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2016.001

MRI-defined cerebrovascular genomics—The CHARGE consortium

S. Debette^{1,2,3,4}; Y. Saba⁵; D. Vojinovic⁶; X. Jian⁷; H. Adam⁸; G. Chauhan^{1,2}; M. Sargurupremraj^{1,2}; S. Kaffashian^{1,2}; J. Ding⁹; J.C. Bis⁹; P. Nyquist¹⁰; K. Mather¹¹; C. Van Duijn¹²; L.J. Launer¹³; M.A. Ikram¹⁴; H. Schmidt¹⁵; W.T. Longstreth^{16,17}; M. Fornage¹⁸; S. Seshadri^{14,16}; For the neuro-CHARGE working group

¹Inserm Center U1219, Bordeaux Population Health, France; ²University of Bordeaux, France; ³Department of Neurology, Bordeaux University Hospital, France; ⁴Department of Neurology, Boston University School of Medicine, MA; ⁵Research Unit for Genetic Epidemiology, Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University of Graz, Austria; ⁶Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands; ⁷Institute of Molecular Medicine and Human Genetics Center, University of Texas Health Science Center at Houston; ⁸IRP/NIH/NIH, Bethesda, MD; ⁹Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle; ¹⁰Department of Anesthesiology and Critical Care Medicine, Neurology, Neurosurgery, and General Internal Medicine, Johns Hopkins University, Baltimore, MD; ¹¹Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia; ¹²Centre for Medical Systems Biology, Leiden, The Netherlands; ¹³Department of Neurology, Clinical Division of Neurogeriatrics, Medical University Graz, Austria; ¹⁴Department of Epidemiology; ¹⁵Department of Neurology, University of Washington, Seattle; and ¹⁶The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA.

ABSTRACT: The CHARGE consortium is an investigator-initiated collaboration to facilitate meta-analyses of genome-wide association studies (GWAS) and genomic analyses based on next generation sequencing (NGS), among multiple large and well-phenotyped population-based cohort studies around the world (<http://www.chargeconsortium.com>). Within the neuro-CHARGE working group, we are presenting an update of ongoing genomic studies on MRI-markers of cerebrovascular disease. Large population-based studies have shown that the burden of cerebrovascular disease extends far beyond that of clinical stroke. MRI-markers of cerebral small vessel disease, such as white matter hyperintensities (WMH), small subcortical brain infarcts, microbleeds, or dilated perivascular spaces, are particularly frequent in older community persons. These markers portend an increased risk of stroke, dementia, and premature death, and were shown to have a high heritability, especially WMH burden. Interestingly recent work has revealed genetic risk variants between WMH burden and stroke. To account for the major role of high blood pressure in the occurrence of WMH, we are performing a GWAS of WMH burden stratified on hypertension status, as well as a joint meta-analysis to account for gene-environment interaction with hypertension. Moreover, based on prior epidemiologic and histologic data suggesting that the pathogenesis of WMH may differ according to their location, we are running separate GWAS for periventricular and deep WMH burden. A GWAS of cerebral microbleeds is being finalized. We are also exploring the role of vascular risk factors in the occurrence of dementia by examining the relation of genetic risk scores for these factors with MRI-markers of brain aging and cerebrovascular disease. Finally, several NGS projects are being conducted to identify rare variants associated with WMH burden and lacunar brain infarcts. Additional projects are also currently being designed, including GWAS of novel MRI phenotypes, such as composite measures of cerebral small vessel disease, as well as lifetime, and epigenomic approaches.

2016.002

Modeling acute lesion volume and topography for genetic analysis (MRI-GENIE study)

Ona Wu, PhD¹; Stefan Winzeck, MS^{1,2}; Steven J.T. Mocking, MS¹; Markus D. Schirmer, PhD³; Mark J.R.J. Bouts, PhD⁴; Elissa C. McIntosh, MA¹; Anne-Katrin Giese, MD^{3,4}; Joseph P. Broderick, MD⁵; Jordi Jimenez-Conde, MD, PhD⁶; Christina Jern, MD, PhD⁷; Brett M. Kissela, MD, MS⁸; Steven J. Kittner, MD, MPH⁹; Dawn O. Kleindorfer, MD¹⁰; Robin Lemmens, MD, PhD¹¹; Arne Lindgren, MD, PhD¹⁰; James F. Meschia, MD¹¹; Braxton D. Mitchell, PhD, MPH^{12,13}; Jonathan Rosand, MD, MS¹⁴; Tatjana Rundek, MD, PhD¹⁴; Ralph L. Sacco, MD, MS¹⁴; Reinhold Schmidt, MD¹⁵; Pankaj Sharma, MD, PhD¹⁶; Agnieszka Slowik, MD, PhD¹⁷; Vincent N.S. Thijs, MD, PhD¹⁸; Daniel Woo, MD, MS¹⁹; Bradford B. Worrall, MD, MS¹⁹; Natalia S. Rost, MD, MPH²⁰

¹Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown; ²University Division of Anaesthesia, Department of Medicine, Cambridge University, UK; ³Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; ⁴Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; ⁵Department of Neurology and Rehabilitation Medicine, University of

Cincinnati College of Medicine, OH; ⁶Department of Neurology, Neurovascular Research Group (NEU-VAS), IMIM-Hospital del Mar (Institut Hospital del Mar d'Investigacions Mèdiques), Universitat Autònoma de Barcelona, Spain; ⁷Institute of Biomedicine, the Sahlgrenska Academy at University of Gothenburg, Sweden; ⁸Department of Neurology, University of Maryland School of Medicine and Veterans Affairs Maryland Health Care System, Baltimore; ⁹KU Leuven—University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), Leuven, Belgium; ¹⁰Vesalius Research Center, Laboratory of Neurobiology, University Hospitals Leuven, Department of Neurology, Belgium; ¹¹Department of Clinical Sciences Lund, Neurology, Lund University; Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden; ¹²Department of Neurology, Mayo Clinic, Jacksonville, FL; ¹³Department of Medicine, University of Maryland School of Medicine, Baltimore; ¹⁴Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, MD; ¹⁵Department of Neurology, Miller School of Medicine, University of Miami, FL; ¹⁶Department of Neurology, Clinical Division of Neurogeriatrics, Medical University Graz, Austria; ¹⁷Institute of Cardiovascular Research, Royal Holloway University of London (ICR2UL), Egham, Ashford and St Peter's Hospitals, UK; ¹⁸Department of Neurology, Jagiellonian University Medical College, Krakow, Poland; ¹⁹Department of Neurology Austin Health, Melbourne Brain Center, Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia; and ²⁰Departments of Neurology and Public Health Sciences, University of Virginia, Charlottesville, VA.

OBJECTIVE: To investigate the relationship between acute ischemic lesion location and volumes and genetics with admission stroke severity and long-term outcome, an automated lesion segmentation algorithm was developed. **BACKGROUND:** One of the primary goals of the MRI-Genetics Interface Exploration study (MRI-GENIE) is to develop clinical MRI scan analysis tools that will advance genetic discovery in stroke. To incorporate lesion volumes and topology into stroke genetic analysis studies, thousands of datasets from multiple centers will need to be analyzed. We therefore developed an automated lesion segmentation algorithm. **DESIGN/METHODS:** Lesion segmentation was performed by randomized classification forests (RF) on a voxel-wise basis. $9 \times 9 \times 1$ patches of the input, consisting of the apparent diffusion coefficient map, isotropic diffusion-weighted image, and non-diffusion-weighted image, resulted in 243 features. Optimal patch size was determined in a 5-fold cross-validation with 50 patients. The final segmentation model was trained on 67 patients and applied to 1,197 independent datasets from the Stroke Genetics Network (SiGN) Repository. The RF outputs were thresholded then further processed using post-hoc regional analyses. All images were spatially normalized to the MNI 1 mm atlas using non-linear co-registration (MNI Autoreg). **RESULTS:** Median (IQR) acute DWI volumes were 4.3 (0–22.0) cm³. The figure shows the incidence map of lesions for all 1,197 subjects (maximum incidence was 138). The frequency of stroke lesions was comparable for both left and right hemispheres and consistent with previously published studies involving single centers. **CONCLUSIONS:** Automatic acute DWI lesion segmentation is feasible. Studies investigating the association between lesion topography and lesion volumes with genetics, admission stroke severity scores and long term functional outcomes in large multi-center datasets are therefore realistic.

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2016.003

Genome-wide association studies of disease severity in neurocritical care: Interim analysis of spontaneous intracerebral hemorrhage volume

Guido J. Falcone¹; Daniel Woo²; Miriam R. Raffeld¹; Alessandro Biffi¹; Chia-Ling Phuah¹; Joshua N. Goldstein³; David L. Tirschwell⁴; Magdy Selim⁵; Scott L. Silliman⁶; Bradford B. Worrall⁷; James F. Meschia⁸; Jordi Jimenez-Conde⁹; Arne Lindgren¹⁰; Christopher D. Anderson¹; Jonathan Rosand¹

¹Center for Human Genetic Research, Massachusetts General Hospital, Boston; ²Department of Neurology, University of Cincinnati College of Medicine, OH; ³Department of Emergency Medicine, Massachusetts General Hospital, Boston; ⁴Stroke Center, Harborview Medical Center, University of Washington, Seattle; ⁵Department of Neurology, Stroke Division, Beth Israel Deaconess Medical Center, Boston, MA; ⁶Department of Neurology, University of Florida College of Medicine, Jacksonville;

2016.002: Modeling acute lesion volume and topography for genetic analysis (MRI-GENIE study)

2016.003: Genome-wide association studies of disease severity in neurocritical care: Interim analysis of spontaneous intracerebral hemorrhage volume

⁷Department of Neurology and Public Health Sciences, University of Virginia Health System, Charlottesville, ⁸Department of Neurology, Mayo Clinic, Jacksonville, FL; ⁹Neurovascular Research Unit, Department of Neurology, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Universitat Autònoma de Barcelona, Spain; ¹⁰Department of Neurology, Skåne University Hospital, Lund, Sweden. **OBJECTIVE:** We report an interim analysis of the first GWAS of ICH volume undertaken by the International Stroke Genetics Consortium. **BACKGROUND:** Spontaneous intracerebral hemorrhage (ICH) carries a grim prognosis and has limited treatment options. ICH volume remains the most potent predictor of outcome. Genome-wide association studies (GWAS) constitute a powerful technique for identifying novel biological pathways and drug targets for human disease. **DESIGN/METHODS:** Two-stage (discovery and replication) cohort study that included subjects from a multicenter US study (discovery) and 2 European studies (replication). We utilized admission head CT to calculate hematoma volume and classify ICH cases as lobar or non-lobar. After genotyping (Illumina 610 platform) and imputation, 7 million genetic variants were available for testing. Association testing was carried out using linear regression. Signals with $p < 5 \times 10^{-8}$ were pursued in replication and tested for association with admission Glasgow Coma Scale (GCS) and 3-month dichotomized (0–2 vs 3–6) modified Rankin Scale (mRS) using ordinal and logistic regression, respectively. **RESULTS:** The discovery phase included 394 ICH cases (228 lobar and 166 non-lobar) and identified 2 susceptibility loci: for lobar ICH volume, a genomic region on 23q13 that encompasses the *PARVB* gene (top SNP rs9614326, $\beta = 1.84$, $p = 4.4 \times 10^{-8}$); for non-lobar ICH volume, an intergenic region on 17p12 (top SNP rs11655160, $\beta = 0.95$, $p = 4.3 \times 10^{-8}$). The replication included 240 ICH cases (71 lobar and 169 non-lobar) and corroborated the association for 17p12 ($p = 0.036$; meta-analysis $p = 2.5 \times 10^{-9}$, p heterogeneity = 0.17) but not for 23q13 ($p = 0.49$). In multivariable analysis, rs11655160 was also associated with lower admission GCS (OR = 0.23, $p = 0.004$) and increased risk of poor 3-month mRS (OR = 1.94, $p = 0.045$). **CONCLUSIONS:** This interim analysis identified 17p12 as a possible susceptibility locus for non-lobar ICH volume. This locus points towards novel biological pathways to be pursued by epidemiologic and bench researchers. Reassuringly, this genetic variant also correlated with admission and 3-month clinical status. These results require further replication. **Study Supported By:** NIH/Neurological Disorders and Stroke.

2016.004

Risk factors for ischaemic stroke: What can we learn from METASTROKE? *Jemma C. Hopewell, PhD; On behalf of the METASTROKE Collaboration of the ISGC*

CTSU, Nuffield Department of Population Health, University of Oxford, UK.

OBJECTIVE: Identify risk factors for Ischaemic stroke. **BACKGROUND:** Mendelian randomization studies, analogous to naturally occurring randomized trials, can be used to determine if risk factors are causally associated with disease, and can thereby offer new insights into biological pathways as well as facilitate discovery of novel therapeutic targets. Under certain conditions, if a genetic variant is associated with the risk factor of interest and is also associated with the disease of interest, then we can conclude that the risk factor is causally associated with the risk of disease. Mendelian randomization has been used extensively in coronary disease research but less so to study ischaemic stroke, mainly due to a lack of available data. **DESIGN/METHODS:** Based on the collaborative efforts of studies contributing to the METASTROKE meta-analyses, genome-wide summary-level association statistics based on >10,000 ischaemic stroke cases are now available. These data provide a unique opportunity to assess the causal nature of risk factor relationships with ischaemic stroke, as well as the importance of ischaemic stroke subtypes. **RESULTS:** We discuss this data resource and illustrate the insights Mendelian randomization studies can offer using the example of a METASTROKE sub-project that examines the impact of life-long differences in genetically determined LDL-cholesterol levels on risk of ischaemic stroke (and its subtypes). **CONCLUSIONS:** We encourage researchers to work with the data available from the METASTROKE collaboration of the ISGC and to thereby advance further our understanding of traditional and emerging risk factors for ischaemic stroke.

2016.005

Genome wide association studies in Han Chinese populations identify novel susceptibility loci for subtypes of ischemic stroke *Tai-Ming Ko, PhD; Tsong-Hai Lee, MD, PhD; Chien-Hsiun Chen, PhD; Yuan-Tsong Chen, MD, PhD; Jer-Yuarn Wu, PhD*

¹Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; and ²Chang Gung Memorial Hospital, Linkou Medical Center, and Chang Gung University College of Medicine, Taoyuan, Taiwan.

OBJECTIVE: Because the etiology of ischemic stroke is heterogeneous, we hypothesize that genetic factors may vary by etiologic subtypes or ethnicities. Thus, we aim to identify genetic factors that contribute to large-artery atherosclerosis LAA or small-vessel occlusion (SVO) based on independent Han Chinese populations. **BACKGROUND:** Although family history studies in ischemic stroke support that genetic factors may be involved in the pathogenesis of 2 major subtypes of ischemia stroke: LAA and SVO, it is still unclear which particular genetic factors contribute to LAA or SVO. **DESIGN/METHODS:** Novel genetic variants that predispose individuals to LAA and SVO were identified by genome-wide association study comprising of 824 individuals (including 444 LAA cases and 380 SVO cases) and 1,727 controls in a Han Chinese population residing in Taiwan. The LAA study was replicated in an independent Han Chinese population comprising of an additional 319 LAA cases and 1,802 controls. The SVO study was replicated in an independent Han Chinese population comprising of an additional 189 LAA cases and 1,265 controls. **RESULTS:** In LAA cases, from 2 independent populations, we identified 5 SNPs, including rs2415317 ($p = 3.10 \times 10^{-8}$), rs934075 ($p = 4.00 \times 10^{-9}$), rs944289 ($p = 3.57 \times 10^{-8}$), rs2787417 ($p = 1.76 \times 10^{-8}$), and rs1952706 ($p = 2.92 \times 10^{-8}$), at one novel locus on chromosome 14q13.3 within PTCSC3 (encoding papillary thyroid carcinoma susceptibility candidate 3) that were associated with LAA stroke at genome-wide significance ($p < 5 \times 10^{-8}$). In SVO cases, from the discovery stage, we identified 2 novel candidate susceptibility

loci on chromosome 3p25.3 (SVO-SNP1, $p = 3.24 \times 10^{-7}$) and chromosome 14 q31.1 (SVO-SNP2, $p = 2.58 \times 10^{-4}$). **CONCLUSIONS:** For LAA, the newly identified SNPs within PTCSC3 gene were found to have genome-wide statistical significance ($p < 5 \times 10^{-8}$) and were shown to be located in a risk locus correlated with papillary thyroid carcinoma. Moreover, the genetic association between PTCSC3 gene and SVO was not identified, which suggested that PTCSC3 is a specific susceptibility locus for LAA. For SVO, we identified 2 novel candidate genetic loci which were valuable for replication by an independent population with SVO. In conclusion, our findings provide insights into the genetic basis of LAA and SVO, which may be applicable in the study of the pathogenesis of ischemic stroke and in the development of alternative therapeutic interventions.

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2016.006

Metabolomic profiles associated with aging in women *Raji Balasubramanian, ScD; Nina Paynter, PhD; JoAnn Manson, MD; J.C. Chen, PhD; Mara Vitalini, DrPH, MPH, RD; Clary Clish, PhD; Christine Albert, MD, MPH; Kathryn Rexrode, MD, MPH*

¹UMASS Amherst School of Public Health and Health Sciences, MA; ²Brigham and Women's Hospital, Boston; ³Harvard TH Chan School of Public Health, Cambridge, MA; ⁴Harvard University, Cambridge, MA; ⁵Wakeforest School of Medicine, NC; ⁶Broad Institute of MIT and Harvard, Cambridge, MA.

OBJECTIVE: To use Metabolomic profiling to identify potential metabolites associated with aging. **BACKGROUND:** Aging is a complex process that results from metabolic activities such as those that generate reactive oxygen species. Metabolomic profiling, an emerging technology, may shed light on metabolites associated with aging and identify key changes associated with premature aging. **DESIGN/METHODS:** A total of 370 metabolites were profiled using liquid chromatography tandem mass spectroscopy (LC-MS) in plasma from 1,153 healthy, control subjects in the Women's Health Initiative. Metabolite levels were log-transformed and standardized, and each considered individually in statistical models. Linear regression models were created with the metabolite as the outcome and age as the primary exposure: <60 (referent), 60–<65, 65–<70, 70–<75, and 75 years and older. Models were adjusted for matching factors in the primary study (race, time period, hysterectomy status, and then additionally for body mass index, medications [antihypertensives, anti-diabetic drugs, aspirin and statins], alternative healthy eating diet score, and creatinine). The likelihood ratio test was used to compare models with age to model without age, with adjustment for false discovery rate (FDR). Significant results were defined as FDR adjusted $p < 0.05$. **RESULTS:** The median age of the women was 68 years (interquartile range 62–72). After multivariate adjustment, 39 metabolites were significantly associated with age >75, including 12 acylcarnitines, 11 free fatty acids, 9 fatty acid derivatives, 2 amino acids, and 5 additional metabolites. Of these, octadecenoyl-L-carnitine (C18:1) had the strongest age association, with levels 0.70 SD (95% CI: 0.48–0.92) higher on average among women >75 years compared to women <60 years of age (referent). C22:0 sphingomyelin (SM), threonine and C24:0 SM were negatively associated with age; levels of these metabolites were on average 0.41 to 0.44 SD lower among the women 75 years and older when compared to the youngest women (linear trend FDR- p value <0.05 for all of these metabolites). **CONCLUSIONS:** Multiple metabolites involved in mitochondrial fatty acid oxidation, as well as several associated with insulin resistance, have a strong relationship with age, even after adjusting for multiple age-associated factors.

2016.007

Genetics of cerebral small vessel disease *Hugh Markus, DM* University of Cambridge.

OBJECTIVE: To increase accuracy in diagnosis of Lacunar stroke using MRI and to confirm the accuracy and heritability of that diagnosis with GCTA. **BACKGROUND:** Although a number of genome wide associations have been detected for Ischemic Stroke these have been primarily with Large Artery and Cardioembolic Stroke and no specific associations have been reported with Small Vessel or Lacunar Strokes. Consistent with this genome-wide complex trait analysis (GCTA) has found that heritability of Small Vessel Disease, as determined using data from the GWAS arrays, is low in contrast to that of large artery disease and cardioembolic stroke, which is higher. This failure to determine genetic associations, and to detect with heritability, for Small Vessel Disease is surprising in view of family history data would suggest genetic risk is particularly important for Small Vessel Disease and the fact that Small Vessel Disease is the most common subtype of single gene stroke disorders. Possible reasons for the lack of success in small vessel disease studies include diagnostic difficulties and phenotypic heterogeneity. **DESIGN/METHODS:** Most GWAS studies to date have not used MRI to confirm Lacunar Stroke, and diagnosis based on Lacunar Syndrome and a diagnosis based on Lacunar Syndrome with CT imaging can be incorrect in as many as a third of cases. We have performed GWAS studies on an MRI confirmed cohort of patients with Small Vessel Disease and have shown that heritability detected using GCTA is greatly increased using this approach. **RESULTS:** It has been suggested that there may be more than one pathology underlying Small Vessel Disease. C. Millerfisher suggested that larger Lacunar Infarcts may be caused by focal atheroma whereas smaller multiple Lacunar Infarcts are due to a diffuse arteriopathy with underlying lipohyalinosis. Using MRI we have subtyped cases into these 2 phenotypes and shown that they appear to have different genetic architecture and heritability. In further studies we have shown that genes involved in oxidative phosphorylation appear to be risk factors for the multiple Lacunar Infarcts/Leukoaraiosis type of Small Vessel Disease but not for the isolated Lacunar Infarct subtype. **CONCLUSIONS:** More recently we have identified, in collaboration with many partners within ISGC, SIGN, and Metastroke, the first locus associated specifically with Small Vessel Stroke on 16q24.2. We have shown it is also associated with white matter hyper-intensities on MRI. We have shown that associations with the gene are stronger if one subtypes cases using MRI.

2016.008

The automated pipeline for white matter hyperintensity detection: The MRI-GENIE study *Markus D. Schirmer, PhD^{1,2}; Adrian Dalca, MSc³; Ramesh Sridharan, PhD³; Anne-Katrin Giese, MD^{4,5}; Joseph P. Broderick, MD⁶; Jordi Jimenez-Conde, MD, PhD⁶; Christina Jern, MD, PhD⁷; Brett M.*

2016.008: The automated pipeline for white matter hyperintensity detection: The MRI-GENIE study

Kissela, MD, MS¹; Dawn O. Kleindorfer, MD²; Robin Lemmens, MD, PhD³; Arne Lindgren, MD, PhD⁴; James F. Meschia, MD⁵; Tatjana Rundek, MD, PhD¹¹; Ralph L. Sacco, MD, MS¹¹; Reinhold Schmidt, MD¹²; Pankaj Sharma, MD, PhD¹³; Agnieszka Slowik, MD, PhD¹⁴; Vincent Thijs, MD, PhD¹⁵; Daniel Woo, MD, MS¹⁶; Bradford B. Worrall, MD, MSc¹⁶; Steven J. Kittner, MD, MPH¹⁷; Jonathan Rosand, MD, MSc^{1,4,18}; Ona Wu, PhD^{1,2}; Polina Golland, PhD³; Natalia S. Rost, MD, MPH¹

¹Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; ²Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital (MGH), Charlestown; ³Computer Science and Artificial Intelligence Lab, MIT; ⁴Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; ⁵Department of Neurology, University of Cincinnati College of Medicine, OH; ⁶Department of Neurology, Neurovascular Research Group (NEUVAS), IMIM-Hospital del Mar (Institut Hospital del Mar d'Investigacions Mèdiques), Universitat Autònoma de Barcelona, Spain; ⁷Institute of Biomedicine, the Sahlgrenska Academy at University of Gothenburg, Sweden; ⁸KU Leuven—University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND); ⁹VIB, Vesalius Research Center, Laboratory of Neurobiology, University Hospitals Leuven, Department of Neurology, Belgium; ¹⁰Department of Clinical Sciences Lund, Neurology, Lund University; ¹¹Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden; ¹²Department of Neurology, Mayo Clinic, Jacksonville, FL; ¹³Department of Neurology, Miller School of Medicine, University of Miami, FL; ¹⁴Department of Neurology, Clinical Division of Neurogeriatrics, Medical University Graz, Austria; ¹⁵Institute of Cardiovascular Research, Royal Holloway University of London (ICR2UL), Egham, Ashford and St Peter's Hospital, UK; ¹⁶Department of Neurology, Jagiellonian University Medical College, Krakow, Poland; ¹⁷Department of Neurology Austin Health, Melbourne Brain Center, Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia; ¹⁸Departments of Neurology and Public Health Sciences, University of Virginia, Charlottesville; ¹⁹Department of Neurology, University of Maryland School of Medicine and Veterans Affairs Maryland Health Care System, Baltimore; and ²⁰Center for Human Genetic Research, Massachusetts General Hospital, Boston.

OBJECTIVE: To extract white matter hyperintensity volumes (WMHv) from clinically acquired fluid-attenuated inversion recovery (FLAIR) images automatically. **BACKGROUND:** WMHv has been shown to be useful as a predictor for stroke. With the emergence of large scale studies, manual segmentation of WMH becomes a labor intensive task that impedes discovery. Machine learning (ML) algorithms and dedicated pipelines can alleviate this burden. **DESIGN/METHODS:** Automated WMHv extraction from multiple subjects using ML algorithms relies on all images occupying the same space, e.g., atlas space. In this work, images are preprocessed using publicly available software. First, each FLAIR image is upsampled using bi-cubic interpolation, in order to reduce the effects of anisotropic voxel sizes. Images are skull-stripped using Robust Brain Extraction (ROBEX). Registration is carried out using the Buckner FLAIR Atlas template and Advanced Normalization Tools (ANTS). Manual WMH outlines of 40 subjects are used for principal component analysis (PCA), in order to learn the spatial distribution of WMH. Using the PCA eigenmaps, areas of leukoaraiosis are identified and WMHv are calculated based on the non-interpolated slices for each subject. **RESULTS:** As part of the MRI-Genetics Interface Exploration study (MRI-GENIE), 2,862 stroke patients from 12 independent sites underwent FLAIR imaging. WMHv were calculated on a subset of 2,372 patients. Results are shown in the figure. **CONCLUSIONS:** In conclusion, by using an automated approach we were able to process a large number of clinical scans, where the extracted WMHv can be used in subsequent analyses. Quality control efforts and extended validation in the additional sites are ongoing.

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2016.009 Genetics of MRI-based traits in patients with ischemic stroke: The MRI-GENIE study

Anne-Karin Giese, MD^{1,2}; Huichun Xu, MD, PhD³; Tushar Dave, MS⁴; Markus D. Schirmer, PhD^{1,4}; Adrian Dalca, MSc⁵; Ramesh Sridharan, PhD⁶; Lisa Cloonan, BA⁷; John W. Cole, MD⁸; Patrick F. McArdle, PhD⁹; Joseph P. Broderick, MD¹⁰; Jordi Jimenez-Conde, MD, PhD⁸; Christina Jern, MD, PhD⁹; Brett M. Kissela, MD, MS¹; Dawn O. Kleindorfer, MD²; Robin Lemmens, MD, PhD¹⁰; Arne Lindgren, MD, PhD¹¹; James F. Meschia, MD¹²; Tatjana Rundek, MD, PhD¹³; Ralph L. Sacco, MD, MS¹³; Reinhold Schmidt, MD¹⁴; Pankaj Sharma, MD, PhD¹⁵; Agnieszka Slowik, MD, PhD¹⁶; Vincent Thijs, MD, PhD¹⁷; Daniel Woo, MD, MS¹⁶; Bradford B. Worrall, MD, MSc¹⁸;

Ona Wu, PhD^{1,4}; Polina Golland, PhD⁵; Steven J. Kittner, MD, MPH¹⁹; Jonathan Rosand, MD, MSc^{1,2,19}; Braxton D. Mitchell, PhD, MPH²⁰; Natalia S. Rost, MD, MPH¹

¹Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; ²Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; ³Department of Medicine, University of Maryland School of Medicine, Baltimore; ⁴Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital (MGH), Charlestown; ⁵Computer Science and Artificial Intelligence Lab, MIT; ⁶Department of Neurology, University of Maryland School of Medicine and Veterans Affairs Maryland Health Care System, Baltimore; ⁷Department of Neurology, University of Cincinnati College of Medicine, OH; ⁸Department of Neurology, Neurovascular Research Group (NEUVAS), IMIM-Hospital del Mar (Institut Hospital del Mar d'Investigacions Mèdiques), Universitat Autònoma de Barcelona, Spain; ⁹Institute of Biomedicine, the Sahlgrenska Academy at University of Gothenburg, Sweden; ¹⁰KU Leuven—University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND); ¹¹VIB, Vesalius Research Center, Laboratory of Neurobiology, University Hospitals Leuven, Department of Neurology, Belgium; ¹²Department of Clinical Sciences Lund, Neurology, Lund University; ¹³Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden; ¹⁴Department of Neurology, Mayo Clinic, Jacksonville, FL; ¹⁵Department of Neurology, Miller School of Medicine, University of Miami, FL; ¹⁶Department of Neurology, Clinical Division of Neurogeriatrics, Medical University Graz, Austria; ¹⁷Institute of Cardiovascular Research, Royal Holloway University of London (ICR2UL), Egham, Ashford and St Peter's Hospital, UK; ¹⁸Department of Neurology, Jagiellonian University Medical College, Krakow, Poland; ¹⁹Department of Neurology Austin Health, Melbourne Brain Center, Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia; ²⁰Departments of Neurology and Public Health Sciences, University of Virginia, Charlottesville; ²¹Center for Human Genetic Research, Massachusetts General Hospital, Boston; and ²²Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, MD.

OBJECTIVE: To delineate the genetic architecture of acute and chronic MRI-detected cerebrovascular phenotypes. **BACKGROUND:** The principal goals of the MRI-Genetics Interface Exploration study (MRI-GENIE) are to develop, validate, and disseminate a clinical MRI analysis platform that will advance genetic discovery in stroke, and to develop novel clinical applications that personalize stroke risk and outcome assessment. Here, we report the initial analysis of 2,181 European ancestry ischemic stroke subjects from the Stroke Genetics Network (SiGN). **DESIGN/METHODS:** Using a novel volumetric analysis pipeline, we analyzed acute DWI lesion volume (DWIv) in 1,159 subjects as a marker for acute cerebrovascular disease and white matter hyperintensity volume (WMHv) in 1,290 subjects as a marker for chronic cerebrovascular disease. Preliminary genome-wide association (GWA) testing per SNP was performed by linear regression modeling for natural log-transformed DWIv and WMHv adjusted for age, sex and PCs. **RESULTS:** On average, subjects were 62.5 ± 14.7 years old, 38.1% were female, 63.9% had hypertension, 22.9% diabetes mellitus, 17.6% coronary artery disease, 15.8% atrial fibrillation, 50.5% had a history of smoking. Of the 2,181 subjects with volumetric data, 20.3% presented with large artery stroke, 15.6% cardioembolic stroke, 15.7% small vessel disease, 7.1% other and 41.3% with undetermined causes of stroke. Both WMHv (median 6.7 cm³, IQR 2.7–14.7 cm³) and DWIv (median 4.0 cm³, IQR 1.3–17.2 cm³) differed significantly across stroke subtypes ($p = 2.747 \times 10^{-12}$ and $p < 2.2 \times 10^{-16}$, respectively). GWA testing for WMHv revealed a putative hit on chromosome 13. No locus reached genome-wide significance in association with DWIv. **CONCLUSIONS:** This preliminary analysis demonstrated: (1) feasibility of extracting the MRI phenotypes from the clinical scans; (2) association of DWIv and WMHv, stroke subtypes and genetic variants. Validation of these findings and in-depth analyses of the genetic architecture of WMHv and DWIv are ongoing.

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Disclosure: None.

2016.010 Novel insight into risk factors for MRI-defined brain infarcts: A trans-ethnic population-based meta-analysis from the CHARGE Consortium

G. Chauhan¹; The METASTROKE consortium; The Stroke Genetics Network (SiGN); The International Stroke Genetics Consortium (ISGC); The Alzheimer's Disease Genetics Consortium (ADGC); UK Young Lacunar Stroke DNA Study; M. Fornage²; L.J. Launer³; M. Ikram⁴; S. Seshadri^{1,5}; W.T. Longstreth, Jr.^{1,6}; S. Debette⁷; The Neurology Working Group of the CHARGE consortium

¹Université de Bordeaux, Centre Inserm U 1219, France; ²Institute of Molecular Medicine and Human Genetics Center, University of Texas Health Science Center at Houston; ³IRP/NIH/NIH, Bethesda, MD; ⁴Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands; ⁵Department of Neurology, Boston University School of Medicine, MA; and ⁶Department of Epidemiology, University of Washington, Seattle.

OBJECTIVE: To identify genetic and vascular risk factors for brain infarcts in a trans-ethnic study. **BACKGROUND:** MRI-defined brain infarcts (BI) are powerful predictors of stroke and dementia. Most are small sub-cortical BI (SSBI) and covert, namely found in those without a history of stroke. While risk factors are generally assumed to be similar to ischemic stroke, large trans-ethnic studies exploring the environmental and genetic determinants of BI are lacking, and recommendations for the management of covert BI are lacking. **DESIGN/METHODS:** Eighteen population-based cohorts contributed 20,949 participants from 5 ethnicities—3,726 with MRI-defined BI and, out of these, 2,021 with SSBI. Associations were tested with vascular risk factors and genetic risk scores (GRS) of vascular risk factors. A trans-ethnic meta-analysis of genome-wide association studies (GWAS) was also performed. Top loci from GWAS were followed up in 3,143 participants from 5 population-based cohorts—1,134 with BI and, out of these, 543 with SSBI—and also checked for association with ischemic stroke (IS) (N = 22,624, of which N = 4,325 had the small vessel disease subtype) and with pathologically defined BI (N = 2,940). **RESULTS:** We observed significant association of MRI-defined BI and SSBI with blood pressure (BP) measurements (p range: 2.6×10^{-5} to 9.4×10^{-23}), smoking (p BI = 4.4×10^{-16} ; p SSBI = 1.2×10^{-7}), type 2 diabetes (p BI = 1.7×10^{-8} ; p SSBI = 2.8×10^{-3}), history of cardiovascular disease (p BI = 1.0×10^{-18} , p SSBI = 2.3×10^{-7}), and history of stroke (p BI = 1.3×10^{-73} ; p SSBI = 3.2×10^{-24}). The strongest associations were with white matter hyperintensity (WMH) burden (p BI = 1.43×10^{-157} ; p SSBI = 3.16×10^{-106}). GRS of BP measurements, but not other vascular risk factors, were significantly associated with BI and SSBI ($p < 0.0022$). Three loci reached genome wide significance for association with BI: FBN2 (log10 Bayesian factor [L10BF] = 6.52; $p = 1.77 \times 10^{-8}$), GRK6P1/ZDDHC20 (L10BF = 7.00; $p = 5.82 \times 10^{-9}$) and SV2B (L10BF = 5.25; $p = 3.36 \times 10^{-7}$). However, we failed to replicate associations of top GWAS loci in the smaller

follow-up samples and could not consistently demonstrate association of these loci with IS and pathologically defined BI. Despite the strong association, the overlap of genetic risk loci for BI and WMH burden was limited. **CONCLUSIONS:** This large trans-ethnic population-based meta-analysis showed significant association with BI and SSBI of WMH burden as well as of blood pressure, smoking, diabetes and history of cardiovascular disease and stroke, but not cholesterol levels or body mass index. Genetic risk loci for BP contribute to increased risk of BI and SSBI. GWAS meta-analysis identified novel genetic risk factors for BI that require validation in larger samples.

Study Supported By: NIH, Neurological Disorders and Stroke, NHMRC, CIHR, European national research institutions, Fondation Leducq.

Disclosure: None.

2016.011

Genetic influences on early neurological instability after ischemic stroke (GENISIS) Ibanez Laura, PhD^{1,*}; Heitsch Laura, MD^{1,*}; Israel Fernandez, PhD²; Caty Carrera²; Daniel Strbian, MD, PhD³; Joan Montaner, MD, PhD²; Carlos Cruchaga, PhD^{1,*}; Jin-Moo Lee, MD, PhD^{1,*}

*Contributed equally; #Shared senior authors; ¹Washington University School of Medicine, St. Louis; ²Hospital Universitari Vall d'Hebron, Barcelona, Spain; and ³University of Helsinki, Finland.

OBJECTIVE: A variety of mechanisms, including hemorrhagic transformation and recanalization, may impact Δ NIHSS24h, but little is known about genetic influences. **BACKGROUND:** Neurologic deficits can be highly unstable in the first hours after an acute ischemic stroke (AIS). Changes within the first 24 hours, measured by NIH stroke scale (NIHSS) and defined here as Δ NIHSS24h (NIHSSbaseline – NIHSS24 hours), substantially influences long-term outcome. **DESIGN/METHODS:** From 2008 to 2016, 1,097 tPA-treated AIS subjects were prospectively enrolled at 3 sites (St. Louis, Helsinki, and Barcelona). NIHSS scores were obtained within 6 hours and again at 24 hours after stroke onset. Genome-wide genotyping was generated for rare and common variants, imputing up to 6 million variants (SNPs). The quantitative trait, Δ NIHSS24h, which follows a normal distribution, was used to measure early improvement/deterioration. **RESULTS:** Genome-wide complex trait analysis (GCTA) indicates that common variants account for 23%–28% of Δ NIHSS24h variability while baseline NIHSS, glucose, age, and site account for only 13%. GWAS for the Finnish and African American (AFA) populations revealed 3 significant loci: in the AFA population, rs34928163 ($p = 3.62 \times 10^{-8}$) associated with Δ NIHSS24h. The direction of association for this variant in the other cohorts was the same. rs59101086, in the *SMAD2* gene region ($p = 3.91 \times 10^{-9}$) was also found associated with Δ NIHSS24h in the AFA population. *SMAD2* is a downstream mediator of TGF- β and has been linked to vascular development and health. *SMAD2* is also related to *FOX2*, which has been linked to small vessel and white matter disease. Finally, rs75697291 ($p = 8.64 \times 10^{-12}$), in *RAB9BP1*, was significantly associated in the Finnish population. This SNP was nominally significant in the Spanish population. **CONCLUSIONS:** In conclusion, GCTA analysis indicated a significant genetic influence on stroke outcome. Our analyses have identified several genome-wide associations and multiple suggestive signals that are cohort-dependent, and therefore will require replication.

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Disclosure: None.

2016.012

FOXF2, a novel risk factor for stroke and cerebral small vessel disease G. Chauhan¹; C.R. Arnold²; A.Y. Chu³; M. Fornage⁴; A. Reyah⁵; J.C. Bis⁶; A.S. Havulinna⁷; The Cervical Artery Dissection and Ischemic Stroke Patients (CADISP) study; The METASTROKE consortium; The Stroke Genetics Network (SiGN); The International Stroke Genetics Consortium (ISGC); O.J. Lehmann⁸; L.J. Launer⁹; M. Ikram¹⁰; P. Carlson¹¹; D.I. Chasman¹²; S.J. Child¹³; W.T. Longstreth, Jr.¹⁴; S. Seshadri¹⁵; S. Debette¹⁶; The Neurology Working Group of the CHARGE consortium

¹Université de Bordeaux, Centre Inserm U 1219, France; ²Alberta Children's Hospital Research Institute and Department of Biochemistry and Molecular Biology, University of Calgary, Canada; ³Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA; ⁴Institute of Molecular Medicine and Human Genetics Center, University of Texas Health Science Center at Houston; ⁵Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden; ⁶Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle; ⁷National Institute for Health and Welfare, Helsinki, Finland; ⁸Department of Ophthalmology, University of Alberta, Edmonton, Canada; ⁹IRP/NIA/NIH, Bethesda, MD; ¹⁰Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands; ¹¹Department of Epidemiology, University of Washington, Seattle; and ¹²Department of Neurology, Boston University School of Medicine, MA.

OBJECTIVE: To identify additional genetic risk factors for stroke by performing a GWAS of incident stroke in longitudinal prospective studies. **BACKGROUND:** Genetic determinants of stroke, the leading neurologic cause of death and disability, are poorly understood and have seldom been explored in the general population. **DESIGN/METHODS:** We performed a genome-wide screen for common genetic variants associated with incident stroke risk in 18 prospective population-based cohorts comprising 84,961 participants, of whom 4,348 experienced stroke. We followed-up variants with $p < 5 \times 10^{-6}$ in the largest available cross-sectional studies (70,804 participants of whom 19,816 experienced stroke). For genome-wide significant findings ($p < 5 \times 10^{-8}$), we explored associations with additional cerebrovascular phenotypes and undertook functional analyses in mouse and zebrafish mutants. **RESULTS:** We replicated 7 of 8 known loci for ischemic stroke and identified a novel locus at chr6p25 (rs12204590, near *FOXF2*) associated with risk of all stroke: odds ratio (OR) = 1.08 (95% CI: 1.05–1.12), $p = 1.48 \times 10^{-8}$ (minor allele frequency 21%). The rs12204590 stroke risk allele also increased MRI-defined white matter hyperintensity (WMH) burden, a marker of cerebral small artery disease, in stroke-free adults ($N = 21,079$; $p = 0.0025$). Consistently, young patients with segmental deletions of *FOXF2* exhibited extensive WMH burden. Conditional (inducible) deletion of *Foxf2* in adult mice resulted in cerebral infarction, reactive gliosis, and microhemorrhage. The zebrafish equivalents of *FOXF2* (orthologs) *foxf2b/foxf2a* were expressed in brain pericytes

and mutant *foxf2b^{-/-}* cerebral vessels showed decreased smooth muscle cell and pericyte coverage. **CONCLUSIONS:** In our study of 155,765 persons in total (24,164 with stroke), we identified common variants near *FOXF2* associated with increased stroke susceptibility. Extensive epidemiologic and experimental data suggest that *FOXF2* mediates this association, potentially via differentiation defects of cerebral vascular mural cells.

Study Supported By: NIH, Neurological Disorders and Stroke, NHMRC, CIHR, European national research institutions, Fondation Leducq.

Disclosure: None.

2016.013

Validation of a novel endophenotype to study the genetics of cerebral edema after hemispheric stroke Rajat Dhar, MD¹; Kristy Yuan, MD¹; Tobias Kulik, MD¹; Yasheng Chen, DSc¹; Laura Heitsch, MD²; Andria Ford, MD¹; Israel Fernandez, PhD³; Caty Carrera, MD³; Joan Montaner, MD³; Jin-Moo Lee, MD, PhD¹

¹Department of Neurology, ²Department of Emergency Medicine, Washington University in St. Louis School of Medicine; and ³Neurovascular Research Laboratory, Vall d'Hebron Research Institute, Barcelona, Spain.

OBJECTIVE: We will apply a novel quantitative biomarker of cerebral edema to evaluate the functional genetic architecture of this important complication of ischemic stroke.

BACKGROUND: Cerebral edema is the leading cause of death and deterioration in patients with hemispheric stroke. There remains significant unexplained heterogeneity in severity of cerebral edema, even amongst patients with similar stroke severity and infarct volume; genetic variations likely underlie heterogeneity in this highly regulated biologic process. Existing biomarkers such as midline shift (MLS) only delineate cerebral edema at its extremes and do not capture the spectrum of this process. **DESIGN/METHODS:** We validated a biomarker that accurately quantifies severity of cerebral edema by measuring change in CSF volume (Δ CSF) from baseline to follow-up CT. We developed an automated machine learning-based algorithm to measure Δ CSF rapidly for multiple scans. We will analyze CT scans at baseline and 24-hour (frequently available for many stroke patients) and integrate this measure of edema with genomic data from our multi-site acute stroke study. **RESULTS:** Δ CSF correlated well with MLS ($r = 0.75$, $p < 0.001$), measured in 20 patients at time of maximal edema. In 33 subjects with scans at baseline and approximately 24 hours, Δ CSF was over half that seen at peak edema and already 32% below baseline (even though MLS remained minimal). Δ CSF within ipsilateral sulci at this early time correlated with subsequent peak MLS ($r = 0.72$) and was greater in those who developed malignant edema (84% vs 47%, $p < 0.001$), even adjusting for NIHSS and infarct volume. Measurement of Δ CSF with the automated algorithm demonstrated strong correlation ($r = 0.89$) with manual delineation in scans from 2 centers. **CONCLUSIONS:** We are now ready to apply Δ CSF as a quantitative endophenotype for cerebral edema in a larger study. CT scans from multiple sites will be uploaded to our central archive for analysis of Δ CSF. We will model residual variability in Δ CSF (adjusting for age, NIHSS and infarct volume) against SNP data in GWAS. We will also evaluate heritability of cerebral edema using GCTA and role of candidate genes.

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Disclosure: None.

2016.014

Sex-specific genome-wide association study of ischemic stroke in the Stroke Genetics Network (SiGN) Myriam Fornage, PhD¹; Jordi Jimenez-Conde, PhD²; Steven J. Kittner, MD³; Hugh S. Markus, MD⁴; Tatjana Rundek, MD, PhD⁵; Sylvia Wassertheil-Smoller, PhD⁶; Stephen Williams, PhD⁷; Kathryn M. Rexrode, MD⁸; For the Stroke Genetics Network (SiGN) and the International Stroke Genetics Consortium (ISGC)

¹The University of Texas Health Science Center at Houston; ²IMIM Hospital del Mar Medical Research Institute, Barcelona, Spain; ³The University of Maryland Medical Center, Baltimore; ⁴University of Cambridge, London, UK; ⁵University of Miami Health System, FL; ⁶Albert Einstein Medical College, New York, NY; ⁷University of Virginia, Charlottesville; and ⁸Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

OBJECTIVE: We aimed to identify sex-specific genetic loci associated with ischemic stroke.

BACKGROUND: Stroke is the second leading cause of death and a major cause of severe disability worldwide. Experimental, epidemiologic, and clinical studies have consistently documented differences between men and women in stroke incidence, prevalence, outcomes, and risk factors. Although stroke heritability has been reported to differ by sex, the genetic variants that may underlie these sex disparities are unknown. **DESIGN/METHODS:** We performed a genome-wide association (GWA) analysis stratified on sex in 12,577 ischemic stroke cases (6,811 men, 5,766 women) and 25,643 stroke-free controls (12,179 men, 13,464 women)

2016.013: Validation of a novel endophenotype to study the genetics of cerebral edema after hemispheric stroke

from the Stroke Genetics Network (SiGN). Testing for heterogeneity of effects between the sexes was conducted using a 1-df χ^2 test. **RESULTS:** We identified a region on chromosome 19q13.2 harboring common SNPs (minor allele frequency (MAF) = 0.11 to 0.25) associated with all ischemic stroke in men (lead SNP $p = 7.4 \times 10^{-8}$; odds ratio [OR] = 0.81) but not in women ($p = 0.38$; OR = 1.03). A common variant (MAF = 0.19) on chromosome 10q22.2 showed evidence of heterogeneity by sex ($p_{\text{het}} = 7.7 \times 10^{-8}$) with sex-specific associations in opposite direction (OR_{men} = 0.86, $p = 4.4 \times 10^{-6}$; OR_{women} = 1.10, $p = 2.8 \times 10^{-3}$). Subtype-specific analyses also identified several sex-specific associations. **CONCLUSIONS:** In this sex-stratified GWA analysis, we identified several loci with sex-specific effects on ischemic stroke and its subtypes. The majority of these loci were in intergenic regions of the genome, suggesting that sex-specific effects on stroke may occur via mechanisms acting on gene regulation. Replications and extension of these findings is planned.

Study Supported By: NIH; National Institute of Neurological Disorders and Stroke.

Disclosure: None.

2016.015

Application of linear mixed models to genetic association studies in common disease *Sara L. Pulit*

Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands.

OBJECTIVE: We sought to explore the advantages of linear mixed models in genome-wide association studies. **BACKGROUND:** Linear mixed models (LMMs) are gaining in popularity in the field of complex trait genetics. Specifically, LMMs are increasingly being used in genome-wide association studies (GWAS), used to test common (minor allele frequency >5%) and low-frequency (minor allele frequency 1%–5%) variation for association to a trait or disease. Typically, GWAS are performed using logistic or linear regression models, which are then corrected for principal components and other fixed covariates meant to eliminate potential confounding. Linear mixed models include a genetic relationship matrix (GRM) that captures structure in the sample including that due to genetic ancestry, family data, or cryptic relatedness. The LMM estimates the contribution of the GRM to variance of the phenotype using a random effects model; fixed effects (like principal components) can additionally be included. Linear mixed models come with the added benefit of increased power and reduced chance of false-positive associations compared to linear and logistic regression. **DESIGN/METHODS:** In a recent genome-wide association study of amyotrophic lateral sclerosis, an LMM was used to account for the structure in the study design, including samples drawn from all over Europe (and thus containing population structure), samples genotyped on various SNP arrays, and unbalanced case-control strata. **RESULTS:** A first analysis of the data, performed with logistic regression and meta-analysis, revealed a total of 4 loci at genome-wide significance. A second analysis using an LMM to correct for stratification in the data revealed a total of 7 loci at genome-wide significance, 6 of which replicated in additional genotyping of independent samples. An 8th locus was also revealed by the LMM at near genome-wide significance ($p = 6 \times 10^{-8}$) that had previously been identified through exome sequencing in ALS. **CONCLUSIONS:** Many genetic datasets in stroke are similarly complex in structure; datasets are often comprised of separately-genotyped cases and controls, samples drawn from multiple populations, and family data or cryptic relatedness. Employing LMMs in future genetic association studies in stroke may help improve power, reduce confounding, and reveal novel loci that increase risk of disease.

Study Supported By: Not applicable.

Disclosure: None.

2016.016

Identifying ischemic stroke risk loci through refinement and expansion of phenotypic definitions *Sara L. Pulit¹; Brad B. Worrall²*; On behalf of the Stroke Genetics Network (SiGN)

¹Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands; and ²Department of Neurology, University of Virginia.

OBJECTIVE: We sought to identify new stroke risk loci by examining phenotypes constructed from the union and intersect of the TOAST and CCS subtyping systems. **BACKGROUND:** Approximately 15 million people have a stroke each year worldwide, and ~80% of these strokes are ischemic, caused by the blockage of an artery leading to the brain. A small number of risk factors, including age, smoking, and hypertension, have been identified. Further, a small number of common genetic variants have been identified as increasing risk of stroke. Importantly, each of these variants have been discovered through analysis of specific stroke subtypes, rather than in analyses of all stroke. Two methods currently exist for subtyping an ischemic stroke: TOAST, originally implemented for diagnosis of stroke in a clinical setting; and CCS, a computerized system that allows for input of clinical observations and data, including imaging. Both CCS and TOAST produce the canonical stroke subtypes—cardioembolic, large artery atherosclerosis, and small artery occlusion—but rely on different input parameters to perform subtyping. **DESIGN/METHODS:** In an effort to evaluate if additional stroke loci can be discovered through refinement or expansion of phenotypic definitions (and building off of previous observations that genetic findings from CCS and TOAST are highly consistent), we performed genome-wide association studies (GWAS) in ~45,000 cases and controls collected by the Stroke Genetics Network (SiGN); all cases in SiGN had a CCS subtype available, while 75% of cases had additionally been subtyped with TOAST. “Intersect” phenotypes were defined using the intersection of TOAST and CCS subtypes (i.e., a case was considered to be of the cardioembolic subtype if both CCS and TOAST had produced this subtype), to test the benefits of phenotypic refinement. Additionally, “union” phenotypes were defined (e.g., a case was considered to be of the cardioembolic subtype if either CCS or TOAST had produced this subtype) to test the benefits of more liberally phenotyping samples. Samples had previously undergone quality control and imputation with sequencing-based reference panels. **RESULTS:** GWAS revealed several novel loci at genome-wide significance in both the union and intersect phenotypes. There appear to be stroke loci

that are robust to liberal inclusion of stroke cases that appear in the “union” phenotype analyses or in both “union” and “intersect” analyses. Other loci are revealed specifically through refinement of phenotyping. **CONCLUSIONS:** We plan to follow up loci in large independent samples made available through the MEGASTROKE effort. This study and its conclusions can potentially inform future genetic efforts in stroke, as they demonstrate the benefits of both liberal sample inclusion (to improve power) and refined phenotype (to reduce phenotypic noise).

Study Supported By: The Stroke Genetics Network (SiGN).

Disclosure: None.

2016.017

Stroke age of onset in the Stroke Genetics Network (SiGN) *Mark Dings^{1,2}; Sara L. Pulit²*; On behalf of the Stroke Genetics Network (SiGN)

¹Center for Molecular Medicine, Department of Genetics, and ²Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands.

OBJECTIVE: The aim of our study is to identify common genetic variants that associate to age of stroke onset. **BACKGROUND:** Stroke is one of the leading causes of death and long-term disability in the world. Approximately 80% of all strokes are ischemic strokes, caused by obstruction of an artery to the brain. A small number of risk factors have been identified, including atrial fibrillation, diabetes mellitus, and hypertension. Additionally, age is an important risk factor for stroke, as risk of stroke rises with age and most strokes occur later in life. A small number of genomic loci, including the recently identified 10q25 locus, have been shown to be associated with age of stroke onset. However, little is known about the full etiology of stroke and of genetic variants that may increase risk of early-onset stroke. **DESIGN/METHODS:** Thus, to identify loci harboring common single nucleotide polymorphisms (SNPs) that associate with age of stroke onset, we performed a genome-wide association study in 13,390 cases collected by the Stroke Genetics Network (SiGN). Cases had previously undergone quality control and imputation with sequencing-based imputation reference panels (described elsewhere). We performed logistic regression in 10 strata of European- and African-ancestry cases, adjusting for principal components and sex. The summary-level results were then combined in an inverse-variance weighted fixed effects meta-analysis. We tested all cases together, as well as stratified into stroke subtype (cardioembolic, large artery atherosclerosis, small artery occlusion, and undetermined) as determined by both the CCS and TOAST classification systems. **RESULTS:** We discovered a locus at genome-wide significance ($p < 5 \times 10^{-8}$) associated to age at onset in small artery occlusion (TOAST) and 6 other loci across the various subtypes worthy of follow-up in additional samples. Analyses in larger independent samples will be necessary to evaluate the robustness of these associations. **CONCLUSIONS:** We have identified several common-variant loci possibly associated to age of stroke onset. Genome-wide associated loci from such an analysis help pinpoint regions of the genome that harbor key risk variants and can yield insights into disease biology and pathogenesis.

Study Supported By: The Stroke Genetics Network (SiGN).

Disclosure: None.

2016.018

Epidemiological study for stroke and dementia in a Japanese community: The Hisayama Study *Toshiharu Ninomiya¹; Jun Hata¹; Yutaka Kiyohara²; Michiaki Kubo²*

¹Kyushu University; and ²RIKEN Center for Integrative Medical Sciences.

OBJECTIVE: To identify susceptible genes of ischemic stroke in Japanese. **BACKGROUND:** Ischemic stroke is a major cause of death and disability and occurs owing to a combination of multiple environmental and genetic risk factors. However, genetic components of common forms of ischemic stroke are largely unknown. **DESIGN/METHODS:** The Hisayama Study is an ongoing, population-based prospective cohort study of cardiovascular disease since 1961 in the town of Hisayama, which is a suburb in the Fukuoka metropolitan area on Kyushu Island, Japan. To identify susceptible genes of ischemic stroke, we performed a large case-control association study and a replication study in a total of 1,112 cases with ischemic stroke and 1,112 age- and sex-matched healthy controls from the residents of the town of Hisayama, Japan, using 52,608 single nucleotide polymorphism (SNP) markers. Furthermore, we investigate the influence of detected SNPs on the development of ischemic stroke, using 14-year follow-up data of a prospective cohort study conducted in 1,683 residents of the town aged 40 years or older. Genomic DNA was extracted from peripheral blood leukocytes, using standard protocols. SNPs were genotyped by using the multiplex PCR-based Invader assay or by direct sequencing of PCR products using ABI3700 capillary sequencers. **RESULTS:** We identified 3 novel candidate genes for ischemic stroke: PRKCH (protein kinase C eta [PKC η]), AGTRL1 (apelin receptor), and ARHGEF10 (Rho guanine nucleotide exchange factor 10). In the functional analyses, we found that PKC η was expressed mainly in vascular endothelial cells and foamy macrophages in human atherosclerotic lesions, and that the kinase activity of PKC η was modified by a nonsynonymous SNP in PRKCH (rs2230500). We also clarified that functional SNPs in AGTRL1 (rs9943582) and ARHGEF10 (rs4376531) affected transcriptional activities owing to the different Sp1-binding affinities. In a 14-year follow-up cohort study of Hisayama residents, all of these SNPs were significantly associated with the development of ischemic stroke. **CONCLUSIONS:** We identified 3 susceptible genes of ischemic stroke in Japan. These findings might contribute to a better understanding of ischemic stroke in the future.

2016.019

Mayo Clinic Florida Cerebrovascular Diseases Registry *Emily S. Edwards, MS¹; Ronald L. Walton, BS²; Dale M. Gamble, MHS¹; Sotheir H. Luke, MPH¹; Owen A. Ross, PhD²; James F. Meschia, MD³*

Table. Affected participants in the ongoing Mayo Clinic Florida Familial CV Registry displayed in descending order of frequency as of April 28, 2016.			
Diagnosis	Female	Male	Grand Total
Definite Ischemic Stroke	121	165	286
Aneurysmal SAH	57	25	82
Unruptured aneurysm	51	13	64
Intracerebral Hemorrhage	22	21	43
Perimesencephalic Subarachnoid Hemorrhage	3	13	16
Moyamoya	12	3	15
Carotid Arterial Dissection	7	7	14
Cavernous Malformation	6	8	14
Transient Ischemic Attack	11	2	13
Arteriovenous Malformation	4	9	13
Vertebral Arterial Dissection	6	4	10
Carotid Stenosis	4	6	10
Probable Ischemic Stroke	5	1	6
Fibromuscular Dysplasia	5		5
Cerebral Venous Thrombosis	1	2	3
Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)	1	1	2
Anoxic Brain Injury		1	1
Angio-Negative SAH		1	1
Intraventricular Hemorrhage		1	1
Leukoaraiosis	1		1
Grand Total	317	283	600

2016.019: Mayo Clinic Florida Cerebrovascular Diseases Registry

¹Neuroscience Research Center, Departments of ²Neuroscience and ³Neurology, Mayo Clinic, Jacksonville, FL. **OBJECTIVE:** To establish a registry and collect DNA and detailed phenotypic information on patients with symptomatic cerebrovascular disease and assemble a broad resource of patients. **BACKGROUND:** Stroke is associated with significant morbidity and mortality. The Global Burden of Disease Study ranked cerebrovascular disease as the second leading cause of death. Standardizing phenotypic data collection and identifying genetic variants that determine risk for cerebrovascular diseases will offer new insight into the development of these conditions and may provide personalized treatment for these diseases. **DESIGN/METHODS:** The registry classifies patients by one or more of 23 cerebrovascular conditions, and includes unaffected family members and healthy controls. Each category has strict requirements for evidence of the disease based on imaging, laboratory results, or diagnostic guidelines. Participants undergo a structured intake assessment of demographic variables, stroke risk factors, stroke symptoms, neurologic impairment, mental status, and functional status pre- and post-symptom onset. A detailed family history is obtained and family members are encouraged to participate. **RESULTS:** We have collected a series of 600 cerebrovascular patients with a broad range of phenotypes (table). Genetic screening and positive test results provide important insight in clinical, genetic, and neuropathologic correlations. Such results help define the frequency, penetrance, and thus pathogenicity of genetic variants. Positive findings will be reported, negative results will prioritize pedigrees for further study. **CONCLUSIONS:** In conclusion, we have established a diverse collection of patients with cerebrovascular phenotypes through the Mayo Clinic hospital. **Study Supported By:** The Mayo Clinic Foundation, James and Esther King Foundation, Florida State, American Heart Association, and the Myron and Jane Hanley Award for Stroke Research. **Disclosure:** O.A. Ross is a member of the editorial board of *PLoS ONE*, *Molecular Neurodegeneration*, and *Parkinsonism and Related Disorders*, and is funded by NIH grants NS078086 and NS072187.

2016.020
Into thin air: Translating murine “collateral gene” variants to humans James E. Faber, PhD; Robert Sealock, PhD; Hua Zhang, MD; Jennifer L. Lucitti, PhD; Brian K. Buckley, BS

Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill. **OBJECTIVE:** Our objective was to review evidence that gene variants may contribute to the wide range in pial (leptomeningeal) collateral flow in patients with AIS. **BACKGROUND:** Studies in 25 inbred strains of mice have shown that extent (number and diameter) of pial collaterals varies by ~50 fold, and that infarct volume after permanent MCAO evidences a 30-fold variation that closely follows these strains (Sealock et al., 2014). Polymorphisms in a novel gene, *Rabep2*, have recently been shown to be responsible for 80% of this variation. Three additional QTL have been identified in mice, and gene targeting studies have identified several other genes in the collaterogenesis pathway. Future studies planned in mice may identify additional candidate genes. **DESIGN/METHODS:** Since the pathway that *Rabep2* drives to control formation of collaterals during embryogenesis (i.e., collaterogenesis) is likely well-conserved among vertebrates, polymorphisms at human *RABEP2* and/or other “collateral genes” may contribute to variation in collateral extent in humans. **RESULTS:** Studies are underway to test for association between collateral status and polymorphisms at *RABEP2* and other candidate collateral genes in AIS patients: GENetic Determinants of Collateral Status in Stroke (GENEDCSS) is a prospective multicenter study begun in 2015, with collateral score as primary endpoint. Likewise, lookup analysis began in 2016 in METASTROKE, CHARGE, SIGN and DeltaNIH, with MRI-GENIE and GISCOME to follow. Studies in mice have shown that aging and other cardiovascular risk factors cause a decline in collateral number and diameter (collateral rarefaction). While these effects are smaller than those due to differences in genetic background, they support adjusting for presence of risk factors in human studies. **CONCLUSIONS:** The above prospective and retrospective studies may identify polymorphisms linked to the wide variability in collaterals in humans. Such findings may lead to a diagnostic biomarker capable of distinguishing subjects with sparse vs abundant collaterals when they are healthy, before stroke, and for use on acute stroke triage. These results could also inform future research aimed at identifying strategies to prevent collateral rarefaction in individuals without stroke, in particular those with cardiovascular risk factors. **Study Supported By:** NIH: NHLBI HL111070, Neurological Disorders and Stroke NS083633, NCTraCS 009P1. **Disclosure:** None.

2016.021
Acute endophenotypes group update Jin-Moo Lee¹; Carlos Cru-
 chaga¹; Laura Heitsch¹; Raj Dhar¹; Steve Kittner²; Stephanie Debette³; Daniel Strbian⁴; Turgut
 Talisumak⁵; Joanna Pera⁶; Agnieszka Slowik⁷; Ganesha Chauhan⁸; Chris Levi⁹; Tatjana Run-
 dek¹⁰; Joan Montaner¹¹; Caty Carrera¹²; James F. Meschia¹³; Jordi Jiménez-Conde¹⁴; Jaume
 Roquer¹⁵; Cristina Gallego-Fabrega¹¹; Elena Muñio¹¹; Natalia Culell¹¹; Jane Maguire^{12,13}; Israel
 Fernandez-Cadenas¹¹

¹Department of Neurology, Washington University in St. Louis; ²Department of Neurology, Baltimore Veterans Administration Medical Center, and University of Maryland School of Medicine; ³Bordeaux University Hospital, Bordeaux Population Health Inserm Center U1219, France; ⁴Department of Neurology Helsinki University Central Hospital, Finland; ⁵Department of Neurology, Jagiellonian University Medical College, Poland; ⁶School of Medicine and Public Health, University of Newcastle, Australia; ⁷Department of Neurology, Miller School of Medicine, University of Miami; ⁸Neurovascular Research Laboratory, Vall d'Hebron Institute of Research, Universitat Autònoma de Barcelona; ⁹Department of Neurology, Mayo Clinic, FL; ¹⁰Department of Neurology, Institut Hospital del Mar d'Investigació Mèdica (IMIM); Universitat Autònoma de Barcelona, Spain; ¹¹Stroke Pharmacogenomics and Genetics, Fundació Docència i Recerca MutuaTERRASSA, Spain; ¹²School of Nursing and Midwifery, Faculty of Health and Medicine, University of Newcastle, Callaghan, Australia; and ¹³Hunter Medical Research Institute, Newcastle, Lambton Heights, Australia.

OBJECTIVE: The objective of this International Stroke Genetics Consortium working group that involves 11 institutions is to join researchers that are interested in the acute phase of stroke. Currently, this Group has 7 projects underway, including Genome-wide Association (GWA), Epigenome Wide Association, neuroimaging and plasma biomarkers studies. **BACKGROUND:** The Acute endophenotypes working group of the International Stroke Genetics Consortium involves 11 institutions that are interested in the analysis of the genetic risk factors associated with acute endophenotypes of stroke, such as neurologic worsening during the first 24 hours after stroke, hemorrhagic transformation after rt-PA treatment or infections post-stroke. **DESIGN/METHODS:** We have revised and updated the different ongoing projects on our acute endophenotype working group. **RESULTS:** The objective of the Hemorrhagic Transformation (HT) project is to find polymorphisms associated with HT after tPA treatment of acute ischemic stroke. Two loci ($p < 10^{-8}$) have been found associated with HT in a cohort of 1,300 ischemic stroke patients with GWAs data. Currently, the project is in the replication phase. An estimated number of 1,300 new patients treated with tPA and 1,100 patients not treated with tPA with HT data will be analyzed in this replication phase. The GENESIS project (Genetics of Neurologic Instability following Ischemic Stroke) aims to determine genetic variants associated with neurologic outcomes within the first 24 hours after stroke onset. Currently 1,500 participants have been genotyped and analyzed, using a novel endophenotype, termed Δ NIHSS24hrs (NIHSS obtained at 24 hours minus first measured NIHSS <6 hours after stroke onset). We will report on the first GWAS analysis on this early cohort. The ultimate goal of the study will be to enroll 7,000 participants for further discovery and replication. Other endophenotypes will be analyzed including initial stroke severity, incident edema (using serial CT scans), and acute laboratory values. The TOTO project (Targeting Optimal Thrombolysis Outcomes) is a prospective cohort study currently recruiting tPA treated patients from Stroke Centers in Australia. This project aims to recruit 1,000 consecutive stroke patients treated with tPA and then to analyze the role of plasma, genetic and neuroimaging biomarkers in order to develop a predictive model for those patients who benefit most and least when given fibrinolytic therapy. **CONCLUSIONS:** Genomic studies of acute endophenotypes promise to reveal novel variants, genes, and pathways involved in early neurologic deterioration and/or improvement after ischemic stroke. These genetic associations may yield novel therapeutic targets and diagnostic tests to guide acute therapeutic decisions.

Study Supported By: NIH, Spanish national grants (ISCIII) and Australian national grants. **Disclosure:** None.

2016.022

Utility of algorithm-based ischemic stroke case ascertainment in electronic health records (EHR)

Johanna W. Bunschoten^{1,3}; Chia-Ling Phuah, MD^{2,3,5}; Alison M. Ayres, BS⁴; Alessandro Biffi, MD⁵⁻⁷; Jonathan Rosand, MD, MS²⁻⁵; Christopher D. Anderson, MD, MMS²⁻⁵

¹Leiden University Medical Center, the Netherlands; ²Division of Neurocritical Care and Emergency Neurology; ³Center for Human Genetic Research; ⁴The J. Philip Kistler Stroke Research Center, Massachusetts General Hospital, Boston; ⁵Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; ⁶Division of Behavioral Neurology and ⁷Division of Psychiatry, Department of Psychiatry, Massachusetts General Hospital, Boston.

OBJECTIVE: To develop and validate an algorithm for accurate phenotyping of ischemic stroke cases in EHR. **BACKGROUND:** Rapid development of both high-throughput genotyping and electronic health records (EHR) provide an unprecedented opportunity to use health-related databases to accelerate medical genetic research. However, precise extraction of detailed phenotypic information from within the morass of EHR data remains challenging. Current ascertainment of stroke cases is resource-intensive, relying heavily on manual chart review for case validation. **DESIGN/METHODS:** We utilized a hospital-based clinical research database to evaluate our query algorithm, based on all ICD-9 discharge diagnosis code(s) for ischemic stroke and excluding intracerebral hemorrhage or TIA. We restricted our search to all inpatient and ER encounters within a 12-month period (January 1, 2009 and December 31, 2009) in single, large tertiary hospital to determine performance characteristics. Ischemic stroke case validation was performed by manual review of medical record data as gold standard. A neurologist adjudicated the final diagnosis in complex cases. **RESULTS:** Applying our algorithm, we abstracted 2,442 ischemic stroke cases out of 105,403 available records. Abstracted cases were predominantly elderly (mean age 71.9 years), White (80.2%), and male (52.9%). Manual review of medical record data validated 1,741 of the abstracted cases as ischemic stroke, yielding a positive predictive value (PPV) of 71.3% (95% CI: 69.5%–73.1%). Major diagnosis misclassifications included intracerebral hemorrhage (28.4%), TIA (15.1%) and tumor (6.0%). **CONCLUSIONS:** In our stroke-accredited tertiary care hospital database, our discharge diagnosis code-based algorithm for ischemic stroke case ascertainment has a PPV of 71.3%. Major limitations include case misclassification and coding inaccuracy. A broader set of search variables beyond ICD-9 diagnoses is needed to improve the performance of algorithm-based stroke case ascertainment in EHR.

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2016.023

Asymptomatic carotid stenosis is associated with gray and white matter damage

Wagner M. Avelar, PhD¹; Anelyssa D'Abreu, PhD¹; Ana C. Coan, PhD¹; Fabrício Oliveira Lima, PhD¹; Rachel Guimarães, PhD¹; Clarissa L. Massuda, PhD¹; Germano P. Oliveira, MD²; Ana T. Guillaumon, PhD²; Augusto A. Filho MD³; Li L. Min, PhD¹; Fernando Cendes, PhD¹

¹Neuroimaging Laboratory, Department of Neurology and the Brazilian Institute of Neuroscience and Neurotechnology (BRANN); ²Department of Surgery and ³Department of Radiology, School of Medical Sciences, University of Campinas-UNICAMP, SP, Brazil.

OBJECTIVE: To assess the white and gray matter changes in patients with asymptomatic carotid stenosis/occlusion. **BACKGROUND:** The prevalence of asymptomatic carotid stenosis (>50%) increases with age, affecting 10% of men 80 years or older. To date, there is still no consensus of whether clinical, angioplasty or endarterectomy is the best therapeutic option. In earlier studies, endarterectomy showed a benefit in reducing the risk of ischemic events in 5 years. The advances in medical treatment though, decreased the absolute risk of stroke to 1% per year and surgical treatment was put in check, since both angioplasty and endarterectomy carry a stroke risk of 2.5 and 1.4% respectively, as shown in the CREST study. Therefore, the indiscriminate use of the procedure does not seem justified in this group of patients and it should only be recommended in selected patients based on age, risk factors, life expectancy and the risks associated with the procedure. However, recent studies have demonstrated cognitive decline in patients with asymptomatic carotid stenosis >70%. We hypothesize that there is damage in brain gray matter in patients with asymptomatic stenosis. **DESIGN/METHODS:** We studied the white and grey matter changes in 25 patients with asymptomatic carotid stenosis/occlusion. The stenosis/occlusion was defined by angiography and all patients underwent brain MRI. Images were analyzed by the method of voxel-based morphometry (VBM) and through diffusion tensor images (DTI). Our control group involved 25 healthy subjects, matched for gender and age. **RESULTS:** We found that both DTI and VBM showed significant abnormalities of white matter compared to controls, and these findings were diffuse and symmetrical in both hemispheres (ipsilateral and contralateral stenosis). Unlike DTI, the VBM analysis also showed gray matter atrophy predominantly in areas corresponding to the anterior circulation in the hemisphere ipsilateral to the stenosis. **CONCLUSIONS:** In Conclusion, our findings suggest that the stenosis/occlusion is associated with asymptomatic alterations (atrophy) of the gray matter in the hemisphere ipsilateral to the stenosis. The role of asymptomatic carotid stenosis in the gray matter damage must be further investigated with longitudinal studies and comparison with neuropsychological evaluation.

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2016.024

Evaluation of brain gray matter in patients with atrial fibrillation

Danilo Santos, MD; Bruno Rigon, MD; Marilise Katsurayama, MD; Fernando Cendes, PhD; Wagner M. Avelar, PhD; Ana C. Coan, PhD

Department of Neurology, FCM, University of Campinas—UNICAMP.

OBJECTIVE: To assess structural gray matter changes in patients with Atrial Fibrillation (AF). **BACKGROUND:** AF is highly prevalent and due to demographic changes it is expected to increase in frequency in the next future. Hemodynamic changes which are common in AF could induce damage to brain tissue leading to cortical atrophy. Indeed, cortical atrophy has been documented in patients with several chronic cardiovascular disorders. However, the assessment of changes in brain gray matter caused by AF is still a matter of debate. Few studies have focused on the analysis of cortical structures using MRI and voxel based morphometric techniques (VBM). **DESIGN/METHODS:** We acquired 3 T MRI images in 10 patients with AF. None of them had any history of neurologic symptoms and neurologic exam was normal. In addition, they were specifically screened to exclude neurologic deficits (CHF), severe heart failure, B12 deficiency, hypothyroidism, chronic alcohol abuse, head trauma and degenerative neurologic diseases. MRIs were evaluated to exclude ischemic lesions and images were compared to those obtained from 30 healthy controls matched for gender and age (± 5 years). Analysis of gray matter changes was performed by VBM technique with the SPM 8 program using a preprocessing protocol which includes standardization for NMI-124 space, targeting gray matter, modulation, and smoothing. Gray matter maps obtained from patients were compared to controls (t test 2 samples; $p < 0.001$, lower limit of 20 grouped voxels). **RESULTS:** We found gray matter atrophy in patients with AF which was mainly concentrated in the occipital pole. **CONCLUSIONS:** In conclusion, we found structural changes in patients with AF. This results may be related to the hemodynamic changes present in these patients, including the occurrence of microembolism. Indeed, similar results were found in patients with CHF. However, the identification of specific mechanisms underlying these abnormalities in patients with AF, without past stroke, still requires further investigation. Here we demonstrate that imaging studies using VBM can contribute to characterize brain structural changes in patients with AF.

Disclosure: None.

2016.025

Copy number variations in intracerebral hemorrhage

Amanda Donatti¹; Rodrigo Seolini¹; Leslie E. Ferreira²; Miriam Maccarrone³; Fábio R. Torres⁴; Paulo H. C. França⁵; Vivian Nagef⁶; Daniel S. Woo⁷; Michelangelo Mancuso⁸; Norberto L. Cabral⁹; Benilton S. Carvalho⁹; Iscia Lopes-Cendes¹

¹Department of Medical Genetics, School of Medical Sciences, University of Campinas—UNICAMP; and the Brazilian Institute of Neuroscience and Neurotechnology (BRANN), Campinas, SP, Brazil; ²Department of Medicine, Regional University of Joinville—UNIVILLE, Joinville Stroke Registry, SC, Brazil; ³Department of Experimental and Clinical Medicine, Neurologic Institute, University of Pisa, Italy; ⁴Department of Neurology and Rehabilitation Medicine, College of Medicine, University of Cincinnati, OH; and ⁵Department of Statistics, Institute of Mathematics, Statistics and Scientific Computing, University of Campinas—UNICAMP, SP, Brazil.

OBJECTIVE: This is an update of a collaborative study which has been conducted over the past 2 years aiming to evaluate the role of copy number variations (CNVs) in patients with intracerebral hemorrhage (ICH). **BACKGROUND:** CNVs are common structural mutations, which have been identified as potentially leading to changes in gene expression. We have previously identified CNVs which are exclusively present in patients with ICH from Brazil and Italy. In the present study we incorporate the preliminary findings of a large cohort from the USA. **DESIGN/METHODS:** Initially, we used DNA samples from 45 patients with ICH and 41 controls from the Univille Stroke Biobank, in Joinville, Brazil. These were subsequently combined with the results obtained in a group of 18 Italian patients and 30 controls. These samples were analyzed using the Genome-Wide Human SNP Array 6.0; Affymetrix Inc. We validated these results using an additional sample of 800 patients with ICH and 542 controls from the USA. Samples from the USA were divided into 2 cohorts: MGH and GER. The MGH cohort has 561 patients with ICH and 504 controls, while the GER cohort has 239 patients with ICH and 504 controls. Data from these samples were sent to our laboratory for analysis. We preprocessed the raw microarray data using the *crmm* and *aroma.affymetrix* package to obtain statistics that would be used later for copy number analysis. The signal-to-noise ratio (SNR) estimated via *crmm* was used as the sample-specific quality metric and SNR >5 was required to include samples in this analysis. We used the *flashpcaR* and *mahalanobisDist* software packages to perform principal component analysis (PCA) and assess the genetic structure of the Brazilian and Italian cohorts. The Genome Alteration Detection Algorithm (GADA) was used to determine the relative copy number for each patient compared to their respective control group. For each individual, we bootstrapped the segment means and estimated robustly their distribution. Segments with means outside the 95% confidence interval built from the aforementioned bootstrap strategy were called significant. We analyzed biological pathways containing genes with CNVs using the METACORE Software. Genes selected by this software were further analyzed at DGV and DECIPHER databases. **RESULTS:** The PCA showed that Brazilian and Italian patients present similar genetic structure ($p = 0.1639$). We identified 384 CNVs present in Brazilian patients. These CNVs were located in 19 different genes potentially related to mechanisms underlying ICH, such as angiogenesis, coagulation, immunology, tissue development and lipid metabolism process. According to DGV database, 4 of these genes (*CDH13*, *FNTA*, *MACROD2*, and *NAALADL2*) had no common CNVs reported in the normal population. In addition, 8 of these genes (*CYP2E1*, *DEFB4B*, *MACROD2*, *NOMO1*, *NSF*, *PRODH*, *RAF1*, and *RB1CC1*) have been described with relation to cardiovascular and neurologic processes. There were 2 CNVs in Italian patients that were also present in Brazilian patients: *MACROD2* and *NSF*. In the USA cohort we identified 16,000 CNVs across all patients. We observed an average of 20 significant alterations (FDR <0.05) per patient in the USA cohort and these alterations were detected, in average, by a set of 50 probes. A preliminary analysis showed that the CNV located in *NSF* is also found in patients of the USA cohort. **CONCLUSIONS:** We describe here patients with ICH and rare CNVs present in genes related to vascular process, coagulation, hypertension and lipid metabolisms. These CNVs were not detected in age-matched control individuals and many of them had never been described in databases of normal individuals of different

ethnic origins. Furthermore, we have demonstrated for the first time that a CNV in *NSF* may influence the risk for ICH. However, additional studies are necessary to definitively link the CNVs found in this study and increased risk of ICH.

Study Supported By: FAPESP, SP, Brazil.

Disclosure: Nothing to disclose.

2016.026

Selection of families with possible monogenic stroke for next generation sequencing

Andreea Ilinca, MD¹; Ulf Kristofferson, MD, PhD²; Maria Soller, MD, PhD²; Arne G. Lindgren, MD, PhD¹

¹Lund University, Skåne University Hospital, Department of Clinical Sciences, Lund, Neurology; and

²Department of Clinical Genetics, Lund University and Regional Laboratories, Region Skåne, Sweden.

OBJECTIVE: We present an algorithm for the systematic selection of families who appear useful for exome sequencing aimed at identifying known or novel monogenic causes of stroke. **BACKGROUND:** Lund Stroke Register is a hospital-based register that consecutively includes all patients (probands) with a first-ever stroke episode from the uptake area of Skåne University Hospital in Lund, Sweden. Our previous study showed that familial clustering of stroke occurred in the families of a subset of probands with ischemic stroke (IS) under age 56 in this population. **DESIGN/METHODS:** We developed and applied selection criteria based on the probability and feasibility to identify a known or novel monogenic cause of the underlying disorder. Affected family members' (FM) clinical features were considered. **RESULTS:** Based on information gathered at study inclusion, we selected probands who had their first-ever stroke before age 56, who had reported a clustering of stroke in their families that was compatible with monogenic inheritance. We excluded probands whom we were unable to contact at the moment of the present study (figure). We generally excluded families where less than 2 FM with stroke were still alive, less than 2 FM agreed to participate or where a known monogenic cause for stroke had been identified. Two additional families were selected because of unusual clinical traits or radiologic features considered highly suggestive of an underlying monogenic cause. Out of 4,467 included in LSR between 2004 and 2015, 20 families with IS of unknown cause were selected. **CONCLUSIONS:** The 20 families selected are now examined by exome sequencing to exclude known monogenic forms of stroke, and to select families with no identified mutation in the probands for further genetic investigation.

Disclosure: The authors report no disclosures.

* as defined in Familial aggregation of stroke amongst young patients in Lund Stroke Register Ilinca A, Kristofferson U, Soller M, Lindgren AG. Eur J Neurol. 2016 Feb;23(2):401-7.

2016.026: Selection of families with possible monogenic stroke for next generation sequencing

2016.027

GODS project: Genetic contribution to functional outcome and disability after stroke. A genome wide association meta-analysis and exome sequencing analysis study

Jiménez-Conde Jordi, MD, PhD¹; Mola-Caminal Marina, MSc^{1,2}; Soriano-Tárraga Carolina, PhD¹; Giralte-Sieinhauer Eva, MD, PhD¹; Rabionet Raquel, PhD¹; Carrera Caty, MSc¹; Díaz-Navarro Rosa Maria, MD³; Fernández-Cadenas Israel, PhD⁴; Krupinski Jurek, MD, PhD⁵; Estivill Xavier, MD, PhD⁶; Vives-Bauzá C, PhD⁷; Roquer Jaume, MD, PhD⁸; On behalf of Genestroke Consortium

¹Department of Neurology, Neurovascular Research Group, IMIM-Hospital del Mar (Hospital del Mar Medical Research Institute), Universitat Autònoma de Barcelona/DCEXS-Universitat Pompeu Fabra; ²Universitat de Barcelona, Spain; ³Centre for Genomic Regulation (CRG), Barcelona; ⁴Neurovascular research laboratory, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona; ⁵Neurology Department, Son Espases Hospital, Palma de Mallorca; ⁶Stroke Pharmacogenomics and Genetics, Fundació Docència i Recerca Mútua Terrassa, Barcelona; ⁷Neurology Unit, Neuroscience Department, Mútua de Terrassa Hospital, Barcelona; and ⁸Laboratori de Neurociències, Fundació d'Investigació Sanitària de les Illes Balears (FISIB), Palma de Mallorca, Spain.

OBJECTIVE: Our aim was to identify genetic variants and genes influencing outcome after stroke. **BACKGROUND:** Variability in functional outcome after ischemic stroke can be influenced by many factors. Irrespective of clinical factors such as age, stroke subtype, vascular stenosis, location of the injury and size of the affected area, inter-individual variation in neuronal recovery is considerable. The individual genetic load may be responsible of this variability in recovery processes. **DESIGN/METHODS:** We conducted a meta-analysis of 4 different GWAs of Spanish ischemic stroke cases from 5 participating centers. After pruning individuals for inclusion criteria and quality controls, excluding patients with lacunar strokes, medical complications or previous functional impairment, we included 1,193 anterior territorial ischemic stroke individuals with precise phenotyping. Vascular risk factors, severity of stroke (NIHSS), and functional status at 3 months after stroke (mRS-3m) were recorded. Association analyses were performed using SNPTEST v2.5.2 and meta-analyzed with METAL. Data were adjusted for NIHSS at discharge, hypertension, age at stroke onset, gender and principal components. Imputation with 1K genomes was done using IMPUTE2. A convergence analysis with exome sequencing analysis from 81 individuals comparing cases with poor and favourable outcome at 3 months after ischemic stroke was also performed. Those variants (MAF > 1%) most strongly associated with functional outcome after ischemic stroke were selected for replication, prioritizing regions showing results consistent between GWAs and exome sequencing data. **RESULTS:** We identified 400 variants associated with mRS-3m at a level of p value $< 1e^{-5}$. The most striking associations were: rs76221407 ($p = 3.8e^{-8}$), rs182008837 ($p = 4.3e^{-8}$) and rs150862264 ($p = 5.2e^{-8}$) in INADL gene. And rs147062844 ($p = 4.9e^{-8}$) and rs28654703 ($p = 4.9e^{-8}$) in GOLGA6B gene. **CONCLUSIONS:** We identified 2 potential loci suggestive of being relevant for functional outcome and the recovery process after stroke. Follow-up studies are warranted to validate these findings in other populations.

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Disclosure: The authors report no disclosures.

2016.028

Stroke survivor's and their family's views on genetic tests as predictive tools for stroke in a personalised approach to primary health care

Jane M. Maguire, RN, PhD^{1,2}; Donna Chen, MD, PhD³; Brad Worrall, MD, PhD³

¹University of Newcastle, Faculty of Health and Medicine, School of Nursing and Midwifery, NSW, Australia; ²Hunter Medical Research Centre, Newcastle, Australia; and ³University of Virginia, Charlottesville.

OBJECTIVE: This project aims to capture the views of stroke patients and their families to provide a novel, clinically relevant perspective in this rapidly advancing field of personalised medicine. **BACKGROUND:** There is little evidence that explores stroke survivors and families level of understanding or acceptance of genetic testing in either a diagnostic or predictive capacity in complex disease largely because these tests are not yet a reality. Increasingly, personalized approaches to management of health breakdowns include options to add more information through genetic profiles. Currently these are mostly limited to diseases with monogenic origins (single gene), for example BRAC1 and BRAC2. The advent of pharmacogenomic evidence implies a role in the prediction of complex disease outcomes, and exploring the views of patients and their families will add greater understanding of their needs when developing applications for any proposed prediction devices or tests. It is now timely to bring the patients voice into this dialogue as currently many research inroads have occurred in the discovery of genetic associations for complex diseases such as stroke and in the not so distant future patients may be asked to make choices around predictive tests. **DESIGN/METHODS:** A qualitative design was used to capture stroke patients (consumers) and their family's views. Face-to-face semi-structured Interviews were conducted with 16 Australian participants, stroke survivors ($n = 8$) and family members ($n = 8$). Patients and their family member were recruited from a private neurology clinic in the community. All strokes were confirmed by imaging and a consulting neurologist. Interviews were conducted in the stroke patients homes and recorded and transcribed verbatim by the lead researcher (J.M.M.). Content analysis was used to explore and describe the views of this consumer group. **RESULTS:** Stroke patients were not concerned with privacy or who had access to their genetic profile information. Overall, there was limited knowledge about where genetics may play a role in framing a personalised management approach to their health needs. Patients were more concerned with providing their information to benefit others and advance the research where possible and were less of the view that their own needs were relevant. Relatives were more concerned with immediate family and the impact of an increased risk on future children's health. In general, all participants held the view that their genetic information should be shared with employers if it meant that the employee would not be able to perform effectively in the position. **CONCLUSIONS:** The views and concerns of the Australian stroke participants and their families are quite different from those reported in the literature for UK and USA.

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2016.029

"Helping stroke physicians choose who to thrombolyse—Targeting Optimal Thrombolysis Outcomes"—Preliminary data

Preliminary data Jane M. Maguire, PhD^{1,2}; Elizabeth Holliday, PhD^{3,4}; Christopher Levi, MD, PhD^{2,5}; John Attia, MD, PhD^{2,3,4}; Simon Koblar, MD, PhD^{6,7}; Jonathan Sturm, MD, PhD⁸; Vincent Thijs, MD, PhD⁹; Jin-Moo Lee, MD, PhD¹⁰; Israel Fernandez-Cadenas, PhD^{11,12}; Andrew Bivard, PhD²; Lisa Linco, PhD²

¹School of Nursing and Midwifery, Faculty of Health and Medicine, University of Newcastle, Callaghan; ²Hunter Medical Research Institute, Newcastle, Lambton Heights; ³School of Medicine and Public Health, University of Newcastle, Callaghan; ⁴Clinical Research Design, IT and Statistical Support Unit, Hunter Medical Research Institute, Newcastle; ⁵Department of Neurology, John Hunter Hospital, Newcastle; ⁶Stroke Research Program, School of Medicine, University of Adelaide; ⁷Adelaide Centre for Neuroscience Research, University of Adelaide; ⁸Neurology Department, Gosford Hospital; ⁹Austin Hospital, Heidelberg, VIC, Australia; ¹⁰Washington University, Department of Neurology, St. Louis, MI; ¹¹Fundació Docència I Recerca Mutua de Terrassa, Mutua de Terrassa Hospital, Terrassa, Spain; and ¹²Vall d'Hebron Institute of Research, Barcelona, Spain.

OBJECTIVE: To develop a set of clinical decision rules to guide decisions in targeting ischaemic stroke patients for thrombolysis. To validate prediction models in independent cohorts from our international collaborative network. **BACKGROUND:** Intravenous thrombolysis with recombinant tissue plasminogen activator alteplase, is the only proven effective drug therapy in acute ischaemic stroke. International guidelines recommend use of basic clinical criteria and imaging approaches that cannot consistently determine risk-to-benefit ratios for individual patients. This uncertainty and a natural tendency for physicians to be risk-averse means only about 5% of potentially eligible Australian patients receive alteplase annually. Some clinical and basic radiologic predictors have previously been combined into scoring systems, and some serum biomarkers have also been associated with response to alteplase therapy. However, considered individually, these scoring systems and biomarkers have insufficient predictive utility for translation into clinical practice. We seek to develop practical clinical decision rules to help clinicians identify those likely to benefit from alteplase thrombolysis, those in whom treatment may be futile, and those most likely to be harmed. **DESIGN/METHODS:** We will recruit a prospective cohort of Australian ischaemic stroke patients treated with intravenous alteplase, incorporating a combination of clinical, basic radiologic, advanced radiologic and biomarker/genetic measures. Primary outcomes: good and poor 90 days functional outcome; and brain haemorrhage. Preliminary data is presented from 300 patients with ischaemic stroke confirmed by CT or MRI and treated with intravenous alteplase within 4.5 hours of symptom onset admitted to 2 NSW academic stroke units between 2010 and 2013. Two outcomes related to tPA responsiveness and one related to safety were assessed. Responsiveness was defined by good functional outcome at 90 days post-stroke (modified rankin Scale [mRS] score ≤ 2), or poor functional outcome (90 days mRS ≥ 5). The safety-related outcome was haemorrhagic transformation (HT), defined as parenchymal haematoma type 2 (PH2) with or without symptomatic intracranial haemorrhage (sICH) within 48 hours. **RESULTS:** Multivariate model basic clinic-radiological variables (DRAGON score, early infarct signs, hyperdense cerebral artery sign, pre-treatment disability, age, baseline glucose, onset to treatment time (OTT) and stroke severity) achieved an AUC of 0.58 (95% CI: 0.52–0.64). AUC increased significantly ($p < 0.0001$) to 0.82 (0.76–0.86) in the full model, which additionally included the advanced imaging predictors "large core" and "collateral circulation" (figure). The optimal cut-point for this model was associated with a sensitivity of 87.7% and specificity of 70.7%.

2016.029: "Helping stroke physicians choose who to thrombolyse—Targeting Optimal Thrombolysis Outcomes"—Preliminary data

CONCLUSIONS: Incorporating radiologic measures into predictive models for tPA efficacy may significantly increase our ability to predict those likely to benefit from tPA administration.

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2016.030

A network approach to identifying new candidates for stroke and stroke subtypes

Stephen R. Williams, PhD¹; Qiong Yang, PhD²; Tushar Dave, MS³; Sudha Seshadri, MD²; Braxton Mitchell, PhD²; Michele M. Sale, PhD¹; Bradford B. Worrall, MD¹

¹University of Virginia; ²Boston University, MA; and ³University of Maryland, Baltimore.

OBJECTIVE: The objective of this study is to use a network analysis approach in assessing gene co-expression data from atherosclerotic and normal carotid tissue to inform candidate gene selection in GWAS of stroke and stroke subtypes. **BACKGROUND:** Identification of the genetic contributors to stroke, stroke subtypes, and stroke risk factors is difficult because of the complex etiology of the disease(s) and difficulty in accessing relevant tissues. Advanced computational techniques that can assess the impact of many genes together rather than the traditional "one gene, one phenotype" approach are lacking in the field of cerebrovascular disease but may be useful to identify new biologically relevant candidates. **DESIGN/METHODS:** We performed a Weighted Gene Co-expression Network Analysis (WGCNA) of carotid atherosclerotic plaques paired with healthy distant carotid tissue from 32 individuals (63 samples total, one removed during QC) (dataset: GSE43292). Using the information acquired from this dataset, we identified candidate genes to investigate in subsequent genetic analysis of stroke and stroke subtypes in SiGN and METASTROKE. **RESULTS:** Sixteen modules of co-expressed genes were detected. Six of these modules were significantly associated with carotid atherosclerosis, with 2 being distinct candidate modules ($r^2 < 0.45$). We investigated the most connected genes in each of the atherosclerosis-associated modules as candidates for genetic analyses. In the 2 top modules, the recently discovered large artery atherosclerotic stroke LAAS candidate TSPAN2 is highly connected as well as many other known stroke genes and novel candidates. In fact, genes with high module connectivity tended to be highly associated with atherosclerosis overall and the top modules enriched for genes known to be important in cell adhesion, inflammation, as well as stroke via pathway and gene enrichment analyses. Top genes were next used to inform genetic evaluation in SiGN and METASTROKE which resulted in new significant associations with stroke and stroke subtypes. **CONCLUSIONS:** In conclusion, our analyses provide a powerful new approach to identify novel clinically relevant candidates for atherosclerosis, stroke, and stroke subtypes using a network-based approach to inform genetic studies.

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2016.031

Identification of novel metabolites associated with stroke in a population of Asian Indian descent

Bishua R. Sapkota¹; Jairam K.P. Vanamala²; Lavanya Reddivari²; Yundi Liang²; Dharambir K. Sanghera¹

¹Department of Pediatrics, College of Medicine, University of Oklahoma Health Sciences Center; and ²Department of Plant Science/Department of Food Science, Penn State University, University Park.

OBJECTIVE: There is an increased interest in identifying small molecule as novel biomarkers and endophenotypes that correlate with different types of strokes using advanced high-throughput comprehensive metabolite profiling techniques; and hence this study aims to identify those novel biomarkers associated with stroke. **BACKGROUND:** Stroke is the leading cause of serious disability in the United States and worldwide. There is a critical need for novel insights into disease pathophysiology of stroke as there are significant limitations in the early detection, prevention and treatment strategies. Here, we have generated an untargeted global metabolomics profile using serum samples of stroke patients and healthy subjects from North Indian Punjabi population. **DESIGN/METHODS:** Screening of the untargeted global metabolite profiling was performed in 46 serum/plasma samples using Waters ultra-performance liquid chromatography/AB Sciex 5600 quadrupole time-of-flight mass spectrometry fitted with an electrospray source. Metabolite values were analyzed to assess mean differences among groups using one-way analysis of variance, to test for linear trend across groups and to examine Pearson's correlations between them and 7 cardiometabolic traits (CMTs). **RESULTS:** Normalized peak intensities of 17 identified metabolites were significantly different between the stroke patients and healthy controls. Of these, 8 metabolites were novel molecules and are currently non-existent in any public database. Among the annotated metabolites, plasma levels of tyramine-O-sulphate ($p = 9.6E^{-6}$), imidazole-4-acetate ($p = 4.5E^{-4}$), pyroglutamate ($p = 1.4E^{-3}$), 2,3-diphosphoglycerate ($p = 7.6E^{-3}$), and patientoside A ($p = 1.2E^{-2}$) were elevated while plasma concentrations of betaine were reduced ($p = 1.7E^{-3}$) in stroke patients, after adjusting for age and gender. Betaine serves as a substrate in the osmoregulatory betaine-homocysteine methyltransferase reaction—is a strong predictor of elevated plasma homocysteine, a risk factor of CVD. Pyroglutamate linked peptides are involved in several neurodegenerative disorders including Alzheimer's disease. Increased concentration of imidazole 4-acetate, a metabolite of histamine metabolism, contributes to vascular pathology through pro-inflammatory mechanism. **CONCLUSIONS:** Our study has identified several metabolites whose concentrations were significantly perturbed in patients with ischemic stroke. To our knowledge, the present study is the first report to identify novel metabolites associated with ischemia in a population of Asian Indian descent. These findings signify the need for future comprehensive investigations for the identification of human metabolite signatures to further illuminate molecular and etiologic mechanisms linked with stroke, and to facilitate development of approaches in the personalized medicine.

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2016.032

The BioBank Japan project, and the largest Asian GWAS of stroke

Yoichiro Kamatani, MD, PhD; Yukihide Momozawa, PhD; Michiaki Kubo, MD, PhD

RIKEN Center for Integrative Medical Sciences, Yokohama, Japan.

OBJECTIVE: We aimed to perform the largest Japanese GWAS of stroke ever to reveal another genomic aspect of stroke occurrence. **BACKGROUND:** There is a heritable component in stroke risk. More than a few genome wide association studies of stroke were reported until now, and successfully identified associated loci, but mostly the analyzed samples were of European origin. The linkage disequilibrium structures behind genomic data of European and that of non-European are different so that GWAS using non-European population including East Asians could lead to identify additional association signals. The BioBank Japan (BBJ) is a large patient-oriented cohort in Japan, aiming to collect DNA, serum and clinical data to investigate 47 common diseases. This project started in 2003, and now data and materials from 200,000 subjects are stored. So far, we have performed several genome wide association studies by using a part of this sample, and also participated in number of international genomic meta-analyses. Currently, we have finished genotyping most of them by using Illumina OmniExpress and Exome BeadChips. **DESIGN/METHODS:** We have selected 16,256 stroke cases registered to BBJ, and performed GWAS with 27,294 controls collected from 3 population based cohort studies genotyped by the same genotyping chips. **RESULTS:** We identified 3 novel genome-wide significant signals. Two of them are known hypertension susceptibility loci, which would reflect causal effect of hypertension to stroke. **CONCLUSIONS:** East Asian GWAS of stroke identified association signals, which have not been reported from several European GWAS. This will facilitate further understanding of the genomic architecture of stroke.

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2016.033

Understanding and leveraging the pleiotropic structure of lacunar stroke to improve SNP detection in GWAS using novel statistical tools

Loes C.A. Rutten-Jacobs, PhD

University of Cambridge, Department of Clinical Neurosciences, Stroke Research Group, Cambridge, UK.

OBJECTIVE: To apply novel statistical techniques leveraging pleiotropy between associated traits to increase the ability to detect novel genetic variants in lacunar stroke GWAS. **BACKGROUND:** Previous GWAS in lacunar stroke have been disappointing, which is likely due to phenotypic heritability and relatively small sample sizes. Leverage of GWAS results from related phenotypes is a promising method to improve detection of associated genetic variants. As an example we discuss the findings of a recently published paper (Liley et al. *PLoS Genet* 2015;11:e1004926) in which such method has been applied to 10 immune mediated diseases. Furthermore we discuss the potential application of this method to stroke and more specific lacunar stroke. **DESIGN/METHODS:** In the example study, a recently developed method was applied, the Bayesian conditional false discovery rate (cFDR). This method tests SNPs for association between the phenotype of interest, conditioned on the significance in the associated phenotype, while allowing some or all controls to be shared. Summary statistics in the form of p values for were obtained for type 1 diabetes, autoimmune thyroid disease, coeliac disease, multiple sclerosis, narcolepsy, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, ulcerative colitis and Crohn's disease. The groups had a varying degree of shared controls. The cFDR method was applied to these datasets in pairwise analysis. **RESULTS:** 59 SNP-disease association were discovered using cFDR which did not reach GWAS significance after genomic control in the individual datasets. Fifty-one of these SNPs have been previously reported using replication studies of larger GWAS, 8 SNPs across 5 diseases were not previously reported. **CONCLUSIONS:** The cFDR method can improve SNP detection in GWAS and provides insight in shared aetiology between phenotypically related traits. Several traits have been consistently associated with lacunar stroke such as hypertension, white matter hyperintensities and Alzheimer's Disease. Leveraging these traits using cFDR might boost statistical power to identify genetic variants for lacunar stroke.

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2016.034

Genome-wide association study for ischemic stroke in Japanese: The Hisayama Study

Toshiharu Ninomiya¹; Jun Hata¹; Yutaka Kiyohara¹; Michiaki Kubo²

¹University of Kyushu; and ²RIKEN Center for Integrative Medical Sciences.

OBJECTIVE: To identify susceptible genes of ischemic stroke in Japanese.

BACKGROUND: Ischemic stroke is a major cause of death and disability and occurs owing to a combination of multiple environmental and genetic risk factors. However, genetic components of common forms of ischemic stroke are largely unknown. **DESIGN/METHODS:** The Hisayama Study is an ongoing, population-based prospective cohort study of cardiovascular disease since 1961 in the town of Hisayama, which is a suburb in the Fukuoka metropolitan area on Kyushu Island, Japan. To identify susceptible genes of ischemic stroke, we performed a large case-control association study and a replication study in a total of 1,112 cases with ischemic stroke and 1,112 age- and sex-matched healthy controls from the residents of the town of Hisayama, Japan, using 52,608 single nucleotide polymorphism (SNP) markers. Furthermore, we investigate the influence of detected SNPs on the development of ischemic stroke, using 14-year follow-up data of a prospective cohort study conducted in 1,683 residents of the town aged 40 years or older. Genomic DNA was extracted from peripheral blood leukocytes, using standard protocols. SNPs were genotyped by using the multiplex PCR-based Invader assay or by direct sequencing of PCR products using ABI3700 capillary sequencers. **RESULTS:** We identified 3 novel candidate genes for ischemic stroke: PRKCH (protein kinase C η [PKC η]), AGTRL1 (apelin receptor), and ARHGEF10 (Rho guanine nucleotide exchange factor 10). In the functional analyses, we found that PKC η was expressed mainly in vascular endothelial cells and foamy macrophages in human atherosclerotic lesions, and that the kinase activity of PKC η was modified by a nonsynonymous SNP in PRKCH (rs2230500). We also clarified that functional SNPs in AGTRL1 (rs9943582) and ARHGEF10 (rs4376531) affected transcriptional activities owing to the different Sp1-binding affinities. In a 14-year follow-up cohort study of Hisayama residents, all of these SNPs were significantly associated with the development of ischemic stroke. **CONCLUSIONS:** We identified 3 susceptible genes of ischemic stroke in Japan. These findings might contribute to a better understanding of ischemic stroke in the future.

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2016.035

Exome array analysis on cerebral venous thrombosis

Ioana Cotlarciuc, MSc, PhD¹; Thomas Marjot, MRCP²; Maurizio Margaglione, MD³; Elvira Grandone, MD, PhD⁴; Antonio Arauz, MD, PhD⁵; Stephanie Debet, MD, PhD⁶; Guillaume Pare, MSc, MD⁷; José M. Ferro, MD, PhD⁸; Jennifer J Majersik, MD, MS⁹; Vincent Thijs, MD, PhD¹⁰; Alessandro Pezzini, MD¹¹; Ida Martinelli, MD, PhD¹²; Jonathan M. Coutinho, MD, PhD¹³; Turgut Tatlisumak, MD, PhD^{14,15,16}; Pankaj Sharma, MD, PhD, FRCPC¹; On behalf of BEAST Consortium

¹Institute of Cardiovascular Research Royal Holloway, University of London (ICR2UL), UK; ²Department of Gastroenterology and Hepatology, University of Oxford, Oxford University Hospitals NHS Trust; ³Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Italy; ⁴Atherosclerosis and Thrombosis Unit, IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo, Foggia, Italy; ⁵Stroke Clinic, National Institute of Neurology and Neurosurgery Manuel Velasco Suarez, Mexico City, Mexico; ⁶Department of Neurology, Bordeaux University Hospital, Bordeaux University, France; ⁷McMaster University, Pathology and Molecular Medicine, Population Health Research Institute and Thrombosis and Atherosclerosis Research Institute, Hamilton Health Sciences; ⁸Department of Neurosciences, Hospital de Santa Maria, University of Lisbon, Portugal; ⁹Department of Neurology, University of Utah, Salt Lake City; ¹⁰Department of Neurology, Austin Health and Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, VIC, Australia; ¹¹Department of Clinical and Experimental Sciences, Neurology Clinic, University of Brescia, Italy; ¹²A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca'Granda — Ospedale Maggiore Policlinico, Milan, Italy; ¹³Department of Neurology, Academic Medical Center, University of Amsterdam, the Netherlands; ¹⁴Department of Neurology, Helsinki University Central Hospital, Finland; ¹⁵Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Sweden; and ¹⁶Department of Neurology, Sahlgrenska University Hospital, Gothenburg, Sweden.

OBJECTIVE: Our study aims to elucidate the genetics of cerebral venous thrombosis (CVT) by assessing the association of genetic variants on CVT risk using a genome-wide association approach. **BACKGROUND:** Cerebral venous thrombosis (CVT) is a rare cerebrovascular condition accounting for less than 1% of all stroke cases and mainly affects young adults. The genetic component of CVT is largely unknown and so far has been assessed only by candidate gene studies. **DESIGN/METHODS:** We have established an international biorepository of highly characterized CVT cases, BEAST (Biorepository to Establish the Aetiology of Sinovenous Thrombosis) which to date includes CVT cases recruited from 10 centres across 7 countries in Europe and one from the USA. Due to differences in the genetic structure, we have analysed the genetic data considering 3 population groups: Finnish, Italian and West European, using matched controls. Genotyping was performed using the Illumina Infinium HumanCoreExome BeadChip for each population group. After performing quality control the Finnish cohort included 161 cases and 208 controls, the Italian cohort 189 cases and 247 controls and the West European cohort 160 cases and 450 controls. We conducted genome-wide association analysis of CVT for each population group using logistic regression adjusting for age and sex under an additive genetic model, and then we meta-analysed the results using an inverse variance fixed effect method. **RESULTS:** We have identified a significant locus located at *ABO* gene at a significant p value $< 5 \times 10^{-8}$ and a suggestive association at *F11* gene ($p < 10^{-6}$). These results suggest strong implications of these regions in CVT, as *ABO* is involved in end-stage coagulation and has been associated previously with venous thrombosis, while *F11* encodes the coagulation factor XI of the coagulation cascade. **CONCLUSIONS:** In conclusion, our study has identified 2 potential novel loci associated with CVT and replication will be conducted to confirm these associations.

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