The Sahlgrenska Academy Study
on Ischemic Stroke

Christina Jern
Stroke

Has many faces…

• Many different symptoms
• Many different causes

Is rather a syndrome than a single disease

Main types:
• ischemic
• hemorrhagic
Etiologic heterogeneity of ischemic stroke

- Small-vessel disease
- Large-vessel disease
- Cardioembolic stroke
The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)

- 600 consecutive patients with ischemic stroke < 70 years
- 600 population controls matched for age and sex
- Classification of etiologic subtype; TOAST and CCS
- Extensive characterisation of risk factors
- Standardized blood sampling 08.30-10.30 am in the acute phase and after 3 months
- Functional outcome by mRS 3 months and 2 years post-stroke
- Continued inclusion with sampling for genetic analyses only; to date approximately 1000 cases

Jood et al, Stroke 2005
Etiologic subtypes in SAHLSIS

Mean age 56 y
Male sex 64%

- Cryptogenic stroke 27%
- Dissection 5%
- Other determined etiology 4%
- Cardioembolic stroke 16%
- Large-vessel disease 12%
- Small-vessel disease 21%
- Undetermined stroke 15%

Jood et al, Stroke 2005
Adjusted ORs and 95% CI of ischemic stroke and different stroke subtypes for current smoking

- All ischemic stroke: n=600
  - OR: 2.78

- Large-vessel disease: n=73
  - OR: 8.30

- Small-vessel disease: n=124
  - OR: 3.97

- Cardioembolic stroke: n=98
  - OR: 2.59

- Cryptogenic stroke: n=162
  - OR: 2.62
Adjusted ORs and 95% CI of ischemic stroke and different stroke subtypes for diabetes

- All ischemic stroke: n=600
  - OR: 3.67

- Large-vessel disease: n=73
  - OR: 11.61

- Small-vessel disease: n=124
  - OR: 3.76

- Cardioembolic stroke: n=98
  - OR: 3.41

- Cryptogenic stroke: n=162
  - OR: 3.13
Adjusted ORs and 95% CI of ischemic stroke and different stroke subtypes for hypertension

- All ischemic stroke: n=600
  - OR: 2.71
- Large-vessel disease: n=73
  - OR: 2.11
- Small-vessel disease: n=124
  - OR: 3.91
- Cardioembolic stroke: n=98
  - OR: 1.68
- Cryptogenic stroke: n=162
  - OR: 2.08
Blood clot formation causes ischemic stroke

Small-vessel disease

Large-vessel disease

Cardioembolic stroke
Increased von Willebrand factor (VWF) levels in overall ischemic stroke and subtypes

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**Graph:**
- **Y-axis:** VWF (IU/dL) median, IQR
- **X-axis:**
  - Control
  - Overall ischemic stroke
  - Large-vessel disease
  - Small-vessel disease
  - Cardio-embolic stroke
  - Cryptogenic stroke

**Legend:**
- Control
- Patients acute phase
- Patients 3-month follow-up

**Statistics:**
- ***p<0.001
- **p<0.01

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Hanson et al, JTH 2011
Differences in VWF levels between subtypes

- **p<0.001
- **p<0.01
- *p<0.05

Hanson et al, JTH 2011
Factor VII Activating Protease (FSAP) activity

Control  Large-vessel disease  Small-vessel disease  Cardio-embolic stroke  Cryptogenic stroke

FSAP activity (mU/mL) median, IQR

- ***p<0.001
- **p<0.01
- *p<0.05

Control  Patients acute phase  Patients 3-month follow-up

Hanson et al, JTH 2012
Cross-talk hemostasis and inflammation
Increased hsCRP levels in overall IS

*** p<0.001 Mann-Whitney U test

Ladenvall et al, Stroke 2006
Association between an interleukin 1 receptor antagonist (IL-1 RA) SNP and overall ischemic stroke

<table>
<thead>
<tr>
<th>Protein</th>
<th>SNP</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>rs3783550</td>
<td>0.5</td>
<td>1.0</td>
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<tr>
<td></td>
<td>rs2856838</td>
<td>1</td>
<td>1.0</td>
<td></td>
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<td></td>
<td>rs1800587</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
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<td>IL-1β</td>
<td>rs1143643</td>
<td>0.5</td>
<td>1.0</td>
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<tr>
<td></td>
<td>rs16944</td>
<td>1</td>
<td>1.0</td>
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<td>IL-1 RA</td>
<td>rs4251961</td>
<td>0.5</td>
<td>1.0</td>
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<td></td>
<td>rs928940</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs454078</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
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<tr>
<td></td>
<td><strong>rs380092</strong></td>
<td><strong>1.21</strong></td>
<td><strong>1.04-1.42</strong></td>
<td><strong>&lt;0.05</strong></td>
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<td></td>
<td>rs452204</td>
<td>0.5</td>
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<tr>
<td></td>
<td>rs315951</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs9005</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Olsson et al, Stroke 2012
Association independent of classic vascular risk factors only for large-vessel disease

Ladenvall et al, Stroke 2006
Hemostasis and inflammation in subtypes

**Hemostasis**
- Fibrinogen
- VWF
- FVII
- FSAP
- tPA
- PAI-1
- TAFI

**Inflammation**
- hsCRP

All major subtypes
Large-vessel disease
Adjusted ORs and 95% CI of ischemic stroke and different stroke subtypes for family history of stroke

- All ischemic stroke: n=600
  - OR: 1.75

- Large-vessel disease: n=73
  - OR: 1.88

- Small-vessel disease: n=124
  - OR: 1.79

- Cardioembolic stroke: n=98
  - OR: 1.27

- Cryptogenic stroke: n=162
  - OR: 1.70

ORs and 95% CI from:
- Polychronopoulos 2002
- Jerrard-Dunne 2003
- Schulz 2004
Adjusted ORs and 95% CI of ischemic stroke and different stroke subtypes for family of MI

- **All ischemic stroke**
  - n=600
  - OR: 1.21

- **Large-vessel disease**
  - n=73
  - OR: 3.25

- **Small-vessel disease**
  - n=124
  - OR: 0.80

- **Cardioembolic stroke**
  - n=98
  - OR: 1.56

- **Cryptogenic stroke**
  - n=162
  - OR: 0.96

References:
- Jerrard-Dunne 2003
- Schulz 2004
- Jood et al, Stroke 2005
http://www.strokegenetics.org
### Association between sequence variants on chromosome 9p21.3 and large-vessel disease

<table>
<thead>
<tr>
<th>Atherosclerotic Stroke, corrected for Age, Sex, Ethnicity, Center, CAD and MI, and vascular risk factors</th>
<th>rs7044859</th>
<th>rs496892</th>
<th>rs564398</th>
<th>rs7865618</th>
<th>rs1537378</th>
<th>rs2383207</th>
<th>rs10757278</th>
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<tbody>
<tr>
<td></td>
<td>A/T</td>
<td>A/G</td>
<td>A/G</td>
<td>A/G</td>
<td>C/T</td>
<td>A/G</td>
<td>A/G</td>
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<tr>
<td>A</td>
<td>969</td>
<td>730</td>
<td>963</td>
<td>961</td>
<td>960</td>
<td>961</td>
<td>951</td>
</tr>
<tr>
<td>A</td>
<td>4,240</td>
<td>3,346</td>
<td>4,175</td>
<td>4,256</td>
<td>4,187</td>
<td>4,244</td>
<td>4,246</td>
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<tr>
<td>0.4979</td>
<td>0.5733</td>
<td>0.6490</td>
<td>0.6394</td>
<td>0.6672</td>
<td>0.5890</td>
<td>0.5047</td>
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<td>0.4896</td>
<td>0.5528</td>
<td>0.6225</td>
<td>0.6181</td>
<td>0.6424</td>
<td>0.5700</td>
<td>0.4694</td>
<td></td>
</tr>
<tr>
<td>1.12</td>
<td>1.16</td>
<td>1.18</td>
<td>1.18</td>
<td>1.19</td>
<td>1.16</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>(1.00–1.24)</td>
<td>(1.02–1.30)</td>
<td>(1.06–1.32)</td>
<td>(1.06–1.32)</td>
<td>(1.06–1.33)</td>
<td>(1.04–1.29)</td>
<td>(0.99–1.23)</td>
<td></td>
</tr>
<tr>
<td>0.0409</td>
<td>0.0181</td>
<td>0.0034</td>
<td>0.0033</td>
<td>0.0031</td>
<td>0.0083</td>
<td>0.0618</td>
<td></td>
</tr>
</tbody>
</table>

Shown are all single nucleotide polymorphisms (SNPs) in the region on 9p21 that showed nominally significant association to atherosclerotic stroke in one of the two screening samples (see Supplementary Table 1). OR = odds ratio; CI = confidence interval; CAD = coronary artery disease; MI = myocardial infarction.

Gschwendter et al
Ann Neurol 2009
Genetic variation at 9p21.3 is associated with large-vessel disease in SAHLSIS

<table>
<thead>
<tr>
<th>Gene Variation</th>
<th>Large-vessel disease OR (95% CI)</th>
<th>Ischemic stroke OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10965227</td>
<td>1.39 (1.00-1.94)</td>
<td>1.00 (0.84-1.20)</td>
</tr>
<tr>
<td>rs1547705</td>
<td>0.99 (0.59-1.65)</td>
<td>0.97 (0.75-1.25)</td>
</tr>
<tr>
<td>rs1333040</td>
<td>0.86 (0.64-1.16)</td>
<td>0.88 (0.76-1.02)</td>
</tr>
<tr>
<td>rs7857345</td>
<td>0.61 (0.44-0.86)*</td>
<td>0.86 (0.77-1.00)</td>
</tr>
<tr>
<td>rs1333045</td>
<td>0.80 (0.60-1.08)</td>
<td>0.88 (0.76-1.02)</td>
</tr>
<tr>
<td>rs10757278</td>
<td>1.12 (0.83-1.50)</td>
<td>1.13 (0.98-1.31)</td>
</tr>
<tr>
<td>rs1537378</td>
<td>0.82 (0.61-1.11)</td>
<td>0.90 (0.78-1.04)</td>
</tr>
</tbody>
</table>

* p<0.01

Olsson et al
Eur J Neurol 2011
Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke

The International Stroke Genetics Consortium (ISGC), the Wellcome Trust Case Control Consortium 2 (WTCCC2), Céline Bellenguez, Steve Bevan, Andreas Gschwendtner, Chris C A Spencer, Annette I Burgess, Matti Pirinen, Caroline A Jackson, Matthew Traylor, Amy Strange, Zhan Su, Gavin Band, Paul D Syme, Rainer Malik, Joanna Pera, Bo Norrving, Robin Lemmens, Colin Freeman, Renata Schanz, Tom James, Deborah Poole, Lee Murphy, Helen Segal, Lynelle Cortellini et al.

Affiliations | Contributions | Corresponding authors

Nature Genetics 44, 328–333 (2012) | doi:10.1038/ng.1081
Received 26 May 2011 | Accepted 15 December 2011 | Published online 05 February 2012
Common variants at 6p21.1 are associated with large artery atherosclerotic stroke


Affiliations  Contributions  Corresponding author

Nature Genetics 44, 1147–1151 (2012)  doi:10.1038/ng.2397
The Stroke Genetics Network (SiGN)

- PI Steven Kittner, Maryland
- GWAS on the ischemic stroke subtypes large-vessel disease, small-vessel disease and cardioembolic stroke
- All stroke cases subtyped uniformly according to CCS
- 817 cases from SAHLSIS
- GWAS with exome content (Illumina HumanOmni5Exome)
- Genetic analyses ongoing at the US National Institute of Neurological Disorders and Stroke
Swedish GWAS on ischemic stroke

- SAHLSIS
- Lund Stroke Register (LSR), PI Arne Lindgren
- Malmö Cost and Cancer Study (MDC), PI Olle Melander
- GWAS with exome content (Illumina HumanOmniExpressExome)
- Genetic analyses ongoing at Broad Institute
Functional outcome 3 months after index stroke

Death or dependency (mRS score >3)

- All ischemic stroke: 22%
- Large-vessel disease: 27%
- Small-vessel disease: 9%
- Cardioembolic stroke: 31%
- Cryptogenic stroke: 20%

Adjusted OR of death and dependency for CRP above 1.61 mg/L at 3 months: 1.79 (1.12-2.85)

Ladenvall et al, Stroke 2006
Two-year survival free of recurrent stroke

Redfors et al, Acta Neurol Scand 2012
Convalescent TAFI AP and two-year outcome

Plasma TAFI Released Activation Peptide, % relative to pooled plasma

No event
n=480

Composite event
during follow-up
n=37

$P < 0.05$

Jood et al, JTH 2012
Long-term (7-year) follow-up in SAHLSIS

- All surviving patients and controls in SAHLSIS have been followed up seven years after inclusion by questionnaires and assessment tools, e.g. SF36 and HAD.

- All surviving patients who were included at the at Sahlgrenska University Hospital and underwent MRI of the brain (n=318) have been invited to participate in a more extensive follow-up.
Long-term (7-year) follow-up in SAHLSIS

<table>
<thead>
<tr>
<th>1. Nurse</th>
<th>2. MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Additional questionnaires and assessment tools</td>
<td>- Neurological exam</td>
</tr>
<tr>
<td>- Cognitive screening</td>
<td>- Recurrent vascular events</td>
</tr>
<tr>
<td>Barrow Neurological Institute</td>
<td>- Cognitive screening</td>
</tr>
<tr>
<td>Screen for Higher Cerebral Functions Scale (BNIS)</td>
<td>Mini Mental State Examination (MMSE)</td>
</tr>
<tr>
<td>- Blood sampling</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>3. MRI of the brain</th>
<th>4. Neuropsychological testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>- White matter disease</td>
<td>- Psychomotor speed</td>
</tr>
<tr>
<td></td>
<td>- Executive functions</td>
</tr>
<tr>
<td></td>
<td>- Attention</td>
</tr>
</tbody>
</table>
Long-term follow-up in SAHLSIS

- Death and vascular events 1998-2010
- Survival rates obtained from the Swedish population register (folkbokföringen) and cause of death from the Swedish Cause of Death Register (dödsorsaksregistret)
- Vascular events from the Swedish Hospital Discharge register
- Vascular events and causes of death verified through review of all medical records, death certificates and autopsy protocols
Long-term follow-up in SAHLSIS

- Median follow-up 8.7 years in controls (SD 1.6) and 8.5 (SD 2.5) in patients
- Person years 5209 for controls and 5100 for cases
- Preliminary results show large differences in mortality, recurrent stroke and myocardial infarction between subtypes
In summary, SAHLSIS focuses

- Ischemic stroke subtypes
- Hemostasis and inflammation
- Genetics including functional characterisation of SNPs
- Short- and long-term post-stroke outcome
  - Recurrent stroke
  - Cognitive function
  - Depression, fatigue etc
  - White matter disease
- Quality of life for both stroke victims and spouses
Thank you for your attention!
Acknowledgements

**SAHLSIS study group**

Christian Blomstrand, MD, Prof
Katarina Jood, MD, PhD
Hans Samuelsson, Assoc Prof
Staffan Nilsson, Assoc Prof
Gunilla Forsberg-Wärleby, PhD
Sandra Olsson, PhD
Ellen Hanson, PhD
Tara Stanne, PhD
Petra Redfors, MD, PhD student
Lukas Holmegaard, MD, PhD student
Annie Pedersén, MD, PhD student

**Collaborators**

Arne Lindgren, MD, Prof
Bo Norrving, MD, Prof
Olle Melander, MD, Prof
Sandip Kanse, Prof
The Stroke Genetics Consortium

[Logos and affiliations]