



UNIVERSITY OF
GOTHENBURG

THE SAHLGRENSKA ACADEMY

Blodbiomarkörer vid stroke - diagnostik och återhämtning

Christina Jern

Professor, universitetsöverläkare

The Sahlgrenska Academy

Varför studera biomarkörer?

Potentiell nytta:

- Ökad kunskap om patofysiologiska mekanismer; diagnostik
- Nya måltavlor för intervention och läkemedelsutveckling
- Förbättrad prognostisk information; mer individanpassat omhändertagande
- Stratifiering

Definition:

“något som kan mätas objektivt och utvärderas som en indikator för normala biologiska eller patologiska processer eller för farmakologiskt svar på en terapeutisk intervention”

Vi studerar:

- Proteinnivåer och RNA i blod
- Genvarianter



Biomarkörer vid stroke

Hittills få utöver neuroimaging som används kliniskt pga bland annat:

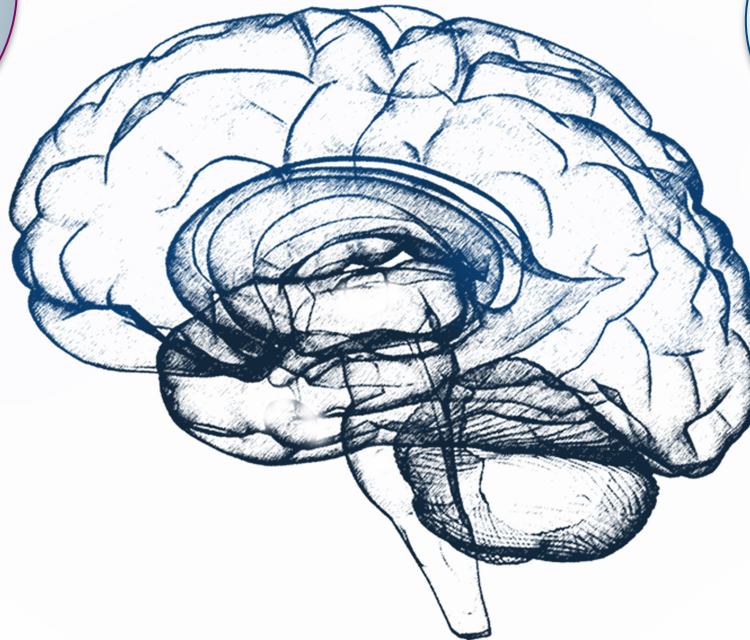
- Hittills svårt att med blodprov/biopsi mäta något som händer i CNS
- Stroke är en mycket heterogen sjukdom – utmaning för design av kliniska studier



Funktionellt utfall (mRS)

Kvarstående
specifika neurologiska
bortfall tex motorisk
påverkan

Depression
Fatigue
Delaktighet
Livskvalitet



Kognitiv
nedsättning

Epilepsi

Ny stroke

Hjärtinfarkt och
andra vaskulära
händelser

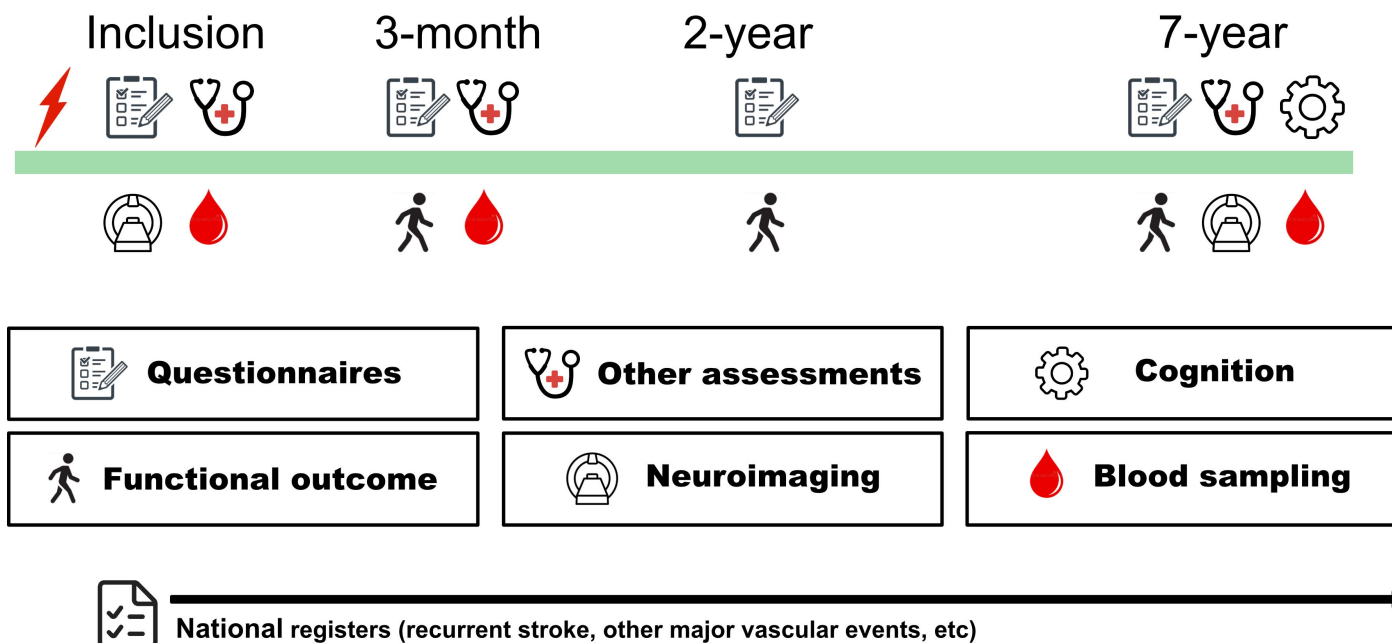
Mortalitet – särskilt av
vaskulär orsak



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The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)



N = 1,090, age median 59 (range 18-69) years, 64% males

Substudy with 7-y follow-up, n=296

Biobanking: whole blood all (DNA)

plasma (EDTA and citrate) and serum, n ≈ 600



Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)

Mycket data...

>3800 besök för forskningspersoner

- Bakgrundsvariabler
 - Neurologiskt utfall
 - Livskvalitet
 - Kognition
 - Depression
 - Hjärntrötthet
 - Ny stroke
 - Analys blodprover
- mm





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SAHLSIS

THE SAHLGRENSKA ACADEMY

Examples of publications on long-term (7-y) outcomes

DOI: 10.1111/ene.15014

ORIGINAL ARTICLE

2021;28:3692-3701

European journal
of neurology
the official journal of the European Academy of Neurology

Cognitive function is an important determinant of employment amongst young ischaemic stroke survivors with good physical recovery

Hans Samuelsson^{1,2,3} | Jo Viken² | Petra Redfors^{2,3} | Lukas Holmegaard^{2,3} | Christian Blomstrand² | Christina Jern^{4,5} | Katarina Jood^{2,3}

DOI: 10.1111/ane.13665

ORIGINAL ARTICLE

2022;146:295-303

Acta
Neurologica
Scandinavica

WILEY

Fatigue 7 years post-stroke: Predictors and correlated features

Annie Pedersen^{1,2} | Emelie Almkvist¹ | Lukas Holmegaard^{3,4} | Cecilia Lagging^{1,2} | Petra Redfors^{3,4} | Christian Blomstrand³ | Katarina Jood^{3,4} | Hans Samuelsson^{3,4,5} | Christina Jern^{1,2}

Acta
Neurologica
Scandinavica

ORIGINAL ARTICLE

2018;138:548-556

Long-term progression of white matter hyperintensities in ischemic stroke

Lukas Holmegaard | Christer Jensen, Petra Redfors, Christian Blomstrand, Christina Jern, Katarina Jood

Seizure: European Journal of Epilepsy 76 (2020) 50-55



Contents lists available at ScienceDirect

Seizure: European Journal of Epilepsy

journal homepage: www.elsevier.com/locate/seizure



Long-term follow-up of post-stroke epilepsy after ischemic stroke: Room for improved epilepsy treatment

Petra Redfors^{a,*}, Lukas Holmegaard^a, Annie Pedersen^b, Christina Jern^{b,1}, Kristina Malmgren^{a,1}

^a Institute of Neuroscience and Physiology, Sweden

^b Institute of Biomedicine, The Sahlgrenska Academy at University of Gothenburg, Sweden





Examples of publications on plasma protein biomarkers and outcomes

Low Circulating Acute Brain-Derived Neurotrophic Factor Levels Are Associated With Poor Long-Term Functional Outcome After Ischemic Stroke

Tara M. Stanne , N. David Åberg, Staffan Nilsson, Katarina Jood, Christian Blomstrand, Ulf Andreasson, Kaj Blennow, Henrik Zetterberg, Jörgen Isgaard, Johan Svensson and Christina Jern

Originally published 14 Jun 2016 | <https://doi.org/10.1161/STROKEAHA.115.012383> | Stroke. 2016;47:1943–1945

Journal of Neurology (2019) 266:2796–2806
<https://doi.org/10.1007/s00415-019-09477-9>

ORIGINAL COMMUNICATION

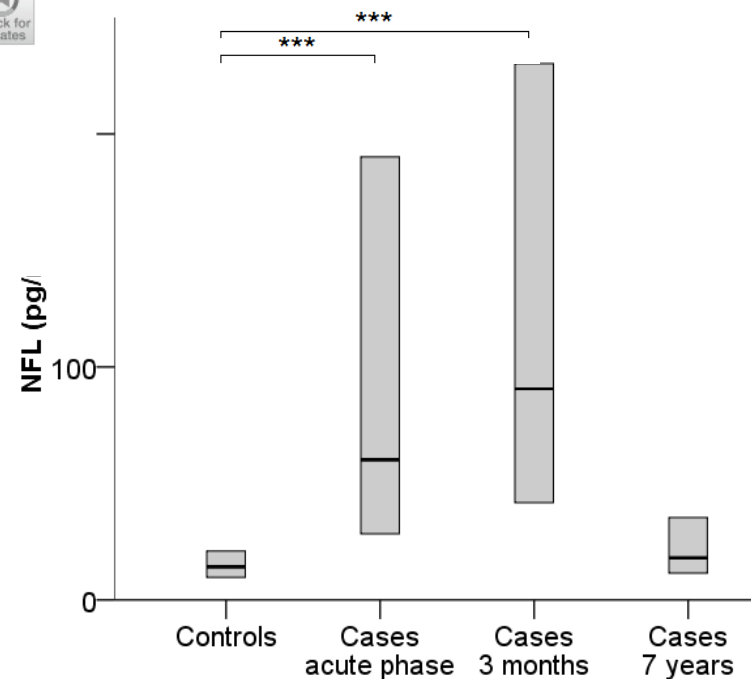


Circulating neurofilament light in ischemic stroke: temporal profile and outcome prediction

Annie Pedersen^{1,2}  · Tara M. Stanne¹ · Staffan Nilsson^{1,3} · Sofia Klasson¹ · Lars Rosengren⁴ · Lukas Holmegaard⁴ · Katarina Jood⁴ · Kaj Blennow^{5,6} · Henrik Zetterberg^{5,6,7,8} · Christina Jern^{1,2}

Serumnivå av NfL;
Horisontella linjer - median
Boxar – interkvartil range

*** P < 0.001

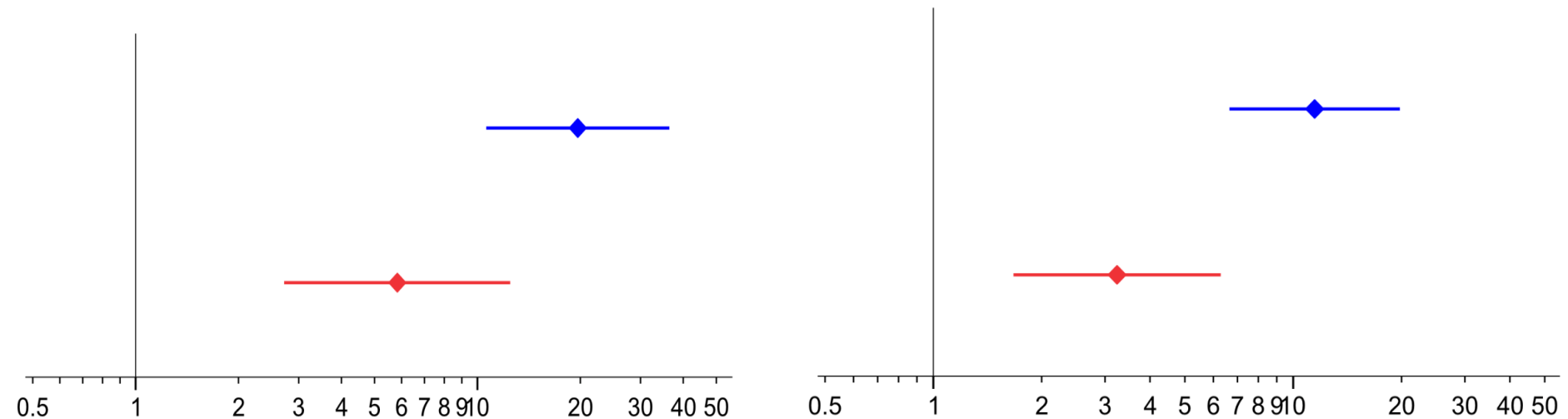




NfL är associerat till funktionellt utfall efter stroke

Serumnivå av NfL 3 mån post-stroke vs mRS 3 mån

Serumnivå av NfL 3 mån post-stroke vs mRS 2 år

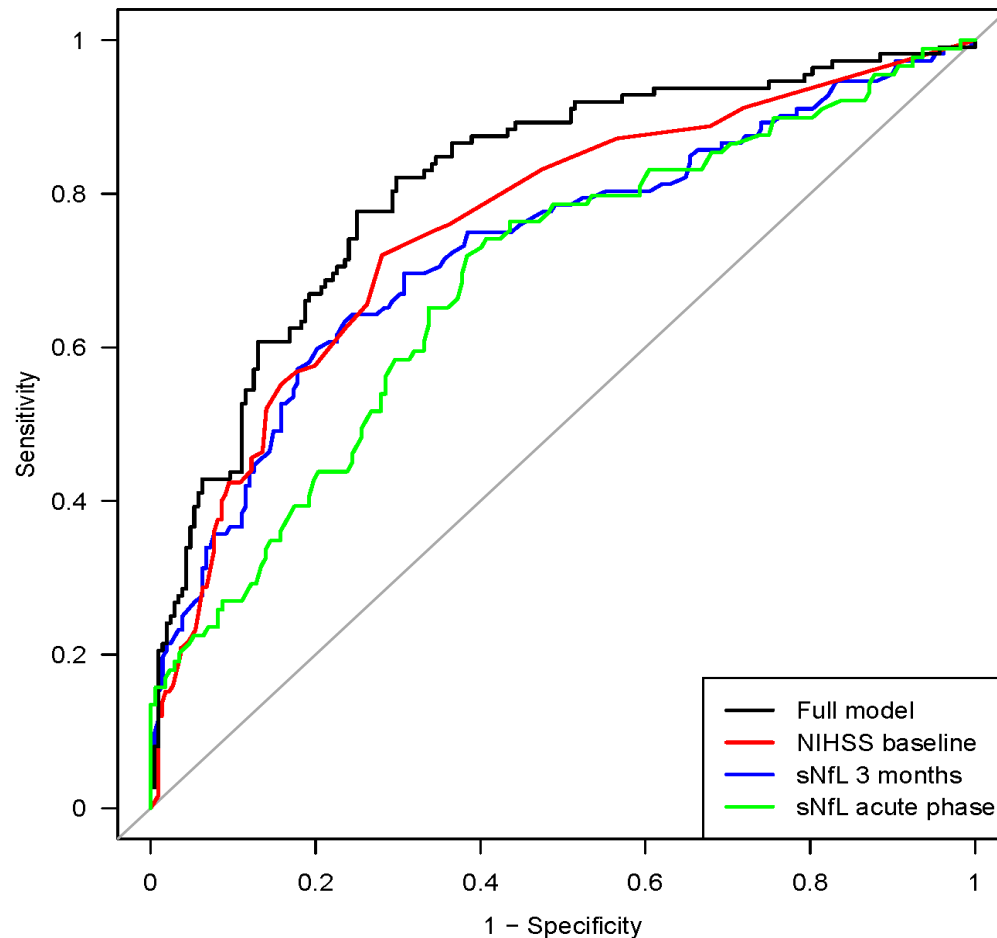


- Univariabel
- Justerad för ålder, tidigare stroke, initial svårighetsgrad (NIHSS) och provtagningsdag



NfL är associerat till funktionellt utfall efter stroke

ROC curves for good vs poor outcome (mRS 0-2 vs 3-6) at 7 years post-stroke.





scientific reports



OPEN

Investigation of 91 proteins implicated in neurobiological processes identifies multiple candidate plasma biomarkers of stroke outcome

Cecilia Lagging^{1,2,6}✉, Sofia Klasson^{1,6}, Annie Pedersen^{1,2}, Staffan Nilsson^{1,3}, Katarina Jood^{4,5}, Tara M. Stanne¹ & Christina Jern^{1,2}

- Acute phase and 3-month levels of 44 and 8 proteins, respectively, associated with 3-month neurological outcome (NIHSS score) after correction for multiple testing and adjustment for age, sex and day of blood draw.
- Three-month levels of 8 proteins were associated with 7-year neurological outcome, and the associations for BCAN and Nr-CAM were independent also of acute stroke severity.



Investigations of associations to cognitive outcome

Same cohort, case only design

N = 205	Acute phase	3 months	7 years
Plasma	X	X	X
Cognitive testing			X

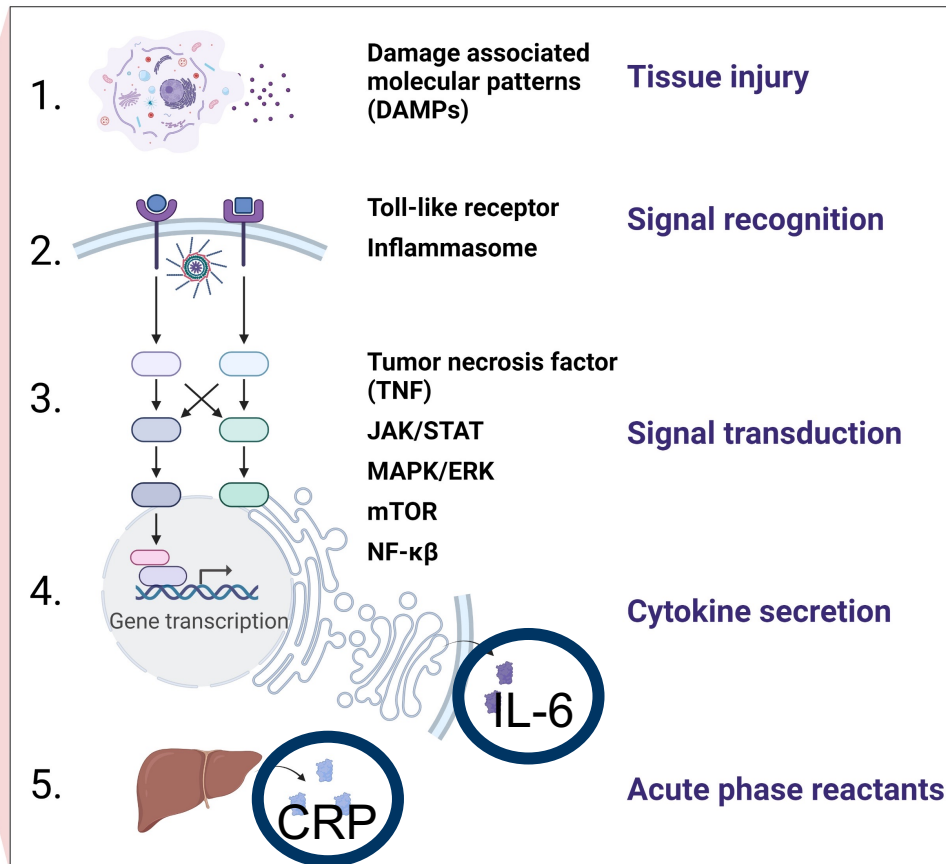


Plasma protein levels vs. 7-year cognition as assessed by the Barrow Neurological Institute Screen (BNIS) for Higher Cerebral Functions

- Nine and 8 proteins in the acute and convalescent phase, respectively, associated with cognitive outcome ($p < 0.05$).
- Associated proteins varied by sampling point, and clustered into separate groups associated to outcome independently of one-another.
- These groups corresponded to different protein classes including brain-specific proteoglycans (BCAN, NCAN); metabolic enzymes (HAGH, NMNAT1); immunological proteins (SIGLEC1, CLEC10A); one neurotrophic factors receptor (GFR-alpha-1), one bone-morphogenic-protein antagonist (VWC2); beta-nerve growth factor (beta-NGF); myostatin (GDF-8); and NfL.



Inflammation- simplified



Created with BioRender.com

- Most clinical studies focus on a few proteins
- IL-6 and hsCRP both "downstream"
- Suggested "upstream" proteins might be closer to the biology
- Hold promise as targets for new therapeutics



SAHLSIS; Inflammation panel (Olink)

Stroke

Volume 53, Issue 9, September 2022; Pages 2847-2858

<https://doi.org/10.1161/STROKEAHA.121.038349>



CLINICAL AND POPULATION SCIENCES

Longitudinal Study Reveals Long-Term Proinflammatory Proteomic Signature After Ischemic Stroke Across Subtypes

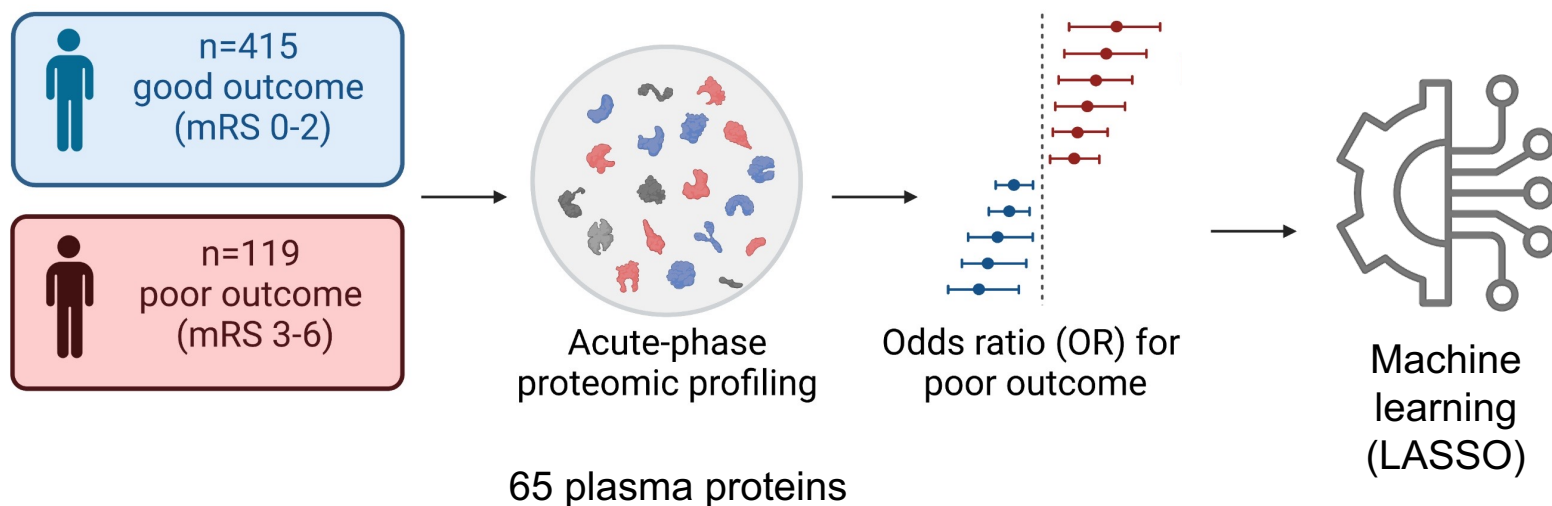
Tara M. Stanne, PhD , Annelie Angerfors, MScEng , Björn Andersson, PhD , Cecilia Brännmark, MD, PhD , Lukas Holmegaard, MD , and Christina Jern, MD, PhD 

n= 600 cases + n= 600 controls; focus ischemic stroke and subtypes



Proteomic profiling identifies novel inflammation-related plasma proteins associated with ischemic stroke outcome

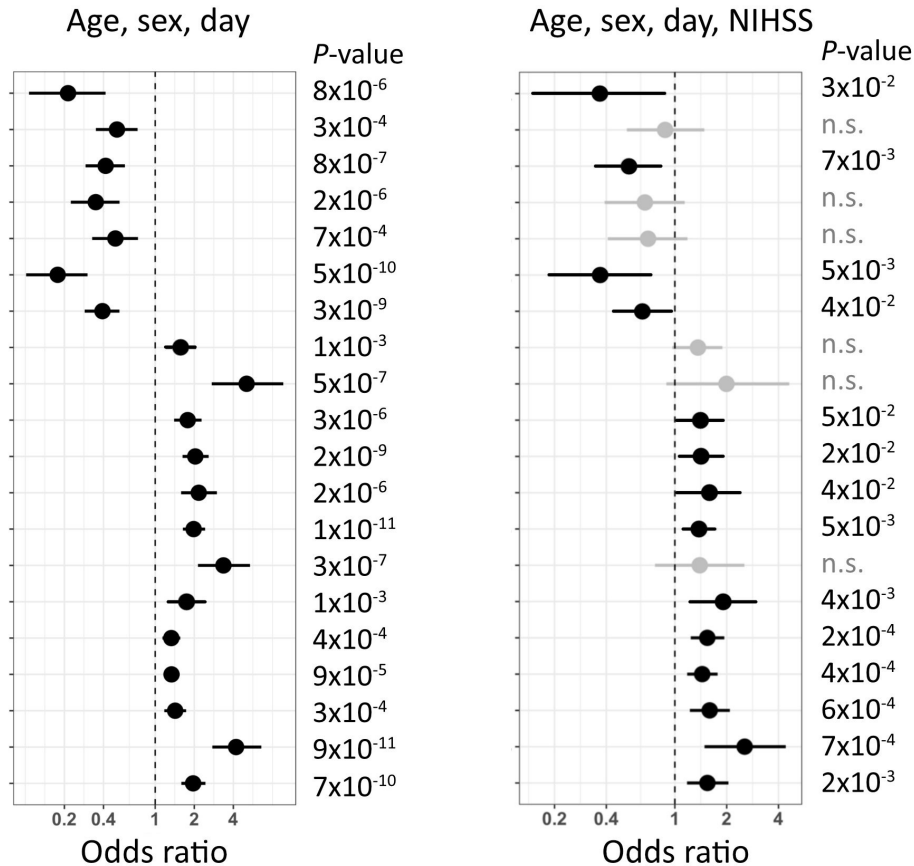
SAHLSIS





Inflammation panel vs 3-month mRS

n=20 proteins with significant associations (FDR <0.05) + n=20 suggestive ($P < 0.05$)



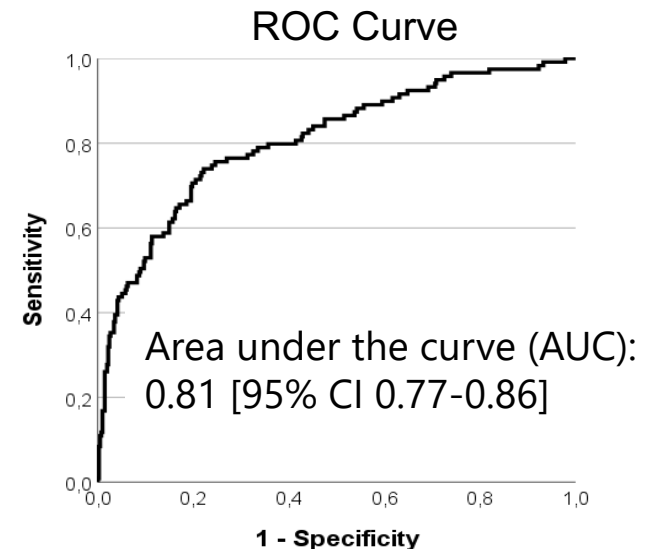
Two main clusters,
consistent with a
role in both
damage and repair



Multiprotein models



Proteins	Name [alias]
TRAIL	Tumor necrosis factor (TNF)-related apoptosis-inducing ligand [TNFSF10]
EN-RAGE	Extracellular newly identified receptor for advanced glycation end-products binding protein [S100A12]
IL-6	Interleukin - 6
OSM	Oncostatin M
Flt3L	Fms -like tyrosine kinase 3 ligand
TNFSF14	TNF superfamily member 14 [LIGHT]
CSF-1	Macrophage colony stimulating factor 1
CCL25	Chemokine (C-C motif) ligand 25 / Thymus-expressed chemokine [TECK]
HGF	Hepatocyte growth factor



AUC for NIHSS ~0.80-0.85
(depending on time point)



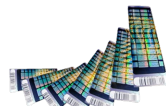
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The Sahlgrenska Academy Study on Ischemic Stroke phase 2 (SAHLSIS2)

- Initiated 2015, now >1 600 cases
- Adult stroke of all ages
- Outcomes through national registers (Riksstroke: mRS at 3 months and 1 year)
- Subgroup cognitive testing at 3 months and 2 years
- Biobanking at index stroke of whole blood (DNA), plasma (EDTA and citrate), serum, Tempus tubes (RNA)

Genomics



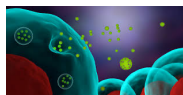
Whole genome arrays (GWAS)

Proteomics



Multiplex assays and targeted analyses

Transcriptomics



RNA sequencing (peripheral blood cells)
Small RNA sequencing (extracellular RNA)
RT-PCR for validation



Association of plasma BD-tau with functional outcome after ischemic stroke

- SAHLSIS N=454 cases and N=55 controls, and SAHLSIS2 N=363 cases
- Plasma tau measured by a novel assay that selectively quantifies BD-tau (Gonzalez-Ortiz F et al, Brain 2023)
- BD-tau more weakly correlated to day of blood draw compared to NfL (r 0.22 vs 0.59; $P < 0.001$)
- BD-tau significantly and independently associated with 3-month functional outcome (8mRS), and the effect size was higher than for NfL
- When both BD-tau and NfL were included in multivariable models only BD-tau remained significant (BD-tau OR 1.70, IQR 1.05 - 1.73, $p < 0.05$ and NfL OR 1.14, IQR 0.76 - 1.72, NS)

- Pilot study on 200 cases with broader protein profiling by OLINK Explore 3072 on acute phase plasma samples (median 3 days after index stroke)
- Binary logistic regression
- Machine learning methods
 - LASSO
 - Random Forest

SAHLSIS2			
	All ischemic stroke	3- month outcome	
		Favorable	Unfavorable
n	200	124	76
Age, median [IQR], years	70 [59-80]	66 [52-74]	79 [70-85]
Male sex, n (%)	123 (61%)	85 (68%)	38 (50%)
Hypertension, n (%)	85 (42%)	41 (33%)	44 (58%)
Diabetes mellitus, n (%)	25 (12%)	8 (6.5)	17 (22%)
Smoker, n (%)	24 (12%)	17 (14%)	7 (9%)
Thrombectomy + intravenous thrombolysis, n (%)	22 (11%)	14 (11%)	8 (10%)
Intravenous thrombolysis only, n (%)	36 (18%)	26 (21%)	10 (13%)
Thrombectomy only, n (%)	15 (8%)	6 (5%)	9 (12%)
Admission stroke severity (NIHSS), median [IQR]	3 [1-8]	2 [0-6]	5 [2-9]
24 h after recanalization therapy†, median [IQR]	2 [1-8]	1 [0-2]	9 [3-14]

† the subset of patients who underwent recanalization therapy (intravenous thrombolysis and/or mechanical thrombectomy) were rescored using NIHSS 24 hours after their procedures.

Proteomics

Olink Explore 3072



8 x 384-plex subpanels

Inflammation I + II

Cardiometabolic I + II

Neurology I + II

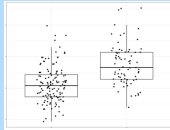
Oncology I + II

15ul
plasma

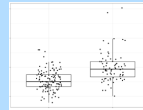


Large number of proteins from each panel significantly associated to functional outcome representing the depicted independent groups

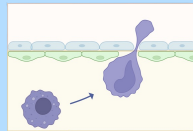
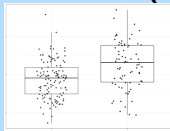
Neuroaxonal
damage
(NfL)



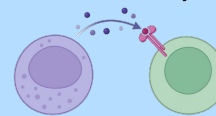
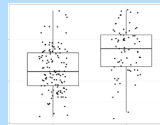
Transcriptional
regulation
(RBFOX3)



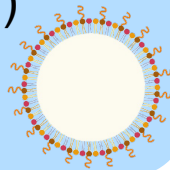
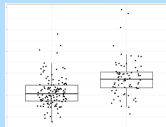
Neutrophil
chemotaxis
(LECT2)



Cytokine
signaling
(TNFRSF11B)

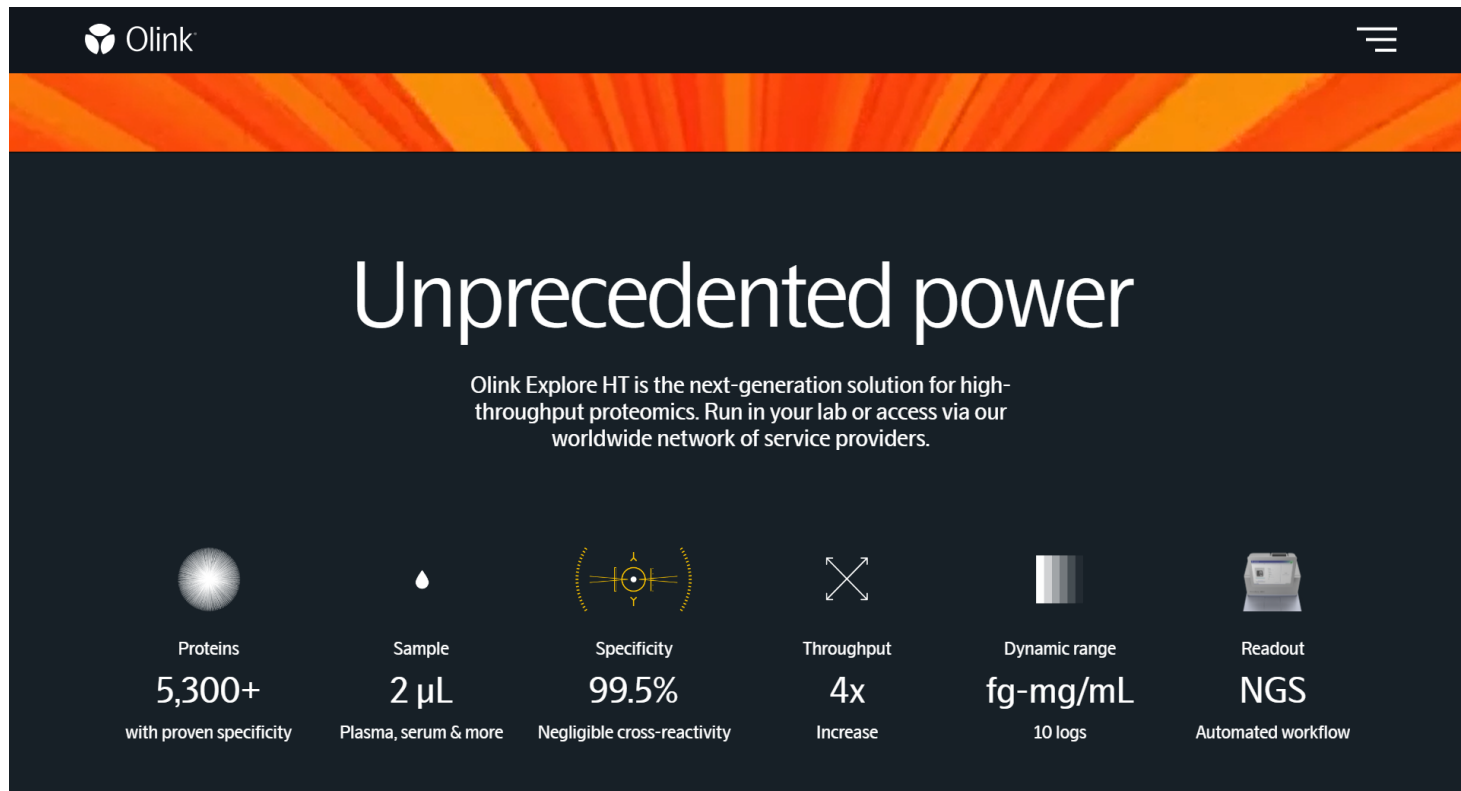


Lipid
metabolism
(PLIN1)



No clinical
variables selected
by either machine
learning method.

Olink Explore HT



The image is a screenshot of the Olink Explore HT website. At the top, there is a dark blue header with the Olink logo on the left and a hamburger menu icon on the right. Below the header is a wide orange and yellow sunburst graphic. The main content area has a dark blue background. The headline 'Unprecedented power' is centered in large white text. Below it, a paragraph states: 'Olink Explore HT is the next-generation solution for high-throughput proteomics. Run in your lab or access via our worldwide network of service providers.' At the bottom, there are six feature cards arranged horizontally. Each card has an icon, a title, a value, and a descriptive note.

Proteins	Sample	Specificity	Throughput	Dynamic range	Readout
5,300+	2 μ L	99.5%	4x	fg-mg/mL	NGS
with proven specificity	Plasma, serum & more	Negligible cross-reactivity	Increase	10 logs	Automated workflow

- We will perform a new pilot on 200 cases from SAHLSIS2 to replicate Explore 3072 and identify further novel biomarkers in coming months....

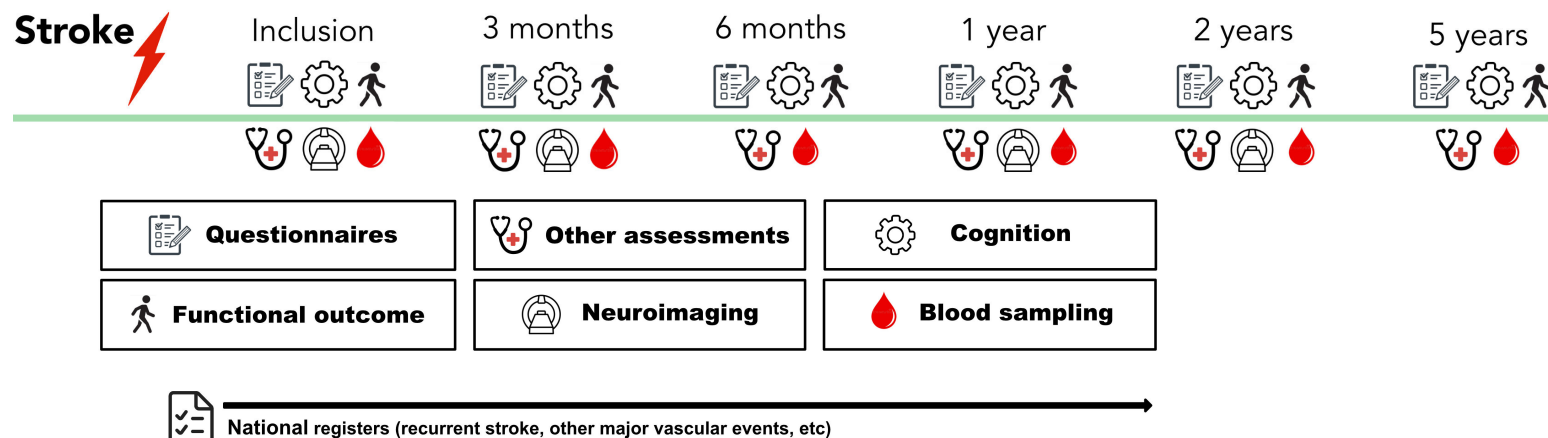


The FIND Stroke Recovery Study

The FIND Stroke Recovery Study (FIND): Rationale and protocol for a longitudinal observational cohort study of trajectories of recovery and biomarkers post-stroke

Cecilia Brännmark, Sofia Klasson, Tara M Stanne, Hans Samuelsson, Margit Alt Murphy, Katharina Stibrant Sunnerhagen, David N Åberg, Oscar Jalnefjord, Isabella M Björkman-Burtscher, Katarina Jood, Turgut Tatlisumak, and Christina Jern. BMJ Open 2023;13(5):e072493.

Overview of study protocol



Primary aims :

- Determine trajectories of global as well as specific domains of cognition and neurological function after stroke.
- Identify clinical variables, as well as neuroimaging and blood biomarkers, predicting post-stroke cognitive and neurological outcomes.



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Ischemisk stroke – hög risk nya vaskulära händelser

THE SAHLGRENSKA ACADEMY
INSTITUTE OF BIOMEDICINE

Thromb Haemost 2016; 116(03): 537-543
DOI: 10.1160/TH15-12-0938



Download PDF

Stroke, Systemic or Venous Thromboembolism

Schattauer GmbH

Haemostatic biomarkers are associated with long-term recurrent vascular events after ischaemic stroke

Annie Pedersen , Petra Redfors , Linnea Lundberg , Ann Gils , Paul J. Declerck , Staffan Nilsson , Katarina Jood , Christina Jern



Stroke 2023 May;54(5):1289-1299.

ORIGINAL ARTICLE

C-Reactive Protein, Interleukin-6, and Vascular Recurrence After Stroke: An Individual Participant Data Meta-Analysis

John J. McCabe¹, PhD*; Cathal Walsh², PhD*; Sarah Gorey³, MB; Katie Harris⁴, PhD; Pablo Hervella, PhD; Ramon Iglesias-Rey, PhD; Christina Jern⁵, PhD; Linxin Li⁶, DPhil; Nobukazu Miyamoto, MD; Joan Montaner⁷, PhD; Annie Pedersen, PhD; Francisco Purroy⁸, PhD; Peter M. Rothwell⁹, FMedSci; Catherine Sudlow¹⁰, PhD; Yuji Ueno¹¹, MD; Mikel Vicente-Pascual¹², MD; William Whiteley¹³, PhD; Mark Woodward¹⁴, PhD; Peter J. Kelly¹⁵, MD

10 studies

8,420 patients with ischemic stroke or TIA

18,920 person years of follow-up

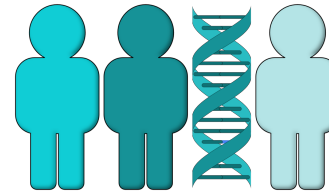
1,407 MACE;

C-Reactive Protein, Interleukin-6, and Vascular Recurrence According to Stroke Subtype: An Individual-Participant Data Meta-analysis.

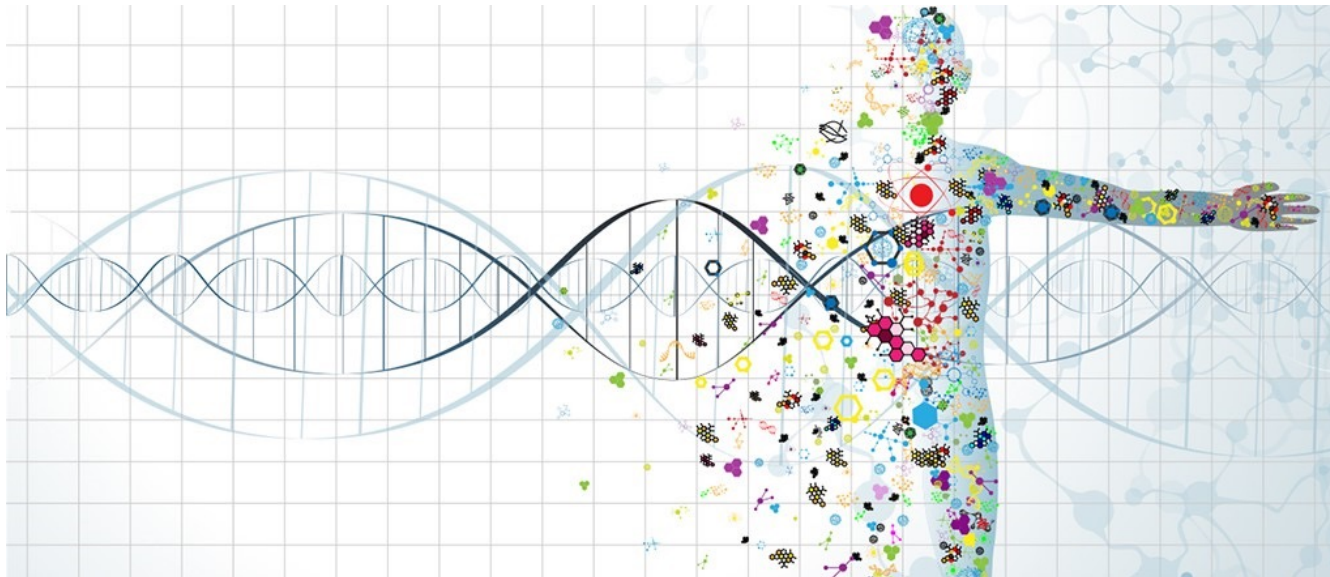
McCabe JJ, et al
Presentation at ESOC 2023
Manuscript submitted



Precisionsmedicin



- Flyttar tonvikten inom medicin från reaktion till prevention
- Stor potential för framtiden – mer individbaserat omhändertagande
- Utmaningar – etiska, sociala och legala aspekter





THANK YOU

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 Lukas Holmegaard, MD, PhD student
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Collaborators, e.g.

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 David Åberg, MD, PhD, Assoc. Prof.

 Marcela Davila, PhD, Assoc. Prof. and coworkers at
 Bioinformatics and Data Centre

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 Staffan Nilsson, Assoc. Prof.

 Henrik Zetterberg, MD, PhD, Prof.
 Kaj Blennow, MD, PhD, Prof.
 Fernando Gonzalez Ortiz, MD, PhD student

**... and all study participants
 and their families**



**Konung Gustaf V:s
 Drottning Victorias
 Stiftelse**



**International
 Stroke
 Genetics
 Consortium
 ISGC**