DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a

The rupture of intracranial aneurysms leads to hemorrhage in over 30,000 Americans annually, with mortality and morbidity exceeding 75%. Because these lesions can be repaired prophylactically, an understanding of the pathogenetic mechanisms and early identification would be of tremendous benefit. But the etiology of cerebral aneurysms remains unclear.

We propose to define the risk factors, incidence of familial aggregation, and patterns of inheritance in patients with cerebral aneurysms. We will pay particular attention to the ethnic background, and establish a blood and tissue bank for molecular analyses. We plan to utilize the large number of patients currently treated at the University of Texas in Houston, to identify 500 patients with cerebral aneurysms and 50 affected families that can be studied in a detailed and consistent manner. A strength of our patient population is the ethnic diversity of those treated: 41% Caucasian, 24% African-American, 26% Hispanic, and 9% Asian. Our hypothesis is that different ethnic groups will have different risk factors, patterns of familial aggregration, and inheritance of cerebral aneurysms. The following are our specific aims:

1. To compare the incidence of familial aneurysms in patients of different ethnic backgrounds and to establish the patterns of inheritance

2. To compare the risk factors for cerebral aneurysm in patients with different ethnic backgrounds

3. To establish a blood and tissue sample bank for linkage and molecular analyses.

succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public

1. Department of Neurosurgery and the University Clinical Research Center, University of Texas-Houston Medical Center
2. Memorial Hermann Hospital, Houston, Texas

information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**
PERFORMANCE SITE(S) (*organization, city, state*)

KEY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

Name	Organization	Role on Project
Dong H. Kim, M.D.	University of Texas Medical School	Prinipal Investigator
Dianna M. Milewicz, M.D.	University of Texas Medical School	Mentor
Seung-Ho Kang, Ph.D	University of Texas Medical School	Statistical Consultant
Gerard Tromp, Ph.D	Wayne State University	Collaborator

Type the name of the principal investigator/program director at the top of each printed page and each continuation page. (For type specifications, see instructions on page 6.)

RESEARCH GRANT

TABLE OF CONTENTS

*Type density and type size of the entire application must conform to limits provided in instructions on page 6.

Appendix (*Five collated sets. No page numbering necessary for Appendix.*) [X] Check if Appendix is included

Number of publications and manuscripts accepted or submitted for publication (*not to exceed 10*) >

Other items (*list*):

Gender/Minority/Pediatric Inclusion for Research

Institutional and Environmental Committment

Prior Research Training/Career Goals and Objectives

Career Development Plan/Training in the Responsible Conduct of Research

Appendix 1 and 2: Consent forms

Biosketch: Drs. Dianna Milewicz and Seung-ho Kang

Letters: 3 sealed letters of recommendation – Drs. Guy L. Clifton, Gayatry Mohapatra, and Linda Noble
Institutional Committent – Drs. L. Maximilian Buja and James T. Willerson
Mentor Letter – Dr. Dianna Milewicz
Collaboration letters: Drs. Gerard Tromp and Seung-ho Kang

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
>Dong H. Kim	>Assistant Professor of Neurosurgery

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Stanford University	B.S.	9/82-6/86	Biological Sciences
University of California, San Francisco	M.D.	9/86-6/90	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Post-graduate education:

7/90-6/91 Harvard Medical School, General Surgery Intern

7/91-6/96 University of California, San Francisco, Neurosurgery Resident

7/96-6/97 University of Florida, Cerebrovascular Surgery Fellow

Appointments: 6/98 - now University of Texas, Houston, Assistant Professor of Neurosurgery and Director, Comprehensive Center for Cerebrovascular Surgery

7/97-6/98 Cornell University Medical College, Assistant Professor

Awards: Valedictorian, Glen A. Wilson High School, 1982
Regents and Alumni Scholar, University of California, 1982
Graduation with Honors, Stanford University, 1986
Meritorious Research, Western Student Medical Research Forum, 1988
Henry Newman Award, San Francisco Neurological Society, 1993

Publications:

1. Kim, DH, Kulick, MI: An in vivo- in vitro investigation of the effect of steroidal and non-steroidal anti-inflammatory agents on collagen synthesis. Stanford University Library, 1986

2. Kim DH, Barbaro NM, Fields HL: Hyperalgesia following naloxone precipitated withdrawal from morphine. Clin Res 36, No. 1, 1988

3. Kim DH, Bederson JB, Fields HL, Barbaro NM: Hyperalgesia occurs with acute withdrawal from spinal or periaqueductal grey administered morphine.
Clin Res 37, No. 1, 1989

4. Kim DH, Fields HL, Barbaro NM: Morphine analgesia and acute physical dependence: rapid onset of two opposing, dose-related processes.
Brain Research 516, 1990.

5. Kim DH, Harsh GR IV: Advances in brain tumor biology, the genetics of astrocytoma. Curr Techniques in Neurosurgery, 1993.

6. Kim DH, Maeda T, Mohapatra G, Park S, Waldman FW, Gray JW, Feuerstein BG: Molecular cytogenetics of malignant gliomas. J Neuro-Oncology 15, Supp, 1993.

7. Kim DH, Mohapatra G, Bollen A, Waldman FM, Feuerstein BG: Chromosomal abnormalities in glioblastoma multiforme and glioma cell lines detected by comparative genomic hybridization. Int J Cancer 60, 1995.

8. Mohapatra G, Kim DH, Feuerstein BG: Detection of multiple gain and losses in ten glioma cell lines by comparative genomic hybridization. Genes, Chrom, Cancer 13, 1995.

9. Kim DH, Gutin P, Noble LJ, Nathan D, Ross G, Nockels RN. Genetically engineered fibroblasts secreting nerve growth factor or brain-derived growth accelerates recovery from acute, traumatic spinal cord injury in the rat. Neuroreport 7, 1996.

10. Mohapatra G, Moore DH, Kim DH, Grewal L, Hyun WC, Waldman F, Pinkel D, Feuerstein BG: Analyses of brain tumor cell lines confirm a simple model of relationships among fluorescence in situ hybridization, DNA index, and comparative genomic hybridization. Genes, Chrom, Cancer 20, 1997.

11. Mautes AEM, Kim DH, Sharp FR, Panter S, Sato M, Maida N, Bergeron M, Guenther K, Noble LJ: Induction of heme-oxygenase-1 (HO-1) in the contused spinal cord of the rat. Brain Res 795 (1-2): 17-24, 1998.

12. Kim DH, Mohapatra G, Feuerstein BG: Genetic tumor markers in gliomas, In: Berger M, Wilson CB, eds. Textbook of Gliomas, 1998.

13. Kim DH, MacDonald J, Day AL: Approaches to terminal basilar bifurcation aneurysms, In: Kaye A, Black P, eds. Operative Neurosurgery, in press.

14. Kim DH, Zipfel G, Day AL: Long-term outcomes from the clipping of basilar apex aneurysms - the results of a modern surgical series. Submitted.

15. Zipfel G, Kim DH, Greenwald D, Day AL: Post-operative peri-mesencephalic and supracerebellar hemorrhage distal to the surgical site. Submitted.

16. Kim DH, Tu C, Cassacia-Bonnefil P, Chao M: An in vitro model of neuronal injury using primary cell cultures: modulation of injury can produce apoptotic or necrotic death. Submitted.

17. Kim DH, Tu C, Cassacia-Bonnefil P, Chao M: Neurotrophins can protect neurons in an in vitro model of injury by signalling through the *trk* receptors. Submitted.

18. Kim DH, Nates J, Malkoff M, Zaidi S: Sub-arachnoid hemorrhag of unknown etiology – heterogeneity of presentation. In preparation.

19. Kim DH, Tu C, Zhao X, Hayes RL: Neurotrophins can inhibit the activation of caspase-3 following neural injury. In preparation.

20. Kim DH, Zhao X. Modulation of cAMP levels can confer neuroprotection following injury. In preparation.

Other Support

Name of Individual: N/A

Active/Pending

Project Number (Principal Investigator):

Source:

Title of Project (*and/or Subproject*):

Dates of Approved/Proposed Project:

Annual Direct Costs / Percent Effort:

The major goals of this project are...

Overlap (*summarized for each individual*):

Active/Pending

Project Number (Principal Investigator):

Source:

Title of Project (*and/or Subproject*):

Dates of Approved/Proposed Project:

Annual Direct Costs / Percent Effort:

The major goals of this project are...

Overlap (*summarized for each individual*):

Active/Pending:

Project Number (Principal Investigator):

Source:

Title of Project (*and/or Subproject*):

Dates of Approved/Proposed Project:

Annual Direct Costs / Percent Effort:

The major goals of this project are...

Overlap (*summarized for each individual*):

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: Dr. Kim has exclusive use of 400 sq. ft. of laboratory space with lab benches, desks, etc. In addition, Dr. Kim has access to over 2000 sq. ft. of shared space in the Department of Neurosurgery, including a tissue culture room with laminar flow. Dr. Kim will be performing many of the assays, particularly at the beginning of the project, in Dr. Milewicz's laboratory. She has use of 850 sq. ft. of space.

Clinical: The inpatient care will be located in Memorial Hermann Hospital, the major teaching affiliate of the University of Texas, Houston. A major tertiary referral center, with 24 Neurological Intensive Care beds, and helicopter-based transfers, Hermann Hospital is one of the largest centers in the country for the treatment of cerebrovascular disease. It is adjacent to the medical school building.

Located in Hermann Hospital is the University Clinical Research Center (CRC). The CRC will provide nursing staff for recruitment of patients into the study, for obtaining blood and tissue samples, for storage and record keeping. The CRC also provides an ideal place to interview asymptomatic members of affected families, and to obtain consent and samples.

Animal: not applicable

Computer: The clinical database will be maintained on a Dell Dimension XPS B, with a Pentium III processor, 34.2 GB hard drive, and Jaz drive for backup. This computer is equipped with Microsoft Access, the database, and has EPI-INFO statistical programs. The clinical data will be backed up weekly, and the Jaz discs will be stored in 2 separate locations, including a fire-proof safe.

Office: Dr. Kim has 300 sq. ft. of office space, and a dedicated secretary.

Other: Spiral CT scanner, with CT angiography software is located at Hermann Hospital for the screening studies. Agreement with Dr. Joel Yeakley, Chief of the Division of Neuroradiology, to provide this study at cost for patients whose screens will be paid through the grant.

Probable funding of Genomic Core Sequencing Laboratory, part of the UCRC.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Dr. Kim's laboratory: Perkin-Elmer 480 DNA thermal cyclers (2), microfuges (2), protein and DNA gel electrophoresis equipment, -20°C and -80°C freezers, refrigerators, CO2 incubators, sterile hood, phase contrast microscope.

Dr. Milewicz's laboratory: Applied Biosystems Prism 310 Genetic Analyzer with GeneScan and DNA Sequencing Analysis software, BioRad SequiGen GT sequencing apparatus, hybridization oven, shaking water bath, IBI UV transilluminator and Quickshoot photo system, liquid nitrogen storage tanks (in two different locations) for DNA and cells, gel dryers and vacuum pump.

SECTION III: Specialized Information

Prior Research Training/Career Goals and Objectives:

My first research experience was in college. During my sophomore year, I began to study the effect of anti-inflammatory agents on wound healing with a plastic surgeon, Michael Kulick. My responsiblities included the regular dosing of the monkey subjects and collagen synthesis assays performed on resected tendons. This work lead directly to an honors thesis.

In medical school, I began working with Howard Fields on the physiology of pain and opiate dependence. I learned stereotactic techniques, intracellular and extracellular recording techniques in the rat brainstem (the periaqueductal grey and nucleus raphe magnus), and pharmacological analyses. Most importantly, I had a fantastic mentor who shared with me his joy of gaining new insights and extending knowledge. By the time I graduated, I was firmly committed to making research a part of my career. Since that time, research has been a part of my daily routine.

As I began by residency training in neurosurgery, I took a year in the laboratory (this was the only time I was in a laboratory full time except for summers between semesters). I was very interested in tumor biology and thought that I might specialize in neuro-oncology. I decided to study genomic changes in glial tumors, and worked with Gayatry Mohapatra and Burt Feuerstein. We were the first to study brain tumors using comparative genomic hybridization, a powerful new technique that detects changes in DNA copy number across the entire genome. I became very familiar with basic genomic techniques, and continued to work on projects in this laboratory for the next 4 years.

As my residency progressed, however, my clinical interest increasingly focused on cerebrovascular disease. As such, my research interest expanded to novel neuroprotective strategies. I became particularly interested in neurotrophins such as nerve growth factor. In the latter part of my training, I designed a study to test the effect of the neurotrophins in the setting of acute spinal cord injury. Fibroblasts genetically engineered to secrete nerve growth factor or brain-derived growth factor (using a retroviral vector) were injected into rat spinal cord contusion sites generated by a weight drop model. This in vivo study showed a definite but limited biological response to the neurotrophins.

In addition to the laboratory studies, I took part in several clinical studies, mostly retrospective reviews. My main interests however centered in the laboratory, and my efforts focused on increasing my expertise with molecular analyses. By the time I completed by residency, I was firmly committed to an academic career, and wanted the majority of my efforts directed towards research.

I completed a fellowship in cerebrovascular surgery, and I am now a faculty member starting my research career. I believe that I have two strengths. The first is my additional training in cerebrovascular surgery. I worked with Dr. Arthur Day in Florida, a renowned surgeon with an extensive aneurysm surgery caseload (>200 aneurysms per year). I gained experience not only with the surgical approaches to these lesions, but in the peri-operative management as well. Second, I do have a good background in genomic technology, particularly for a clinician.

As I began the process of starting a research program, my priority was to combine my clinical interest and expertise with a laboratory project centered on patient-oriented research. This grant is a result of that process. This project takes advantage of the strengths here at the University of Texas – a very high volume of patients

with cerebral aneurysms, the presence of a Clinical Research Center, and expertise in medical genetics (Dr. Milewicz). In addition, I will have the resources of both Dr. Milewicz's laboratory and my own laboratory.

If I get funding and salary support, my Chairman has agreed to allow 75% effort for this project. Part of that effort will include coursework and tutorials so that I can become knowledgeable in areas that I have little experience. These include statistical analyses and study design, epidemiology, and medical genetic analyses (see the section below).

My goal is to become an independent investigator. I hope to establish a long-term project identifying the genetic changes involved in cerebral aneurysms, and test the usefulness of such findings in the treatment of patients: as screening tools, prognostic indicators, and as the basis for understanding the pathogenetic mechanisms.

Career Development Plan/Training in the Responsible Conduct of Research

My plan is to enhance my expertise in two areas through a combination of coursework and tutorials. I am planning to take courses in two areas: human genetics and clinical epidemiology/statistical analysis. I plan on taking 5 course over 2 years, one course at a time as most of my effort will be directed towards the research project. In addition, I will have continuous tutorials with my Mentor, Dr. Milewicz, and my Statistical Consultant, Dr. Kang as the project progresses. In addition, I will be working directly in Dr. Milewicz's laboratory, particularly at the beginning, to enhance my knowledge of the basic molecular techniques.

First Year:

1. PH 1610 4 credits (School of Public Health Course)
Introduction to Biometry – includes study design, data description, probability, distribution of random variables, applications of the binomial and normal distributions, estimation and confidence intervals, hypothesis testing, contingency tables, regression, and analysis of variance. Additional topics include introduction to statistical computing and data management, distribution free statistical methods, demographic measures, and life tables.

2. PH 2610 4 credits
Introduction to Clinical Epidemiology – observational studies, case-series and case-control studies, cross-sectional and cohort studies, diagnosis, causality, ethical guidelines in research, hypothesis testing with Type 1 and Type 2 errors.

3. GS110032 2 semester hours (Graduate School Course)
Introduction to Genomics and Bioinformatics – topics include genetic mapping, physical mapping, sequencing under the subject of structural genomics, basic algorithms of bioinformatics with special reference to DNA/protein sequence-based analysis in the context of genomics, and the latest advancement in functional genomics.

Year 2:

4. GS110013 3 semester hours

Genetics and Human Disease – introduces principles and methods of human genetic analysis with special reference to the contribution of genes to the burden of disease. The aim is to describe the analytical processes whereby genetic mechanisms are inferred and genes located on chromosomes.

5. GS110033 3 semester hours

Methods in Genetic Epidemiology – offers practical experience in the analysis of genetic marker data. The course will cover the basic theory behind linkage analysis and will focus on learning analysis techniques and computer packages.

As part of the course, Introduction to Clinical Epidemiology, I will be learning about the ethical conduct of research. To complete my training in the responsible conduct of research, I will also be taking a survey course designed specifically for clinicians. A result of a K30 grant from the NIH, the Medical School runs a Clinical Research Curriculum that meets every Wednesday evening for a year. In addition to lectures on the basic design and analysis of clinical trials, many sections focus on ethical issues, regulatory issues, and possible conflicts of interest.

RESEARCH PLAN

a. Hypothesis and Specific Aims

The rupture of intracranial aneurysms leads to hemorrhage in over 30,000 Americans annually, with mortality and morbidity exceeding 75%. Prophylactic surgical repair of these lesions can now be achieved with a low complication rate. Therefore, an understanding of the pathogenetic mechanisms and early identification of those at risk would reduce the morbidity and mortality of this disorder.

The etiology of cerebral aneurysms remains unclear, but there is evidence for genetic and environmental contributions. A genetic basis is supported by familial aggregation of the disorder. Ten percent of patients with a cerebral aneurysm report an aneurysm in a first-degree family member. Screening of asymptomatic first degree-relatives detects aneurysms in another 10%. There is also an association with polycystic kidney disease, a known genetic disorder. An environmental contribution is supported by the finding that smoking is a known risk factor for cerebral aneurysms. However, studies on hypertension, alcohol and oral contraceptive use have yielded contradictory results.

Although aneurysm rupture can be a catastrophic event, screening of the general population is not feasible given the costs and risks involved. Currently, screening is recommended only for families with a known aneurysm history. However, this is based on studies performed exclusively on Caucasian populations, with the majority centered on Northern European families. Is screening indicated for Hispanic or African-American families? Are there other factors that point to high risk? There are already indications that ethnic differences exist. A population based study found that African-Americans had 2.1 times the risk of aneurysm rupture when compared to caucasians. However, no other study has examined ethnic differences in regards to inheritance patterns or risk factors.

We propose to define the differences in risk factors, familial aggregation, and patterns of inheritance in cerebral aneurysm patients with different ethnic backgrounds. We will utilize the large number of patients currently treated at the University of Texas in Houston to identify 500 patients with cerebral aneurysms and 50 affected families that can be studied in a detailed and consistent manner. All asymptomatic relatives of patients with a familial history will be screened.

A strength of our patient population is the ethnic diversity of those treated: 41% Caucasian, 24% African-American, 26% Hispanic, and 9% Asian. We will enroll patients with both sporadic aneurysms and a family history. Our hypothesis is that different ethnic groups will have different risk factors, patterns of familial aggregration, and inheritance of cerebral aneurysms. In addition, we will establish a blood and tissue bank of samples collected from these patients. In combination with the clinical data, this will provide a powerful resource for linkage analyses and the testing of candidate genes to identify those that contribute to cerebral aneurysms in different ethnic groups.

Aim 1: To compare the incidence of familial aneurysms in patients of different ethnic backgrounds and to establish the patterns of inheritance.

We will be able to compare the incidence of familial aggregation of cerebral aneurysms in different ethnic groups. These findings will have immediate clinical consequences. Does the current screening recommendations apply to all ethnic groups? Is the yield of the screening test the same for different ethnic groups? Pedigrees will be constructed in an on-going manner and the modes of inheritance determined by visual analysis. Differences in the familial aggregation or pattern of inheritance between the ethnic groups

would signify differences in the genetic basis. If there are subsets of families with a clearly defined mode of inheritance of cerebral aneurysms, these data will provide important leads for the proposed molecular analyses in Aim 3.

Aim 2: To compare the risk factors for cerebral aneurysm in patients with different ethnic backgrounds.

The data obtained and compared will include the known risk factor of smoking and the controversial risk factors of hypertension, alcohol, and oral contraceptive use. In addition, we will examine hypercholesterolemia, diabetes mellitus, and known cardiovascular disease. This data will be important for three reasons. First, it will clarify the contradictory results noted in previous studies since this analysis will be largest such study to date. Second, we may note ethnic differences in the risk factors involved, as well as differences between sporadic and familial cases. Third, we will need this risk factor information to clarify the data on the familial aggregation. For example, if the incidence of familial aggregation is highest in African-Americans, we will be able to determine whether or not this results from a higher incidence of a risk factor being present in this population.

Aim 3: To establish a blood and tissue sample bank for molecular analyses.

Blood samples (to extract DNA and store serum) and skin biopsies (to explant fibroblasts) will be obtained from all enrolled patients. In addition, blood samples will be obtained from family members for molecular analyses. For this project, we plan two approaches. First, we will test markers with linkage to cerebral aneurysms detected by other laboratories. We have a formal collaboration with a group performing sib-pair analyses of Finnish patients with familial aneurysms (Dr. G. Tromp, Wayne State University). We can determine if these markers extend to the general population, and to patients with different ethnic backgrounds.

Second, we can test specific candidate genes. One such candidate is FBN1, which encodes fibrillin, a major component of microfibrils. Mutations in FBN1 have already been associated with some patients with thoracic aortic aneurysms. Because we have fibroblast cell cultures as well as DNA, we can test for both defects in fibrillin-1 cellular metabolism and mutations in FBN1. With our tissue bank, we can easily test other known genes, such as the components of the elastin system, and candidate genes that will be identified by the linkage studies.

b. Background and Significance

Clinical Background

The rupture of intracranial aneurysms leads to non-traumatic subarachnoid hemorrhage (SAH) in over 30,000 Americans annually (1). Despite steady advances in diagnostic techniques, surgical and endovascular repair, and peri-operative management, SAH remains a devastating condition. Mortality exceeds 25%, and significant morbidity among the survivors exceeds 50% (2). Patients that present in good neurological condition following minor bleeds generally have good outcomes; patients with more severe hemorrhages either die or remain with permanent neurological deficits. Population based incidence rates for SAH vary from 6-16 per 100,000, with the highest rates reported for Japan and Finland (3-5). Unlike other types of stroke, the incidence of SAH has not declined over time, despite the marked improvement in blood pressure control in the general population (6,7). Currently, there are no known behavioral modifications that can prevent SAH (8).

Because the prophylactic repair of these aneurysms can now be achieved with extremely low morbidity, an understanding of the pathogenetic mechanisms and early identification of those at risk would provide a tremendous benefit. A recent meta-analysis examined 733 patients undergoing elective surgical repair of unruptured, asymptomatic aneurysms from 1966 to 1992. The results improved significantly over time, but even with inclusion of data from the 1960's, the overall mortality was 1%, and morbidity 4.1% (9). More recent series report even better outcomes, with almost 100% making good recoveries following repair of normal sized aneurysms (10). In addition, the advent of endovascular embolization has allowed for safer treatment of aneurysms that are associated with a higher surgical risk, such as those in the posterior circulation (11).

However, significant clinical questions remain unanswered. Since aneurysm rupture can be a catastrophic event, should the general population be screened? Given the costs of the screening procedures available as well as the associated risks, such an approach is currently not feasible (discussed below). Are there populations at higher risk for cerebral aneurysms? The only population determined to have a higher risk are patients with a strong family history for cerebral aneurysms (where two or more first-degree relatives are affected). Screening in such cases has a high yield and it is current clinical practice to screen all the members of such families (17-19, 25, 36, 37). But even here, information is limited. With the exception of one study in Japan, the data are derived from screens performed on primarily homogeneous Caucasian populations, mostly involving Northern European families. We do not know whether screening is indicated in families with other ethnic backgrounds.

The etiology of cerebral aneurysms remains unclear but there is evidence for both genetic and environmental influences. As with many disorders, the aneurysm itself may be the end-point for a variety of pathological processes. Understanding the different pathogenetic mechanisms involved may lead to more specific screening tests and therapies tailored to that particular patient. To reach such a goal, a comprehensive approach is necessary, involving large numbers of patients, including those of different ethnic backgrounds, combining detailed clinical and historical data with molecular analyses.

Genetic Basis of Cerebral Aneurysms

Several lines of evidence suggest a significant genetic contribution to the etiology of cerebral aneurysms. First, there is an association of cerebral aneurysms with a genetic disorder. The incidence of cerebral aneurysms is increased in patients with Autosomal Dominant Polycystic Kidney Disease (12). From autopsy series it is known that 25% of patients with Autosomal Dominant Polycystic Kidney Disease harbor intracranial aneurysms at time of death. In 20% of these patients, rupture of an aneurysm is the cause of death (13, 14). This condition shows genetic heterogeneity: at least 3 different genes can be responsible for Autosomal Dominant Polycystic Kidney Disease.

There is also a significant subset of cerebral aneurysm patients with familial aggregation. Since the first report in 1954 by Chambers et al (15), over two hundred families have been identified to have two or more members with cerebral aneurysms (reviewed in 16). These families did not have a history of another heritable disorder such as Autosomal Dominant Polycystic Kidney Disease. In general, approximately 10% of patients who present with a SAH have such a family history. Reports range from a low of 5% in a mail-in survey performed in Sweden (17), to a high of 20% in a community-based study of Rochester, Minnesota (18). Most series report a familial incidence of 9-11% (19). In addition, 6 pairs of monozygotic twins with SAH have been reported (20), further suggesting a genetic basis to this disorder.

The cerebral aneurysms that arise in these families behave differently when compared to the sporadic cases. The aneurysms rupture at a younger age (by over 5 years), and may rupture at a smaller size (16). They affect women more often than men, and involve the middle cerebral artery more often than the anterior cerebral artery. Affected family members have a distinct predilection for rupture within the same decade. In one study, 80% of

sisters had a SAH within 10 years of each other (a mean of 6 years). Similarly, all six reported twins had bleeds in the same decade, most within 5 years (19). First degree family members had a 7 times greater risk of SAH than second degree relatives (21).

The pattern of inheritance is not known. To date, few studies have published pedigrees or attempted segregation analysis. Most reports include 1 or 2 families, and a variety of possible transmission patterns have been reported (9, 16, 23, 24). One author attempted a synthesis of all the pedigrees published in the literature, but his analysis was confounded by the inconsistent and incomplete generation of pedigrees (16). Most relied on the history-taking only to determine the affected family members. Such an approach does not detect those members who harbor unruptured and asymptomatic aneurysms. More recent, larger series have performed screening tests, but these pedigrees were not analyzed (22, 25). While there can be significant genetic heterogeneity, it is likely that there will be subsets of families that demonstrate consistent patterns of inheritance, particularly if the ethnic background is taken into account.

There are already indications that there are ethnic differences in the prevalence of cerebral aneurysms. A population based study examined the risk of SAH in blacks compared to whites in the Greater Cincinnati metropolitan area. Blacks were found to have 2.1 times the risk of SAH than whites (52). In addition, incidence rates for SAH can vary in different countries, with the highest rates reported for Japan and Finland (3-5).

Risk Factors

Smoking is a known risk factor for SAH. A prospective study involving over 100,000 women found a lower relative risk in former smokers when compared with current smokers. In addition, increased duration from smoking cessation was correlated with decreased SAH risk (60).

However, the finding for other risk factors has been contradictory. Hypertension is clearly a risk factor for hemorrhagic stroke overall, but the data specifically for SAH is less consistent. Several cohort studies have suggested that hypertension leads to increased risk, but several case-control studies have failed to reproduce the link (reviewed in 8). With the marked improvement in the blood pressure control of the general population, there has been a corresponding decrease in the overall stroke rate, but there has been no change in the incidence of SAH (7, 62-64).

Similarly, one study noted an increased risk of SAH for moderate users of alcohol (<150 g/wk), with a larger risk for heavy users (>150 g/wk), but another study actually noted a decreased risk in moderate users (reveiewed in 65). Such contradictory results are also noted in over 20 studies looking at oral contraceptive use. A recent meta-analysis concluded a possible, very small increase in risk with oral contraceptives (66).

As with the studies on familial aggregration, the analysis of risk factors have been hampered by small patient populations. The largest reported analysis involved fewer than 300 patients (67). The Framingham Study only identified 36 cases of SAH as of the last report (68). In addition, data on the risk factors in different ethnic populations are entirely absent.

In summary, an understanding of the pathogenetic mechanisms and early identification of those at risk for cerebral aneurysms would reduce the morbidity and mortality from SAH. The etiology of cerebral aneurysms remains unclear but there is evidence for genetic and environmental contributions. We currently know that familial aggregation of cerebral aneurysms occurs in 10% of Caucasion patients, but do not know the incidence in patients with other ethnic backgrounds. Similarly, smoking is a known risk factor, but the analysis has not been extended to specific ethnic groups. Because the population-based rates of SAH are different in the United States, Japan, and Finland, significant ethnic differences are likely to be found.

Molecular Basis for Vascular Disorders

At present, no specific genetic defect associated with cerebral aneurysms has been identified. One approach for finding genetic defects is to test candidate genes for association. The work to date has centered on COL3A1, the gene for type 3 collagen. Initial studies reported reduced production of type 3 collagen in some patients with cerebral aneurysms (27). However, DNA sequencing analyses have failed to note any mutations (28,29). A recent study analyzed the involvement of matrix metalloproteinases 3 and 9, and plasminogen activator inhibitor-1. Again, polymorphisms of these genes in Finnish patients did not associate with cerebral aneurysms (69).

A candidate that we plan to test is the protein fibrillin-1 (fib1) which is a major component of microfibrils. Structures of the extracellular matrix, microfibrils are widely distributed in the tissues, particularly in the vascular structures, often closely associated with elastin. Fib1 is generated from profibrillin, a 359 kD glycoprotein that is encoded by the gene FBN1 (26). Profibrillin is secreted and proteolytically processed to the 20 kD fib1, then deposited into the extracellular matrix in the form of microfibrils. Defects in fib1 may play a role in the pathogenesis of at least some familial aneurysms for two reasons.

1. Mutations in FBN1 is the cause of Marfan syndrome, an autosomal dominant disease affecting the cardiovascular, ocular, and skeletal systems. The cardiovascular features are the life-threatening complication of the disorder, with major morbidity from the rupture of vascular abnormalities such as dissection of the aorta (26). Although SAH is not a common manifestation of this disease, a high incidence of cerebral vascular abnormalities such as fusiform dilatations, dissections, and aneurysms has also been described (12). Screening of patients with Marfan's syndrome for asymptomatic intracranial aneurysms has not been reported.

   A total of 137 mutations, distributed throughout the FBN1 gene, have been characterized in affected individuals. The majority are missense mutations or deletions or that alter a single amino acid. Others are splicing errors or large genomic deletions that remove one exon while maintaining the reading frame (26, 30-32). One mutation produces the full profibrillin molecule, but a change in its amino acid sequence disrupts its normal processing to fib1, stopping its incorporation into microfibrils (33).

2. Some patients with thoracic aortic aneurysms (TAAs) have mutations in the FBN1 gene. Given that aortic aneurysms are a prominent feature of Marfan syndrome, patients with TAAs, but not Marfan syndrome, were screened for mutations in the FBN1 gene (34, 35). In a subset of such patients, mutations were noted in FBN1. The reasons why these patients have such mutations but do not develop Marfan syndrome is not known.

In previous work done in Dr. Milewicz's laboratory, the contribution of genetic factors to the development of TAAs were investigated by comparing the prevalence of TAAs in first–degree relatives of patients referred for surgical repair of TAA (51). First-degree relatives of the TAA patients demonstrated a higher prevalence of both TAAs when compared with the control group (relatives of the spouse). This study supported the role of genetic factors in the etiology of TAA, and provided critical pedigrees for the genetic analysis that followed. Blood for DNA harvest had been obtained from these families. In addition, skin biopsies provided explant fibroblast cultures for the study of protein synthesis and processing.

Using these resources, the hypothesis that FBN1 mutations can be associated with sporadic TAAs was tested. It was determined that fibroblasts from up to 40% of TAA patients had defects in the synthesis,

secretion, or processing of fib1. Screening for mutations was performed using Single Stranded Conformational Polymorphisms analysis (SSCP), and aberrantly migrating bands were sequenced directly. Different FBN1 mutations were found in a subset of TAA patients (34). One finding was of a missense mutation in exon 27. Dermal fibroblasts were used to study the effect of this mutation on fib 1 cellular processing. This mutation decreased the amount of fib1 deposited into the pericellular matrix.

Another approach is to perform genome-wide linkage analyses using polymorphic markers. The rapid progress of the Human Genome Project and the identification of single nucleotide polymorphisms is rapidly expanding the power for such studies. Currenty, there are two NIH funded projects attempting such linkage (CRISP Database). One project is mapping susceptibility loci in cerebral aneurysm families from the Utah Population Database (5R01NS37737-02). The other study, based at Wayne State University with Dr. Gerardus Tromp, proposes to perform sib-pair linkage analyses of cerebral aneurysm families identified in Finland (5R01NS34395-02). Both of these projects are centered on overwhelmingly Caucasian populations.

We have established a collaboration with Dr. Tromp. Markers that are identified in the Finnish patients will be tested in our population. The results of his study will provide new markers to test, and we will be able to determine whether the same markers are linked in familial patients with other ethnic backgrounds, and whether they generalize to patients without a family history. In addition, these markers are likely indicate new candidate genes. We will have an ideal resource for both clinical and molecular analyses.

c. Preliminary Studies/Progress Report

In 1999, a total of 115 patients were admitted to the University of Texas, Houston with a diagnosis of spontaneous SAH. In 96 of these patients, the bleeding was the result of aneurysm rupture. An additional 27 patients were admitted with a diagnosis of cerebral aneurysm, detected due to symptoms (such as changes in the visual fields) or found incidentally as a result of a screening test (CT or MRI). The ethnic background was 41% Caucasian, 24% African-American, 26% Hispanic, and 9% Asian.

Nine patients had a positive family history in that they had a first degree relative with a known aneurysm. One interesting patient presented with both a cerebral and a thoracic aneurysm. Her mother also had a thoracic aneurysm, while a maternal grandmother may have died from a cerebral aneurysm.

The treatment of patients with SAH is well-established. The aneurysm is obliterated by surgical or endovascular means, and peri-operative management centers on the treatment of vasospasm and hydrocephalus – common complications following hemorrhage. However, the general approach to those with a family history is more complex. Should the asymptomatic family members be screened? By what methodology? If an aneurysm is detected, should it be prophylactically treated?

Our current approach is to recommend screening to all first-degree relatives in families with 2 or more affected members. This is based on the results of screening from large series of affected families. In a Finnish study of 21 families, the prevalence of aneurysms in asymptomatic relatives was 10% (36). Two other studies, based in the Netherlands and Japan, found an incidence of 8% and 13.9%, respectively (25, 37). All three groups concluded that screening was indicated.

The gold standard for aneurysm detection remains the cerebral angiogram. But this is an invasive procedure that can result in significant morbidity such as stroke (the complication rate is 1%). For that reason, alternative methods are desirable. We currently use the helical CT-based angiogram, or CTA. This study is quite sensitive,

and can detect aneurysms as small as 2 mm, and certainly those larger than 5 mm. Comparison studies in patients who have also had cerebral angiography show a >90% sensitivity rate, with no false positives (38-40). The risks are negligible, and an added benefit is that the cost of screening is quite low. The real cost of performing this study is under $500, compared to cerebral angiography which can cost 10-20 times that amount (J. Yeakley, M.D., personal communication). If the CTA is positive, then this finding is confirmed with angiography.

It is our current practice to inform all members of affected families of these data, and to recommend screening with CTA. As these members have direct experience with the result of rupture, the vast majority will proceed with a screening study. Because the test is being ordered as a result of standard medical practice, the expenses are covered by insurance. A problem arises for those members without any coverage – generally 15% of our patient population. In such cases, we have helped them enroll in national programs such as Medicaid or Medicare if they qualify, or referred them to the local county facility for the test. The majority will have a negative screen. As de novo aneurysms can be detected later, and the general incidence increases with age (12), we recommend a repeat test every 5 years.

In approximately 10% of those screened, an asymptomatic aneurysm is detected. It is generally believed that the annual incidence of rupture in this situation is 1-2%. Yasui et al followed 234 patients with untreated aneurysms for an average of 7 years, and found a hemorrhage rate of 2.3% per year (41). Similarly, Juvala et al followed 142 patients with 181 aneurysms for at least 10 years. They found a bleed rate of 1.4% per year. When hemorrhage occurred, it was fatal in 52% of patients (42). Both groups recommended prophylactic repair in all patients unless a specific contraindication existed.

Weibers et al observed 130 patients with 161 unruptured aneurysms for an average of 8.3 years, and also found the rate of hemorrhage to be 1.4% per year (43,44). Moreover, a finding by these investigators was that all the aneurysms that proceeded to bleed had a diameter greater than 10 mm. This is a controversial result given that the average size of all aneurysms at rupture is less than 10 mm in size (45). Indeed, of the 1092 patients enrolled into the Cooperative Aneurysm Study, the median diameter of a ruptured aneurysm was 7 mm, and 71% of ruptured aneurysms were under 10 mm in size (46). However, very few aneurysms that ruptured were under 5 mm in size.

These different findings can be reconciled if the number of small aneurysms greatly exceeds the number of large aneurysms. While the rate of hemorrhage may be significantly lower for aneurysms under 10 mm in size, their greater numbers are reflected in the average size of all ruptured aneurysms. In fact, autopsy series do note the greater numbers of smaller aneurysms (47). A recent, multi-center trial probably confirms the higher hemorrhage rate for aneurysms greater than 10 mm. The International Study of Unruptured Intracranial Aneurysms involved 2621 patients in 53 participating centers (48). Patients who were known to harbor aneurysms, but were not treated for any reason, were retrospectively recruited into the study, then followed for SAH. This study found the annual incidence of rupture to be only 0.05% per year in those aneurysms under 10 mm in size, but a rupture rate 11 times higher when the size was 10 mm or greater. Based on these considerations, we currently have the following treatment paradigm.

1. Asymptomatic aneurysms less than 5 mm in size

- recommendation: no treatment
- follow-up study in one year
- re-consideration of the aneurysm enlarges

2. Aneurysms of 5 to 9 mm in size

- discussion of the relevant data regarding the natural history, risks and benefits of surgery vs. observation
- recommendation: no treatment
- follow-up study in one year
- re-consideration of the aneurysm enlarges

3. Aneurysm 10 mm or larger

- discussion of the relevant data regarding the natural history, risks and benefits of surgery vs. observation
- recommendation: surgical or endovascular treatment

In practice, this paradigm is necessarily individualized for each patient, as multiple factors enter into each treatment decision. First, there are anatomical features on an aneurysm that indicate a much higher risk of future bleeding. Examples include the presence of daughter aneurysms and significant irregularity of the aneurysm walls (49). Second, there are characterics of the patient that must be considered. What is the age of the patient? What is the life expectancy? Are there co-morbidities such as severe cardiac disease? The overall risk of future rupture and the possible morbidity of treatment both change with increasing age. In addition, the psychological response of the patient to the diagnosis plays an important role. Some are terrified of “brain surgery,” while others do not wish to live with “a bomb in my brain.” Each decision is made together with the patient, after taking all of these factors into account, and after the patient understands completely the risks of both an interventional approach as well as the natural history of the disorder.

d. Research Design and Methods

Our hypothesis is that different ethnic groups will have different risk factors, patterns of familial aggregration and modes of inheritance. We plan to test this hypothesis by studying a large number of patients and affected families, with complete screens and consideration of ethnic backgrounds. Currently, we treat over 100 patients per year with cerebral aneurysms at the University of Texas in Houston. As 10% of these patients are likely to have a family history, we plan to enroll 500 patients and 50 affected families over the next 5 years. A strength of our patient population is the ethnic diversity of those treated: 41% Caucasian, 24% African-American, 26% Hispanic, and 9% Asian.

In the first part of this project, we will gather detailed clinical data, including the family history and incidence of possible risk factors such as hypertension. In affected families, we will screen all asymptomatic members. Pedigrees will be established. With these data, we plan to establish the risk factors, incidence of familial aggregation, and patterns of inheritance in patients with cerebral aneurysms. We plan to define the differences in those with different ethnic backgrounds. We should be able to establish which patients should be screened: those with a family, history, certain risk factors or specific ethnic backgrounds. Because we will have data on familial aggregation as well as risk factors for the same group of patients, we will also determine whether a specific family develops aneurysms as a result high susceptibility to a risk factor, or to another, unknown cause.

The second part of this project involves blood and tissue samples from every patient and family member for molecular analyses to identify specific genes that may be involved in cerebral aneurysm formation. We plan to define the differences in the genetic basis for cerebral aneurysms in patients with different ethnic backgrounds.

Case definition:

All patients will have a confirmed diagnosis of cerebral aneurysm with angiography, the "gold standard." Those asymptomatic family members that are screened using CTA will undergo angiography only if a positive result is noted. The diagnosis of a cerebral aneurysm on angiogram is straightforward. The minimum size of a detectable aneurysm is 2-3 mm. The only potential diagnostic dilemma arises from infundibula, a widening of the take-off of a vessel. Most commonly seen with the posterior communicating artery, it is controversial whether infundibula represent pre-aneurymal lesions or normal variants. Patients with infundibula will not be classified as having an aneurysm.

Definition of race/ethnicity:

We will determine the race and ethnicity of each patient, and trace the racial backgound to all 4 grandparents, including the countries of origin. We will note those patients with mixed ethnic backgrounds as well. For example, an "African-American" patient may have a Caucasian parent. "Hispanic" patients will be divided into those with Mexican or Central American ancestry and those with Cuban, Puerto Rican, or other origin. For example, Mexicans are more likely to be related to the original Native American population, whereas Cubans are often of a mixed Caucasian/African ancestry.

Recruitment:

All patients with a cerebral aneurysm will be approached to participate by the investigator, co-investigator or a member of nursing staff from the Clinical Research Center. For those who cannot speak English, translator services at the participating institution will be utilized. The patient will be asked to consent to a detailed questionnaire of the medical, social, and family history. They will be informed that such information will be entered into a confidential database. If the patient is unable to give consent due to cognitive deficits or coma, then a family member will be approached to give consent. The consent form is attached as Appendix 1.

Affected families will be identified when the patient notes a history of cerebral aneurysm in a first-degree relatives (for a total of two in the family). In such cases, the other family members will be approached by the investigator, co-investigator or the Clinical Research Center nursing staff. They will be asked to enroll in the study, to provide a detailed medical and social history. As a matter of normal practice, they will be informed of a 10% risk for having an aneurysm, and screening with CT angiography will be recommended. If a positive finding is noted, treatment recommendations will follow the guidelines noted above. Although we will gather data from these individuals as part of the study, the medical recommendations given these individuals and any treatment rendered will not deviate from standard care (specified in detail in the section above). The rationale, risks and benefits will be discussed.

Each enrollee will be asked to provide a 20 cc sample of blood and a 3 mm specimen of skin. If the patient goes to surgery, the specimen will be taken during the operation. Otherwise, patients and family members will be seen in the Clinical Research Center to undergo venipuncture and punch biopsy. A separate informed consent will be obtained for these procedures (Appendix 2).

Data collection and management:

The data management system will be the ACCESS relational database. The data will be maintained in separated, relational tables such as Demographics, Aneurysm Characteristics, and Ethnic Background. The data

can be joined for subsequent analyses by key. The data entry programs will be designed to note data inconsistencies at the point of entry. Data quality checks will be run quarterly.

Statistical Analyses:

To determine the expected sample size, we estimated the available number of patients with cerebral aneurysms in the University of Texas at Houston. This yielded approximately over 100 patients per year whose ethnic diversity is 41% Caucasian, 24% African-American, 26% Hispanic and 9% Asian. The primary null hypothesis in this study is that there is no difference among the ethnic groups with respect to the rate familial aggregation of cerebral aneurysms.

Since the sample size is already determined given the study design and subject accrual rate, we chose to assess the minimal detectable differences given the sample sizes of 500 patients, $\forall$=0.05 and 1-$\exists$=0.80. A sample of 500 patients (41% Caucasians and 59% Non-Caucasians) will have 80% power to detect the minimal difference of 7% (the difference in the rates of familial aggregation of cerebral aneurysms between Caucasians patients of 10% and non-Caucasians patients 17%) based on the arcsine transformation of the binomial proportions. The following table shows the power to be achieved with different combinations of the rate of familial aneurysms using 500 patients (41% Caucasians and 59% non-Caucasians) and $\forall$=0.05.

Table 1. Power Calculations for the rate of cerebral aneurysms

Caucasians	Non-Caucasians	Power
10%	15%	60%
10%	20%	93%
10%	25%	99%

A sample of 325 patients (41% Caucasians and 24% African-American among 500 patient) will have 80% power to detect the minimal difference of 12% (the difference in the rates of cerebral aneurysms between Caucasians patients of 10% and African-American patients 22%) with a 0.017 significance level with a Bonferroni's correction ($\forall$=0.05/3, where 3 is the number of pairwise comparisons).

The primary analyses will consist of comparing the rate of cerebral aneurysms among different ethnic groups. The chi-square test will be employed to test the incidence rates among the ethic groups. The logistic regression will be used to evaluate and compare the influence of combinations of risk factors on the rate of cerebral aneurysms. SAS software will be used for data analysis. We will use SAS PROC FREQ and SAS PROC LOGIST. Missing values may occur for some individuals. Missing values will be imputed using appropriate techniques after a careful examination of collected data sets. For example, missing values can be imputed using individual averages or sample averages corrected by some risk factors.

Aim 1: To compare the incidence of familial aneurysms in patients of different ethnic backgrounds and to establish the patterns of inheritance.

The incidence of a positive familial history of cerebral aneurysms will be determined in patients with SAH and cerebral aneurysms. Logistic regression will be used to compare the incidence in different ethnic groups, after adjusting for risk factors. Given the large sample size, the power of the study is high (see statistical section above).

These findings will have immediate clinical consequences. Does the current practice recommendation of screening of family members apply across all ethnic groups? We may be inappropriately extrapolating data derived from Northern European populations to patients with other ethnic backgrounds. Is the incidence of familial aggregation the same across all ethnic groups? Is the yield of screening the same across different ethnic groups? A particularly high incidence in one group may necessitate more careful screening protocols. A difference in incidence would also signify possible differences in the genetic basis. Given that rates of SAH differ in various countries, significant differences between ethnic groups are likely to be found.

Pedigrees will be constructed in an on-going manner as the screening results become available. Possible modes of inheritance will be determined by visual analysis, then compared with the results from other families of similar and different ethnic backgrounds. If there are notable differences between the ethnic groups in the mode of inheritance of cerebral aneurysms, segregation analyses will be pursued.

If there are subsets of families with a clearly defined mode of inheritance, these data will provide important leads for the proposed molecular analyses in Aim 3. They may point to specific regions of the genome (e.g. the X-chromosome) that can be targeted more intensely.

Aim 2: To compare the risk factors for cerebral aneurysm in patients with different ethnic backgrounds.

The data specifically obtained and compared will include the known risk factor of smoking and the controversial risk factors of hypertension, alcohol, and oral contraceptive use. In addition, we will examine hypercholesterolemia, diabetes mellitus, and cardiovascular disease. We will adjust for the effects of age and gender using logistic regression.

1. In this study, we will divide subjects into current smokers, former smokers, non-smokers with a smoking spouse, and non-smokers without a smoking spouse. The level of smoking will be quantified using pack-years, and the duration since the last cigarette noted.
2. Because patients who present with an aneurysmal bleed are invariably hypertensive as a result of the SAH, we will rely on the patient's history, gathered from family and the primary care physician. We will determine the maximal systolic and diastolic pressures, the time since diagnosis, and the number and dosage of antihypertensive medications prescribed.
3. The use of alcohol will be estimated in g/wk, with the duration noted, as well as the time since last use.
4. Any history of oral contraceptive use, its formulation and duration noted.
5. History or hypercholesterolemia, diabetes mellitus, and cardiovascular disease will be determined from the patient, family, or the primary care physician.

This data will be important for three reasons. First, it will clarify the contradictory results noted in previous studies. The result of this analysis will be largest such study to date. Second, we may note ethnic differences in the risk factors involved, as well as differences between sporadic and familial cases. Third, we will need the risk factor information to clarify the data on the familial aggregation. For example, if the incidence of familial aggregation is highest in African-Americans, we will be able to determine whether that is the result of increased susceptibility to smoking, or another genetic factor.

Aim 3: To establish a blood and tissue sample bank for molecular analyses.

Blood samples (to extract DNA) and skin biopsies (to explant fibroblasts) will be obtained from all enrolled patients. Blood samples will be obtained from family members. The combination of the detailed clinical and epidemiological data, coupled with blood and tissue samples will provide an powerful resource for molecular analyses. A strength of our project is that we will have enrolled patients with sporadic aneurysms as well as a familial aggregation, and patients with all ethnic backgrounds. In addition, we will be able to study both the gene itself and the protein product. With the rapid identification of single nucleotide polymorphisms, the power to perform linkage analyses has increased dramatically. Francis Collins, director of the Human Genome Project, has announced that the first complete sequence will be available in May of 2000. Investigators with access to a large, well-defined patient populations will be in key positions to establish linkage and identify genes of interest.

Blood samples will be obtained by venipuncture and separated by centrifugation. The buffy coat will be extracted, lysed, and the DNA extracted by ethanol precipitaion. The DNA will be frozen at -80ºC. The skin biopsies will be used to culture and freeze fibroblasts. The cells can be reconstituted at any point in the future. The explanted fibroblasts allow us the determine both the synthesis and processing of specific proteins of interest. All samples will be divided and stored in two different locations for safety.

For this project, we plan two approaches. First, we will test molecular markers with linkage to cerebral aneurysms detected by other laboratories. We have a formal collaboration with Dr. Tromp to study the markers identified in Finnish patients (see letter). We can determine if associated markers extend to different ethnic populations. In addition, we can easily test markers identified by other investigators.

Second, we can test specific candidate genes. Initially, we will test the hypothesis that fib1 defects are present in patients with cerebral aneurysms. We will first determine whether the fibroblasts from affected individuals synthesize, secrete, process and deposit fib1 in the matrix similar to cells from unrelated controls. We propose to screen a limited number of patient samples for these studies, approximately 25 samples. A standard number of cultured fibroblasts (250,000) are plated then pulsed with radiolabeled cysteine. The media, cell lysate, and matirx are collected and the proteins separated by SDS gel electrophoresis. The level of labeling is quantitated by scanning densitometry of radioautographic films (50). Using the protein product itself as a screen can save a significant amount of work depending on the gene being studied. For example, FBN1 has 65 exons, making sequence analysis time-consuming.

Those patients that show abnormalities in the cellular processing of fib1 will then be examined for mutations in FBN1. We will use genomic DNA. Each FBN1 exon will be individually amplified and screened for mutations using SSCP. Aberrantly migrating bands will then be sequenced in both sense and antisense directions. Our mutation detection rate has been quite high when using this protocol. As a positive control, we will simultaneously screen DNA from 2 patients with classic Marfan syndrome (from our DNA bank). Because Marfan syndrome patients do not have genetic heterogeneity, these data will allow us to estimate the mutation detection rate.

Once a DNA sequence variation that alters the amino acid sequence is detected, the entire family will be studied to confirm that the putative mutation is present in affected individuals (segregation analysis). Genomic DNA from at least 50 unrelated individuals will also be studied to confirm that the change does not represent a polymorphism found in the general population.

The studies described for fib1 will be extended to other candidate genes. Immediate examples include components of elastin system, major contributors to the integrity of the vessal wall. We also expect the identification of additional candidates as the linkage analyses proceed.

Anticipated Problems:

Because of our large patient population, we do not anticipate problems enrolling 100 patients per year with cerebral aneurysms. We currently treat well over 120 patients per year with such lesions. We have experience with other clinical trials in this population, and well over 90% of patients consented to enroll. In addition, we do not anticipate problems with family members. It is our current practice to recommend screening in affected families. As these members have direct experience with the result of rupture, the vast majority will proceed with a screening study. We have on occasion had difficulty obtaining screening studies for patients without any medical insurance. This grant requests funds specifically to complete the screens in such situations.

Patient drop-out is not an issue as this is not an outcomes study. Once the basic demographic and risk factor data is generated, the screenings completed, and the tissue samples given, the enrollee's involvement in the trial is completed. We will maintain contact with these patients, to inform them of findings if necessary as detailed above.

One possible problem may be a referral bias as we are an academic, tertiary care center. In general, any SAH is considered a neurosurgical emergency, and most hospitals tend to send the patient to a tertiary center. In addition, we transfer many patients from small, outlying hospitals due to helicoptor transport available. In these hospitals, neurosurgical call coverage is not available so that we receive all patients, not just the most complex cases. Therefore, we estimate that our patient population reflects the general population and is not a severely selected group.

For the analysis of risk factors, the design of the study is that of a case series. For this reason, we can determine significant associations, but cannot prove cause and effect. For this reason, we plan to perform definitive, cohort studies if positive associations are noted.

A more significant problem arises in defining the ethnic background of our patients. By careful history taking, we hope to clearly establish the ethnic background of each individual. But there will be cases where the patient has a mixed heritage. For example, a patient with a Caucasian and African-American parent is usually classified as "African-American," but his genetic background is mixed. We will note all such situations, and analyze the results accordingly. If patients of such mixed parentage constitutes a significant minority of our patients, it would change the overall number of patients needed for the study to have adequate power. We are prepared to increase the enrollment as necessary, until statistically significant results are obtained, or the study proves the null hypothesis with power.

Because the DNA extraction methods, culture of fibroblasts, and DNA and protein assays are established and functioning in Dr. Milewicz's laboratory, we are confident that the analyses will proceed expeditiously. During the course of the grant, it is our plan for DH Kim to gain the necessary expertise to establish such a program in his own laboratory.

We will be analyzing protein synthesis and processing in skin fibroblasts. While it is likely that any defect identified also exists in the cerebral vasculature, we will confirm any such findings directed from aneurysm samples in future studies.

Time table: (please see career development plan as well)

0-12 months - enroll 100 patients, gather clinical data and samples.
- establish system for efficient extraction of DNA and storage,

culture and freezing of fibroblasts.
-course work and intensive tutorials for DH Kim:
statistical methods and basic epidemiology with S Kang,
genetic epidemiology, pedigree and linkage analysis with D Milewicz

13-24 months - analyze initial data. Modify recruitment, data management, data parameters depending on the initial experience.
-continue coursework and tutorials
-begin visual inspection of pedigrees
-begin to identify first families for testing of candidate genes
- learn the specific protocols involved in the molecular analyses in Dr. Milewicz's laboratory

25-36 months – complete course work and tutorials
-begin risk factor analysis, interim analyses to determine final sample size
-begin work testing of candidate genes
-establish protocols and capability for molecular analyses in Dr. Kim's laboratory

37-60 months – expanded linkage analysis and testing of candidate genes
-completion of risk factor analyses
-completion of incidence analyses, results of screening tests
-apply for RO1 grant

e. Human Subjects

1. Five hundred patients with cerebral aneurysms, and first-degree relatives in patients with a familial history. The age range will include all patients with such a diagnosis. Usually rare under age 20, patients have presented with a SAH into their 80's. As this study involves the gathering of clinical data, and blood and skin specimens only (i.e. no changes in treatment, or testing of interventions), no patient will be excluded due to poor health status or other concurrent diseases. Patients of both genders, and all ethnic groups will be included. Children will not be included as aneurysms are rare in pediatric populations.
2. The clinical data will be gathered from the patient, family members, and the medical chart. The data will be entered on separate forms (outside the medical record), and coded for confidentiality. This data will not be available to anyone outside the study. Blood and skin samples will be specifically obtained for the study, after consent, and will not be taken from existing specimens.
3. All patients will be approached to participate by the investigator, co-investigator or a member of nursing staff from the Clinical Research Center at Hermann Hospital. Whenever possible this will be carried out preoperatively in the patients room. For patients who can not speak English, every effort will be made to utilize translator services at the participating institution in order to obtain informed consent. Those patients who can not provide informed consent will not be included in the study (there will be no waiver). In order to provide subjects with the best choices for participation in this study, we have developed two consent forms. Each is attached for review as an appendix.
4. There is little risk to participate in this study. Burning pain is associated with the injection of Lidocaine and a small scar at the site of biopsy can occur. The site of biopsy may become infected although this has not been a complication in our experience. Additionally, venipuncture is associated with pain at the site of the insertion of the needle and occasionally a small bruise. All charges for these studies will be covered through the grant. The enrollees will not bear any cost of the screening studies. Any genetic information derived as part of the study could engender risk to the person's ability to get medical or life insurance. The study subjects will be protected from this by coding the information and maintianing strict anonymity as the molecular analyses proceeds. If information of found that could be of use to the patient, this will only be

relayed confidentially to the patient, by the principal investigator, and be maintained outside the medical records of the enrollees.

5. As stated above, strict confidentiality will be maintained, and all records coded. In addition, they will be maintained outside the medical record. If a complication occurs due to the venipuncture or skin biopsy, although extremely unlikely, full access to the necessary treatment will be overseen by the principal investigator. We do not need to monitor the data for safety to patients as no experimental intervention or treatment is planned.
6. The information obtained from this study of patients with cerebral aneurysms will not benefit the individual directly. First degree relatives of aneurysm patients will benefit if an asymptomatic aneurysm is detected, but this is not a specific benefit to the study as the screening would proceed anyway. Those relatives without medical insurance may have an easier time obtaining the screening study. The molecular analyses may provide prognostic information that could be of use to the enrollees. It is reasonable to proceed with the study because the risk to the patients and first-degree family members is low. Complication rates from venipuncture and skin biopsy are low. In addition, the possible complications are minor and will not affect the health of the enrollee. We expect to gain valuable information regarding the ethnic differences in the risk factors and familial aggregation of cerebral aneurysms that may change the current clinical practice. Furthermore, we may be able to identify genes that play a role in the pathogenesis of cerebral aneurysms.

f. Vertebrate Animals

Not applicable

g. Literature Cited

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h. Consortium/Contractual Arrangements: Not applicable

i. Consultants: See attached letters from Drs. G. Tromp and S. Kang

Gender/Minority/Pediatric Inclusion for Research

1. National distribution of the disease to be studied with regard to women and minorities.

	American Indian or Alaskan Native n (%)	Asian or Pacific Islander n (%)	Black, not of Hispanic origin n (%)	Hispanic n (%)	White, not of Hispanic origin n (%)	Other or Unknown n (%)	Total n (%)

Female	(0.4)	(1.4)	(6.3)	(4.3)	(38.7)	(0)	(51)
Male	(0.4)	(1.4)	(5.5)	(4.5)	(37.1)	(0)	(49)
Total	(0.8)	(2.8)	(11.8)	(8.8)	(75.8)	(0)	(100)

Source: U.S. Bureau of census 1990

2. Study Population

	American Indian or Alaskan Native n (%)	Asian or Pacific Islander n (%)	Black, not of Hispanic origin n (%)	Hispanic n (%)	White, not of Hispanic origin n (%)	Other or Unknown n (%)	Total n (%)
Female	(0.0)	(4.6)	(12.2)	(13.3)	(20.9)	(0.0)	(51)
Male	(0.0)	(3.4)	(11.8)	(12.7)	(20.1)	(0.0)	(49)
Total	(0.0)	(9.0)	(24.0)	(26.0)	(41.0)	(0.0)	(100)

Source: Data of admissions to Memorial Hermann Hospital, 1999.

Our local population has a higher percentage of ethnic minorities, with the exception of Native Americans, then the national demographic. The will facilitate our study as we are looking for ethnic differences.

3. Etimated study population for the study.

	American Indian or Alaskan Native n (%)	Asian or Pacific Islander n (%)	Black, not of Hispanic origin n (%)	Hispanic n (%)	White, not of Hispanic origin n (%)	Other or Unknown n (%)	Total n (%)
Female	(0)	(23)	(61)	(66.5)	(104.5)	(0.0)	(255)
Male	(0)	(17)	(59)	(63.5)	(100.5)	(0.0)	(240)
Total	(0)	(40)	(120)	(130)	(205)	(0.0)	(495)

The pediatric population is not included for study because cerebral aneurysms are rarely found in this population.

Appendix 1: Consent Form #1

THE GENETIC BASIS OF CEREBRAL ANEURYSMS
INFORMED CONSENT

INVITATION TO PARTICIPATE IN A RESEATCH STUDY:

You are being asked to take part in a research study because you or a member of your family has a history of a brain aneurysm. Your taking part is voluntary and you may refuse to take part or stop taking part at any time without penalty, loss of benefits or change in your or your family member's present or future care.

PURPOSE OF STUDY:

The purpose of this study is to see if brain aneurysms run in families, and to look at the risk factors and patterns of inheritance.

DESCRIPTION OF STUDY:

We are hoping to enroll 50 families. You will be asked questions about your medical history, and whether you or other members in you family have aneurysms. An aneurysm is a balloon-like weakness in an artery. In the brain, the aneurysm can rupture leading to uncontrolled bleeding.

BENEFITS:

The research may benefit you because it may find if you or members of your family are at risk for having a brain aneurysm. If an aneurysm is found, it can be treated.

RISK:

This research should present no risk to you.

COMPENSATION:

There is no payment for taking part in the study. If you suffer an injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment, and professional services will be available to you, just as they are to the community in general. You should report any injury to Dr. Dong H. Kim at his office (713) 704-6445 and to the Committee for the Protection of Human Subjects at (713) 500-5827.

CONFIDENTIALITY/ANONYMITY:

Information about you will be treated with confidentiality. Such information will not be a part of your medical record, or be made available to other physicians or insurance companies. The results of the study will be reported to the University of Texas Basic Research Committee, again confidentially. In the event of any

publication regarding this study, your identity will not be revealed. You understand that representatives of the Food and Drug Administration (FDA) and the sponsor of this research may review your research and/or medical data for the purpose of verifying research data. The FDA representatives can see personal identifiers in our records; however, your identity will be masked from any copies that may possibly be made. You will not be identified in any reports or publications resulting from the study.

FOR FURTHER INFORMATION ABOUT THIS RESEARCH STUDY:

Dr. Dong H. Kim will be glad to answer any further questions about this research study. Dr. Kim can be reached at 713-704-6445. You may also address questions about the study or your rights as a research subject to the University of Texas Committee for the Protection of Human Subjects at (713) 500-5827.

BEFORE YOU SIGN THIS DOCUMENT:

By signing below, you are agreeing to take part in this research study. Make sure that any questions have been answered to your satisfaction and that you have a thorough understanding of the study. If you decide to take part in this research study, a copy of this document will be given to you.

This study has been approved by the Committee for the Protection of Human subjects of the University of Texas Houston Health Science Center and Hermann Hospital (CPHS) as HSC-MS-99-274.

_____

Subject Signature

_____

Date

_____

Printed Name of Individual Obtaining Consent

_____

Signature of Individual Obtaining Consent

Appendix 2: Consent form #2

THE GENETIC BASIS OF CEREBRAL ANEURYSMS
INFORMED CONSENT

INVITATION TO PARTICIPATE IN A RESEATCH STUDY:

You are being asked to take part in a research study because you or a member of your family has a history of a brain aneurysm. Your taking part is voluntary and you may refuse to take part or stop taking part at any time without penalty, loss of benefits or change in your or your family member's present or future care.

PURPOSE OF STUDY:

The aim of this study is to identify changes in the walls of blood vessels. We hope to identify reasons why you or family member developed an aneurysm by looking at the material in the walls (proteins) or the genetic material that make up the walls (DNA).

DESCRIPTION OF STUDY:

One circle of skin about 1/8th inch round and $1/8^{th}$ inch deep will be taken by means of a small punch instrument from a region of the upper arm that has been numbed with a local anesthetic. The skin is numbed by the injection of about a quarter milliliter (about a twentieth of teaspoon full) of a local anesthetic called xylocaine. In addition, a small amount of blood (about 4 tablespoons) will be drawn from a vein in the arm. The technique of blood drawing is common practice in hospitals. The needle stick is accompanied by slight discomfort and a bruise may form at the site of needle entry. When ever possible the skin and blood samples will be obtained at the time of surgery. The sample will be used to grow cells for further study at a later time and may be shared with other investigators who are performing similar studies.

All such samples will be coded to maintain confidentiality. No information will be available to you, other physicians, or insurance companies. We will not break the code unless a finding is made that may benefit you directly. For example, we may find a gene that is associated with the presence of an aneurysm. This information may allow us to inform you of your chances of developing an aneurysm, either higher or lower. If this happens, you will be contacted directly by Dr. Kim. You will have the results as well as the meaning explained to you in great detail. Again, this information will be confidential, no written notices will be sent to you, and this will not be a part of your medical record, or be available to any other persons. You will be asked to keep us current on your address and phone numbers so that we can get in touch will you. We will also contact you yearly to update your address.

BENEFITS:

The research to be done on the samples may not help you directly. The results of the study may be of benefit to you or members of your family, as described above.

RISK:

Blood sampling requires about 20ml (4 tablespoonfuls) of blood per sample. Risks of blood sampling are burning, pain or bruising. There may be slight discomfort and the possibility of a scar with the skin biopsy which is reasonably expected. The skin biopsy site could get infected.

COMPENSATION:

There is no payment for taking part in the study. If you suffer an injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment, and professional services will be available to you, just as they are to the community in general. You should report any injury to Dr. Dong H. Kim at his office (713) 704-6445 and to the Committee for the Protection of Human Subjects at (713) 500-5827.

CONFIDENTIALITY/ANONYMITY:

Information about you will be treated with confidentiality. Such information will not be a part of your medical record, or be made available to other physicians or insurance companies. The results of the study will be reported to the University of Texas Basic Research Committee, again confidentially. In the event of any publication regarding this study, your identity will not be revealed. You understand that representatives of the Food and Drug Administration (FDA) and the sponsor of this research may review your research and/or medical data for the purpose of verifying research data. The FDA representatives can see personal identifiers in our records; however, your identity will be masked from any copies that may possibly be made. You will not be identified in any reports or publications resulting from the study.

FOR FURTHER INFORMATION ABOUT THIS RESEARCH STUDY:

Dr. Dong H. Kim will be glad to answer any further questions about this research study. Dr. Kim can be reached at 713-704-6445. You may also address questions about the study or your rights as a research subject to the University of Texas Committee for the Protection of Human Subjects at (713) 500-5827.

BEFORE YOU SIGN THIS DOCUMENT:

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This study has been approved by the Committee for the Protection of Human subjects of the University of Texas Houston Health Science Center and Hermann Hospital (CPHS) as HSC-MS-99-274.

_____ _____

Subject Signature Date

_____ _____

Printed Name of Individual Obtaining Consent Signature of Individual Obtaining Consent

All Key Personnel for the Current Budget Period

Name	Degree(s)	SSN	Role on Project *(e. g. PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Not applicable					