Proteomic study in the acute phase of stroke

Farmacogenómica y Genética Neurovascular
Institut de Recerca de Sant Pau

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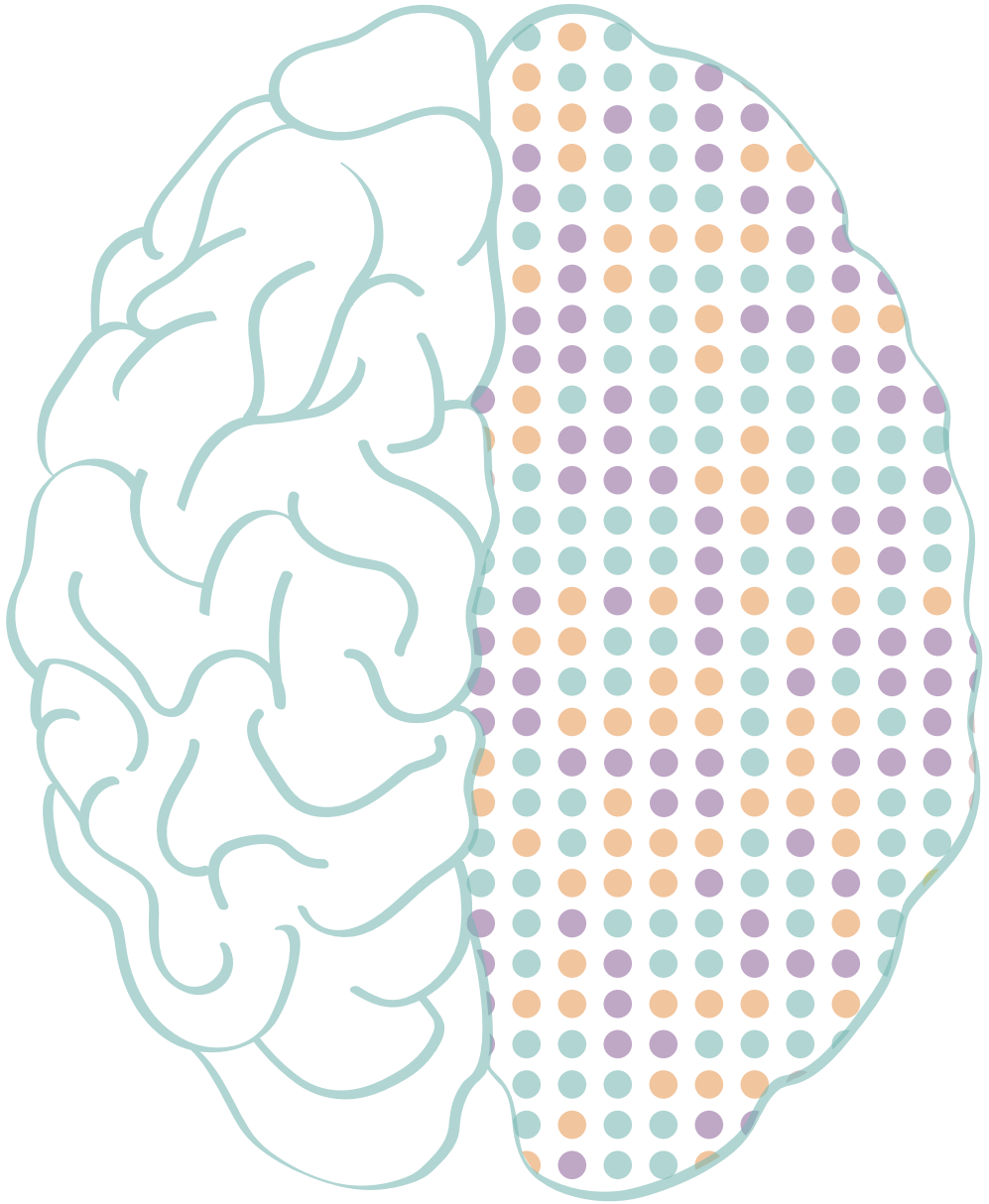
04/03/2024



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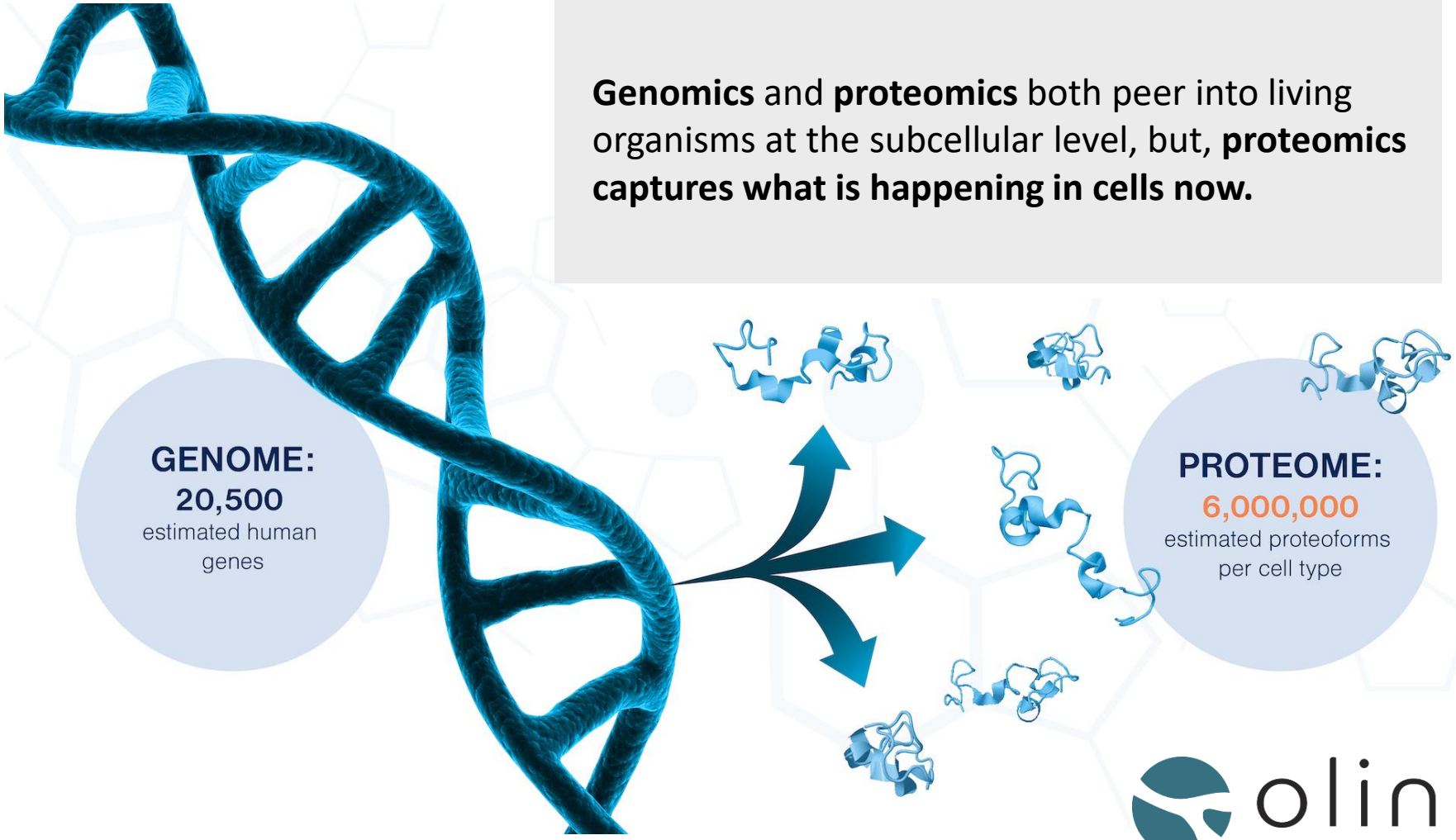
Proyectos de Investigación de Medicina Personalizada de Precisión de la Acción Estratégica en Salud del Instituto de Salud Carlos III

Introduction

Genomics and **proteomics** both peer into living organisms at the subcellular level, but, **proteomics captures what is happening in cells now.**



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Introduction

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Large-scale plasma proteomics comparisons through genetics and disease associations

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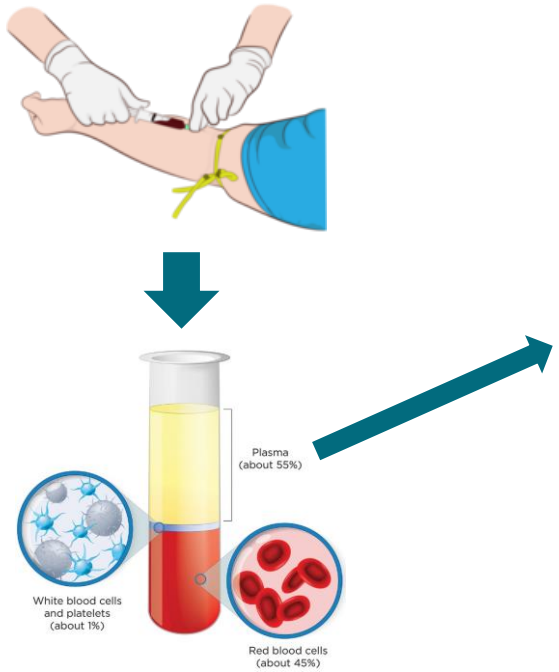
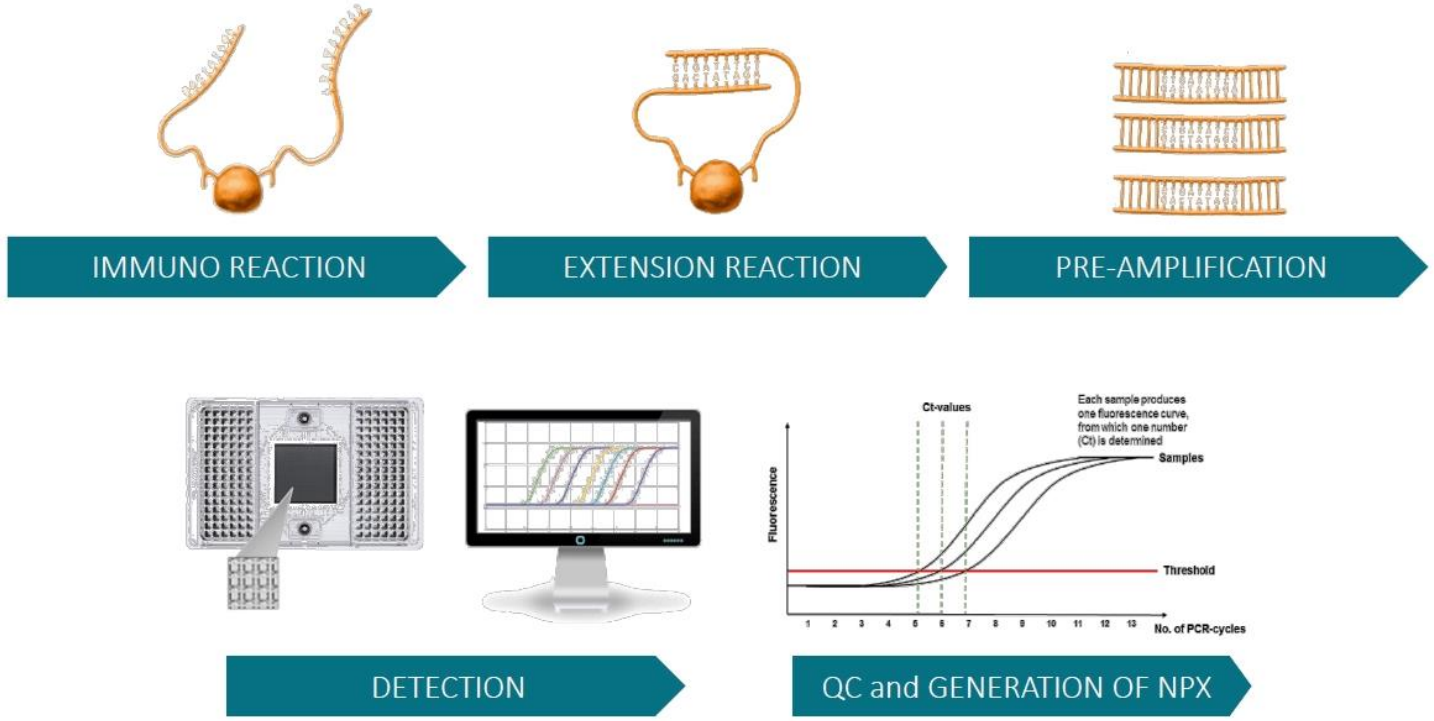
Abstract

High-throughput proteomics platforms measuring thousands of proteins in plasma combined with genomic and phenotypic information have the power to bridge the gap between the genome and diseases. Here we performed association studies of Olink Explore 3072 data generated by the UK Biobank Pharma Proteomics Project[1] on plasma samples from more than 50,000 UK Biobank participants with phenotypic and genotypic data, stratifying on British or Irish, African and South Asian ancestries. We compared the results with those of a SomaScan v4 study on plasma from 36,000 Icelandic people[2], for 1,514 of whom Olink data were also available. We found modest correlation between the two platforms. Although *cis* protein quantitative trait loci were detected for a similar absolute number of assays on the two platforms (2,101 on Olink versus 2,120 on SomaScan), the proportion of assays with such supporting evidence for assay performance was higher on the Olink platform (72% versus 43%). A considerable number of proteins had genomic associations that differed between the platforms. We provide examples where differences between platforms may influence conclusions drawn from the integration of protein levels with the study of diseases. We demonstrate how leveraging the diverse ancestries of participants in the UK Biobank helps to detect novel associations and refine genomic location. Our results show the value of the information provided by the two most commonly used high-throughput proteomics platforms and demonstrate the differences between them that at times provides useful complementarity.

Introduction





Olink

Pairs of antibodies labelled with DNA oligonucleotides bind to the target antigen in solution, allowing hybridization and extension by DNA polymerase. This newly created DNA barcode fragment is amplified by standard PCR before transfer to the integrated microfluidic chip (IFC), which is loaded onto the instrument for qPCR and data readout.

Background

There is a lack of proteome-wide studies in stroke. Proteomic data could be very useful to find potential treatments and to understand the biological mechanisms associated with stroke.

Objective

Objective

To perform a proteome-wide study in stroke to analyse multiple phenotypes to identify the biological pathways associated with stroke risk, stroke outcome and acute treatment response.

Methodology

Cohort description

This study involved **535 plasma samples** from 9 Spanish hospitals.

The cohort included:

- **398 ischaemic strokes**
- **45 haemorrhagic strokes**
- **45 mimics**
- **40 population controls**
- **7 CADASIL**

inibic — Mª Del Mar Castellano

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MULTICENTRIC STUDY

Methodology

ANALYSIS

a) Risk factors, biomarkers of stroke diagnosis.

- Ischaemic stroke vs. control. **IIB-Sant Pau.**
- Ischaemic stroke vs mimics. **IBIS**
- Haemorrhagic stroke vs controls. **IBIS**
- Stroke (ischaemic + haemorrhagic) vs. mimics. **IBIS**
- Ischaemic vs. haemorrhagic stroke. **IBIS**
- Stroke aetiologies (CES, AT, SVD, UND). **IBIS**

b) rt-PA/thrombectomy.

- Haemorrhagic transformation yes/no. **IIB-Sant Pau**
- Futile recanalisation. **IIB-Sant Pau/IBIS**

c) Evolution.

- dNIHSS, baseline NIHSS. **IIB-Sant Pau.**
- dmRS, mRS third month. **IIB-Sant Pau.**
- Post-stroke infections. **IIB-Sant Pau.**
- Post-stroke temperature. **Albacete.**
- Post-stroke cognitive impairment. **IIB-Sant Pau.**

d) Other

- Pre-stroke infections. **Albacete**

Institut de Recerca Sant Pau

IBiS INSTITUTO DE BIOMEDICINA DE SEVILLA

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Ischaemic stroke vs. control

Inclusion criteria

1. Patients with an episode of ischemic stroke, Mimic or population controls.
2. Patients with plasma for proteomic study and GWAS data, DNA or blood for genetic study.
3. Patients over 18 years of age.
4. Patients of European ethnic origin.
5. Samples collected less than 10 years ago.
6. In ischemic stroke, the plasma was collected during the first 6 hours from symptom onset and before treatment.

Methodology

Objectives

1. Understanding the biological mechanisms by which Ischaemic stroke occurs.

- **Analysis**

Associated variables

Logistic regression

- Age
- Sex

1. **39 (Population controls) vs 383 (IS)**
2. **84 (Population controls + Mimics) vs 383 (IS)**

Statistical analysis

Linear regression with **Limma**

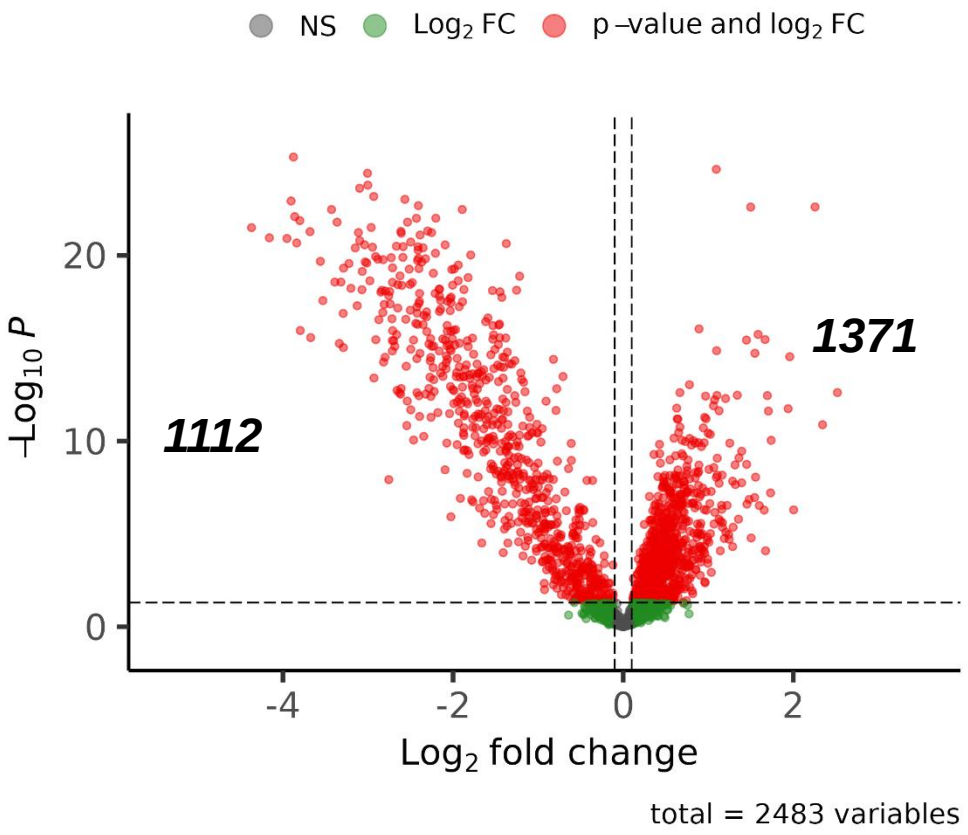
NPX ~ Phenotype + clinical variables

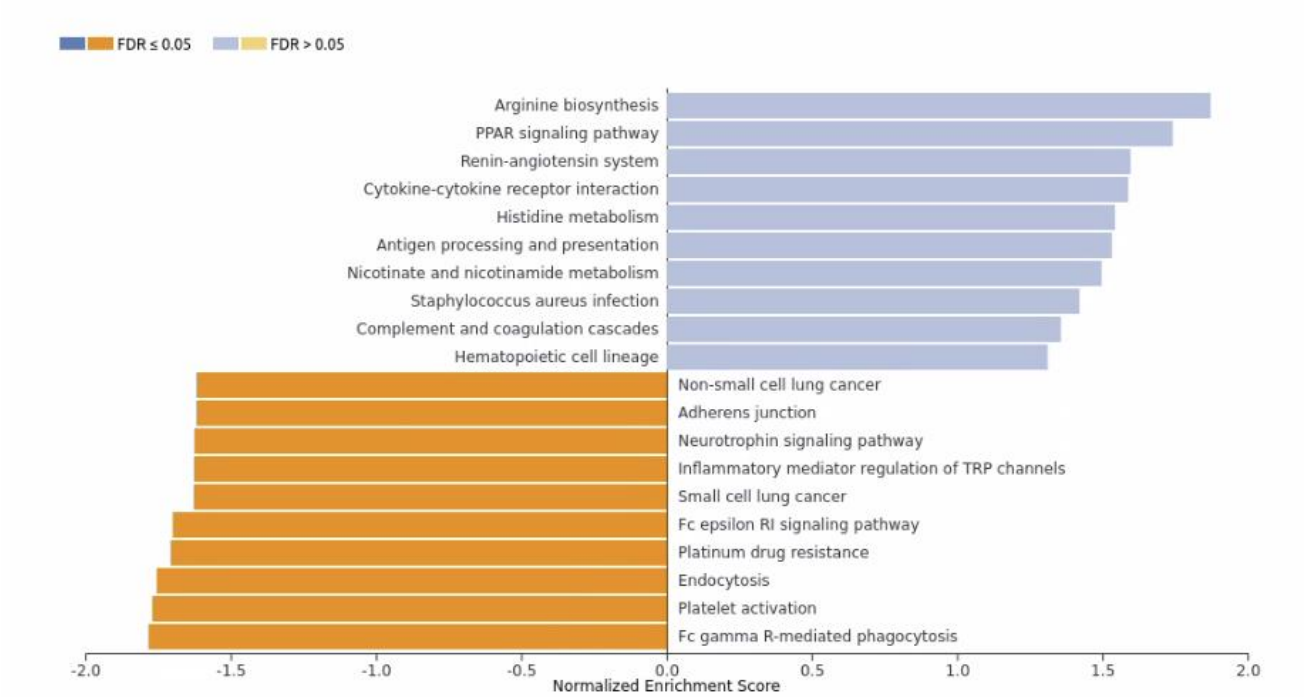
GSEA (Gene set enrichment analysis)

Results

1. 39 (Population controls) vs 383 (IS)

- **Linear regression**
- **Covariates: Sex + age**
- **2483 Proteins**
- **1507 proteins pass the FDR cut-off**

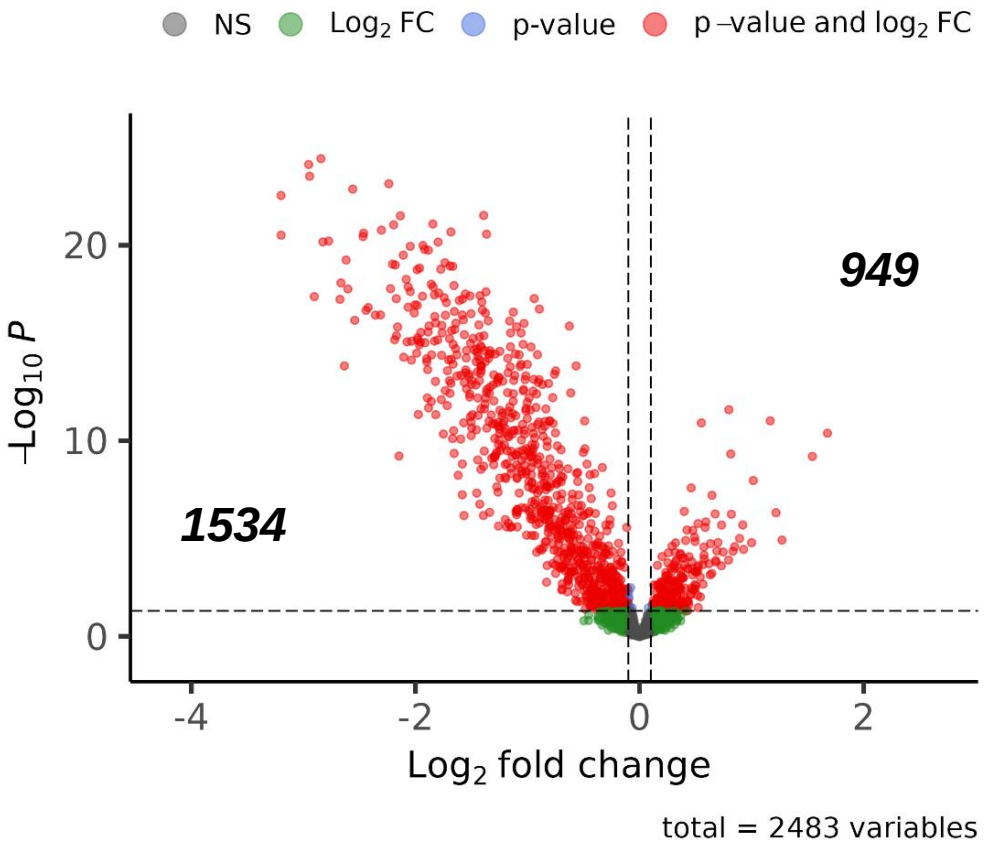


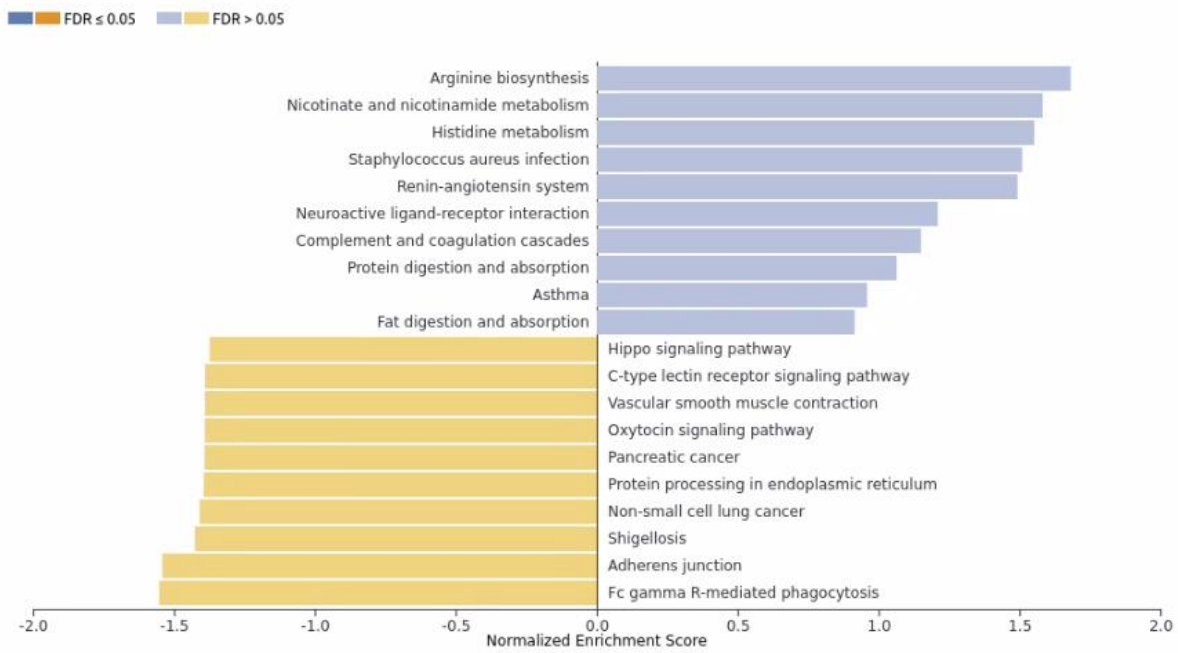


Results

2. 84 (Population controls + Mimics) vs 383 (IS)

- **Linear regression**
- **Covariates: Sex + age**
- **2483 Proteins**
- **1007 proteins pass the FDR cut-off**



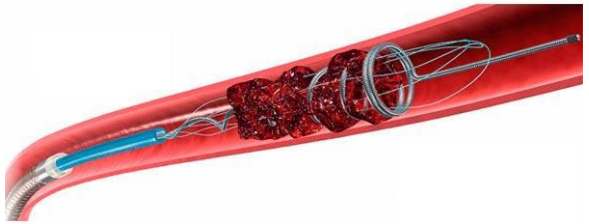


Hemorrhagic transformation

Introduction

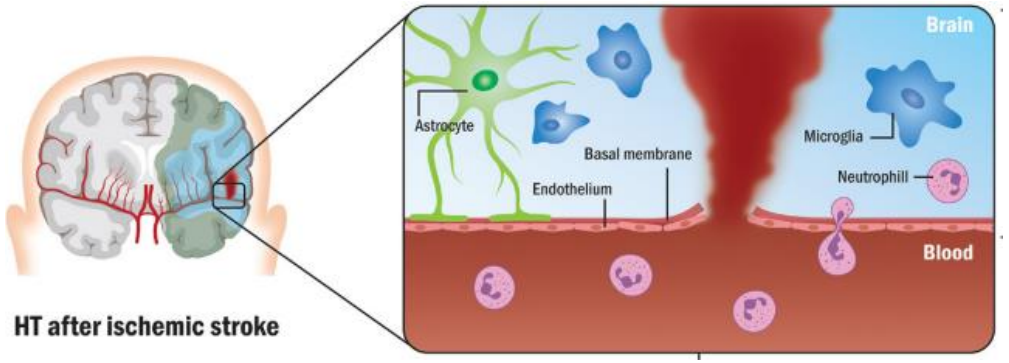
Reperfusion treatments

- Thrombolytic therapy with rtPA (tissue plasminogen activator)
- Mechanical thrombectomy



Hemorrhagic Transformation (HT)

- **There is extravasation of blood, generally into the infarcted zone.**
- 15-20% of patients treated with rtPa suffer from HT.

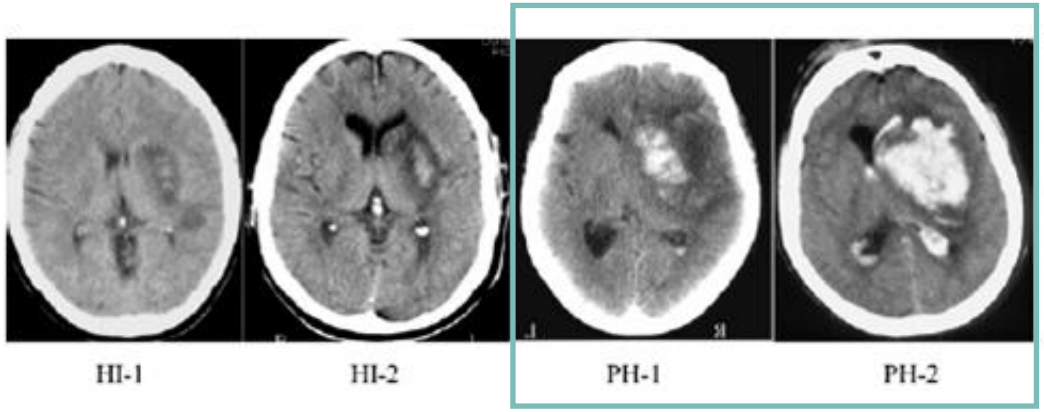


Introduction

Parenchymal hemorrhage

- **Parenchymal Hematoma Type 1 (PH-1):** Bleeding occurs in <30% of infarcted tissue with moderate mass effect.
- **Parenchymal Hematoma Type 2 (PH-2):** Bleeding involves >30% of infarcted tissue and greater mass effect.

- The frequency of PH1 and PH in CT at 22-36 hours is 2.6-2.5%. They worsen the functional and vital prognosis of the patients *(Wahlgren N, et al., Lancet, 2007).*



Class	Type	Description
1	Hemorrhagic transformation of infarcted brain tissue	
1a	HI1	Scattered small petechiae, no mass effect
1b	HI2	Confluent petechiae, no mass effect
1c	PH1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect
2	Intracerebral hemorrhage within and beyond infarcted brain tissue	
	PH2	Hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect
3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage	
3a	Parenchymal hematoma remote from infarcted brain tissue	
3b	Intraventricular hemorrhage	
3c	Subarachnoid hemorrhage	
3d	Subdural hemorrhage	

Heidelberg bleeding classification (Ji Man Hong, et al., frontiers in Neurology, 2021)

Introduction

Risk factors for HT

Associated factors	High risk	Low risk
Clinical features		
Age (21)	Old	Young
Sex (21)	Male	Female
Weight (66)	Obese	Normal weight
Temperature (67)	Fever	Normothermia
Glucose (68)	Hyperglycemia	Normoglycemia
Blood pressure (68)	Hypertensive	Normotensive
Variability of blood pressure (69)	Yes	No
Stroke severity (21)	Severe stroke (≥22 on NIHSS)	Mild stroke (1–5 on NIHSS)
Size/type of infarct (21)	Large/embolic territorial (MCA, ACA, PCA, cerebellar)	Small/lacunar or small vesseldisease
Atrial fibrillation (21)	Yes	No
Congestive heart failure (22)	Yes	No
Renal impairment (70)	Yes	No
Previous stroke (21)	Yes	No
Diabetes (21)	Yes	No
Platelet count (16)	Low	No
Previous antiplatelet treatment (68)	Yes	No
OTT (66)	Late (≥ 180 min)	Early (< 180 min)
ERT (71)	Late (> 6 h)	Early (≤ 6 h)
Biochemical factors		
MMP-9/c-Fn (70)	High	Low
Fibrinogen (16)	Low	High
Ferritin (28)	High	Low
S100B (72)	High	Low
TAFI (73)	High	Low
PAI-1 (73)	Low	High
VAP-1/SSAO activity (70)	High	Low
APC (28)	High	Low
PDGF-CC (74)	High	Low
Genetics		
Leukocyte mRNA (MCFD2, VEGI/AREG, MARCH7, SMAD4) (75)	Low/High	High/Low
A2M (76)	High	Low
Factor FXII (76)	Low	High
Factor FXIII V34L (77)	High	Low
Imaging findings		
Early signs of ischemia (21)	Yes	No
Focal hypodensity, edema, mass effect on baseline (20)	Yes	No
Leukoaraiosis (22)	Yes	No
Collateral flow (29)	Low	High
ADC value (80)	Low	High
Cerebral blood flow or volume (28)	High	Low
Infarct volume on DWI (25)	Large	Small

Risk factors for HT (Ji Man Hong, et al., frontiers in Neurology, 2021)

Introduction

Previous studies have shown that there is a genetic component associated with response to reperfusion treatments.

- In a recent study using the GWAS in a cohort of 1,904 Ischemic strokes treated with rTPA a locus in the ***ZBTB46* gene** was associated with the occurrence of HT post rtPA *(Carrera et al. Brain 2021).*
- In another published meta-analysis involving 216 cases of PH and 1818 controls (Ischemic strokes treated with rTPA /mechanical thrombectomy), the ***RP11-362K2.2: RP11-767I20.1 gene*** was associated with the onset of PH *(Muiño et al. Journal Clinical Medicine 2021)*.
- There is a **modest genetic-clinical score** to predict the risk of severe HT (*Del Rio-Espinola et al. Ann of Neurol 2012*) validated (*Carrera et al. Neurology 2019*).

Inclusion criteria

1. Patients with an episode of ischemic stroke treated with rt-PA and/or thrombectomy.
2. Patients with plasma for proteomic study and GWAS data, DNA or blood for genetic study.
3. Patients over 18 years of age.
4. Patients of European ethnic origin.
5. Samples collected less than 10 years ago.
6. In ischemic stroke, the plasma was collected during the first 6 hours from symptom onset and before rt-PA or thrombectomy treatment.

Methodology

- **Analysis**

Objectives

1. Understanding the biological mechanisms by which PH occurs.
2. Finding potential biomarkers for PH.

Associated variables

Logistic regression

1. **264 (No HT) vs 65 (PH)** →

2. **306 (Including HT) vs 65 (PH)** →

Statistical analysis

Linear regression with **Limma**

NPX ~ Phenotype + clinical variables

↓

GSEA (Gene set enrichment analysis)

Linear regression with **Limma**

Protein ~ Phenotype

↓

Logistic regression

Phenotype ~ Proteins

Phenotype ~ clinical variables

Phenotype ~ Proteins + clinical variables

Results

Objectives

1. Understanding the biological mechanisms by which PH occurs.

- **Analysis**

Associated variables

Logistic regression

- Age
- Sex
- Reperfusion treatment
- TOAST
- Diabetes

1. **264 (No HT) vs 65 (PH)**

Statistical analysis

Linear regression with **Limma**

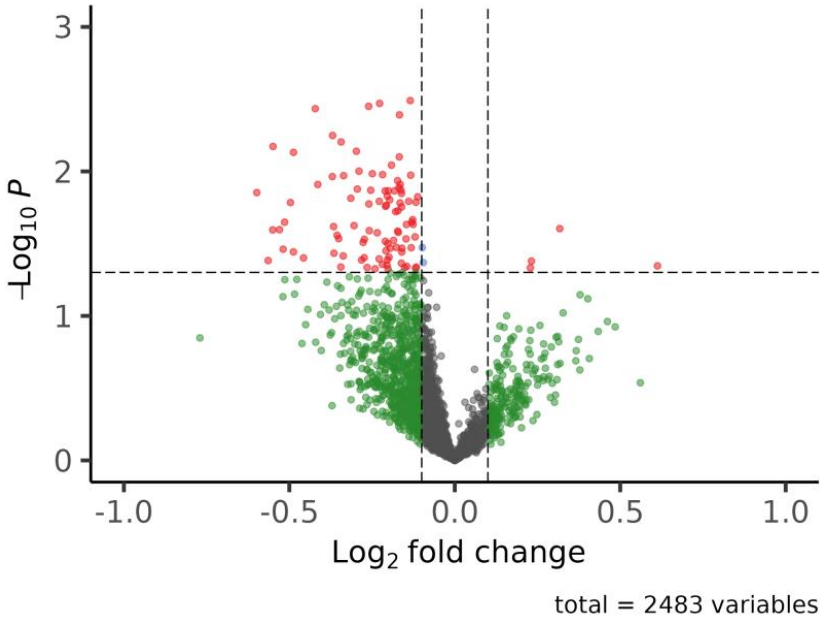
Phenotype ~ Age + Sex + Treatment + TOAST + Diabetes

GSEA (Gene set enrichment analysis)

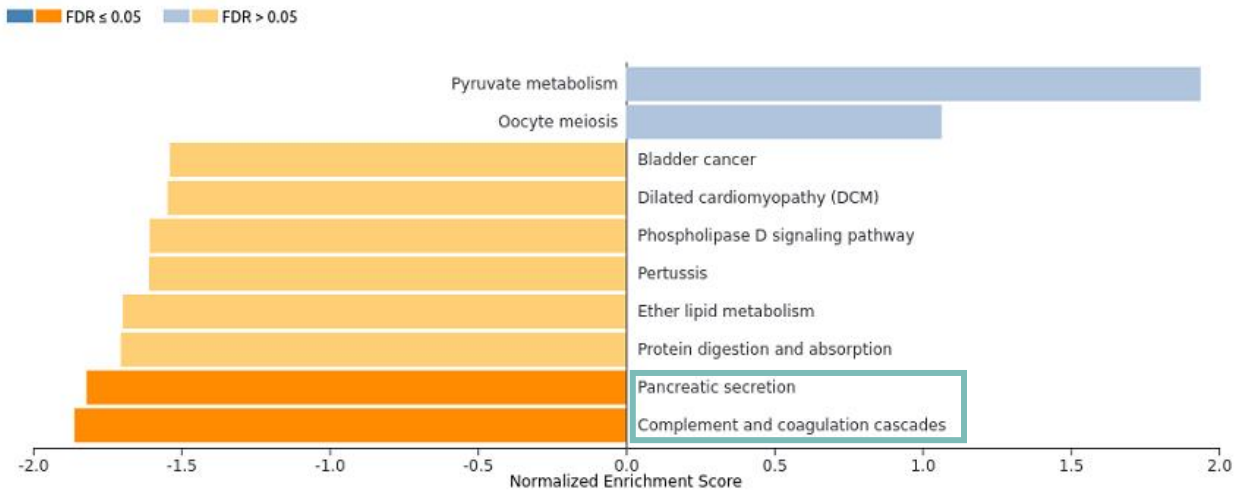
Results

1. 264 *(No HT)* vs 65 (PH)

- **Linear regression**
- **2483 Proteins**
- **No protein passes FDR cut-off**



- **GSEA (Gene set enrichment analysis)**



Results

- **Analysis**

Objectives

1. Understanding the biological mechanisms by which PH occurs.
2. Finding potential biomarkers for PH.

Associated variables

Logistic regression

- Age
- Sex
- Reperfusion treatment
- TOAST
- Diabetes

1. **264 (No HT) vs 65 (PH)**

2. **306 (Including HT) vs 65 (PH)**

Statistical analysis

Linear regression with **Limma**

Phenotype ~ Age + Sex + Treatment + TOAST + Diabetes

GSEA (Gene set enrichment analysis)

Linear regression with **Limma**

Protein ~ Phenotype

Logistic regression

Phenotype ~ Proteins

Phenotype ~ Age + Sex + Treatment + TOAST + Diabetes

Phenotype ~ Proteins + Age + Sex + Treatment + TOAST + Diabetes

3 Potential biomarkers

Next steps

1. We continue with the analysis of the evolution of stroke.

2. We are going to genotype the 535 patients with Axiom Spain Biobank Array.

3. We are collecting the replication of the analysis (N=200).

Conclusions

Our goal is to analyse stroke risk, outcome and acute treatment response.

Pathway analysis identified that cell-cell adherents junctions, endocytosis, platelet activation and inflammation pathways were over-represented in IS.

Pathway analysis identified that the complement and coagulation cascades are overrepresented in patients with PH.

Our study identifies three proteins as potential biomarkers for PH.

In brief, we will genotype the discovery cohort.

We are collecting the replication cohort.

THANK YOU FOR YOUR ATTENTION

Funded by Previctus Project.
Proyectos de Investigación de Medicina Personalizada de Precisión de la Acción Estratégica en Salud del Instituto de Salud Carlos III

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https://strokemics.com/previctus/