Genetics of Ischemic Stroke

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Tissue Ischemia
Ischemic thresholds vary from tissue to tissue

Muscle

Brain

Ischemia

Muscle

Brain

Tissue Ischemia
Ischemic thresholds vary from tissue to tissue
Ischemic thresholds
Clinical Research in the 1940’s

EFFECTS OF ANOXIA ON NERVE CELL FUNCTION

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IMMEDIATE EFFECT OF ANOXIA ON BRAIN FUNCTION

One study is particularly outstanding in describing the effect of anoxia on the brain. Rossen et al. (1943) exposed human volunteers to acute cerebral ischemia by inflating a pneumatic cuff positioned around the neck (see Fig. 1). The initial events were the following: a fixation of the eyes after 5-6 sec., loss of consciousness and of muscle tone 1/2 - 1 second hereafter, accompanied by appearance of large amplitude, slow waves in the electroencephalogram. Usually the cuff was deflated at this moment but if the ischemia period were prolonged, tonic and clonic convulsions were often seen after 15-20 seconds. It should be noted that no subject was harmed by the procedure and that periods of ischemia up to 100 sec. were well tolerated by the few schizophrenic patients included in the group.


Fig. 1. Apparatus for producing acute cerebral ischemia in humans worn by its designer, Mr. J.P. Anderson (by permission).
Genetics of Ischemic Stroke

- Stroke Definition & Subtypes
- Stroke Risk
  - Risk Factors & Causes of Stroke
  - MEGASTROKE GWAS
  - Stroke Risk Biomarker Discovery
- Stroke Outcomes
  - Brain Injury Mechanisms and Outcomes after Stroke
  - GENISIS GWAS
Stroke Definition

• The abrupt loss or lack of blood flow to a region of the brain caused by the blockage of an artery (ischemic stroke) or bleeding into or around the brain (Intracranial hemorrhage).

• Stroke subtypes
  ▫ Intracranial hemorrhage
  ▫ Ischemic stroke
Stroke

Ischemic Stroke

Intracranial Hemorrhage
- Intraparenchymal
- Subarachnoid
- Subdural
- Epidural
Stroke Etiologies

• Stroke is the end result of multiple underlying diseases (etiologies)
• Important to determine etiology because preventative treatment depends on underlying etiology
Stroke

Ischemic Stroke

Primary Hemorrhage
- Intraparenchymal
- Subarachnoid

20%

Large Artery Atherosclerosis

85%

15%

Intraparenchymal
Subarachnoid

Thrombus
Stroke

Ischemic Stroke

Primary Hemorrhage
- Intraparenchymal
- Subarachnoid

20% Large Artery Atherosclerosis
25% Small Vessel Occlusion
85% Ischemic Stroke
15% Primary Hemorrhage

Lacunar Infarcts

- Small deep infarcts
- Small vessel occlusion
- Caused by:
  - arteriosclerosis
  - microembolus
  - mural plaque blocking origin
- Common in basal ganglia, thalamus, pons

Autopsy specimen (Okazaki, ‘88)
Stroke

Primary Hemorrhage
- Intraparenchymal
- Subarachnoid

Ischemic Stroke

Large Artery Atherosclerosis

Small Vessel Occlusion

Cardioembolism
- Atrial Fibrillation
- Valve disease
- Ventricular thrombi
- Many others

85%
15%
20%
25%
20%

Intraparenchymal
Subarachnoid
Stroke

Ischemic Stroke

Primary Hemorrhage
- Intraparenchymal
- Subarachnoid

Large Artery Atherosclerosis: 20%
Small Vessel Occlusion: 25%
Cardioembolism: 20%
Unknown Etiology: 30%
Other Determined Etiology: 5%

Prothrombic states
Dissections
Arteritis
Migraine/vasospasm
Drug Abuse
Many more

TOAST Criteria—Research classifications for Underlying causes of stroke.
Risk factors

• Unmodifiable Risk Factors
  ▫ Age (2x per decade >55 years)
  ▫ Gender (30% higher for men)
  ▫ Race (African-American highest)
  ▫ Personal or family history (stroke or TIA)
  ▫ Diabetes
    • Can cause circulatory problems that lead to stroke
    • High blood sugar exacerbates stroke

• Modifiable Risk Factors
  ▫ Hypertension (consistently > 140/90)
  ▫ Heart disease (atrial fibrillation, or prior MI)
  ▫ High risk lifestyle factors
    • Smoking
    • Alcohol
The Genetic Architecture of Stroke Risk

• Case-control GWAS
  ▫ Patients with stroke
  ▫ Stroke-free subjects

• Individual GWAS
  ▫ 1000’s of patients

• Meta-Analysis
  ▫ 10,000’s patients
Case-Control GWAS

Human Genome
- $3.2 \times 10^9$ base pairs
- 25,000 genes
- 500,000-5,000,000 SNPs

P-value threshold chosen to correct for multiple comparisons (all SNPs in LD $\approx 1,000,000$)
Known GWAS loci
METASTROKE, NINDS SiGN, & CHARGE

- METASTROKE
  - 10,307 cases
  - 17,326 controls
  - Malik et al. NEUROLOGY ABO (IS)

- NINDS SiGN
  - 12,612 cases
  - 32,473 controls
  - Pulit et al. LANCET NEUROL TSPAN2 (LAS)

- CHARGE
  - 4,338 incident cases
  - 84,961 participants
  - Chauhan et al. LANCET NEUROL FOXF2 (SVD)
**Known Stroke GWAS loci**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Association</th>
<th>p-value</th>
<th>Nearest Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12204590</td>
<td>6:1337393</td>
<td>All</td>
<td>1.48x10^{-8}</td>
<td>FOXF2</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>rs12646447</td>
<td>4:111699326</td>
<td>Cardioembolic</td>
<td>4.72x10^{-24}</td>
<td>PITX2</td>
<td>Homeodomain TF</td>
</tr>
<tr>
<td>rs532436</td>
<td>9:136149830</td>
<td>Ischemic</td>
<td>4.3x10^{-08}</td>
<td>ABO</td>
<td>Blood grouping</td>
</tr>
<tr>
<td>rs2107595</td>
<td>7:19049388</td>
<td>Large Vessel</td>
<td>2.5x10^{-10}</td>
<td>HDAC9</td>
<td>Histone deacetylase</td>
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<tr>
<td>rs2723334</td>
<td>4:111688752</td>
<td>Cardioembolic</td>
<td>8.4x110^{-24}</td>
<td>PITX2</td>
<td>A-fib-associated</td>
</tr>
<tr>
<td>rs12932445</td>
<td>16:73069888</td>
<td>Cardioembolic</td>
<td>1.2x10^{-08}</td>
<td>ZFHX3</td>
<td>Zinc-finger homeodomain TF</td>
</tr>
</tbody>
</table>

- GWAS reveal genetic variants related to underlying causes of stroke
- Power of GWAS is increased when ischemic strokes are broken down into underlying causes (TOAST criteria)
  - Cardioembolic, larger artery atherosclerosis, small vessel, other known, unknown
Mega-Stroke GWAS

- Meta-analyze the 16 European GWAS (N=443,096/34,217)
- Meta-analyze the 3 East-Asian GWAS (N=46,119/17,591)
- Meta-analyze the 2 South-Asian GWAS (N=7,578/2,385)

Trans-ethnic meta-analysis

COMPASS, meta-analysis of AA GWAS (N=12,303/3,804)

INTERSTROKE Latin American GWAS (N=1,247/555)

Strokes - 58,552
Controls - 510,343

All meta-analysis is done using fixed effect inverse variance weighting using METAL.
Mega-Stroke GWAS

Martin Dichgans & Stephanie Debette, personal communication

- 22 novel loci
- 10 known loci
## Mega-Stroke GWAS

**Martin Dichgans & Stephanie Debette, personal communication**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Related vascular trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASZ1</td>
<td>White matter hyperintensities on brain MRI</td>
</tr>
<tr>
<td>WNT2B</td>
<td></td>
</tr>
<tr>
<td>TSPAN2</td>
<td></td>
</tr>
<tr>
<td>PMF1-BGLAP</td>
<td></td>
</tr>
<tr>
<td>RGS7</td>
<td>Carotid plaque</td>
</tr>
<tr>
<td>KCNK3</td>
<td>Carotid IMT</td>
</tr>
<tr>
<td>TM4SF1</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>PITX2</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>ANK2</td>
<td>Systolic BP</td>
</tr>
<tr>
<td>EDNRA</td>
<td>Diastolic BP</td>
</tr>
<tr>
<td>FGA-FGB-FGG</td>
<td>LDL levels</td>
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<td>LOC100505841</td>
<td>HDL levels</td>
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<tr>
<td>BNIP-NKX2-5</td>
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<tr>
<td>FOXF2-FOXQ1</td>
<td></td>
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<tr>
<td>SLC22A7-ZNF318</td>
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<tr>
<td>HDAC9</td>
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<tr>
<td>CDK6</td>
<td></td>
</tr>
<tr>
<td>Chr9p21</td>
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<tr>
<td>LINC01492</td>
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<td>ABO</td>
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<tr>
<td>SH3PXD2A-OBFC1</td>
<td></td>
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<tr>
<td>MMP12-MMP13</td>
<td></td>
</tr>
<tr>
<td>PDE3A</td>
<td></td>
</tr>
<tr>
<td>Chr12q24</td>
<td></td>
</tr>
<tr>
<td>TBX3-TBX5</td>
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</tr>
<tr>
<td>LRCH1</td>
<td></td>
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<tr>
<td>FURIN-FES</td>
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<tr>
<td>ZFHX3</td>
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<tr>
<td>Chr16q24</td>
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</tr>
<tr>
<td>PRPF8-SCARF1</td>
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<tr>
<td>ILF3</td>
<td></td>
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<tr>
<td>SMARCA4-LDLR</td>
<td></td>
</tr>
</tbody>
</table>

- **P-value**
  - <1.30 x 10^-04
  - <5 x 10^-08
Stroke Risk
Biomarker Discovery
Intermediate phenotypes

- Intermediate endophenotypes may provide more power than regular phenotypes
- More directly affected by genetic variation
- Provide a biological model relating the genetic variation with the trait
Intermediate phenotypes: Stroke

- We do have access to plasma levels of 180 different analytes (RBM Discovery Map panel) in more than 800 people

- Several of these analytes may be important to stroke risk and outcomes and were selected for genetics studies

- We also have access to GWAS data as well as exome-chip or whole-exome data
Available datasets

Table 8: Demographics WU-ADRC and ADNI samples

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample #</th>
<th>Age (range)</th>
<th>Male (%)</th>
<th>APOE ε4+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WU-ADRC</td>
<td>controls</td>
<td>239</td>
<td>71±7 (53-91)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>100</td>
<td>74±7 (53-90)</td>
<td>46</td>
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<tr>
<td>ADNI</td>
<td>controls</td>
<td>85</td>
<td>76±5 (62-92)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>481</td>
<td>75±6 (57-89)</td>
<td>66</td>
</tr>
</tbody>
</table>

Age represents age at lumbar puncture
Intermediate phenotypes: Stroke

List GWASed analytes (55):
- Alpha 1-Antichymotrypsin (AACT)
- Alpha-1 Antitrypsin (AAT)
- Angiotensin Converting Enzyme (ACE)
- Adiponectin (Adipo)
- Apolipoprotein E (ApoE)
- Beta-2-Microglobulin (B2M)
- Brain-Derived Neurotrophic Factor (BDNF)
- B-Lymphocyte Chemoattractant (BLC)
- Bone Morphogenetic Protein 6 (BMP6)
- Complement C3 (C3)
- Clusterin (CLU)
- Ciliary Neurotrophic Factor (CNTF)
- Complement Factor H (CFHR1)
- C-Reactive Protein (CRP)
- Cystatin C (CystC)
- Epidermal Growth Factor (EGF)
- Epidermal Growth Factor Receptor (EGFR)
- E-Selectin (Esel)
- Factor VII (Factor7)
- FASLG Receptor (FAS)
- Fas-Ligand (FASL)
- Fibroblast Growth Factor 4 (FGF4)
- Fibrinogen (Fibrino)
- Ferritin (FRTN)
- Heparin-Binding EGF-Like Growth Factor (HBEGF)
- Intercellular Adhesion Molecule 1 (ICAM1)
- Interleukin-6 receptor (IL6r)
- Insulin
- Leptin
- Monocyte Chemotactic Protein 1 (MCP1)
- Monocyte Chemotactic Protein 2 (MCP2)
- Monocyte Chemotactic Protein 3 (MCP3)
- Monocyte Chemotactic Protein 4 (MCP4)
- Macrophage-Derived Chemokine (MDC)
- Monokine Induced by Gamma Interferon (MIG)
- Macrophage Inflammatory Protein-1 alpha (MIP1a)
- Macrophage Inflammatory Protein-1 beta (MIP1b)
- Macrophage Inflammatory Protein-3 alpha (MIP3a)
- Matrix Metalloproteinase-2 (MMP2)
- Matrix Metalloproteinase-9 (MMP9)
- Matrix Metalloproteinase-9 total (MMP9tot)
- Neuronal Cell Adhesion Molecule (NrCAM)
- Plasminogen Activator Inhibitor 1 (PAI1)
- Platelet-Derived Growth Factor B (PDGFBB)
- Resistin (Resist)
- Thrombospondin-1 (TP1)
- Tissue Inhibitor of Metalloproteinases 1 (TIMP1)
- Tumor Necrosis Factor alpha (TNFa)
- Tumor Necrosis Factor Receptor-Like 2 (TNFR2)
- Thrombopoietin (TMBP)
- Vascular Cell Adhesion Molecule-1 (VCAM1)
- Vascular Endothelial Growth Factor (VEGF)
- Protein S
- von Willebrand Factor (vWF)
- Brain Natriuretic Peptide (NT-proBNP)
Intermediate phenotypes: Stroke

**Results:**

- We did find genome-wide significant hits for 24 analytes: AAT, ACE, ApoE, BLC, CFHR1, CRP, CystC, EGFR, Esel, Factor7, FGF4, IL6r, Leptin, MCP2, MCP4, MDC, MIP1a, MIP1b, NrCAM, PDGFBB, TMBP, VCAM1, VEGF, vWF.
### Results GWAS plasma levels

<table>
<thead>
<tr>
<th>Analytes</th>
<th>GWAS hit gene</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT</td>
<td>PPP4R4, SERPINA1, 6, 9, 10, 11</td>
<td>6.95E-12</td>
</tr>
<tr>
<td>ACE</td>
<td>ABO, ACE</td>
<td>1.28E-43</td>
</tr>
<tr>
<td>ApoE</td>
<td>APOE</td>
<td>3.01E-30</td>
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<tr>
<td>BLC</td>
<td>CACNG2, DDAH1</td>
<td>2.36E-08</td>
</tr>
<tr>
<td>CFHR1</td>
<td>CFH</td>
<td>5.52E-142</td>
</tr>
<tr>
<td>CRP</td>
<td>APOE</td>
<td>8.99E-12</td>
</tr>
<tr>
<td>EGFR</td>
<td>SLC35F4</td>
<td>6.14E-08</td>
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<tr>
<td>Esel</td>
<td>ABO</td>
<td>4.48E-53</td>
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<tr>
<td>Factor7</td>
<td>WDR11-AS1</td>
<td>2.45E-26</td>
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<tr>
<td>FGF4</td>
<td>GALNTL6</td>
<td>4.54E-10</td>
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<tr>
<td>IL6r</td>
<td>IL6R</td>
<td>4.65E-105</td>
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<tr>
<td>Leptin</td>
<td>LOC387723, CTAGE7P, GLRX3</td>
<td>7.99E-09</td>
</tr>
<tr>
<td>MIP1b</td>
<td>CCR3</td>
<td>1.89E-10</td>
</tr>
<tr>
<td>NrCAM</td>
<td>NRCAM</td>
<td>2.59E-10</td>
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<tr>
<td>TMBP</td>
<td>DHX8, ARL10</td>
<td>9.84E-09</td>
</tr>
<tr>
<td>VCAM1</td>
<td>RAMP1, UBE2F</td>
<td>2.55E-08</td>
</tr>
<tr>
<td>VEGF</td>
<td>LPP</td>
<td>5.29E-08</td>
</tr>
<tr>
<td>vWF</td>
<td>ABO</td>
<td>4.63E-08</td>
</tr>
<tr>
<td>CystC</td>
<td>CST3, CST4, CST9, CST9L</td>
<td>2.66E-08</td>
</tr>
<tr>
<td>MCP2</td>
<td>CCL8</td>
<td>2.45E-13</td>
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<tr>
<td>MCP4</td>
<td>DIAPH3, LINC00311, MIR5093</td>
<td>7.55E-10</td>
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<td>MDC</td>
<td>Hrh4</td>
<td>8.94E-08</td>
</tr>
<tr>
<td>MIP1a</td>
<td>CCL18</td>
<td>3.80E-15</td>
</tr>
<tr>
<td>PDGFBB</td>
<td>PAG1</td>
<td>7.46E-08</td>
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</tbody>
</table>
Results

- We performed GWAS (Common variants) for 146 proteins levels
- We found 56 GWAS hits (28 Novel) with 47 analytes
- 39 of those hits have also been reported to be associated with other complex treats
  - Heart disease, T2D, or MS
  - Can we use this data to find novel biomarkers for complex traits including stroke and stroke outcomes?
CD40 and MS

a  Plasma CD40 levels

b

- log10(p-value)

- 20
  - 15
  - 10
  - 5
  - 0

0 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Chromosome

rs6032660

- log10(p-value)

0 5 10 15 20

0 20 40 60 80 100

Recombination rate (cM/Mb)

Position on chr20 (Mb)

13 genes omitted
CD40 and MS

![Graph showing log CD40 levels for cases and controls.]
Single Variant Approach

- The SiGN study is a case control study aimed to find new genetic variants involved in Stroke risk.
- 16,851 stroke cases and 32,473 controls.
- Comparison of the p values from SiGN with the p values for each of the 146 analytes.

- We have found that stroke genetic architecture overlaps with the genetic architecture of Selectin E plasma levels.
• Mega-Stroke Results Exploration (from the ISGC meeting in Boston)

• Main Finding:

<table>
<thead>
<tr>
<th>SNP</th>
<th>P value stroke</th>
<th>P value Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs635634</td>
<td>All: 5.9x10^{-09} LAA: 0.0004 CE: 2.7x10^{-07} SAO: 0.44</td>
<td>ACE: 7.8x10^{-08} SelE: 1.13x10^{-52} ICAM1: 4.5x10^{-05} vWF: 2.3x10^{-5}</td>
</tr>
</tbody>
</table>
**SELECTIN E**

- The involvement of Selectin E with stroke and cardiovascular disease has been *previously reported*.
- Our findings suggest that Selectin E may be part of the causal pathway of the stroke event.
- Previous studies showed that administration of Selectin E to animal models decreased atherosclerosis.

- **All together suggest that Selectin E may be an endophenotype for stroke and a potential new drug target to prevent stroke**
Next Steps

• Stroke
  ▫ Test the weighted PRS with the MEGASTROKE results
  ▫ Replicate the results
  ▫ Functional Studies

• Measure additional analytes (Somalogic) in additional plasma samples with genetic data
  ▫ Perform Mendelian Randomization and
  ▫ PRS for Stroke, AD, PD other complex traits (GWAS catalog) to identify novel biomarkers for other diseases
Stroke Risk vs. Stroke Outcomes

Risk = Underlying cause

Stroke & Brain Injury

Disability

P-value

<1.30 x 10^-04

<5 x 10^-08
Genetics of Acute Ischemic Stroke

- Most stroke genetics studies to date have focused on disease risk (case-control design)
- Few genetic studies examining outcomes after stroke.
  - Important because may shed light on mechanisms involved in ischemic brain injury
    - Injury mechanisms
    - Repair/recovery mechanisms
  - Genetic variants involved in ischemic brain injury might be expected to alter outcomes after stroke
Stroke Outcome

Injury mechanisms

Recovery mechanisms

Neurological Outcome

Days

Months

NIHSS score

Time after stroke onset (days)

Fibrin

Plasminogen

NINDS tPA study, NEJM, 1995
First 24 hrs impacts long-term outcome and is highly unstable

Table. Variance in 90d mRS explained by ΔNIHSS (Partial R²)

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS₀₂h</td>
<td>0.25</td>
</tr>
<tr>
<td>ΔNIHSS₂h-24h</td>
<td>0.31</td>
</tr>
<tr>
<td>ΔNIHSS₂₄h-10d</td>
<td>0.09</td>
</tr>
<tr>
<td>ΔNIHSS₁₀d-9₀d</td>
<td>0.06</td>
</tr>
<tr>
<td>ΔNIHSS₂₄hrs</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Acute Stroke: mechanisms

Injury mechanisms

Recovery mechanisms

Days

Months

Neurological Outcome

Mechanisms Derived from Animal Models

Human Stroke Endophenotypes

Dirnagl, Iadecola, Moskowitz, Trends Neurosci, 1999
Capturing mechanisms using genetics
Creative endophenotypes

- Carefully chosen endophenotypes can be used to capture specific mechanisms
- Example: Early neurological change
  \[ \Delta \text{NIHSS}_{24\text{hrs}} = \text{NIHSS}_{6\text{hrs}} - \text{NIHSS}_{24\text{hrs}} \]
  - Neurological improvement (+ \( \Delta \text{NIHSS} \))
    - Recanalization / reperfusion
    - Collateral flow
    - Endogenous neuroprotective mechanisms
  - Neurological deterioration (- \( \Delta \text{NIHSS} \))
    - Hemorrhagic transformation
    - Edema
    - Inflammation
Using Endophenotypes for GWAS

• **Quantitative endophenotypes provide more power than dichotomous traits**
  ▫ Fewer subjects may be needed to find associations compared to dichotomous traits
  ▫ Examples: ΔNIHSS_{24hrs}, hemorrhage volume, edema (midline shift) etc.

• **GWAS for quantitative traits is distinct from case-control design**
  ▫ Uses only cases (stroke patients without controls)
Quantitative Endophenotypes

Are there genotypes that correlate with the quantitative trait?

Every SNP will have a p-value for correlation with quantitative trait.

No controls; only cases with a range of values (trait)
Quantitative Endophenotypes

CSF ptau levels

$APOE$

$P = 5.30 \times 10^{-33}$

Deming et al, 2017
CSF ptau levels

\[ \text{CSF ptau: } \beta = 0.04, \ p = 7.6 \times 10^{-10} \]

\[ \beta = 0.04, \ p = 9.1 \times 10^{-3} \]

\[ \beta = 0.04, \ p = 3.1 \times 10^{-3} \]

\[ \beta = -0.05, \ p = 2.9 \times 10^{-10} \]

\[ \beta = -0.05, \ p = 5.1 \times 10^{-2} \]

\[ \beta = -0.05, \ p = 1.5 \times 10^{-2} \]

Progression
\[ p = 0.525 \]

\[ p = 0.666 \]

GMNC associated with brain volume in recent imaging study (Adams et al, 2016)

Deming et al, 2017
GENISIS
Genetics of Early Neurological Instability after Ischemic Stroke

Washington University
- Laura Heitsch, MD
- Laura Ibanez, PhD
- Raj Dhar, MD
- Yasheng Chen, PhD
- Carlos Cruchaga, PhD
- Jin-Moo Lee, MD, PhD

Vall D’Hebron Hospital
- Joan Montaner, MD
- Israel Fernandez, PhD
- Caty Carrera, PhD

Grant Funding:
- NIH SPOTRIAS P50 NS55997 (JML)
- NIH R01 NS085419 (JML, CC)
- AHA Career Development Grant (LH)
- NIH K23 NS099487 (LH)
- NIH KL2 TR000450 (RD)
- AAN/ABF/Genentech Research Fellowship (CP)
Genetics of Early Neurological 

InStability after Ischemic Stroke
GWAS of $\Delta$NIHSS$_{24h}$

**Patient Selection Criteria**

**Inclusion Criteria:**
1. Ischemic stroke
2. Age $\geq$ 18 years
3. Arrival to hospital within 6 hours of onset
4. Treatment with IV tPA in 0-4.5 window OR not treated with IV tPA

**Exclusion Criteria:**
1. Non-ischemic stroke
2. Treatment with intra-arterial intervention (thrombolysis or thrombectomy)

**Study Cohort:** 4 cohorts (N = 2317) recruited between 2012-2016
- St. Louis (n=617)
- Barcelona (n=1198)
- Helsinki (n=391)
- Krakow (n=111)

**Quantitative Endophenotype**

\[ \Delta\text{NIHSS}_{24h} = \text{NIHSS}_{6h} - \text{NIHSS}_{24h} \]
## GENISIS
### all cohorts clinical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>GENISIS cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>2318</td>
</tr>
<tr>
<td>Age</td>
<td>70.82 (13.86)</td>
</tr>
<tr>
<td>Race (AA)</td>
<td>8.08%</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>45.68%</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>29.15%</td>
</tr>
<tr>
<td>DM</td>
<td>27.29%</td>
</tr>
<tr>
<td>HTN</td>
<td>70.95%*</td>
</tr>
<tr>
<td>HLD</td>
<td>51.69%</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>40.89%*</td>
</tr>
<tr>
<td>Statin</td>
<td>35.87%*</td>
</tr>
<tr>
<td>Baseline glucose</td>
<td>136.69 (53.51)</td>
</tr>
<tr>
<td>Baseline NIHSS</td>
<td>9.29 (7.00)</td>
</tr>
</tbody>
</table>

### GENISIS all cohorts clinical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>GENISIS cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>154.96 (26.45)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>82.05 (16.61)</td>
</tr>
<tr>
<td>tPA</td>
<td>65.28%</td>
</tr>
<tr>
<td>OTN (min)*</td>
<td>147.21 (69.28)</td>
</tr>
<tr>
<td>Delta NIHSS</td>
<td>2.43 (5.78)</td>
</tr>
<tr>
<td>TOAST</td>
<td></td>
</tr>
<tr>
<td>Cardioembolism</td>
<td>40.24%</td>
</tr>
<tr>
<td>Large Artery</td>
<td>12.65%</td>
</tr>
<tr>
<td>Small vessel</td>
<td>9.95%</td>
</tr>
<tr>
<td>Other Determined</td>
<td>4.17%</td>
</tr>
<tr>
<td>Undetermined</td>
<td>32.99%</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>9.76%</td>
</tr>
</tbody>
</table>
GENISIS
all cohorts NIHSS distributions

Baseline NIHSS Distribution  ΔNIHSS Distribution

BL NIHSS  ΔNIHSS
## GENISIS

Multivariate Analysis of $\Delta$NIHSS (n=2280)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial R-Square</th>
<th>Model R-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline NIHSS</td>
<td>0.1065</td>
<td>0.1065</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.0175</td>
<td>0.1241</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tPA</td>
<td>0.0109</td>
<td>0.1349</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline glucose*</td>
<td>0.0052</td>
<td><strong>0.1401</strong></td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*inverse glucose used
### GENISIS

#### Separate Cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Barcelona</th>
<th>Helsinki</th>
<th>St Louis – EuA</th>
<th>St Louis - AA</th>
<th>Krakow</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1198</td>
<td>391</td>
<td>430</td>
<td>187</td>
<td>111</td>
</tr>
<tr>
<td>Age</td>
<td>74.62 (12.58)</td>
<td>65.31 (13.98)</td>
<td>68.69 (13.69)</td>
<td>63.21 (14.70)</td>
<td>70.10 (12.62)</td>
</tr>
<tr>
<td>Baseline NIHSS</td>
<td>10.26 (7.24)</td>
<td>6.95 (5.94)</td>
<td>9.12 (6.98)</td>
<td>8.97 (6.73)</td>
<td>8.40 (6.10)</td>
</tr>
<tr>
<td>tPA treatment</td>
<td>64.83%</td>
<td>48.85%</td>
<td>79.35%</td>
<td>79.23%</td>
<td>51.35%</td>
</tr>
<tr>
<td>Delta NIHSS</td>
<td>2.66 (5.67)</td>
<td>2.33 (5.95)</td>
<td>1.93 (6.11)</td>
<td>2.43 (6.04)</td>
<td>2.23 (4.42)</td>
</tr>
<tr>
<td>TOAST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>39.51%</td>
<td>43.96%</td>
<td>41.53%</td>
<td>37.16%</td>
<td>35.92%</td>
</tr>
<tr>
<td>Large Artery</td>
<td>12.00%</td>
<td>15.42%</td>
<td>13.69%</td>
<td>9.29%</td>
<td>11.65%</td>
</tr>
<tr>
<td>Small Vessel</td>
<td>10.91%</td>
<td>7.71%</td>
<td>8.82%</td>
<td>3.83%</td>
<td>2.91%</td>
</tr>
<tr>
<td>Other</td>
<td>4.03%</td>
<td>8.23%</td>
<td>1.62%</td>
<td>15.30%</td>
<td>1.94%</td>
</tr>
<tr>
<td>Undetermined</td>
<td>33.56%</td>
<td>24.68%</td>
<td>34.34%</td>
<td>34.43%</td>
<td>47.57%</td>
</tr>
</tbody>
</table>
PCAs All population

PC1

PC2

BCN
Finland
Krakow
WUSTL
European PCs
MANTRA: Trans-ethnic Meta-analysis of GWAS

- Traditional GWAS approaches have utilized European descent populations to avoid problems associated with genetic heterogeneity
- MANTRA takes into account similarities in allelic effects between closely related populations, while allowing for heterogeneity between more diverse ethnic groups.
- Improves power for GWAS
- Statistical approach is different from traditional GWAS—significance is expressed as log10 Bayes’ Factor
\[ \Delta \text{NIHSS} = \text{SNP} + \text{Age} + \text{Gender} + \text{Glucose}_{BL} + \text{NIHSS}_{BL} + \text{PC1} + \text{PC2} \]
Chr2 rs1350101

- Rs1350101 falls in **STK17B** intron
- Serine/threonine 17b
  - aka, Drak2, DAP kinase-related apoptosis-inducing protein kinase 2
  - Inhibits TGF-β signaling
chr10 rs7900948

- Rs7900948 falls in a gene desert
- Nearest gene REEP3
  - Receptor Accessory Protein 3
  - Microtubule binding protein required for cell division and nuclear envelope reassembly
Genetics of Early Neurological InStability after Ischemic Stroke

- **Chr19 rs11666532**
  - Rs116666532 falls within **NLRP11** intron
  - NLR family pyrin domain containing 11
  - NLRs, NOD-like receptors
    - Intracellular sensors of PAMPs (pathogen-associated molecular patterns)
  - Little known about NLRP11, but NLRs are involved in inflammasome activation
Inflammasome

Genetics of Early Neurological Instability after Ischemic Stroke

Debbie Maizels/Nature Publishing Group
**Chr21 rs7276690**

- Rs7276690 falls in intron of **RCAN1**
- Regulator of calcineurin 1
  - aka, DSCR1, Down syndrome candidate region 1
  - Induced by cellular calcium overload and oxidative stress
  - Potent inhibitor of calcineurin
    - Attenuates inflammatory cascades
RCAN1 Function

https://www.pancreapedia.org/molecules/rcan1

Pritchard & Martin, Chpt 10 in Down Syndrome, Ed Subrata Kumar Dey, 2013
RCAN1 and focal ischemia

RCAN1 Knockout

RCAN1 Overexpression

Sobrado et al, J Inflamm, 2012

Brait et al, PLOS ONE, 2012
Summary of GENISIS

- GWAS has identified four genome-wide associations, in proximity to genes related to inflammation and apoptosis
  - Needs replication
    - Accumulating samples at 1500/year—3000 samples in 2 years
    - Currently have almost 5000 samples
  - Functional studies
    - KO mice exist for two of the genes
    - Test in experimental stroke (mouse MCAO) models

- Additional lines of study
  - Is influence of candidate SNPs tPA-dependent or independent?
  - Are there SNPs that are associated with extreme phenotypes
    - Extreme deterioration
    - Extreme improvement
  - Are there other endophenotypes that might inform other mechanisms involved in early outcome after stroke?
Summary & Conclusions

• We are using GWAS to understand the genetic architecture of:
  ▫ Stroke Risk (underlying causes of stroke)
    • 30 loci—truly defining a genetic architecture
  ▫ Stroke Outcome (Brain injury or repair mechanisms)
    • 4 loci (smaller numbers)
    • Further define genetic mechanism using endophenotypes
      • Neurological Worsening
      • Neurological Improvement

• “Genetic Overlap” approaches can be used to identify potential biomarkers for stroke risk or outcome
Wash U Collaborators

- Laura Heitsch  Emergency Medicine
- Raj Dhar  Neurology
- Yasheng Chen  Neurology/Radiology
- Chia-Ling Phuah  Neurology
- Carlos Cruchaga  Psychiatry
- Laura Ibanez
Genetics of Early Neurological Instability after Ischemic Stroke