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Research Paper

Metabolomics Predicts Neuroimaging Characteristics of Transient Ischemic Attack Patients☆

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ABSTRACT

Background: Neuroimaging is essential for the diagnosis and prognosis of transient ischemic attack (TIA). The discovery of a plasmatic biomarker related to neuroimaging findings is of enormous interest because, despite its relevance, magnetic resonance diffusion weighted imaging (DWI) is not always available in all hospitals that attend to TIA patients.
Methods: Metabolomic analyses were performed by liquid chromatography coupled to mass spectrometry in order to establish the metabolomic patterns of positive DWI, DWI patterns and acute ischemic lesion volumes. We used these methods with an initial TIA cohort of 129 patients and validated them with a 2nd independent cohort of 152 patients.
Findings: Positive DWI was observed in 115 (40.9%) subjects and scattered pearls in one arterial territory was the most frequent lesion pattern (35.7%). The median acute ischemic lesion volume was 0.33 (0.15–1.90) cm^3. We detected a specific metabolomic profile common to both cohorts for positive DWI (11 molecules including creatinine, threoninyl-threonine, *N*-acetyl-glucosamine, lyso phosphatidic acid and cholesterol-related molecules) and ischemic lesion volume (10 molecules including lysophosphatidylcholine, hypoxanthine/threonate, and leucines). Moreover lysophospholipids and creatinine clearly differed the subcortical DWI pattern from other patterns.
Interpretation: There are specific metabolomic profiles associated with representative neuroimaging features in TIA patients. Our findings could allow the development of serum biomarkers related to acute ischemic lesions and specific acute ischemic patterns.

1. Introduction

Magnetic resonance diffusion weighted imaging (DWI) remains the best neuroimaging technique to detect acute ischemia, above all since the new tissular definition of transient ischemic attack (TIA) has become essential in the evaluation of TIA patients (Easton et al., 2009). According to a recent meta-analysis, despite transient clinical symptoms, one out of three patients with definite TIA has an acute DWI lesion (Brazzelli et al., 2014). Moreover, DWI has been shown to be an important predictor of early stroke recurrence (Purroy et al., 2004) and it has been proposed to add to clinical prognostic scales like ABCD2I (Giles et al., 2011) and ABCD3I (Merwick et al., 2010). Furthermore, not only the presence but also the patterns of DWI are important both for the etiological classification and for patient prognosis (Purroy et al., 2011). However, despite the increased availability of magnetic resonance imaging (MRI) not all TIA patients undergo DWI. Therefore, the discovery of a plasmatic biomarker related to neuroimaging findings is of enormous interest.

The use of metabolomics on TIA patients has started a new era in biomarker discovering for clinical purpose (Jove et al., 2015a). Metabolomics allows the study of the complete set of low-molecular-weight intermediates (metabolites), which vary according to the pathologic state of the cell, tissue, organ, or organism and are context-dependent (Jove et al., 2014; Mauri-Capdevila et al., 2013).

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The aim of the present study was to perform a metabolomic analysis to find new biomarkers associated with the presence of acute DWI lesion and the volume and patterns of these lesions. As previously (Jove et al., 2015a), results were validated in an independent cohort.

2. Methods

2.1. Subjects

This study was approved by the ethics committee of the Arnau de Vilanova University Hospital. The main methodology has been previously described (Jove et al., 2015a). We prospectively recruited two independent cohorts of consecutive TIA patients who were attended to by a neurologist during the first 24 h after the onset of symptoms. Both cohorts shared the same methodology but were recruited at different times (Fig. 1). We excluded 12 patients with contraindications to MRI from the original study. We therefore analyzed 129 patients from cohort 1 and 152 patients from cohort 2. TIA was defined according to the classical definition as acute onset of focal cerebral or monocular symptoms lasting <24 h and thought to be attributable to a brain ischemia (Anon, 1990). In order to avoid ethnic differences in the observed metabolomic profiles, all the patients included were Caucasian in origin. Patients were classified etiologically according to the Trial of ORG 10172 (TOAST) (Adams et al., 1993). Undetermined territory included patients without higher brain function disturbance such us aphasia, hemianopsia, neglect, or vertebrobasilar symptoms. Vertebrobasilar TIA was characterized by the following symptoms: bilateral or shifting motor or sensory dysfunction, complete or partial loss of vision in homonymous fields, dizziness, vertigo, or any combination thereof (Purroy et al., 2011).

2.2. Neuroimaging Protocol

A MRI was acquired using a 1.5-T whole-body system with a 24-mT/m gradient strength, 300 ms rise time, and an echo-planar–capable receiver equipped with a gradient overdrive (Philips Intera 1.5 T, MRI scanner). The images obtained included axial T2-weighted turbo spin-echo (TR/TE: 4800/120), T1-weighted spin-echo (TR/TE: 540/15), axial turbo fluid-attenuated inversion recovery (TR/TI/TE: 8000/2200/120), and echo-planar diffusion images (TR/TE: 3900/95). The field of view was 230 mm and the matrix was 256 × 256 in all sequences. The DWI were obtained with a single-shot spin-echo echo-planar pulse sequence with diffusion gradient b values of 1000 s/mm2 along orthogonal axes over 20 axial sections, using 6 mm thick sections, and an interslice gap of 1 mm. Tissue abnormality (positive DWI) was defined as areas of high signal intensity on isotropic DWI reflecting an acute ischemic lesion. Patterns of DWI were determined according to a previous definition (Purroy et al., 2011): DWI normality, scattered pearls in one arterial territory (SPOT), multiple vascular territories, a single cortical lesion in one vascular territory and a subcortical pattern. Two Neuroradiologists blinded to clinical features established the presence and patterns of DWI abnormalities. The interobserver agreement (kappa value) is 1.0 for identifying positive DWI and 0.98 for identifying DWI patterns. Furthermore, OsiriX V.4.0 imaging software (Rosset et al., 2004) was used to calculate the total volume of DWI abnormality. We manually outlined the respective abnormalities using the OsiriX closed polygon tool, thereby creating a region of interest (ROI). ROIs in between the segmented slices were interpolated automatically. The resulting DWI abnormality volume was then determined.

2.3. Metabolomic Analysis

For non-targeted metabolomic analysis, plasma samples were obtained in the morning in order to avoid diurnal variations and metabolites were extracted with methanol according to previously described methods (Wikoff et al., 2008). Briefly stated, 90 μl of cold methanol (containing phenylalanine-C13 as an internal standard) were added to 30 μl of plasma, incubated for one hour at −20 °C and centrifuged for three minutes at 12,000g. The supernatant was recovered, evaporated

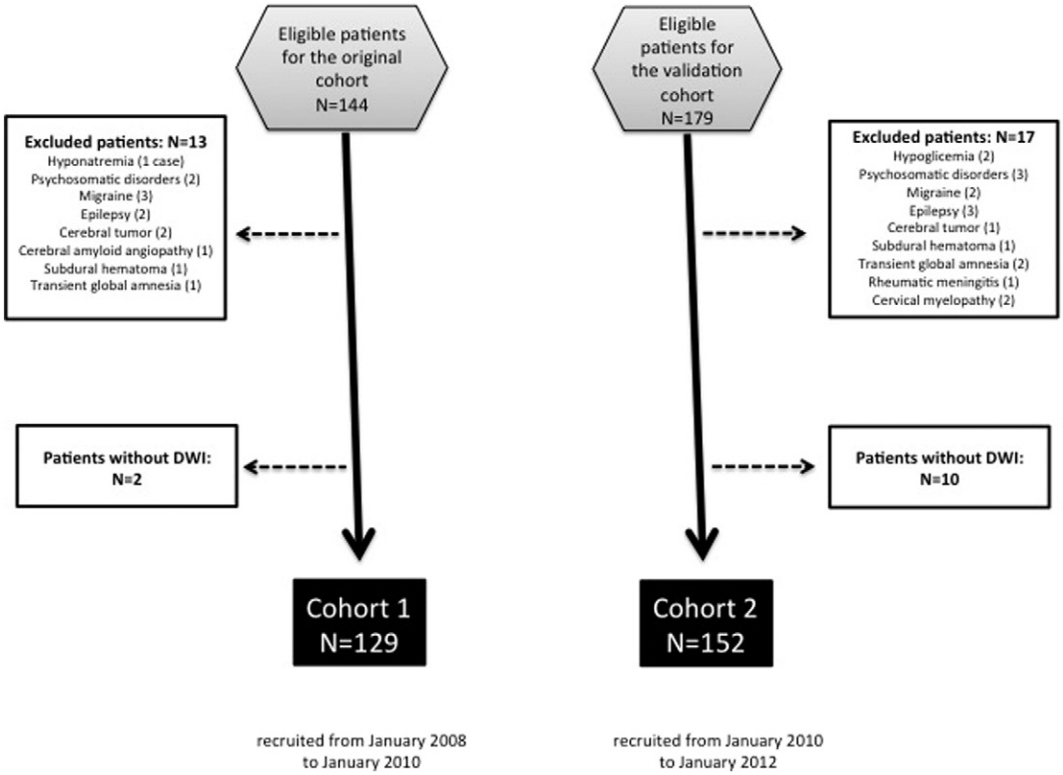

Fig. 1. Patient inclusion chart.