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Blood gene expression profiling to identify potential new biomarkers for ischemic stroke etiological classification

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Introduction: Ischemic stroke is a cerebrovascular disease characterized by the disruption of blood flow into the brain due to an arterial occlusion. Depending on the source of the clot that blocks blood flow, ischemic stroke etiologies can be classified as cardioembolic (CE), large artery atherthrombotic (LAA), small vessel disease (SVD) or undetermined (UND). The use of biomarkers (proteins or genes) might facilitate the accurate identification of each etiology thus contributing to prescribe the most appropriated secondary treatment in order to avoid recurrences. Our aim was to identify differentially expressed genes associated with CE or LAA etiology in ischemic stroke patients.

Methods: Gene expression discovery phase: Eight LAA and 17 CE stroke patients matched by age and neurological severity were included in the discovery phase. All of them underwent reperfusion therapy. Pure RNA was isolated from white blood cells fraction from blood samples (RiboPure and GLOBINclear kits, Ambion, USA). RNA concentration was determined by RiboGreen (Invitrogen, USA) and pooled by mixing equal amounts of RNA from different patients with the same stroke etiology in each pool. Four LAA and 7 CE pools were obtained with a RIN number of 5-7 (Bioanalyzer 2100 platform, Agilent, UK). RNA was retrotranscribed to cDNA (Ovation Pico WTA, Nugen, USA) and hybridized in GeneChip Human Exon 1.0 ST Arrays (Affimetrix, USA). Gene expression validation phase: Differentially expressed genes (p-value≤0.005 and logFC ≥ |1|) were selected as candidates to be analyzed by RT-PCR using TaqMan probes (Applied Biosystems, USA) in individual samples from an independent cohort of 23 LAA and 46 CE stroke patients matched by age and neurological severity. Results are expressed as Relative Quantification ($RQ=2^{-\Delta\Delta Ct}$) to endogenous genes *GAPDH* and *PPIA*, and a calibrator sample. Circulating protein analysis: Genes that showed the same trend in the validation phase were analyzed at a protein level through ELISA assay (Cusabio) in 33 CE, 17 LAA, 10 small vessel disease (SVD) and 8 healthy controls. Pearson-Chi squared test was used for the Univariant analyisis and logistic regressions were performed for the multivariate analysis using RQ levels as cut-off points for considered genes. Integrated discrimination improvement (IDI) was employed to compare predictive models in etiological classification. A p-value<0.05 was considered statistically significant and p-value<0.2 a statistical trend.

Results: The genes *SIRPB2, DYRK2, NECAP1, IFIT2, CCDC75, IFIT1* were upregulated in CE whereas *GOLPH3L, CYB5a, CPN1 and TMEFF1* were upregulated in LAA stroke patients (p-value≤0.005 and logFC ≥ |1|). These were mainly related to processes of carbohydrate metabolism and inflammation processes. After RT-PCR analysis *GOLPH3L* was significantly upregulated in LAA (p<0.05); and *CYB5a* and *TMEFF1* showed the same trend (p<0.5). Only *IFIT1* was upregulated in CE (p<0.5). After logistic regression multivariate analysis, *GOLPH3L*>0.4524, *CYB5a*>3.2453 and dyslipidemia remained baseline predictors of LAA etiology (OR: 3.919 [1.092-14.068], p=0.036; OR: 2.620 [0.699-9.829], p=0.153; and OR: 2.849 [0.915-8.870], p=0.071), respectively). Moreover, *GOLPH3L*>0.4524 and *CYB5a*>3.2453 helped to distinguish LAA from CE etiology (Integrated Discrimination Index 13%, p=0.002). No difference was found when circulating related protein concentration was analyzed in CE, LAA, SVD patients and healthy controls.

Conclusion: Gene expression analysis is an interesting approach to identify potential new biomarkers or therapeutic targets related to ischemic stroke etiology. However, improvements in this strategy are needed such as gene expression analysis in specific cell subpopulations or at later time-points to avoid acute phase processes. Although the pooling strategy reduces interindividual variability a validation step in individual samples is needed. The presence of common underlying pathophysiologic processes is an important challenge to deal with in new ischemic stroke etiology biomarkers discovery.

Biography

Víctor Llombart graduated in Universitat Rovira i Virgili (URV) (Tarragona, Spain) in the specialty of Biotechnology in 2010. One year later, he obtained a Master of Science Degree in Immunology in the Universitat Autònoma de Barcelona (UAB) and Universitat de Barcelona (UB) (Barcelona, Spain). During 2011, he collaborated with the Neuroimmunology Research group of Vall d'Hebrón Institute of Research (VHIR) (Barcelona, Spain). Since 2012 he has been developing his Ph.D. in Internal Medicine in Neurovascular Research Laboratory from VHIR. His Ph.D. is focused on the discovery of new potential biomarkers for the diagnosis of stroke through proteomic based techniques such as Stable Isotope Labelling in Cell Culture (SILAC) and Matrix-Assisted Laser Dessorption/Ionization imaging by Mass Spectrometry (MALDI-IMS). He has published 4 original articles (1 of them as first author), 1 review, developed a patent and collaborated in the public website http://stroke-biomarkers.com. He has also collaborated as a reviewer in the journal Translational Proteomics, and has participated in Spanish Society of Neurology with an oral communication and a poster in 2012 and 2013 respectively.

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