nonlobar ICH, whereas APOE ε2 or ε4 genotype was associated with lobar ICH. This suggests that there are different pathophysiological processes for ICH based on location. In addition, this suggests that there may be a protective association of hypercholesterolemia that varies by hemorrhage location.

APOE ε2 or ε4 genotype has been associated with lobar ICH in other studies as well, and it appears to play a role in the pathogenesis of amyloid angiopathy (Biffi et al., 2010; Charidimou et al., 2015; Woo et al., 2004; Zhang et al., 2014). Cerebral amyloid angiopathy is the result of the deposition of an amyloidogenic protein in cortical and leptomeningeal vessels. The accumulation of amyloid correlates with loss of smooth muscle cells that are found within the walls of blood vessels (Rensink et al., 2003). Damage to smooth muscle cells can result in thinning of the cell wall, which can result in hemorrhage (Frösen, 2014).

Charidimou and colleagues (2015) studied cerebral amyloid angiopathy in patients with and without lobar ICH. Researchers found that the subjects with cerebral amyloid angiopathy presenting with lobar ICH were more likely to have the APOE ε2 genotype, but in non-hemorrhagic cerebral amyloid angiopathy cases, APOE ε4 was enriched. This suggests that the APOE ε4 genotype may be a protectant against hemorrhage whereas APOE ε2 increases the risk of hemorrhage.

Location of SAH in the Brain

SAH occur less frequently than ICH. Incidence and outcome are based on artherogenic risk factors (i.e., hypertension, smoking, etc.) and the size and location of the aneurysm. Only about 50% of those who experience a SAH survive (van Gijn, Kerr, & Rinkel, 2007). Familial aggregation of SAH occurs in 6% to 9% of SAH cases, and those with familial SAH have worse outcome than those cases that are sporadic (Bromberg et al., 1995).

Nieuwkamp and colleagues (2014) studied the clinical and radiological heterogeneity of aneurysmal SAH based on associated risk factors. Subjects were divided into two groups: those with artherogenic risk factors (hypertension and smoking) (n = 113) and those without (n = 29). Even though researchers purposely included only young adults (ages 18-40), and an overwhelming large percentage (nearly 80%) were either diagnosed with hypertension and/or were smokers, resulting in a small sample size of those without artherogenic risk factors. Nevertheless, researchers found that the middle cerebral artery was the site of aneurysm for most SAH cases regardless of group. Interestingly, subjects with artherogenic risk factors were less often women. In addition,

the atherogenic risk factor group had more often-larger aneurysms, multiple aneurysms, and a poorer outcome.

Research Question and Rationale

The causes of hemorrhagic strokes are multifactorial but appear to have a strong familial link. The low incidence and high mortality rates make it difficult to determine a specific genetic source. However, several genes are associated with hemorrhagic stroke and it is the documented biological risk factors that may help researchers determine if there is a specific gene or group of genes that increases the risk of hemorrhagic stroke within families.

Each gene has its own purpose and plays an important role in maintaining balance within the vascular system. The CCM pathway genes and COL4A1/COL4A2 genes both relate to cell integrity. Mutations in either of these gene groups significantly increase the risk of hemorrhages. For COL4A1/COL4A2, increased blood flow, as in exercising or during the stress of natural birth process, increases the risk even more, demonstrating that the interaction between genes and environment is an important component of disease outcome. Hormone replacement therapy or having a first pregnancy at an older age is associated with decreased risk of SAH, supporting a yet undetermined association between hormones and acquisition of hemorrhagic stroke.

Hypertension is a primary risk factor for hemorrhagic stroke. Higher stages of hypertension significantly increase the risk of ICH. As blood pressure builds, it can cause already weakened blood vessels to rupture resulting in a hemorrhagic stroke. The ACE gene is part of the renin-angiotensin-aldosterone system (RAAS) and is important in

the regulation of blood pressure. RAAS increases renin production. Renin release is triggered when blood pressure is low, when there is sympathetic nerve cell action (i.e., fight or flight response), or when salt levels are low. A dominant ACE genotype is significantly associated with hypertension, as well as ICH and can result in smooth muscle cell death and collagen deposition.

The association between cholesterol levels and hemorrhagic stroke is not as clear as the relationship between hypertension and hemorrhagic stroke. ApoE is one of the key lipoproteins of lipoprotein complexes that regulate the metabolism of lipids by directing their transport, delivery, and distribution from one tissue or cell type to another through apoE receptors and proteins associated with lipid transfer and lipolysis. Higher levels of cholesterol are associated with higher levels of apoE protein. The APOE ε4 variant is associated with faster cholesterol metabolism resulting in higher cholesterol levels; whereas, the APOE ε2 variant is associated with slower cholesterol metabolism resulting in lower cholesterol level. Both APOE ε4 and ε2 have been associated with lobar ICH and play a role in the pathogenesis of amyloid angiopathy.

Limited incidences of hemorrhagic stroke make researching risk factors challenging. Identifying a family with a strong history of hemorrhagic stroke is the first step in the research process. With ASHA's position statement on the importance of prevention of communication problems, it is the responsibility of those in the field of speech-language pathology to expand the traditional role of identification and treatment of communication disorders to include efforts to eliminate the underlying cause of those disorders. Because communication disorders result from hemorrhagic stroke, the best

way to treat them is to eliminate the source. Therefore, determining the risks of hemorrhagic stroke and preventing them is the first place to start. It is unlikely that there is one gene that guarantees an individual will acquire a hemorrhagic stroke; instead, it is more likely that several genes interact with each other and the environment that would result in hemorrhagic stroke. Thus, it is expected that there will be differences in the environmental and biological risk factors within families with a history of hemorrhagic stroke and between families with hemorrhagic stroke.

This study is designed to answer the following research questions related to familial hemorrhagic stroke:

Question 1: What are the similarities and differences of the biological risk factors associated with hemorrhagic stroke, such as hypertension, diabetes, and/or hypercholesterolemia within and between families with hemorrhagic stroke?

Question 2: What are the similarities and differences of the environmental factors associated with hemorrhagic stroke, such as exercise, alcohol consumption, mediation use, etc. within and between families affected by hemorrhagic stroke?

Question 3: Are there differences between the location of hemorrhagic stroke between and within families?

CHAPTER III

METHODS

The purpose of this study was to determine the risk factors associated with hemorrhagic stroke within families with a history of hemorrhagic stroke and between families with a history of hemorrhagic stroke. The risk factors of hemorrhagic stroke were determined by administration of a family medical and social history questionnaire. In addition, participants completed the *Health Promoting Lifestyle Profile II (HPLPII)* and the *Perceived Stress Scale (PSS)*. The rationale for use of these participants is that because hemorrhagic strokes aggregate within families, there is a greater chance that a genetic commonality can be found within the same family and possibly between families.

Participants

Participants were recruited through stroke support groups in the southeast, social media and Casa Colina Hospital and Medical Center in Pomona, California. Individuals who had a hemorrhagic stroke at age 65 years or younger and had at least one other family member with a hemorrhagic stroke at 65 years or younger were invited to participate in the study. First- and second-degree relatives of the person with hemorrhagic stroke were also invited to participate in the study.

Inclusion Criteria

1. Hemorrhagic stroke at the age of 65 or younger
2. At least one first or second degree relative who had a hemorrhagic stroke at the age of 65 or younger
3. Understands and reads English well enough to read and answer questionnaire questions

Exclusion Criteria

1. Presence of other neurological or neurodegenerative disease

Data Collection Procedures

The current study was approved by the Institutional Review Board at the University of North Carolina at Greensboro and Institutional Review Board at Casa Colina Hospital and Centers for Healthcare. An informed consent was secured from each participant before enrolling in the study.

Recruitment flyers were emailed or mailed to 14 stroke support group facilitators in the southeast. Two stroke support group facilitators requested additional information, 1 facilitator reported no stroke support group members with a hemorrhagic stroke and declined to assist with recruitment, and no information was obtained from the remaining 11 stroke support group facilitators. Flyers and emails were sent to speech-language pathologist and physical therapist working at 4 hospitals and 4 universities and community colleges in the southeast. Information was also shared on social media (Facebook). Seven individuals initially contacted researchers for possible inclusion in the study. Initial interviews determined 3 of the individuals had an ischemic stroke rather

than a hemorrhagic stroke and were eliminated. The remaining 4 individuals had a hemorrhagic stroke or had a first or second degree relative with a hemorrhagic stroke and were asked to contact their first and second degree relatives to determine if family members would be willing to participate in the study. Four families were recruited for the study for a total of 14 individuals (8 with hemorrhagic stroke, 6 without hemorrhagic stroke). In addition, 2 individuals from Casa Colina were recruited and filled out initial questionnaires but researchers were not able to contact them for follow-up questions. Therefore, there was not sufficient data available to include them in the study.

Family, Medical and Social History Questionnaire

The purpose of administering a medical history and social questionnaire in this study was to establish personal and family history of hemorrhagic stroke as well as risk factors associated with hemorrhagic stroke. Participants were asked if they had a first or second degree blood relative who had a hemorrhagic stroke at or before the age of 65. Once identified, participants were asked at what age the family member had the hemorrhagic stroke and if the family member had a history of hypertension, high cholesterol, or diabetes. The medical history portion of the questionnaire asked specific questions about history of high blood pressure, high cholesterol, and diabetes. Participants without a hemorrhagic stroke were asked to obtain their blood pressure, cholesterol and blood glucose levels from their physician and share this information with researchers. Participants who had a hemorrhagic stroke were asked to obtain the same information with the addition of the location of their hemorrhage, blood pressure, cholesterol and blood glucose levels prior to their hemorrhagic stroke and share this

information with researchers. The social history portion of the questionnaire asked specific questions related to cigarette smoking, alcohol consumption, and exercise habits, as well stress level prior to the hemorrhagic stroke. Non-hemorrhagic stroke participants were asked the same questions as they pertain to their current history.

Definitions

High blood pressure was defined as systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg based on current guidelines (James et al., 2014) or currently taking medication for high blood pressure (i.e., thiazide diuretics, angiotensin converting enzyme inhibitors, calcium blockers, beta blockers or angiotensin receptor blockers).

High cholesterol was defined as blood cholesterol level ≥ 200 based on current guidelines (Stone et al., 2014) or currently taking medication to lower cholesterol levels, such as statins or cholesterol absorption inhibitors.

Diabetes Mellitus was defined based on current guidelines of A1C ≥ 6.5, fasting plasma glucose ≥126 mg/dL (American Diabetes Association, 2012) or taking medication to lower blood glucose levels such as, insulin, biguanides, or meglitinides.

High risk medications were defined as any medication that interferes with the blood's ability to clot, such as anticoagulant and/or antiplatelet.

Health Promoting Lifestyle Profile II

The Health Promoting Lifestyle Profile II (HPLPII) (Walker, Sechrist, & Pender, 1983) is a self-report questionnaire. It was administered to determine the health-promoting lifestyle habits of participants. The HPLPII consists of 52 questions related to lifestyle habits and gives an overall health-promoting lifestyle score and six subscales:

health responsibility, physical activity, nutrition, spiritual growth, interpersonal relations, and stress management. The options for the questions are given on a four-point Likert scale ("never" (1), "sometimes" (2), "often" (3), and "routinely" (4)) and asked the respondents to indicate how often they adopt specific health-promoting behaviors. Non-hemorrhagic stroke participants were asked to answer each question based on their habits for the past 6 months. Participants with hemorrhagic stroke were asked to answer the same questions, to the best of their recollection, of their habits 6 months prior to their hemorrhagic stroke. Overall, the score for health-promoting lifestyle and behavioral aspects was calculated using the mean of responses for all 52 items and for each subscale (eight or nine items).

Perceived Stress Scale

The Perceived Stress Scale (PSS) (Cohen, Kamarck, Mermelstein,1983) is a self-report measure of stress. It consists of 10 questions related to how individuals perceive their own stress in the past month. Participants with hemorrhagic stroke were asked to answer the questions, to the best of their knowledge, of their feelings or thoughts occurring one month prior to their hemorrhagic stroke. Non-hemorrhagic stroke participants were asked to respond to questions with events occurring in the last month. Responses: never, almost never, sometimes, fairly often, and very often were added together to determine an overall PSS score. Questions 1, 2, 3, 6, 9, and 10 were scored 0 to 4, respectively. Questions 4, 5, 7, and 8 were scored 4-0, respectively. The PSS has a range of scores between 0 and 40 with higher scores indicating more perceived stress. Scores ranging from 0-13 would be considered low stress, 14-26 would be considered

moderate stress, and 27-40 would be considered high-perceived stress. Each person handles stress differently. One person may find the exact same event very stressful, another person may not find it stressful at all. The PSS allows participants to rate their own perceived stress.

Data and Statistical Analyses

This study had retrospective and prospective elements. That is, participants with hemorrhagic stroke were asked to recall environmental and medical information prior to their stroke, whereas, non-hemorrhagic stroke participants answered the same basic questions relevant to their current state. Nonparametric statistical analysis was preformed to compare biological risk factors and environmental risk factors associated with hemorrhagic stroke within and between families with a history of hemorrhagic stroke and to measure the strength and direction of association that exists between variables where appropriate. Visual representation, such as pedigree charts, bar charts and scatter plots, were used for data comparison. All necessary statistic procedures were completed using IBM SPSS V.20 software, Armonk, NY.

CHAPTER IV

RESULTS

The aim of the study was to identify similarities and differences of the biological risk factors associated with hemorrhagic stroke, such as hypertension, diabetes mellitus, and/or high cholesterol, and environmental risk factors, such as exercise, alcohol consumption, smoking, and perceived stress within and between families with a history of hemorrhagic stroke. Health and family history questionnaires were obtained from 4 families with a history of hemorrhagic stroke to determine if there were commonalities within the same family or among the different families. In addition, the HPLPII was administered which gave an overall total score and 6 subtest scores: health responsibility, physical activity, nutrition, spiritual growth, interpersonal relations, and stress management. PSS was used to determine perceived stress.

Participant demographics by family for gender, current age, age at time of hemorrhagic stroke, race and familial relationship are shown in Table 1.

Table 1. Participant Demographics

Family	Participant	Relationship to Proband	Sex	Race	Current Age	Hemorrhagic Stroke (Y/N)	Age at Hemorrhagic Stroke
Family 1	P.M. (II $_3$)	Proband	F	White	65	Yes	55
	G.M. (I $_2$)	Mother	F	White	Deceased	Yes	62
	W.M. (III $_2$)	Daughter	F	White	50	No	---
	S.M. (III $_3$)	Daughter	F	White	45	No	---
Family 2	A.D. (II $_1$)	Proband	M	White	73	Yes	65
	B.D. (III $_1$)	Son	F	White	45	Yes	40
	G.D. (III $_2$)	Daughter	F	White	39	No	---
	S.D. (III $_3$)	Daughter	F	White	37	No	---
Family 3	H.N. (III $_1$)	Proband	M	White	62	Yes	59
	B.H. (II $_4$)	Aunt	F	White	84	Yes	64
Family 4	M.C (II $_1$)	Proband	F	AA	Deceased	Yes	62
	S.C. (III $_2$)	Daughter	F	AA	49	No	---
	I.C. (III $_3$)	Daughter	F	AA	48	Yes	41
	T.C. (III $_4$)	Daughter	F	AA	48	No	---

Family History

Family 1

P.M. is participant II $_3$ on the pedigree shown in Figure 1. She is a 65-year-old white female who had a left frontal lobe hemorrhagic stroke at the age of 55. P.M. reported a history of hypertension and diabetes mellitus prior to her hemorrhagic stroke. She took medication for regulation of both. She did not take aspirin or any other anticoagulant medication. P.M. reported that she did not participate in strenuous exercise 4 or more hours per week. She reported that she did have general stress; she did not smoke but did drink alcohol (1-30 drinks per month) prior to her stroke. She had 4

children. One son died at the age of 22 in a car accident, one son, currently 53 years of age with a history of hypertension, did not participate in the study. P.M.'s oldest daughter, W.M. is participant III $_2$ on the pedigree in Figure 1. W.M. is a 50-year old female who reported no history of hypertension, diabetes mellitus or high cholesterol. She did not smoke cigarettes but did drink alcohol (1-30 drinks a month). She reported no general stress. W.M.'s BMI is 21.8. P.M.'s youngest daughter, S.M., participant III $_3$ on the pedigree in Figure 1, reported a recent diagnosis of hypertension, but no history of diabetes mellitus or high cholesterol. She did not smoke cigarettes, drink alcohol or do moderate or strenuous exercise 4 hours per week or more. She did report general stress.

P.M.'s mother, G.M., participant I $_2$ on the pedigree shown in Figure 1, had a hemorrhagic stroke at the age of 62 that resulted in death. G.M. had a history of hypertension, diabetes and high cholesterol. Her BMI was 26.5. She reportedly had a history of hypertension, diabetes mellitus and high cholesterol. She did not smoke cigarettes, drink alcohol or exercise. She had 4 children (2 males, 2 females).

P.M. had two older brothers who are deceased. Both brothers died prior to the age of 65, one with complications due to a heart transplant and the other from an unknown cause. She has one living sister age 62. No information was available about the sister.

Family 2

A.D. is participant II $_1$ on the pedigree in Figure 2. She is a 73-year old female with right hemisphere frontal lobe hemorrhagic stroke at age 65. A.D. reported a history of hypertension and high cholesterol but not diabetes mellitus prior to her stroke. She took medication for both hypertension and high cholesterol as well as 350 mg baby

aspirin prior to her stroke. She reported that she did exercise, did not smoke cigarettes or drink alcohol prior to her stroke, but did have general stress prior to her stroke. Her BMI at the time of her stroke was 33.3. A.D. had a younger brother who died in his 60s of heart disease. Her mother died in her 80s of a stroke although she did not know the type (ischemic or hemorrhagic). Her father also died of a heart attack in his 70s. She has four children.

A.D.'s oldest son, B.D. is participant III $_1$ on the pedigree in Figure 2. B.D. had a right hemisphere, frontal lobe hemorrhagic stroke at the age of 40. A.D. did not go to the doctor regularly prior to his stroke and had no knowledge of his medical history. However, to his knowledge, he did not have hypertension, high cholesterol or diabetes mellitus. Currently, he has hypertension and takes medication for this. He did not report exercise or general stress prior to his stroke. He did drink alcohol (>30 drinks a month) and smoked cigarettes (>1 pack a day). At the time of his stroke, B.D.'s BMI was 26.2. He has no children.

A.D.'s three other children were the progeny of a different father than B.D. and were therefore half-siblings to him. G.D., a 39-year old female, is the eldest daughter of A.D. She is participant III $_2$ on the pedigree in Figure 2. She reported no history of hypertension, high cholesterol or diabetes mellitus. She did not exercise. She did drink alcohol (1-30 month), did not smoke, and reported no general stress. G.D.'s BMI is 33.3. She has no children.

S.D. is participant III $_3$ on the pedigree in Figure 2. She is the youngest daughter of A.D. and half-sibling to B.D. and full sibling to G.D. S.D. reported a history of

hypertension and high cholesterol, but not diabetes mellitus. She did not exercise. She did drink alcohol (1-30 per month), and smoke cigarettes (>1 pack per day). She also reported general stress. S.D.'s BMI is 36.0. She has no children.

A.D.'s 35-year old youngest son reportedly has hypertension but no other information was available.

Family 3

Family 3 consisted of two participants. H.N. is participant III $_1$ on the pedigree in Figure 3. He is a 62-year-old white male who had a right hemisphere hemorrhagic stroke at the age of 59. He reported episodic hypertension, not medicated, prior to his stroke. He reported no high cholesterol or diabetes mellitus prior to his stroke. He did participate in moderate or strenuous exercise 4 hours or more per week. He drank alcohol (1-30 month). He did not smoke cigarettes. He reported general stress prior to his stroke. He had no children. According to H.N., both his maternal grandfather and great-grandfather had strokes at an early age. H.N.'s parents both have hypertension. His father has been taking blood pressure medication since he was in his mid-30s.

B.H. is participant II $_4$ on the pedigree in Figure 3. She is the 84-year old paternal aunt to H.N. B.H. had a hemorrhagic stroke at the age of 64. She reported a history of hypertension, unmedicated prior to her stroke. She did not have diabetes mellitus or high cholesterol. She did not exercise prior to her stroke. She did drink alcohol (1-30 per month) and did not smoke cigarettes. B.H. did not have any biological children. B.H. has father had an aneurysm in his brain at the age of 65 and died during surgery to repair it. Her mother died of cardiac arrest in her mid-70s.

Family 4

M.C. is participant II $_2$ on the pedigree in Figure 4. M.C. was an African American female who had a hemorrhagic stroke at the age of 56. She died at the age of 62 with breast cancer. According to M.C.'s daughters, S.C. and I.C., participants III $_2$ and III $_3$, respectively, M.C. had a history of hypertension and high cholesterol but not diabetes mellitus prior to her stroke. She reportedly did not smoke cigarettes or drink alcohol. Her BMI was 22.7 at the time of her stroke. M.C. had 7 siblings all with a history of hypertension. M.C.'s mother, represented on the pedigree in Figure 4 as I $_2$, had a stroke at the age of 68 that resulted in death. M.C. had 4 daughters. The oldest three daughters participated in the study. Her youngest daughter, age 46, did not, but reportedly has hypertension.

M.C.'s oldest daughter, S.C., is a 49-year-old with a history of hypertension and high cholesterol but not diabetes mellitus. She did not smoke. She did report that she did drink alcohol (1-30 drinks per month). S.C.'s BMI is 26.6. She has 3 children.

I.C. had a right hemisphere hemorrhagic stroke at the age of 41. She had a history of hypertension and high cholesterol but not diabetes mellitus prior to her stroke. She did participate in moderate or strenuous exercise 4 hours per week, did not smoke cigarettes or drink alcohol. She did not report general stress prior to her stroke. I.C.'s BMI was 18.5 at the time of her stroke. She was taking blood pressure and high cholesterol medication as well as aspirin prior to her stroke. She has 2 children.

T.C. is the identical twin of I.C. She is 48 years old with a history of hypertension but not high cholesterol or diabetes mellitus. She reported that she participates in

moderate or strenuous exercise 4 hours or more per week and does not smoke cigarettes, drink alcohol or have general stress. Her BMI is 23.6. She has 2 children.

Biological and Environmental Risk Factors—by Family

Table 2 shows the biological and environmental risk factors for all participants. Hypertension was more prevalent than diabetes mellitus or cholesterol with 78.6% (11/14) of participants reporting a history of hypertension. The youngest participant with a diagnosis of hypertension was 35 years of age and the oldest participant was 84 years of age. Of the 3 remaining participants, 1 participant with a hemorrhagic stroke was unaware of his history. The other 2 remaining were non-hemorrhagic stroke participants who reported no history of hypertension. Family 4 had the highest percentage with 100% (4/4). Family 3 also had 100% (2/2) with episodic hypertension; however, neither participants was taking medication to regulate blood pressure at the time of their hemorrhagic stroke. In Family 1, 75% (3/4) reported hypertension. Family 2 had 50% (2/4) that reported hypertension; however, one participant with hemorrhagic stroke reported no knowledge of hypertension prior to the hemorrhagic stroke as he did not go to the doctor regularly.

High cholesterol was present in 42.9% (6/14) participants. Of those, Family 4 had the highest percentage with 75% (3/4). Family 2 had 50% (2/4) reporting high cholesterol although one family with a hemorrhagic stroke did not go to the doctor on a regular basis prior to his stroke so information was not available on him. Family 1 reported 25% (1/4) with high cholesterol and Family 3 had no participants with high cholesterol.

Diabetes mellitus was present in 14.3% (2/14) participants. Family 1 accounted for both incidences of diabetes mellitus.

General stress, as measure by the self-identification on the questionnaire was present in 41.7% (6/14) of participants. No information was available on 2/14 (1 from Family 1 and 1 from Family 4) as these were deceased. Family 1 had 75% (3/4) reported general stress. The remaining person in Family 1 was deceased so no information was obtained about stress. Family 2 had 50% (2/4) participants who reported general stress and Family 3 had 50% (1/2) who reported general stress. No participants from Family 4 reported general stress.

Alcohol use was reported in 57.1% (8/14) participants. Alcohol consumption was reported as 1-30 drinks per month by all but one participant. He reported >30 drinks per month. There were only 2/14 (14.3%) who reported smoking. Both of these were in Family 2 and also reported alcohol use.

Only 28.6% (4/14) reported that they participated in moderate or strenuous exercise 4 hours or more per week. Two were in Family 4 and 1 was in Family 3 and 1 was in Family 2.

Biological and Environmental Risk Factors—with Hemorrhagic Stroke

All participants 87.5% (7/8) with a hemorrhagic stroke reported hypertension prior to their stroke with the exception of one participant who did not go to the doctor regularly. Five of the eight (62.5%) participants were taking medication to regulate blood pressure. Two participants reported episodic hypertension and were not taking medication to regulate blood pressure at the time of their stroke.

High cholesterol was reported by 50% (4/8) of the participants with hemorrhagic stroke. Again, one participant did not go to the doctor regularly so did not have any knowledge of his cholesterol levels; however, he does not currently have high cholesterol.

Two participants (2/8, 25%) with hemorrhagic stroke reported taking an aspirin daily. Interestingly, these two participants, one from Family 1 and one from Family 4, both also had high cholesterol.

Diabetes mellitus was reported in 25% (2/8) participants with hemorrhagic stroke. Both of these were in Family 1.

Exercise was reported in 37.5% (3/8) participants with hemorrhagic stroke. Only 1 participant (1/8, 12.5%) smoked cigarettes and he smoked >1 pack/day. He is also the only participant who reported consumption of >30 alcoholic beverages a month.

General stress was reported in 37.5% (3/8) participants; however, information was not available regarding general stress on 2 participants who were deceased.

Biological and Environmental Risk Factors—without Hemorrhagic Stroke

Hypertension was the most prevalent biological risk factor among participants without hemorrhagic stroke with 83.3% (5/6). Only 2/6 (12%) participants reported high cholesterol and no participants reported a history of diabetes mellitus.

Only 16.7% (1/6) reported strenuous exercise 4 or more hours per week. Cigarette smoking was reported in 33.3% (2/6) participants. Both smokers were from Family 2. Alcohol use was reported in 66.7% (4/6) participants. General stress was reported in 50% (3/6) participants.

Location of Hemorrhagic Stroke

Of the 8 participants with hemorrhagic stroke, 2 were deceased, and 1 did not know the location of her hemorrhagic stroke. Interestingly, 4/5 of the remaining participants with a hemorrhagic stroke reported that their hemorrhagic stroke occurred in the right hemisphere of the brain. Of the remaining 4 participants with hemorrhagic stroke, 3 reported a frontal lobe lesion and 1did not report a lobe location. One participant with hemorrhagic stroke reported that his hemorrhagic stroke was a SAH. None of the remaining participants reported type of hemorrhagic stroke.

Figure 1. Family 1 Pedigree

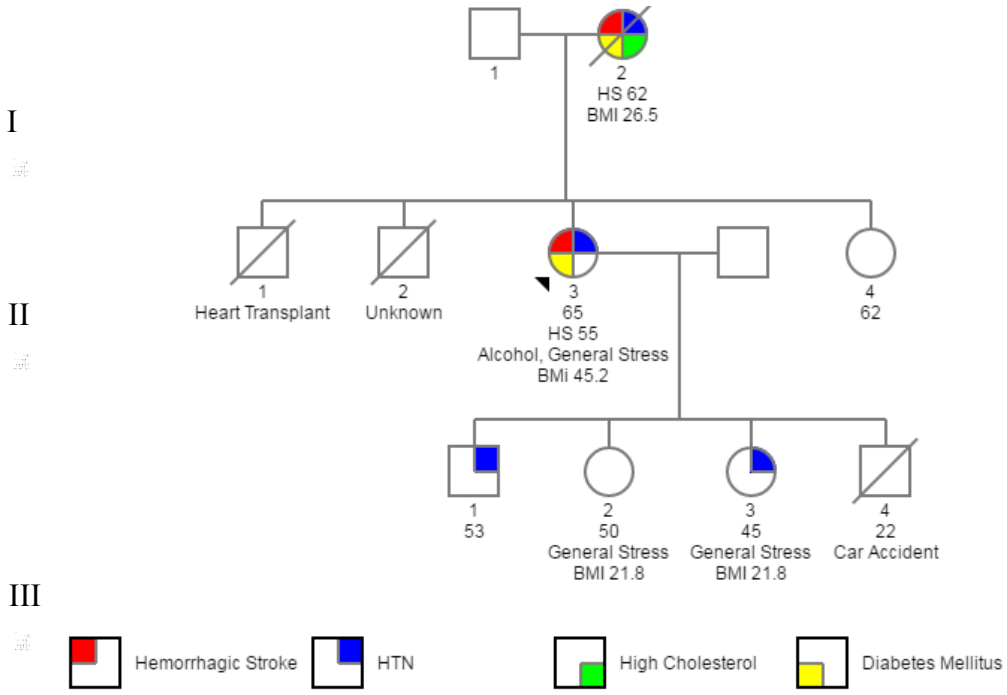


Figure 2. Family 2 Pedigree

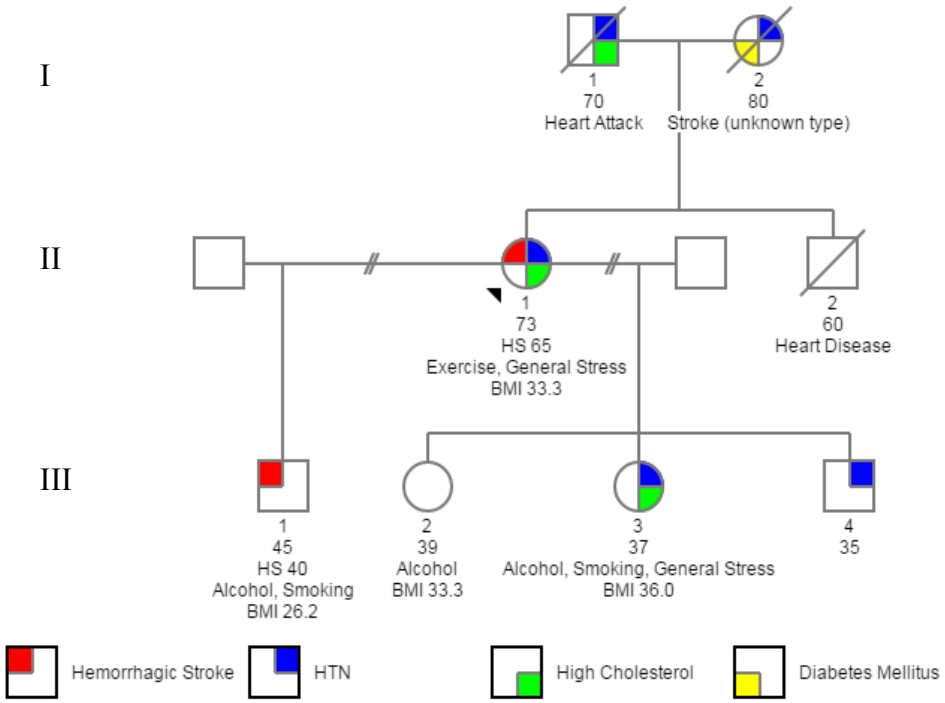


Figure 3. Family 3 Pedigree

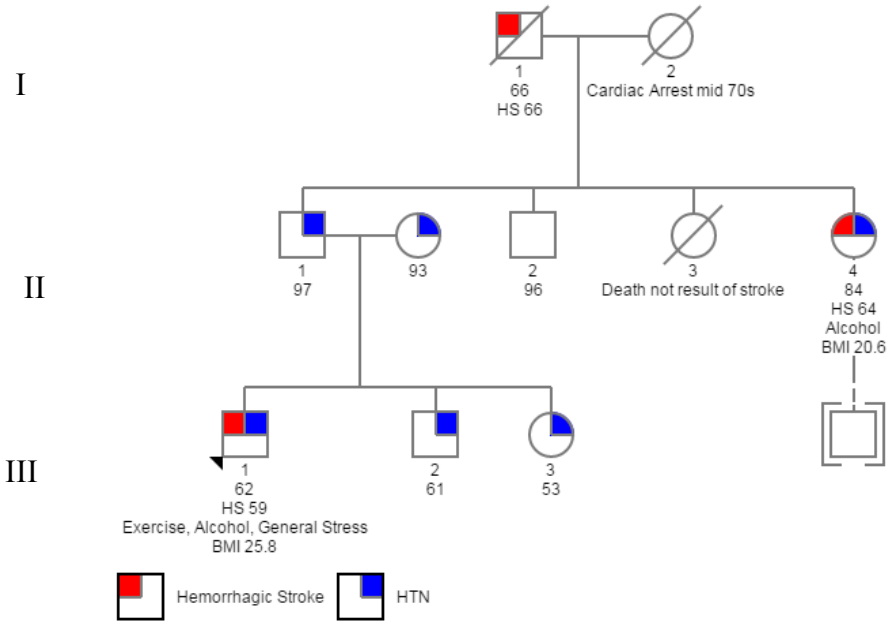


Figure 4. Family 4 Pedigree

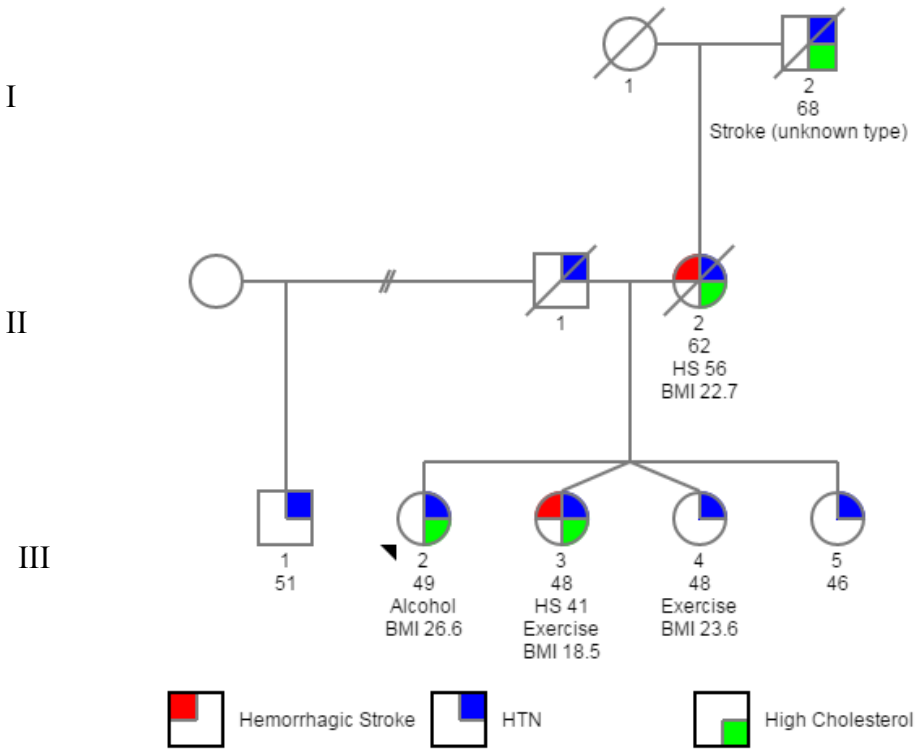


Table 2. Biological and Environmental Risk Factors

Family	Participant	HS	HTN	HC	DM	Exercise	Smoke	Alcohol	GS	BMI
Family 1	P.M. (II $_3$)	+	+	-	+	-	-	+	+	45.2
	G.M. (I $_2$)	+	+	+	+	-	-	-	un	26.5
	W.M. (III $_2$)	-	-	-	-	-	-	+	+	21.8
	S.M. (III $_3$)	-	+	-	-	-	-	-	+	21.8
Family 2	A.D. (II $_1$)	+	+	+	-	+	-	-	+	33.3
	B.D. (III $_1$)	+	un	un	un	-	++	++	-	26.2
	G.D. (III $_2$)	-	-	-	-	-	+	+	-	33.3
	S.D. (III $_3$)	-	+	+	-	-	+	+	+	36.0
Family 3	H.N. (III $_1$)	+	+*	-	-	+	-	+	+	25.8
	B.H. (II $_4$)	+	+*	-	-	-	-	+	-	20.6
Family 4	M.C (II $_1$)	+	+	+	-	-	-	-	un	22.7
	S.C. (III $_2$)	-	+	+	-	-	-	+	-	26.6
	I.C. (III $_3$)	+	+	+	-	+	-	-	-	18.5
	T.C. (III $_4$)	-	+	-	-	+	-	-	-	23.6

HS = hemorrhagic stroke, HTN = hypertension, HC = high cholesterol, DM = diabetes mellitus, GS = general stress, BMI = body mass index, *= episodic hypertension, medication was not taken at time of stroke, un=unknown, ++= smoked >1 pack cigarettes/day and >30 alcoholic beverages/month

HPLPII

Table 3 and Table 4 shows scores on the HPLPII by participant and HPLPII means scores and standard deviation by family. Fisher's exact test indicated no

significant difference between participant's HPLPII score or between families' HPLPII scores.

Mann Whitney U Test was performed to compare biological and environmental risk factors with HPLPII scores among all participants. There was a significant difference in HPLPII—physical activity score and presence of high cholesterol (p=.048), HPLPII—nutrition score and presence of high cholesterol (p=.048), and HPLPII—nutrition scores and reported general stress (p=.04). No other significance in distribution of HPLPII scores were observed among all participants.

HPLPII scores for participants without hemorrhagic stroke were compared. Kolmogorov-Smirnov goodness-of-fit test showed a significant difference between individual participants without hemorrhagic stroke on the HPLPII—nutrition score ($\bar{x}$=2.74, SD=.381, p=.021).

No significant difference was found when HPLPII scores for participants with hemorrhagic stroke were calculated. However, there was a significant difference in BMI between participants with hemorrhagic stroke and those without hemorrhagic stroke ($\bar{x}$=27.35, SD=8.485, p=.046).

Spearman rho correlation was computed to access the relationship between HPLPII scores and the biological and environmental risk factors associated with hemorrhagic stroke. There was a positive correlation between HPLPII—Physical Activity score and high cholesterol (r_s=.648, n=12, p=.031); HPLPII—Physical Activity score and HDL (r_s=.965, n=7, p=.000) and HPLPII—Health Responsibility score and Diabetes (r_s=.583, n=12, p=.047).

PSS

Table 5 shows PSS means and standard deviation by family. Fisher's exact test revealed a significant difference between individual with hemorrhagic stroke when compared to those without hemorrhagic stroke (p=.026).

Figure 5 shows PSS total scores by individual and family. A Mann-Whitney U Test indicated that PSS scores were significantly higher for participants with hemorrhagic stroke ($\bar{x}$=24.33) than for participants without hemorrhagic stroke ($\bar{x}$=15.67), U=4.0, p=.028). PSS scores were examined for association with environmental and biological risk factors and no significant association was found. However, there was a significant association between participants with hemorrhagic stroke PSS scores and HPLPII—Nutrition scores (p=.174, Fisher's exact test) compared to participants without hemorrhagic stroke. Spearman rho correlation was computed to assess the relationship between PSS scores and the biological and environmental risk factors of hemorrhagic stroke. There was a positive correlation between PSS score and age at time of stroke (rs=.928, n=6, p=.008). No other significant correlations were identified related to PSS.

A spearman rho correlation was computed to assess the relationships between the biological risk factors for hemorrhagic stroke and the environmental risk factors for hemorrhagic stroke. There was a negative correlation between systolic blood pressure and alcohol use (rs= -.753. N=10, p=.012), and diastolic blood pressure and alcohol use (rs= -.753. N=10, p=.012). There was a positive correlation between glucose levels and strenuous exercise 4 or more time a week (rs=.760, n=8, p=.028). No other significant correlations were indicated.

Table 3. HPLPII Scores by Participant

	HS	TS	HS	PA	Nu	SG	IR	SM
Family 1								
P.M. (II 3)	+	2.75	3.56	1.38	2.89	3.00	3.00	2.75
G.M. (I 2)	+	X	X	X	X	X	X	X
W.M. (III 2)	-	2.25	1.44	1.75	2.56	2.67	2.89	2.13
S.M. (III 3)	-	2.25	1.78	2.13	2.33	2.67	2.78	1.78
Family 2								
A.D. (II 1)	+	2.56	2.67	2.25	3.56	2.44	2.78	1.88
B.D. (III 1)	+	1.87	1.11	1.50	1.78	2.67	2.67	1.63
G.D. (III 2)	-	2.65	2.56	2.25	3.22	2.22	2.67	3.00
S.D. (III 3)	-	2.19	1.67	1.13	2.56	3.22	2.67	1.75
Family 3								
H.N. (III 1)	+	3.08	2.22	3.38	2.67	3.89	3.22	3.13
B.H. (II 4)	+	1.60	1.00	1.13	1.78	1.89	2.22	1.25
Family 4								
M.C. (II 2)	+	X	X	X	X	X	X	X
S.C. (III 2)	-	2.57	2.00	1.25	3.22	3.33	3.44	2.00
I.C. (III 3)	+	3.54	3.33	3.63	2.89	3.78	3.22	4.00
T.C. (III 4)	-	2.69	1.77	2.00	2.56	3.67	3.67	2.25

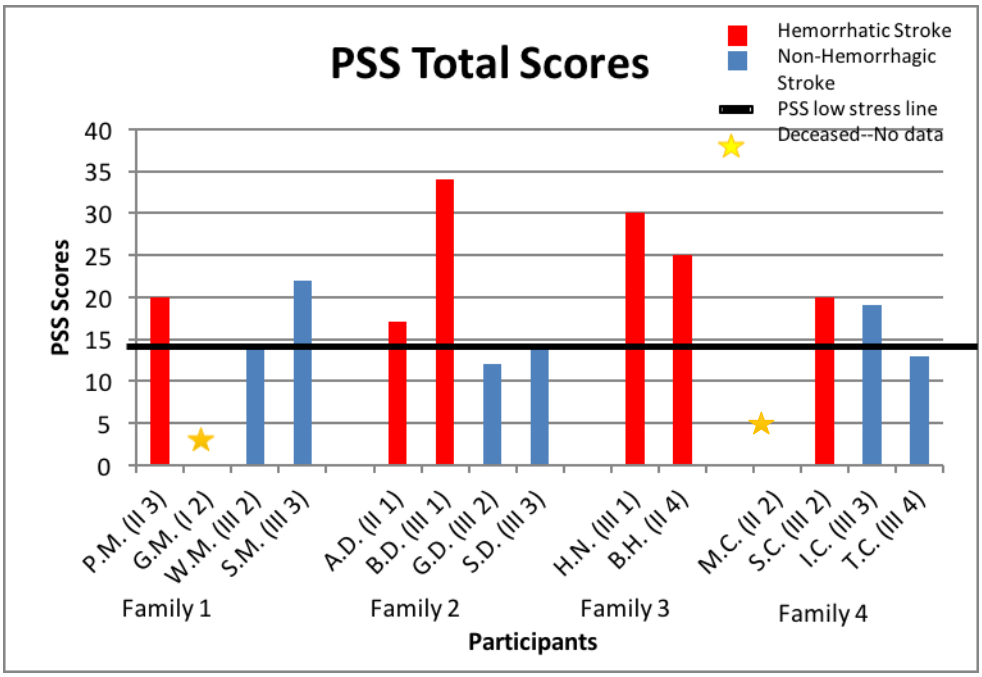
HPLPII = Health Promoting Lifestyles II;TS=HPLPII Total Score; HR= HPLPII Health Responsibility; PA= HPLPII Physical Activity; Nu = HPLPII Nutrition; SG = HPLPII Spiritual Growth; IR = HPLPII Interpersonal Relation; SM = Stress Management; X=no information—deceased; HS = hemorrhagic stroke

Table 4. Descriptive Statistics of HPLPII Scores by Family

		Family 1 n=3		Family 2 n=4		Family 3 n=2		Family 4 n=3	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TS	12	2.42	.289	2.32	.359	2.34	1.047	2.93	.520
HR	12	2.26	1.139	2.00	.745	1.61	.863	2.37	.842
PA	12	1.75	.375	1.78	.561	2.26	1.591	2.29	1.217
Nu	12	2.59	.281	2.78	.785	2.23	.629	2.89	.330
SG	12	2.78	.191	2.67	.430	2.89	1.414	3.59	.235
IR	12	2.89	.110	2.70	.055	2.72	.707	3.44	.225
SM	12	2.21	.504	2.07	.632	2.19	1.329	2.75	1.089

HPLPII = Health Promoting Lifestyles II; TS=HPLPII Total Score; HR= HPLPII Health Responsibility; PA= HPLPII Physical Activity; Nu = HPLPII Nutrition; SG = HPLPII Spiritual Growth; IR = HPLPII Interpersonal Relation; SM = Stress Management

Figure 5. PSS Scores



PSS = Perceived Stress Scale; low perceived stress = 0-13, moderate perceived stress = 14-26, high perceived stress = 27-40

Table 5. PSS Descriptive Statistics

		Family 1 n=3		Family 2 n=4		Family 3 n=2		Family 4 n=3	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PSS Score	12	18.67	4.16	19.25	10.05	27.5	3.54	17.33	3.79

PSS = Perceived Stress Scale; low perceived stress = 0-13, moderate perceived stress = 14-26, high perceived stress = 27-40

CHAPTER V

DISCUSSION

The aim of this research study was to identify biological and environmental risk factor associated with hemorrhagic stroke by examining the similarities and differences between and within families with a history of hemorrhagic stroke. The resulting biological risk factors associated with hemorrhagic stroke in this study were somewhat similar to expectations based on current literature. Hypertension was the biological risk factor most associated with hemorrhagic stroke followed by high cholesterol and then diabetes mellitus. The environmental factors considered for this study, exercise, smoking, alcohol consumption, and general stress, were more ambiguous.

Hypertension was prevalent in all four families and was the most salient biological risk factor among all study participants (87.7%, 12/14). The penetrance of hypertension in the general population is approximately 32% for ages 40-59 and 65% for ages 60 and older (Yoon, Fryar & Carroll, 2015). The youngest participant in this study was 37 and the oldest, at time of stroke, was 65. Interestingly, 5/8 participants with hemorrhagic stroke had a diagnosis of hypertension prior to their stroke and were taking medication for blood pressure management. Two participants with hemorrhagic stroke had episodic hypertension prior to their stroke, but were not on medication for blood pressure management, and one participant with hemorrhagic stroke did not go to the doctor regularly and had no medical records identifying him with hypertension. All non-

hemorrhagic stroke participants who had a diagnosis of hypertension were taking medication for blood pressure management. These results support previous literature that hypertension is a major risk factor for hemorrhagic stroke. It is compelling that a large portion of the non-hemorrhagic stroke family members also has hypertension in a greater percentage than what would be expected in the general population. Hypertension, like hemorrhagic stroke, aggregates within families. It is unclear if hypertension is the precipitance in this study or if there is an underlying, unknown variable. Unfortunately, no information was available regarding age of diagnosis of high blood pressure. Also, it is unclear if any of the participants taking medication for blood pressure control were actually managing their blood pressure.

High cholesterol was reported in 42.9% (6/14) participants, which is much higher than the general population at 12.1% (National Center for Health Statistics, 2016). All participants with high cholesterol reported taking medication for lowering cholesterol levels. Family 2 and Family 4 reported higher incidences of high cholesterol (2/4 and 3/4 respectively), whereas Family 3 (0/2) reported no history of high cholesterol, and Family 1 had only 1/4 participant with high cholesterol. Of the 8 participants with hemorrhagic stroke, 4 reported high cholesterol prior to their stroke. Cholesterol levels were obtained by 2/4 participants. Of these, both reported LDLs within the normal range, 96 mg/dL and 121 mg/dL. Although Sturgeon and colleagues (2007) found that low LDL was significantly associated with ICH, participants with hemorrhagic stroke in this study did not. Only one participant with hemorrhagic stroke reported his type of hemorrhagic stroke as SAH. No other study participants reported the type of hemorrhagic stroke.

There is no evidence to support that high cholesterol is unequivocally association with hemorrhagic stroke type; however, the high proportion of participants with high cholesterol in this study is indicative of high cholesterol clustering within families, which may be a risk factor for hemorrhagic stroke among those families.

As in previous studies (Howard et al. 2013; Kissela et al., 2002; Matsukawa et al., 2012), diabetes mellitus was not associated with hemorrhagic stroke in this study. It was reported in only 2/14 (14.3%) participants, and both of these cases were in participants with hemorrhagic stroke within the same family (Family 1).

Moderate or strenuous exercise 4 hours or more per week was reported in 4/14 (28.6%) participants. Of these, 3 (one each from Family 2, Family 3, Family 4) had a hemorrhagic stroke and 1(from Family 4) did not. It is interesting that 3/8 (37.5%) of participants with hemorrhagic stroke reported moderate or strenuous exercise 4 hours or more per week. However, the amount of time that passed between participants' last exercise session and the event of their hemorrhagic stroke was unknown. Exercise increases blood pressure. In mouse models, mutations of the COL4A1/COL4A2 gene significantly increased the risk of hemorrhagic stroke. In addition to this, the test subjects that were exposed to more strenuous exercise exhibited an increased risks of hemorrhagic stroke. Lee and colleagues (1999) found that the relative risk of hemorrhagic stroke was borderline significant with increased frequency of vigorous exercise (p for trend=0.07 and 0.68). Results here suggests that there could possibly be a stronger link between exercise and hemorrhagic stroke for those individuals with a strong family history of hemorrhagic stroke.

Alcohol consumption was reported in all families (8/14, 57.1%) and cigarette smoking in Family 2 (3/4) only.

Stress is a difficult concept to define as each individual perceives stress differently. What one person may consider a very stressful situation, another may not consider stressful at all. On the health history questionnaire, general stress was reported in all families (6/12, 50%). PSS scores were significantly higher for participants with hemorrhagic stroke (all reported moderate to high perceived stress) than those without hemorrhagic stroke (all reported low to moderate perceived stress). This strongly suggests that perceived stress may be related to hemorrhagic stroke. As stress is known to increase blood pressure, it may be an important consideration for future research.

The greatest variability in HPLPII scores occurred on the HPLPII—Nutrition score and HPLPII—Physical Activity score. Since nutrition and physical activity habits are closely related, it is not surprising that these two areas would have the greatest variability.

Limitations and Future Research

There are several limitations that should be considered in this study. First, the sample size was quite small with only 4 families participating. Results may not be generalized beyond the specific population from which the sample was drawn, however, it is a starting point and has demonstrated that is feasible to complete a multigenerational, multi-family study on hemorrhagic stroke.

All information provided for this study was self-reported from participants. Participants filled out questionnaires and were requested to obtain their medical records

and share those with researchers. Unfortunately, many participants had difficulty obtaining copies of their medical records from their physician's office. Some information was not reported in the medical records and therefore some information was not completed on the questionnaires. In one instance, the participant requested their medical records, but their medical records were not received. In another instance, the participant reported that it had been 20 years since her hemorrhagic stroke and her medical records were not available. In all other missing cases, participants either did not request their medical records or refused to share their medical records with researchers. Researchers did not have access to the medical records so information obtained on the medical history questionnaire could not be verified.

For those participants with hemorrhagic stroke, it had been at least 3 to as long as 20 years since their stroke. Participants may have forgotten specific information related to their hemorrhagic stroke or their personal habits or their perceived stress prior to their stroke. Even though they were requested to fill out the HPLPII and PSS to the best of their knowledge with information prior to their stroke, they could have failed to remember information.

Another limitation to this study was that the pedigrees discussed were incomplete. All first and second-degree relatives were not listed, as their information was not available. Again, participants obtained information about family member's medical history. The information given may have been incorrect, inaccurate and/or incomplete. The one disease that all participants knew and discussed was hypertension. This could be because a majority of participants had a diagnosis of hypertension, which could make

them hyperaware of others with the same disease or they could have over projected the diagnosis of hypertension while under projecting other medical diagnosis, such as high cholesterol and diabetes mellitus.

Age of onset of disease is as important as the disease itself. This information was not asked by researchers and thus not given. The age at which a disease is first diagnosed is important especially as it pertains to hypertension which can often be controlled with medication. As hypertension was the biological risk factor most closely associated with hemorrhagic stroke in this study, understanding when someone was diagnosed and what regiment the individual is doing to regulate blood pressure would add a lot to the knowledge.

Future studies could focus on how communication between family physicians and patients occur. Precision medicine requires that correct and thorough information be provided by patients and interpreted by the physician. Clearly, based on the incomplete information provided by participants, there is a gap in information and/or understanding of medical and family history. It is unclear if participants in this study did not truly understand the risk factors of hemorrhagic stroke or if they were never informed of the risks. It could be that physicians do not understand that patients with a strong family history of hemorrhagic stroke have an increased risk of hemorrhagic stroke, because they are not given complete information from patients. This could create a failure to identify high risk populations.

There does not seem to be one clear biological or environmental factor that can be identified as the “cause” of hemorrhagic stroke. Instead, there appear to be different

influences that differ by family. High blood pressure seems to be the most common element. BP can change throughout the day due to stress, posture changes, exercise or sleep. Because a large portion of participants had a diagnosis of hypertension, it would be fortuitous to consider elements associated with hypertension for those with a family history of hemorrhagic stroke more closely, especially perceived stress. In addition, more multi-family, multi-generational pedigrees need to be completed for comparison.

This study identified 4 families with a strong history of hemorrhagic stroke. In the future, these families could be utilized for genetic testing. Genetic testing can reveal variations in genes, chromosomes or proteins that may have an effect on a person's health. Completing genetic testing on family members with hemorrhagic stroke and comparing the results with non-effected members may show variations of a gene, protein or chromosome between the groups that may lead researchers to a better understanding of the cause(s) of hemorrhagic stroke within families.

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APPENDIX A

PARTICIPANT QUESTIONNAIRE FOR HEMORRHAGIC STROKE

PARTICIPANTS

1. Please complete the following chart by circling your response.

Ethnicity	Do you have hypertension?	Do you have high cholesterol?	Do you have Diabetes Mellitus?	Do you take high-risk medications?
American Indian or Alaska Native Asian Black/African American Hispanic/Latino Native Hawaiian or Other Pacific Islander White Other	YES NO	YES NO	YES NO	YES NO

2. Sex: Male Female

3. Prior to your stroke did you do moderate or strenuous exercise 4 hours or more per week? For example, did you do an activity such as fast walking or aerobics where your heart beats faster than normal, where you can talk but not sing?

   YES NO

4. Prior to your stroke, did you drink alcohol? YES NO

   If yes, how much/often:

   _____1-30 drinks per month _____More than 30 drinks per month

   _____More than 5 drinks per day at least 1 time a month

5. Prior to your stroke, did you smoke cigarettes? YES NO

   If yes, how much: _____More than 1 pack/day _____Less than 1 pack/day

6. Prior to your stroke, did you experience unexpected general stress at home and/or in the workplace? YES NO

7. How many children do you have? _____

8. How many siblings do you have? _____

9. How many aunts/uncles do you have? _____

10. Do you have a first-degree blood relative (mother, father, brother, sister) or second-degree blood relative (aunt, uncle, grandparent, grandchildren) who has had a stroke before the age of 65? YES NO

11. If you answered yes to number 9, please complete the chart.

Relationship	Age at stroke	High blood pressure	Diabetes Mellitus	High cholesterol
Mother				
Father				
Child 1				
Child 2				
Child 3				

Child 4				
Sibling 1				
Sibling 2				
Sibling 3				
Sibling 4				
Mother's mother				
Mother's father				
Father's mother				
Father's father				
Aunt 1				
Aunt 2				
Aunt 3				
Aunt 4				
Uncle 1				
Uncle 2				
Uncle 3				
Uncle 4				

APPENDIX B

QUESTIONNAIRE FOR NON-HEMORRHAGIC PARTICIPANTS

1. Please complete the following chart by circling your response.

Ethnicity	Do you have hypertension	Do you have high cholesterol	Do you have Diabetes Mellitus	Do you take high risk medications
American Indian or Alaska Native Asian Black/African American Hispanic/Latino Native Hawaiian or Other Pacific Islander White Other	YES NO	YES NO	YES NO	YES NO

2. Sex: Male Female

3. Do you do moderate or strenuous exercise 4 hours or more per week? For example, did you do an activity such as fast walking or aerobics where your heart beats faster than normal; where you can talk but not sing? YES NO

4. Do you drink alcohol? YES NO

   If yes, how much/often:

   _____1-30 drinks per month _____More than 30 drinks per month

   _____More than 5 drinks per day at least 1 time a month

5. Do you smoke cigarettes? YES NO

   If yes, how much: _____More than 1 pack/day _____Less than 1 pack/day

   If no, have you ever smoked? YES NO

6. Do you experience unexpected general stress at home and/or in the workplace?

   YES NO

7. How many children do you have? _____

8. How many siblings do you have? _____

9. How many aunts/uncles do you have? _____

10. Do you have a first-degree blood relative (mother, father, brother, sister) or second-degree blood relative (aunt, uncle, grandparent, grandchildren) who has had a stroke before the age of 65? YES NO

11. If you answered yes to number 9, please complete the chart.

Relationship	Age at stroke	High blood pressure	Diabetes Mellitus	High cholesterol
Mother				
Father				
Child 1				
Child 2				

Child 3				
Child 4				
Sibling 1				
Sibling 2				
Sibling 3				
Sibling 4				
Mother's mother				
Mother's father				
Father's mother				
Father's father				
Aunt 1				
Aunt 2				
Aunt 3				
Aunt 4				
Uncle 1				
Uncle 2				
Uncle 3				
Uncle 4				

APPENDIX C

MEDICAL CHART REVIEW FOR PARTICIPANTS WITH HEMORRHAGIC STROKE

ID Number	At time of admittance	History prior to stroke
Age at time of hemorrhagic stroke		
Location of hemorrhagic stroke		
Blood pressure		
Cholesterol level		
Blood glucose levels		
High risk medications		
BMI		

APPENDIX D

MEDICAL CHART REVIEW FOR NON-HEMORRHAGIC PARTICIPANTS

ID Number	
Age at time of hemorrhagic stroke	
Blood pressure	
Cholesterol	
Blood glucose levels	
High risk medications	
BMI	

APPENDIX E

PRECEIVED STRESS SCORE (PSS)

Perceived Stress Scale (PSS)

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

	Never	Almost never	Some-times	Fairly often	Very often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	☐	☐	☐	☐	☐
2. In the last month, how often have you felt that you were unable to control the important things in your life?	☐	☐	☐	☐	☐
3. In the last month, how often have you felt nervous and "stressed"?	☐	☐	☐	☐	☐
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	☐	☐	☐	☐	☐
5. In the last month, how often have you felt that things were going you way?	☐	☐	☐	☐	☐
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	☐	☐	☐	☐	☐
7. In the last month, how often have you been able to control irritations in your life?	☐	☐	☐	☐	☐
8. In the last month, how often have you felt that you were on top of things?	☐	☐	☐	☐	☐
9. In the last month how often have you been angered because of things that were outside of your control?	☐	☐	☐	☐	☐
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	☐	☐	☐	☐	☐

APPENDIX F

HEALTH PROMOTING LIFESTYLE PROFILE II (HPLPII)

Health-Promoting Lifestyle Profile II

DIRECTIONS: This questionnaire contains statements about you *present* way of life or personal habits. Please respond to each item as accurately as possible, and try not to skip any item. Indicate the frequency with which you engage in each behavior by circling:

	Never	Sometimes	Often	Routinely
1. Discuss my problems and concerns with people close to me.	N	S	O	R
2. Choose a diet low in fat, saturate fat, and cholesterol.	N	S	O	R
3. Report any unusual signs or symptoms to a physician or other health professional.	N	S	O	R
4. Follow a planned exercise program.	N	S	O	R
5. Get enough sleep.	N	S	O	R
6. Feel I am growing and changing in positive ways.	N	S	O	R
7. Praise other people easily for their achievements.	N	S	O	R
8. Limit use of sugars and food containing sugar (sweets).	N	S	O	R
9. Read or watch TV programs about improving health.	N	S	O	R
10. Exercise vigorously for 20 or more minutes at least three times a week (such as brisk walking, bicycling, aerobic dancing, using a stair climber).	N	S	O	R
11. Take some time for relaxation each day.	N	S	O	R
12. Believe that my life has purpose.	N	S	O	R
13. Maintain meaningful and fulfilling relationships with others.	N	S	O	R
14. Eat 6-11 servings of bread, cereal, rice and pasta each day.	N	S	O	R
15. Question health professionals in order to understand their instructions.	N	S	O	R
16. Take part in light to moderate physical activity (such as sustained walking 30-40 minutes 5 or more times a week).	N	S	O	R
17. Accept those things in my life which I cannot change.	N	S	O	R
18. Look forward to the future.	N	S	O	R
19. Spend time with close friends.	N	S	O	R
20. Eat 2-4 servings of fruit each day.	N	S	O	R
21. Get a second opinion when I question my health care provider's advice.	N	S	O	R
22. Take part in leisure-time (recreational) physical activities (such as swimming, dancing, bicycling).	N	S	O	R
23. Concentrate on pleasant thoughts at bedtime.	N	S	O	R
24. Feel content and at peace with myself.	N	S	O	R

	Never	Sometimes	Often	Routinely
25. Find it easy to show concern, love and warmth to others.	N	S	O	R
26. Eat 3-5 servings of vegetables each day.	N	S	O	R
27. Discuss my health concerns with health professionals.	N	S	O	R
28. Do stretching exercises at least 3 times per week.	N	S	O	R
29. Use specific methods to control my stress.	N	S	O	R
30. Work toward long-term goals in my life.	N	S	O	R
31. Touch and am touched by people I care about.	N	S	O	R
32. Eat 2-3 servings of milk, yogurt or cheese each day.	N	S	O	R
33. Inspect my body at least monthly for physical changes/danger signs.	N	S	O	R
34. Get exercise during usual daily activities (such as walking during lunch, using stairs instead of elevators, parting car away from destination and walking).	N	S	O	R
35. Balance time between work and play.	N	S	O	R
36. Find each day interesting and challenging.	N	S	O	R
37. Find ways to meet my needs for intimacy.	N	S	O	R
38. Eat only 2-3 servings from the meat, poultry, fish, dried beans, eggs, and nuts group each day.	N	S	O	R
39. Ask for information from health professionals about how to take good care of myself.	N	S	O	R
40. Check my pulse rate when exercising.	N	S	O	R
41. Practice relaxation or mediation for 15-20 minutes daily.	N	S	O	R
42. Am aware of what is important to me in life.	N	S	O	R
43. Get support from a network of caring people.	N	S	O	R
44. Read labels to identify nutrients, fats, sodium content in packaged food.	N	S	O	R
45. Attend educational programs on personal health care.	N	S	O	R
46. Reach my target heart rate when exercising.	N	S	O	R
47. Pace myself to prevent tiredness.	N	S	O	R
48. Feel connected with some force greater than myself.	N	S	O	R
49. Settle conflicts with other through discussion and compromise.	N	S	O	R
50. Eat breakfast.	N	S	O	R
51. Seek guidance or counseling when necessary.	N	S	O	R
52. Expose myself to new experiences and challenges.	N	S	O	R