NeuroImage 82 (2013) 586–594

Contents lists available at ScienceDirect

NeuroImage

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

# Metabolomic approach to human brain spectroscopy identifies associations between clinical features and the frontal lobe metabolome in multiple sclerosis

Lisa K. Vingara [a,1], Hui Jing Yu [b,2], Mark E. Wagshul [c,3], Dana Serafin [d], Christopher Christodoulou [d], István Pelczer [a], Lauren B. Krupp [d], Mirjana Maletić-Savatić [d,*,4]

[a] Department of Chemistry, Princeton University, Princeton, NJ 08540, USA
[b] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA
[c] Department of Radiology, Stony Brook University Medical Center, Stony Brook, NY 11794, USA
[d] Department of Neurology, Stony Brook University Medical Center, Stony Brook, NY 11794, USA

ARTICLE INFO

*Article history:*
Accepted 29 May 2013
Available online 7 June 2013

*Keywords:*
Magnetic resonance spectroscopy (MRS)
Metabolomics
Relapsing–remitting multiple sclerosis
Multivariate statistics

ABSTRACT

Proton magnetic resonance spectroscopy ($^1$H-MRS) is capable of noninvasively detecting metabolic changes that occur in the brain tissue in vivo. Its clinical utility has been limited so far, however, by analytic methods that focus on independently evaluated metabolites and require prior knowledge about which metabolites to examine. Here, we applied advanced computational methodologies from the field of metabolomics, specifically partial least squares discriminant analysis and orthogonal partial least squares, to in vivo $^1$H-MRS from frontal lobe white matter of 27 patients with relapsing–remitting multiple sclerosis (RRMS) and 14 healthy controls. We chose RRMS, a chronic demyelinating disorder of the central nervous system, because its complex pathology and variable disease course make the need for reliable biomarkers of disease progression more pressing. We show that in vivo MRS data, when analyzed by multivariate statistical methods, can provide reliable, distinct profiles of MRS-detectable metabolites in different patient populations. Specifically, we find that brain tissue in RRMS patients deviates significantly in its metabolic profile from that of healthy controls, even though it appears normal by standard MRI techniques. We also identify, using statistical means, the metabolic signatures of certain clinical features common in RRMS, such as disability score, cognitive impairments, and response to stress. This approach to human in vivo MRS data should promote understanding of the specific metabolic changes accompanying disease pathogenesis, and could provide biomarkers of disease progression that would be useful in clinical trials.



## Introduction

### *The challenge of finding biomarkers for multiple sclerosis*

Multiple sclerosis (MS) is an immune-mediated demyelinating disorder affecting the central nervous system; it is one of the most frequent causes of disability in young adults (Kurtzke and Wallin, 2000). Most patients suffer a relapsing–remitting (RR) disease course that over time transitions into insidious progression. The pathogenic mechanisms that underlie the relapsing phase and lead to the transition from RR to secondary progression remain poorly understood (Frohman et al., 2006); clinically, the unpredictability and variability in symptoms complicate disease management and render prognosis particularly elusive.

Knowledge of metabolic changes, which are a reflection of the underlying biochemistry, could provide biomarkers that would greatly improve the prospects of managing MS, and provide insight into the disease process itself. Ideally, MS biomarkers would be acquired noninvasively and would reflect disease-related pathogenic processes.

*Abbreviations:* MRS, magnetic resonance spectroscopy; $^1$H-MRS, proton magnetic resonance spectroscopy; RRMS, relapsing–remitting multiple sclerosis; CTWM, control white matter, i.e., MRS of white matter from healthy controls; NAWM, normal-appearing white matter, i.e., MRS from the frontal lobe in RRMS patients; NELES, periventricular non-enhancing lesions in RRMS patients.

* Corresponding author at: 1250 Moursund Street, Suite 1250, Houston, TX 77030, USA. Fax: +1 832 825 1243.
*E-mail address:* maletics@bcm.edu (M. Maletić-Savatić).
[1] Current affiliation: Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University, Portland, OR 97239, USA.
[2] Current affiliation: BioClinica, Inc. Newtown, PA 18940, USA.
[3] Current affiliation: Gruss MRRC, Department of Radiology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.
[4] Current affiliation: Department of Pediatrics, Baylor College of Medicine, Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX 77030, USA.

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.05.125

Such biomarkers could foster early diagnosis and perhaps distinguish between those patients who present with clinically isolated syndrome, but never develop MS, from those who will develop a RR disease. Biomarkers, or specific patterns of biomarkers, would also make it possible to quantify patient response to treatments, improving the quality and specificity of clinical trials.

*The strengths and limitations of conventional MRI and MRS*

The ability of conventional magnetic resonance imaging (MRI) to identify demyelinating inflammatory plaques within the white matter offers a fairly noninvasive way to track disease progression by monitoring lesion burden, though there is often only a loose correlation between changes revealed by conventional MRI and clinical status (Bakshi et al., 2008). MRI can improve diagnosis of MS by distinguishing it from disorders with a similar clinical presentation, but here again it is not foolproof: T2 lesions occur in other neurological disorders and have been documented in asymptomatic aging brains (Bakshi et al., 2008; Vernooij et al., 2008), while some patients with clinically definite MS display no MRI abnormalities (Fazekas et al., 1999).

Neuroimaging methods such as magnetic resonance spectroscopy (MRS) can reveal metabolic changes in white matter that appears healthy by conventional MRI (De Stefano and Filippi, 2007), and therefore might provide a more precise means of diagnosing and following the disease course. However, MRS has its own limitations: MRS studies typically evaluate independent changes in only a small handful of major metabolites (Poullet et al., 2008; Sajja et al., 2009) such as N-acetyl groups (mainly N-acetyl aspartate (NAA)), choline-containing compounds (Cho), creatine and phosphocreatine (Cr + PCr), and myo-inositol (mI). While changes in these specific metabolites have been reported at various stages of MS (Arnold et al., 1994; Chard et al., 2002; Narayana, 2005), such targeted analyses have failed to develop an MS-specific metabolic signature. More importantly, these targeted analyses rely on prior knowledge about the metabolite's presence to calculate and compare group means—however, they overlook a vast amount of potentially valuable information that might be contained in smaller but abundant metabolites such as lipids, lactate, aspartate (Asp), glutamine (Gln), and glutamate (Glu), which are difficult to quantify using current methods.

*Metabolomic techniques can circumvent these limitations*

To circumvent these limitations, we turned to techniques developed in the emerging field of metabolomics. Metabolomics uses a non-targeted approach to obtain an accurate representation of the metabolome, the collection of small molecules that reflect the processes which take place in living biological systems (Griffin, 2003; Lindon and Nicholson, 2008; Smolinska et al., 2012). In contrast to the traditional approach of interrogating a specific subset of small molecules based on a predetermined hypothesis, similar to testing for high cholesterol as an indicator of heart disease, the aim of metabolomics is to acquire a functional biochemical profile that encompasses all detectable small metabolites (specifically identified or not) and trace changes in the profile over the course of development or disease to generate new hypotheses. Whether the goal is to assess the metabolic effect of diet, a response to drug therapy or differences in populations, the question is simple: what has changed?

Metabolomic analysis can be performed on any biological matrix—blood, tears, urine, cerebrospinal fluid or biopsied tissue in vitro, or living tissue in vivo—using mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy (Hassan-Smith et al., 2012; Lindon and Nicholson, 2008). The resulting high-density datasets are analyzed with multivariate statistical modeling to identify metabolites that correlate with functional changes in a given system (Griffin, 2003). Metabolomic-type analysis can overcome the sorts of signal distortions that can occur with MRS, providing previously unavailable information about living tissue, in vivo. Unlike other quantification tools, metabolomic analysis of the full resolution spectra has the advantage of not requiring a priori knowledge such as the line shapes of the metabolite resonances. Therefore, the resonances that can be identified are not limited to the user's input criteria, and changes in small resonances can be extracted.

Untargeted metabolic profiling has been proven feasible for a variety of human diseases (Griffin, 2003). One of the most significant applications has been the use of NMR-based metabolomics on sera for rapid, accurate, and noninvasive assessment of coronary artery disease (Brindle et al., 2002). Other applications include the detection of oral squamous cell carcinoma using plasma (Zhou et al., 2009), epithelial ovarian cancer with sera (Odunsi et al., 2005), the characterization of inflammatory bowel disease using urine samples (Williams et al., 2009), and distinguishing multiple sclerosis patients from controls using cerebrospinal fluid samples (Hassan-Smith et al., 2012; Rajalahti et al., 2010). In vivo, a lot of work and much success have been in the area of distinguishing brain tumor type and grade using metabolic profiling in combination with other MR measures (Galanaud et al., 2006; Preul et al., 1996).

In our study, we extend multivariate statistical analyses to in vivo MRS spectra obtained from individuals with RRMS and healthy controls. We identify a metabolomic model of RRMS that distinguishes between spectra from three tissue types in vivo: the white matter of the frontal lobe in healthy controls (CTWM); the frontal lobe in RRMS patients, which appears normal by conventional MRI (normal-appearing white matter, NAWM); and the periventricular non-enhancing lesions in RRMS (NELES). We validate this metabolomic model by predicting a set of spectra not used in the model-building procedure and achieve excellent assignment of tissue type. We also show, for the first time, that the untargeted metabolomic techniques applied to in vivo MRS data can identify metabolic perturbations that correlate with clinical features common in RRMS.

## Subjects and methods

*Subject selection*

The study was designed to focus solely on RRMS subjects whose clinical information is summarized in Supplementary Table 1. We recruited 27 individuals (22 females, age 38.6 ± 10.1 years; age range: 23–62 years) who met diagnostic criteria for RRMS (Polman et al., 2011) through the outpatient offices of the Multiple Sclerosis Comprehensive Care Center in Stony Brook, NY. RRMS subjects had to be clinically stable, which we defined as at least two months since the last relapse, ambulatory with at most bilateral assistance, and able to tolerate neuroimaging. Participants could be on or off disease-modifying therapy, but their medications had to have been stable for at least two months prior to evaluation, and participants were imaged no sooner than four weeks after their last steroid dose. Subjects with an Extended Disability Status Scale (EDSS) (Kurtzke, 1983) greater than 6.5 were excluded from the study.

The control group consisted of 14 subjects (13 females; age 31.1 ± 9.1 years; range: 21 to 51 years) recruited from a community sample of healthy volunteers who had no history of neurological disorders. All participants gave written consent to participate in the study, which was approved by the Institutional Review Boards of Stony Brook and Princeton Universities.

*Subject evaluations*

Neuropsychological measures were included on a subgroup of participants who consented for cognitive testing (Table 1). Neuropsychological measures included: the Rey Auditory Verbal Learning Test (RAVLT), a list learning task that assesses verbal learning and memory (Nici, 2000); the Symbol Digit Modality Test (SDMT), a measure of working memory and cognitive processing speed (Parmenter et al.,