

Genetic Predictors for Stroke in Adults with Sickle Cell

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A. Specific Aims:

We have documented genetic associations with risk of infarctive stroke in children with SCA, but little is known about genetic risk factors for stroke in adults. Our preliminary data suggest that genes influencing stroke risk in adults differ from those conferring risk in children with SCA. Stroke in adult patients with SCA had not been systematically studied, and data on the prevention and treatment of stroke in SCA after childhood are lacking. We postulate that the genetic determinants of stroke in SCA are sub-type specific, and involve many of the same pathways contributing to stroke in the general population. The objectives of this exploratory proposal are to extend our findings in children to adult SCA patients with stroke, and evaluate the effect of multiple candidate markers on stroke risk, with particular emphasis on stroke phenotype.

- 1) Identify genes influencing stroke risk in adults with SCA, using a candidate gene approach. As stated in the original proposal, we aim to enroll 400 eligible adult subjects with SCA and genotype patient DNA samples using a multilocus approach to simultaneously assess 114 variants among 73 candidate genes. Genotyping assays for this exploratory study include a novel MALDI-TOF mass spectrometry assay to interrogate several other recently identified candidate stroke markers. We will phenotypically characterize both case and control subjects using cerebral MRI/MRA data. We will test for associations between multiple genetic markers and specific stroke subtypes in adults with SCA.
- 2) Determine risk haplotypes and gene-gene interactions that influence stroke risk in adult SCA. After identification of a possible risk allele (Specific Aim #1) within or near a given candidate gene locus, additional SNPs at that locus will be surveyed to study regions in linkage disequilibrium and identify possible risk haplotypes within the locus. Such haplotype tagging may help to uncover the variation responsible for stroke risk associated with a particular candidate gene locus. Haplotype-based analysis also provides a more powerful approach to define risk alleles identified in Specific Aim #1. Together, these analyses will strengthen the power of any single SNP associations found in Specific Aim # 1 and provide a basis for further investigations to find causative gene variants.
- 3) Probe for associations between candidate genes and neurocognitive deficits in adults with SCA. Neurocognitive testing and quantitative MRI may detect SCA patients with subclinical vasculopathy who are at risk for cognitive dysfunction and eventual dementia. The association between candidate gene markers and neurocognitive impairment will be examined in a subset of SCA patients who will already have had specialized brain imaging studies and extensive neurocognitive testing performed as part of enrollment in another existing study. Using these surrogate measures of subclinical vasculopathy, we will test for genetic associations with pathways leading to silent infarcts in SCA. Identification of genetic risk factors for an intermediate phenotype such as neurocognitive impairment in the absence of lesions on MRI, may aid in early detection and intervention prior to the development of infarctive lesions by conventional neuroimaging.

B. Studies and Results (describe studies directed toward specific aims)

Specific Aim #1: To date, 57 subjects from our local institution (Northern California CSCC) have been consented for this study; blood samples and accompanying cerebral MRI/MRA data have been collected on these subjects. We have extracted DNA from these samples and have performed initial genotyping for candidate SNPs included on the RMS candidate gene panel (linear array). Genotyping results for these samples have been entered and stored in a dedicated Filemaker Pro database. In the past year, we have successfully established collaborations with both international (Dr. Yasser Wali, Sultan Qaboos University, Oman) and national (Dr. Mark Gladwin, NIH and Dr. Samir Ballas, Thomas Jefferson

University) investigators. IRB approval has been obtained by these investigators and Materials Transfer Agreements for the collection of subject samples and clinical data are in place. These collaborations not only serve the purpose of achieving adequate size and representation of African-Americans in the study population, but also of studying a more genetically homogeneous SCA population outside of the U.S. Neuroimaging studies have been performed on most of these patients, thus minimizing the outside costs associated with this study. The MaldiTOF and DNA sequencing assays for the additional candidate genes outlined in the proposal have been developed and validated using our previously tested samples.

Specific Aim #2: Although no formal analyses have yet been performed, we have developed the statistical models to be applied to our genotyping data, with modifications for the loglinear and haplotype analyses that are planned.

Specific Aim #3: We have enrolled and collected samples from 17 subjects participating in the Neurocognitive Study.

C. Significance (of findings to the scientific field and impact on health)

The significance of the findings from this study will not be known until full genotyping and statistical analyses have been performed on all of the samples. The efficiency of our genotyping assays will be maximized by batching samples and performing statistical analyses on a complete dataset. Collection of samples from the other institutions listed in the protocol has been hampered by the limited funding available to perform the requisite neuroimaging studies. Nonetheless, the total number of samples to be obtained from the two participating sites and our own will still provide sufficient power to detect larger effect sizes, as shown in the research plan. If significant genetic associations are found and confirmed, these markers could be used to tailor clinical and therapeutic decisions to an individual's risk profile. Identification of genes influencing specific stroke subtypes may also lead to a better understanding of the distinctive pathophysiologies of stroke and more effective therapies for stroke prevention in these patients.