Whole Exome Sequencing of Intracranial Aneurysm

Tatiana Foroud, PhD; for the FIA Study Investigators*

The risk of intracranial aneurysm (IA) is increased among individuals with first-degree relatives with history of IA.¹ A variety of approaches have been used to identify genes that contribute to the risk of IA.²⁻⁴ Genomewide association studies have identified and replicated associations on chromosome 4q31.23 (EDNRA), 8q12.1 (SOX17), 9p21.3 (CDKN2A/CDKN2B/CDKN2BAS), 10q24.32 (CNNM2), 12q22, 13q13.1 (Kl/STARD13), 18q11.2 (RBBP8), and 20p12.1.⁵⁻⁶ An alternative approach to gene discovery is to identify rare variants of relatively large individual effect. The most efficient approach to identify this type of disease-producing variant is through the study of families having a large number of members with IA. In many cases, the segregation of the disease in these families seems to be consistent with Mendelian inheritance.

Multiplex IA families were recruited through the international Familial Intracranial Aneurysm (FIA) study.⁷ Rigorous review of all available medical records and detailed subject interviews were performed to classify subjects with regard to the likelihood that they had an IA. In addition, unaffected first-degree family members, with risk factors increasing the likelihood that they might have an unruptured IA (smoking, hypertension, >30 years), were offered magnetic resonance angiography. These data were available from >400 multiplex FIA families. After careful review, 7 families were selected for whole exome sequencing. These 7 families had the largest number of family members with a definite IA who also had DNA available for genetic study. These families also seemed to be consistent with either autosomal-dominant or autosomal-recessive inheritance. Whole exome sequencing was completed for a total of 50 individuals, both affected and unaffected, within these 7 families (Figure 1).

Whole exome sequencing was performed at the Center for Inherited Disease Research. Exon capture was performed using the Agilent SureSelect 50Mb Human All Exon Kit, and sequencing was done on the Illumina HiSeq 2000. Each sample achieved a mean of 76X coverage of exonic bases. An average of 91% of targeted bases was covered by ≥8 sequencing reads.

Detailed review of the quality of the sequencing data was performed using the Genome Analysis Toolkit (GATK, version 1.2.9). A series of filters were used to remove variants that met criteria that would suggest that they were unlikely to contribute to IA. These filters resulted in the removal of SNPs that have frequency >3% in white populations and those not consistent with Mendelian inheritance within the family. The remaining variants were prioritized using the following criteria. Genes were given higher priority if (1) they were related to selected categories and genes of interest in the Gene Ontology (GO) database; (2) more affected individuals within the family carried the variant; (3) any controls sent from the family of interest did not carry a variant within the gene; (4) the variants within the gene were predicted to be deleterious by ≥1 protein prediction programs (SIFT, Polyphen2, MutPred for single nucleotide variants; SIFT-indel and an internal program for indels); or (5) they were within a highly conserved region as defined by GERP or could not be assessed by ≥1 of these programs. Thus, we included variants that were not inherited by all affected individuals in the family of interest, which also allowed for locus heterogeneity within our prioritization scheme. The process for prioritization is summarized in Figure 2.

Ninety-six candidate genes were identified which met our prioritization criteria. A quarter of the candidate genes are also part of relevant GO categories, such as vascular process in circulatory system, vasculature development, vasculogenesis, basement membrane, or collagen. Plans are ongoing to sequence the 96 candidate genes in a series of ≥400 additional familial IA cases. This will allow additional novel variants to be identified. Sequence data from publicly available controls, such as the Exome Sequencing Project will be compared with the sequencing results from the IA cases to identify those genes having a greater number of potentially deleterious variants in IA cases as compared with controls (ie, burden analysis). In addition, preliminary knockdown studies of the most promising candidate genes will be undertaken in zebrafish.

Appendix

Clinical Centers

University of Alabama at Birmingham: W. Fisher (principal investigator [PI]) and H. Forson (coordinator); Clinical Trials Research Unit, University of Auckland and Auckland City Hospital, New Zealand: C. Anderson (PI), E. Mee (PI), C. Howe (coordinator), and S. Vos (coordinator); Royal Perth Hospital, Sir Charles Gairdner Hospital, Royal Adelaide Hospital, Royal Melbourne Hospital, Alfred Hospital, Westmead Hospital, Royal North Shore Hospital, Royal Prince Alfred Hospital, Australia: C. Anderson (PI), G. Hankey (PI), N. Knuckey (PI), J. Laidlaw (PI), P. Reilly (PI), N. Dorsch (PI), M. Morgan (PI), M. Besser (PI), J. Rosenfeld (PI), K. Athanasiadis (coordinator), Royal North Shore Hospital, Royal Prince Alfred Hospital, Australia: C. Anderson (PI), G. Hankey (PI), N. Knuckey (PI), J. Laidlaw (PI), P. Reilly (PI), N. Dorsch (PI), M. Morgan (PI), M. Besser (PI), J. Rosenfeld (PI), K. Athanasiadis (coordinator),

Received March 28, 2013; accepted April 8, 2013.
From the Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN.
*A list of all FIA study investigators is given in the Appendix.
Correspondence to Tatiana Foroud, PhD, Indiana University School of Medicine, 410 W 10th St, Indianapolis, IN 46202. E-mail tforoud@iupui.edu


© 2013 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.113.001174
Figure 1. Pedigrees used for whole exome sequencing. IA indicates intracranial aneurysm.

Figure 2. Variant prioritization.
G. Steinberg (PI), D. Luu (coordinator), and M. Coburn (coordinator); University of Texas at Houston: M. Malkoff (PI) and A. Wojner (coordinator); University of Virginia: N. Kassel (PI), B. Worrall (co-PI), and G. Radakovic (coordinator); University of Washington: D. Tirschwell (PI) and P. Tanzi (coordinator); Washington University: C. Derdeyn (PI) and M. Catanzaro (coordinator); and University of Manitoba: A. Kaufmann (PI) and D. Gladish (coordinator).

Clinical Coordinating Center
Joseph P. Broderick, MD (Principal Investigator); Laura Sauerbeck, RN, MS (FIA Study Manager); Jeanne Sester (Research Associate); and Charlie Moomaw (Data Manager).

Executive Committee
Craig Anderson, MD; Joan E. Bailey-Wilson, PhD; Joseph P. Broderick, MD (Chair); Robert Brown, Jr, MD; E. Sander Connelly, MD; Ranjan Deka, PhD; Tatiana Foroud, PhD; John Huston, MD; Nerissa Ko, MD; Carl Langefeld, PhD; Irene Meissner, MD; Guy Rouleau, MD, PhD; Laura Sauerbeck, RN, MS; Bradford Worrall, MD; and Daniel Woo, MD, MS.

Imaging Phenotyping Committee
John Huston, MD; David Kallmes, MD; and Harry Cloft, MD.

Medical Record Phenotyping
Joseph P. Broderick, MD; Robert Brown, Jr, MD; Daniel Woo, MD, MS; Irene Meissner, MD; David Wiebers, MD; Dawn Kleindorfer, MD; Matthew L. Flaherty, MD; Jason Mackey, MD, MS; Shannon Kohake, MD; Sharyl Martini, MD, PhD; and Felipe De Los Rios La Rosa, MD.

Genetic Analysis
Janice Farlow, BS; Tatiana Foroud, PhD; Daniel Koller, PhD; Hai Lin, BS; and Yunlong Liu, PhD.

Center for Inherited Disease Research
Kimberly Doheny, PhD; Kurt Hetrick, MS; Hua Ling, PhD; and Elizabeth Pugh, PhD.

Disclosures
None.

Sources of Funding
This study was supported by R01NS39512, HHSN268200782096C, and T32GM077229.

References
Whole Exome Sequencing of Intracranial Aneurysm
Tatiana Foroud

Stroke. 2013;44:S26-S28
doi: 10.1161/STROKEAHA.113.001174

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/6_suppl_1/S26

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/