Structural and metabolic measurements in Rett patients with MECP2 mutation

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Target Audience: Researchers from a broad range of backgrounds interested in clinical application of advanced and multidisplinary imaging techniques.

Purpose: The Rett syndrome is a monogenic postnatal developmental disorder that affects normal brain development during early childhood in females, with an incidence of 1 in 10,000-15,000 live births. Patients with RTT appear to reach developmental milestones seemingly normally until about 6-18 months when signs and symptoms of disease begin to appear. This typically includes stereotypic hand movements, motor coordination deficits, seizures, language and learning disabilities and mild to severe cognitive impairments[1,2]. Other clinical hallmarks include ataxia, spasticity, respiratory abnormalities and autonomic dysfunction[3]. The principal aim of this study is to identify specific biomarkers of RTT syndrome using neuroimaging techniques that could be used as index of disease progression. The importance of identifying specific biomarkers of RTT syndrome also derives from the possibility to monitor the response to an experimental drug treatment through the modification of such factors.

Methods: 14 patients (mean age of 9.6 yrs + 5.9, range 2.4-23.5 yrs) who met the revised diagnostic criteria for typical and atypical RTT and revealed *MECP2* mutations participated to the study. They were characterized by different clinical assessments (severity, epilepsy, speech, respiratory abnormalities). The sample included also 14 matched healthy controls (mean age of 10.4 yrs + 5.8, range 2.2-24.1 yrs). Younger female controls (5 subjects) were retrospectively selected from our database. They underwent an MRI examination because of various reasons (headache, head trauma, cataract, dizziness). Inclusion criteria were the lack of neurological, behavioural or developmental disorders. Other controls (8 female subjects) were healthy volunteers. All patients and the five younger controls were sedated with a general anaesthesia with a halogenated while spontaneously breathing. Parents of both patients and controls received information about all aspects of the study and gave their written consent. MRI data were acquired using a GE 1.5T HDxt Signa system. The MR protocol included a whole brain, 3D high resolution, T1-weighted FSPGR (isotropic voxel size=1mm^3) for voxel-based morphometry (VBM) technique and pseudo-Continuous Arterial Spin Labeling sequence for the measurement of cerebral perfusion (3D pCASL, post-labelling delay=1025 ms; FOV=240mmx; spiral=512x8; slice thickness= 4mm; no spacing; 3 NEX). Data pre-processing of MR images and statistical analysis were realized by the software package Matlab (The MathWorks, Inc) and by using the SPM8 software for voxel-based morphometry (VBM) and statistical comparison between different groups in different conditions[4]. Since this study includes subjects with very different ages, DARTEL algorithm[5] was integrated in the analysis. Smoothed, modulated and normalized data were used for statistical analysis. ASL images were coregistered to the high-resolution T1-weighted volumetric images and quantitative maps of Cerebral Blood Flow (CBF) were obtained from 'labeled' and 'control' images using literature parameters[6,7]. Finally, applying the specific affine transformations of each subject obtained by DARTEL procedure, each CBF map was transformed into the study-specific template and normalized into the MNI space. The normalized modulated and smoothed Gray Matter (GM)/White Matter (WM) image segments in each group were entered into a voxel-wise two-sample t-test analysis in SPM8 as well as the quantitative maps of CBF. Since, the two groups of subjects present a wide range of age, we used this factor as covariate in the statistical analysis.

Results: The statistical comparison between RTT patients and controls showed a diffuse reduction of GM volume in RTT patients with respect to controls, in particular bilaterally in the Cingulate Gyrus and Medial Frontal Gyrus (BA 24-32-6) and in the insula (BA13) (Figure 1). Analogously, RTT patients showed a reduction of WM volume globally in the whole brain. Considering the epilepsy of patients as parameter of discrimination, we performed two separate statistical analyses considering 8 RTT patients without epilepsy (RTT-noEp), and 6 RTT patients with epilepsy (RTT-Ep) each with their respective controls. In the two separate analyses the results found for all subjects were confirmed, indicating the reduction of GM and WM volumes in RTT patients, independently from the presence or absence of epilepsy. Statistical comparison of CBF maps between patients and controls reveals a diffuse state of hyperperfusion in RTT patients respect to controls, in both gray and white matter (t-test, p-uncorr<0.001) (Figure 2). The separate statistical analyses considering only the 8 RTT no-epileptic subjects with their controls did not reveal any significant effect on CBF. On the contrary, the 6 RTT epileptic patients showed a very extend pattern of hyperperfusion with respect to their controls, including preferentially the frontal and temporal lobes in the grey matter, the corpus callosum and the centrum semiovale in the white matter, as well the cerebellum and the pons (Figure 3). Finally, considering patients with different respiratory patterns (forceful, apneustic, feeble) with respect to their controls, we found a specific significant increase of CBF in the brainsteam in patients with forceful and apneustic respiratory pattern, without a general increment of CBF at the level of whole brain.

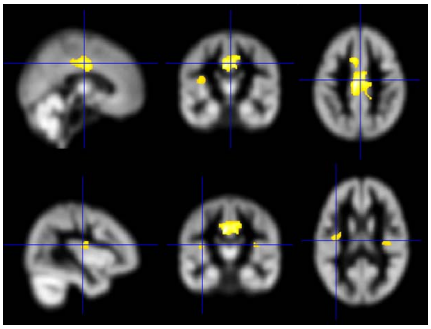

Fig1. Areas of GM volume reduction in RTT patients

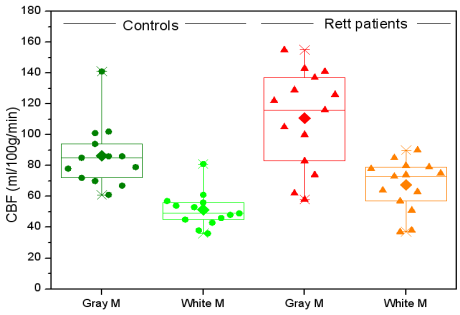

Fig2. Single subject CBF data in RTT patients and controls

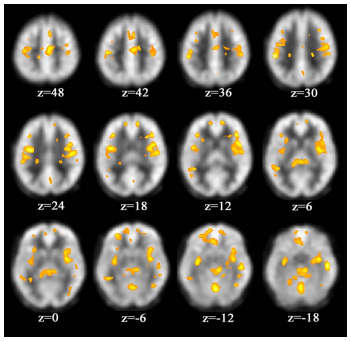

Fig3. Areas of CBF increase in 6 RTT-Ep

Discussion and Conclusion: MRI findings regarding global reductions of GM and WM volumes in RTT patients are in agreement with literature[8,9], and in particular with the reported predominant involvement of frontal and anterior temporal regions and with the relative preservation in the posterior occipital and posterior temporal regions[10,11]. Literature results were obtained in groups of subjects older than 5 years[10,12]. Moreover, our inclusion in the sample of patients of younger than 5 years has allowed extending the results in a larger range of age. To our best knowledge, only one MRI study investigated Arterial Spin Labeling technique in RTT patients[12], but authors did not reach any conclusion, since the ASL technique was not applied to any age-matched control and the quantification of CBF was not performed. The investigation of a functional/metabolic parameter like CBF has allowed also to identify different profiles in the groups of RTT subjects, differentiating epileptic patients from no-epileptic ones and suggesting that this clinical aspect of the disease could have a crucial role in the development and functional activity of many cerebral areas. The increased CBF observed in brainstem of RTT group as function of their abnormal respiration patterns could provide further useful information to understand pathophysiological mechanisms responsible for these aspects.

References [1] Chahrour M & Zoghbi HY. Neuron. 2007. [2] Hagberg B et al, Eur J Paediatr Neurol. 2002. [3] Williamson SL & Christodoulou J. Eur J Hum Genet. 2006. [4] Ashburner J & Friston KJ. NeuroImage 2000. [5] Ashburner J. NeuroImage 2007. [6] Xu G et al. NMR Biomed. 2010. [7] Garcia DM, et al . Magn Reson Med 2005. [8] Subramaniam B et al. Neurology 1997. [9] Armstrong DD. Brain & Development 2001. [10] Carter JC et al. AJNR 2008. [11]Mahmood A et al. AJNR 2010. [12]Naidu S et al. Brain & Development 2001.