PREDICTUS - USE OF SYSTEM BIOLOGY TO PREDICT AND DIAGNOSE TRANSITORY ISCHEMIA AND ISCHEMIC TOLERANCE

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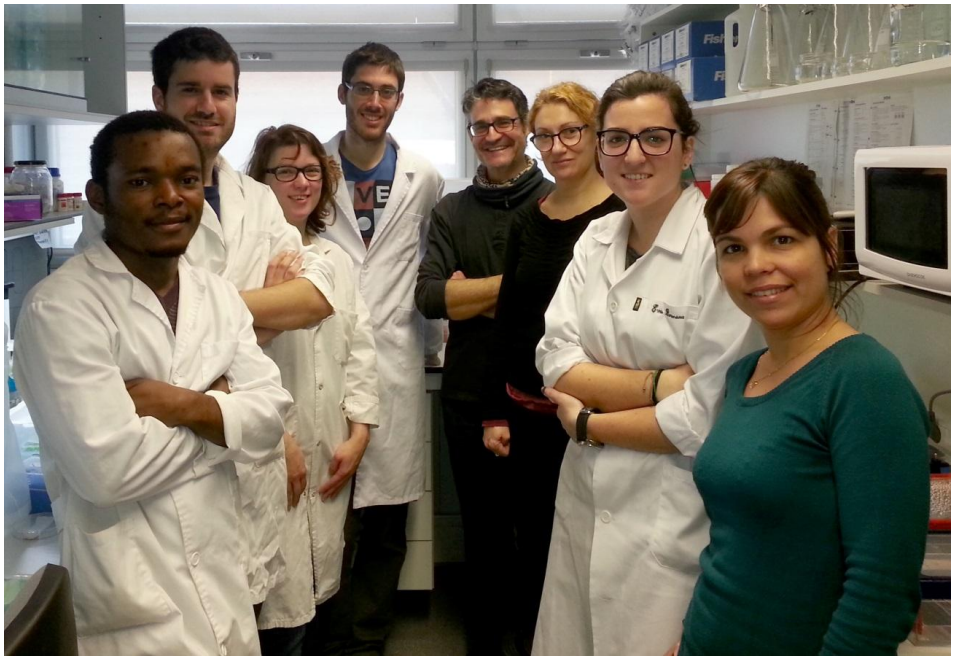

1. Abstract

Stroke is the leading cause of acquired neurological disability. Transient ischemic attacks (TIA) preceded stroke in almost 20% of cases. These "warning events" provide a great opportunity for prevention. For instance, the risk of suffering a stroke is particularly high during the few days after symptoms onset. But TIA patients are a very heterogeneous group in terms of symptoms, risk factors, underlying pathology and early prognosis. Any method to target extensive diagnostic and treatment intervention to patients who really need it is of maximum interest. Paradoxically, it has been well documented in animal models that transient periods of ischemia have a protective effect against later episodes of permanent cerebral ischemia (ischemic tolerance). This phenomenon has not been formally proven in human clinical practice. To fill those gaps, we have performed a multidisciplinary and translational project that has developed an animal model of TIA, ischemic stroke and ischemic tolerance through medial cerebral artery compression. Moreover after metabolomic analysis, we have identified new biomarkers related to stroke recurrence after TIA and large artery disease (3 lysophosphatidylcholines). Moreover, we have recognized a metabolomics signature in those ischemic stroke patients with a recent previous TIA (ischemic tolerance) in human. Finally an analysis of SNPs has identified patients under antithrombotic treatment that present risk of early stroke event.

2. Results

HUMAN SUBSTUDY

Stroke recurrence biomarkers

Metabolomic analysis performed in two consecutive and independent cohorts of TIA patients (131 and 162 subjects) revealed biomarkers related to stroke recurrence (SR), early stroke recurrence and large artery disease (LAA). Lysophosphatidylcholine (LysoPC)(16:0) and LysoPC(20:4) arose as a potential stroke recurrent biomarkers. Finally, a potential LAA biomarker, the LysoPC(22:6) was also described.

Biomarkers related to cardioembolic etiology among TIA pacients

TIA patients have a higher proportion of undetermined etiology than ischemic stroke

patients. Therefore, the etiological classification of patients with transient ischaemic attack (TIA) is a difficult endeavour. Interestingly, after the quantification of concentrations of interleukin-6 (IL-6), tumour necrosis factor-alpha, neuron-specific enolase, high-sensitivity C-reactive protein, IL-1-a and the N-terminal pro-B type natriuretic peptide (NT-proBNP) in the serum of 140 patients with TIA we identified, how high levels of NT-proBNP determined during the first 3 months after a TIA were associated with cardioembolism and the diagnosis of atrial fibrillation.

Biomarkers related to neuroimaging findings

Diffusion weighted imaging (DWI) is very sensitive to detect acute brain ischemia. For this reason it is the preferred neuroimaging modality among TIA patients. Stroke recurrence is associated with positive DWI and specific DWI pattern like scattered pattern. Again, after the study of the two TIA cohorts, we observed specific metabolomic patterns related to positive DWI, DWI lesion patterns and lesion volume.

Human ischemic tolerance

Among 246 ischemic stroke patients, 9.4% had a previous recent TIA within 7 days (ischemic tolerance). Interestingly, patients with previous TIA had significantly smaller infarctions than patients without. Moreover, we observed a metabolomic signature that was related to recent previous TIA.

Stroke acenocoumarol pharmacogenetic study

The main stroke prevention treatment among atrial fibrillation patients is oral anticoagulation such as acenocoumarol (AC). Genes involved in AC metabolism may suffer variations in a single nucleotide (SNP). These SNPs can affect AC target enzymes (VKORC1), enzymes responsible for AC degradation (CYP2C9) or enzymes responsible for vitamin K inactivation (CYP4F2). We genotyped these SNPs in a cohort of patients with AF treated with AC who suffered a stroke (n=36) and a matched cohort of long term anticoagulated (>2 years) AF patients without any vascular event (n= 44). DNA was extracted from blood, and genotyping was done by DNA sequencing. No differences were observed in the vascular risk profile and SNP frequency of both groups. However, diabetes mellitus and CYP4F2 and rs2108622 polymorphism were significantly associated with early stroke. Therefore, patients presenting these polymorphisms could be good candidates to be treated with new oral anticoagulants in order to prevent early ischemic stroke events.

ANIMAL SUBSTUDY

Development of animal model of stroke

We established three mouse models: TIA, stroke and ischemic tolerance. For this purpose, we developed a practical mouse model by compressing the distal middle cerebral artery (MCA) with a blunted micropipette for 7 minutes (TIA model) and for 25 minutes (stroke model). Plasma was collected at basal time and at 6h, 12h, 24h and 48h for metabolomic analysis. Moreover, for the development of ischemic tolerance, mice underwent 5 consecutive occlusions of 2 minutes with a gap of 5 minutes. After a recovery of 24 hours, 60 minutes occlusion was performed. Just before stroke (basal) and 1h, 3h, 6h, 12h and 24h post stroke ischemic cortical brain tissue and plasma were obtained for mRNA and metabolomics assay. Sham model consisted of surgery only mice. As in the other models, the skin and temporalis muscle were dissected until the squamous part of temporal bone was clearly exposed, then a $3mm^2$ aperture was performed. Subsequently, we compressed an area just next to MCA without any drop in cerebral blood flow.

Metabolomics signature of transient ischemia, persistent ischemia and ischemic tolerance

Metabolomic analysis allowed us to separate between different times on same treatment (except for SHAM) and SHAM vs TIA vs stroke. Further, one way ANOVA after Benjamini-Hochberg correction identified 38 metabolites that separate SHAM from TIA and stroke, and 26 metabolites with a Log of Fc≥10 characteristics uniquely of TIA or stroke.

Ischemic tolerance genetic expression

Furthermore, we focus on changes in gene expression during the process of ischemic tolerance. mRNA of the affected area was extracted from all the mice in order to compare expression changes of early and later genes. For each time point we compared SHAM vs conditioned mice and used a specific Hypoxia array to find relevant genes. Among interesting genes we found Serpine 1 (implied on angiogenesis), c-Fos and Lgals3, previously involved in ischemia resistance.

3. Scientific and social interest of the project

Stroke is a leading cause of mortality and incapacity all around the world. The world stroke campaign says that every six seconds, regardless of age or gender, someone somewhere will die from stroke. One in six people worldwide will have a stroke in their lifetime. Therefore any strategy that changes the natural history of the cerebrovascular disease will be of huge social interest. Our project contributed to finding new biomarkers that could improve early TIA etiology diagnosis and early stroke risk stratification after TIA. Therefore these new biomarkers, together with clinical variables, could be extremely useful for primary care and emergency department physicians to guide triage decisions in order to avoid stroke recurrence. Considering the high prevalence of stroke, it is probable that most of these patients will be attended in hospitals or by general practitioners without access to MRI or vascular imaging. Consequently, the identification of a metabolomic signature associated with neuroimaging findings is of huge interest.

Furthermore, we hope that our findings related to ischemic tolerance will help to guide novel neuroprotective therapies in stroke patients. Moreover, atrial fibrillation is one of the main risk factors for ischemic stroke. Our pharmacogenetic study identified patients that would be benefit from direct antithrombotic therapy rather than acenocoumarol treatment.

4. Bibliography

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