GENESIS
Genetics of Early Neurological Instability after Ischemic Stroke

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Financial Disclosures

• I have no relevant financial interests to disclose.

Scientific Disclosures

• I am not a geneticist.
• I am not a genetic statistician.
• I am not a computer scientist.
• I am a simple stroke neurologist.
Wash U Collaborators

• Laura Heitsch  Emergency Medicine
• Raj Dhar  Neurology
• Yasheng Chen  Neurology
• Chia-Ling Phuah  Neurology
• Carlos Cruchaga  Psychiatry
• Laura Ibanez
Tissue Ischemia
Ischemic thresholds vary from tissue to tissue

Muscle

Brain
Ischemic thresholds
Clinical Research in the 1940’s

EFFECTS OF ANOXIA ON NERVE CELL FUNCTION

Anker Jon Hansen
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Panum Institute, University of Copenhagen
Denmark

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.

Ischemic thresholds
Why is the brain so exquisitely sensitive to ischemia?
### Clinical Trials (1990s)

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Mechanism</th>
<th>Drug Name</th>
<th>Trial Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glutamate antagonists</strong></td>
<td><strong>AMPA antagonists</strong></td>
<td>YM872</td>
<td>Phase II: ongoing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZK-200775 (MPQX)</td>
<td>Phase IIa: abandoned</td>
</tr>
<tr>
<td><strong>Competitive NMDA antagonists</strong></td>
<td><strong>CGS 19755 (Selfotel®)</strong></td>
<td></td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td><strong>NMDA Channel blockers</strong></td>
<td><strong>aptiganel (Cerestat®)</strong></td>
<td></td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td></td>
<td>dextrophan</td>
<td></td>
<td>Phase II: abandoned</td>
</tr>
<tr>
<td></td>
<td>dextromethorphan</td>
<td>abandoned</td>
<td></td>
</tr>
<tr>
<td></td>
<td>magnesium</td>
<td>Phase III: no efficacy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NPS 1506</td>
<td>Phase Ib/IIa: suspended</td>
<td></td>
</tr>
<tr>
<td></td>
<td>remacemide</td>
<td>Phase III in cardiopulmonary bypass: borderline efficacy</td>
<td></td>
</tr>
<tr>
<td><strong>NMDA glycine site antagonist</strong></td>
<td><strong>ACEA 1021 (Licostinel®)</strong></td>
<td></td>
<td>Phase I: abandoned</td>
</tr>
<tr>
<td></td>
<td>GV 150526</td>
<td></td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td><strong>NMDA polyamine site antagonist</strong></td>
<td><strong>SL 82-0715 (eliprodil)</strong></td>
<td></td>
<td>Phase III: abandoned</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Mechanism</th>
<th>Drug Name</th>
<th>Trial Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA agonists</td>
<td>↓ excitation, ↓ glutamate release</td>
<td>clomethiazole (Zendra®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Opiate antagonists</td>
<td>↓ glutamate release</td>
<td>nalmefene (Cervene®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Serotonin agonists</td>
<td>↓ glutamate release</td>
<td>Bay x 3702 (Repinotan®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Voltage-gated calcium channel antagonists</td>
<td>↓ Ca^{2+} influx</td>
<td>nimodipine (Nimotop®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>flunarizine (Sibelium®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Voltage-dependent potassium channel agonists</td>
<td>↓ Ca^{2+} influx</td>
<td>BMS-204352</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Sodium channel antagonists</td>
<td>↓ excitation, ↓ glutamate release</td>
<td>Fosphenytoin (Cerebryx®)</td>
<td>Phase III: no efficacy</td>
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<tr>
<td></td>
<td></td>
<td>BW619C89</td>
<td>Phase II: abandoned</td>
</tr>
<tr>
<td>Free radical scavengers</td>
<td>↓ free radical-mediated injury</td>
<td>tirilazad mesylate (Freedox®)</td>
<td>Phase III: abandoned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ebselen</td>
<td>Phase III: borderline efficacy</td>
</tr>
<tr>
<td>Phosphatidylcholine precursor</td>
<td>Membrane stabilizer</td>
<td>citicoline (Ceraxon®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Growth factors</td>
<td>Anti-apoptotic?, ↑ NMDA receptor inactivation</td>
<td>Fibroblast growth factor (Fiblast®)</td>
<td>Phase II / III: abandoned</td>
</tr>
<tr>
<td>Leukocyte adhesion inhibitor</td>
<td>Reduction of leukocyte infiltration</td>
<td>anti-ICAM antibody (Enlimomab®)</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hu23F2G</td>
<td>Phase III: no efficacy</td>
</tr>
<tr>
<td>Unknown</td>
<td>↓ glutamate release, ↓ neuronal excitability, or ↓ NO-mediated injury</td>
<td>lubeluzole (Prosynap®)</td>
<td>Phase III: no efficacy</td>
</tr>
</tbody>
</table>

Clinical Trials

Neuroprotectants

- Over 120 phase II or phase III clinical trials completed (1990s - 2000s)
- 0 showed efficacy
- Negative trials had major impact on pharmaceutical industry
- Currently, Big Pharma is no longer interested in “neuroprotection” as a target for ischemic stroke.
- Thus, most acute ischemic stroke trials are aimed at recanalization
  - Thrombolysis or thrombectomy devices
Why did all of the clinical trials fail?

- **Animal modelers**: Clinical trials were poorly designed
  - Treatment windows were too long (some up to 24 hrs after onset)
  - Unable to achieve preclinical efficacious drug-doses in humans
    - Side effects
    - BBB penetration
  - No evidence that drug target in the brains of humans was engaged
  - Clinical endpoints were not aligned with endpoints in animal models (TTC staining)

- **Clinical researchers**: Animal models are irrelevant to human disease
  - Rodent models do not reflect human heterogeneity
    - Highly controlled homogeneous populations (cloned strains)
    - Use of young animals (unlike human stroke)
    - Limited comorbidities (otherwise healthy)
    - Uniform etiology (suture induced, unlike human stroke—multiple etiologies)
    - Uniform stroke syndrome (usually MCAO)
  - Rodent biology is different from human biology
    - Agyrencephalic brains
    - Little white matter
Problem:
- >1200 targets reported (efficacy in cell or animal models)
- Only 50 of these targets tested in phase II or III human trials (120 trials)
- Cost of human trials:
  - phase II -- $20 million
  - phase III -- $200 million
Start with Human experiments: Bedside to Bench

• Use experiments of nature (genetic mutations) to examine genetic influences stroke outcome in humans
• Find novel mechanisms, OR
• Find known mechanisms (discovered in cell or animal models) that are relevant to human stroke
Genetics of Ischemic Stroke

- Current stroke genetics studies focus on disease risk (case-control design)
  - Have more to do with stroke etiology
  - Indeed, these studies show that the genetics of stroke risk dependent on etiology
- How to design genetic studies to shed light on endogenous neuroprotective mechanisms?
  - Mutations in genes involved in endogenous neuroprotection might be expected to alter outcomes after stroke
Case-Control GWAS

Human Genome
- 3.2 x10^9 base pairs
- 25,000 genes
- 500,000-5,000,000 SNPs

P-value threshold chosen to correct for multiple comparisons (all SNPs in LD ≈ 1,000,000)
Known GWAS loci
META-STROKE, NINDS SiGN, & CHARGE

**META-STROKE**
- 10,307 cases
- 17,326 controls

**NINDS SiGN**
- 12,612 cases
- 32,473 controls

**CHARGE**
- 4,338 incident cases
- 84,961 participants

- Malik et al. *NEUROLOGY*  
  - ABO (IS)

- Pulit et al. *LANCET NEUROL*  
  - TSPAN2 (LAS)

- 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^{-08}</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>8.4x10^{-24}</td>
<td>PITX2</td>
<td>A-fib-associated</td>
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<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

22 novel loci
10 known loci

Martin Dichgans & Stephanie Debette, personal communication
### Genetics of Early Neurological Instability after Ischemic Stroke

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

**P-value**

- $<1.30 \times 10^{-04}$
- $<5 \times 10^{-08}$

---

**Image Diagram:**

- White matter hyperintensities on brain MRI
- Carotid plaque
- Carotid IMT
- Atrial fibrillation
- Coronary artery disease
- Systolic BP
- Diastolic BP
- LDL levels
- HDL levels
- Venous thromboembolism

---

**Note:** The image includes a diagram of the human body with various vascular traits and genetically associated loci highlighted.
Genetics of Acute Ischemic Stroke

- Current stroke genetics studies focus on disease risk (case-control design)
  - Have more to do with stroke etiology
  - Indeed, these studies show that the genetics of stroke risk dependent on etiology

- How to design genetic studies to shed light on mechanisms involved in ischemic brain injury?
  - Mutations in genes involved in ischemic brain injury might be expected to alter outcomes after stroke
Stroke Outcome

Injury mechanisms

Recovery mechanisms

Neurological Outcome

Days

Months

Genetics of Early Neurological InStability after Ischemic Stroke

NIHSS score

Time after stroke onset (days)

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 $R^2$)

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td>NIHSS$_{2h}$</td>
<td>0.25</td>
</tr>
<tr>
<td>ΔNIHSS$_{2h-24h}$</td>
<td>0.31</td>
</tr>
<tr>
<td>ΔNIHSS$_{24h-10d}$</td>
<td>0.09</td>
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<tr>
<td>ΔNIHSS$_{10d-90d}$</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>0.71</td>
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</tbody>
</table>
Acute Stroke: mechanisms

Injury mechanisms
Recovery mechanisms

Days
Months

Neurological Outcome

Mechanisms Derived from Animal Models

Human Stroke Endophenotypes

Excitotoxicity
Peri-infarct depolarizations
Inflammation
Apoptosis

Minutes
Hours
Time

Early Recanalization
Hemorrhagic Transformation
Edema

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?

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

Every SNP will have a p-value for correlation with quantitative trait.
Quantitative Endophenotypes
GENISIS
Genetics of Early Neurological Instability after Ischemic Stroke

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- Laura Heitsch, MD
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- 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

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- NINDS SPOTRIAS P50NS55997
- AHA Career Development Grant
- EMF Career Development Grant
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**

$\Delta$NIHSS$_{24h} = \text{NIHSS}_{6h} - \text{NIHSS}_{24h}$
# GENESIS

## all cohorts clinical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>GENISIS cohorts</th>
</tr>
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<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>
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<tr>
<td>Baseline NIHSS</td>
<td>9.29 (7.00)</td>
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<table>
<thead>
<tr>
<th>Variable</th>
<th>GENISIS cohorts</th>
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<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>
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<tr>
<td>Delta NIHSS</td>
<td>2.43 (5.78)</td>
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</tbody>
</table>

**TOAST**

- Cardioembolism: 40.24%
- Large Artery: 12.65%
- Small vessel: 9.95%
- Other Determined: 4.17%
- Undetermined: 32.99%
- Hemorrhage: 9.76%
GENISIS
all cohorts NIHSS distributions

Baseline NIHSS Distribution

ΔNIHSS Distribution

BL NIHSS

ΔNIHSS
GENYSIS
all cohorts NIHSS distributions

<table>
<thead>
<tr>
<th></th>
<th>Worsening</th>
<th>Little Change</th>
<th>Improvement</th>
</tr>
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<tbody>
<tr>
<td>Recanalization</td>
<td>0%</td>
<td>62%</td>
<td>100%</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>66%</td>
<td>16%</td>
<td>6%</td>
</tr>
<tr>
<td>PH2</td>
<td>66%</td>
<td>3%</td>
<td>0%</td>
</tr>
</tbody>
</table>
## GENESIS

### Variables used in Multivariate Model

<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>

### Variable                  | GENISIS cohorts |
<table>
<thead>
<tr>
<th></th>
<th></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

**Multivariate Analysis of ΔNIHSS (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
Genome-wide Complex Trait Analysis (GCTA)

Genetics of Early Neurological Instability after Ischemic Stroke

- Clinical Variables: 14%
- Genetics: Common Variants: 24%
- Unknown: 61%
# 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 (years)</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>
Baseline NIHSS distribution

Barcelona

Krakow

Helsinki

St Louis - EuA

St Louis - AA
ΔNIHSS distribution

Barcelona

St Louis - EuA

Krakow

Helsinki

St Louis - AA

ΔNIHSS distribution

Count

0 6 12 18 24 30 36

0 -6 -32 -28 -24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36

ΔNIHSS

Count

0 6 12 18 24 30 36

0 -6 -32 -28 -24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36

ΔNIHSS

Count

0 6 12 18 24 30 36

0 -6 -32 -28 -24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36

ΔNIHSS

Count

0 6 12 18 24 30 36

0 -6 -32 -28 -24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36

ΔNIHSS

Count

0 6 12 18 24 30 36

0 -6 -32 -28 -24 -20 -16 -12 -8 -4 0 4 8 12 16 20 24 28 32 36

ΔNIHSS
PCAs All population

InStability after Ischemic Stroke
European PCs

InStability after Ischemic Stroke
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.
MANTRA
Manhattan Plot

ΔNIHSS = SNP + Age + Gender + Glucose_{BL} + NIHSS_{BL} + PC1 + PC2

rs11670808
rs1350101
rs7900948
rs6517243

Cluster Dendrogram

Population:
- African American
- Finnish
- Korean
- Japanese
- European American

InStability after Ischemic Stroke
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
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
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

- Relative quantification of TG and mTRA mRNA
- Relative quantification of 14-3-3 mRNA
- Relative quantification of Cx43 mRNA

Sobrado et al, J Inflamm, 2012

RCAN1 Overexpression

- Time Suspended (s)
- Neurological Score
- Neutrophils
- Total Infarct

Brait et al, PLOS ONE, 2012
Summary of GWAS

- GTCA indicate that there is a significant genetic influence on neurological instability (ΔNIHSS)
- 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
Additional Sites

**Spanish Network**
Joan Montaner, MD, PhD

**Finnish Network**
Daniel Strbian, MD, PhD

**Polish Network**
Agnieszka Slowik, MD, PhD

**Providence Health Network**
Ted Lowenkopf, MD

**Barnes-Jewish Hospital Network**
Jin-Moo Lee, MD, PhD

**Mexican Site**
Antonio Arauz, MD

**Costa Rican Site**
Miguel Barboza, MD

**Brazilian Site**
Ischia Lopes-Cendes, PhD

**Sacramento Site**
Yekaterina Axelrod, MD

**Australian Network**
Jane Maguire, RN, PhD
Summary of GWAS

- GTCA indicate that there is a significant genetic influence on neurological instability ($\Delta$NIHSS)
- 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
  - 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 neurological outcome after stroke?
Other Acute Stroke Endophenotypes

- Hemorrhagic transformation
- Recanalization
  - TCD, CTA, MRA
- Post-stroke cerebral edema

3 hrs

48 hrs

175 cc
CSF Volume

61 cc
Infarct Volume

ΔCSF = 114 cc
-65% / 48 hrs
Novel Endophenotype
CSF Volumetrics - surrogate for edema

Hypothesis: a CT-based measure of change in CSF volume ($\Delta$CSF) will provide an accurate quantitative biomarker of cerebral edema.

Baseline Head CT
121 ml

24-hr Head CT
71 ml

Peak Edema
65 ml
Kinetics of $\Delta$CSF edema formation

Stroke onset

Baseline Head CT

24-hr Head CT

Peak Edema

Change in CSF Volume (ml)

114 ml

53 ml

10 ml
Validation of $\Delta$CSF$_{24\text{hr}}$

$\Delta$CSF$_{24\text{hr}}$ vs. peak MLS

$\Delta$CSF$_{24\text{hr}}$: malignant vs. non-malignant edema

% change from baseline to early FU CT in volume of IL sulci

% change from baseline to early FU CT in volume of IL sulci

% Volume

Time from Onset

No ME

Malignant Edema
Automation

Machine learning algorithm to segment CSF

Geodesic Active Contour

Random Forest

Training Sets → Randomize → Set 1 → Set 2 → Set N → Combine → Output
Automated CSF Segmentation Validation

Hounsfield Unit Thresholding
Random Forest
Random Forest + Geodesic Active Contour

- Under-segmented
- Over-segmented
- Overlap
Automated CSF Segmentation Validation

Hounsfield Unit Thresholding

Random Forest

Random Forest + Geodesic Active Contour

\[ Y = 0.948 \times X + 23.88 \quad R^2 = 0.200, P = 0.005 \]

\[ Y = 1.266 \times X + 26.12 \quad R^2 = 0.906, P < 0.0001 \]

\[ Y = 1.003 \times X + 2.742 \quad R^2 = 0.904, P < 0.0001 \]
Automated CSF Segmentation
Characterization of 150 patients
Imaging-Genomic Pipeline

• Automated calculation of $\Delta \text{CSF}_{24\text{hr}}$ as quantitative endophenotype for cerebral edema
  ▫ Using CT scans obtained at baseline and 24-hour
  ▫ Rate of edema formation
• Acquiring baseline and 24-hour CT scans from
  ▫ Wash U, Barcelona, Helsinki, Krakow
  ▫ Estimate at least 2,000 sets of CT scans
• Identify genetic variants associated with $\Delta \text{CSF}_{24\text{hr}}$
  ▫ Adjusting for covariates (age, NIHSS)
  ▫ Determine genetic influences on cerebral edema
    • SNPs, Genes, Pathways
Summary & Conclusions

- We are using GWAS to understand the genetic architecture of early neurological outcome after acute ischemic stroke
  - Four GWAS hits, some in credible genes suggest feasibility of approach, but will require replication
    - Genes involved in inflammation and apoptosis
    - Hope to find mechanisms involved in rapid neurologic improvement or deterioration
- Carefully defined quantitative endophenotypes might provide means to understand mechanisms of complex phenotypes like ischemic brain injury
  - Neurological Worsening
    - Edema formation, hemorrhagic transformation, inflammation
  - Neurological Improvement
    - Recanalization/fibrinolysis, endogenous neuroprotection, collateral flow
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Joanna Pera, MD, PhD

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Portland Medical Center
St. Peter Hospital
Sacred Heart Medical Hospital
Little Company of Mary Center
St. Joseph Medical Center
Swedish Medical Center

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Washington University School of Medicine in St. Louis
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Laura Heitsch, MD
Laura Ibanez, PhD
Raj Dhar, MD
Yasheng Chen, Phd
Boone Hospital Center
Allyn Sher, MD

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KL2 (LH)
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- Raj Dhar  Neurology
- Yasheng Chen  Neurology/Radiology
- Chia-Ling Phuah  Neurology
- Carlos Cruchaga  Psychiatry
- Laura Ibanez
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  ▫ Christian Burrell
  ▫ Derek Holder

• Stroke Research Coordinators
  ▫ Jill Newgent
  ▫ Jenny Babka
  ▫ Rosmy George

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  ▫ Crystal Young
Genetics of Early Neurological Instability after Ischemic Stroke
Sample Size and GWAS hits

Visscher et al, Am J Hum Genetics, 2012