

In vivo Quantitative Imaging and Postmortem Protein Analysis Reveal CNS Hypomyelination in the Restless Legs Syndrome

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Introduction: Restless Legs Syndrome (RLS) is a sensorimotor disorder with a prevalence of 5-10% in western countries [1]. The disorder is characterized by uncomfortable leg sensations with an irresistible urge to move the legs, causing chronic sleep disruption at night. Evidence from a number of analytical approaches has suggested that insufficient brain iron may underlie the pathophysiology of this syndrome [2-3]. Iron is essential for normal myelination in the brain. Disruption of iron availability in the brain can affect oligodendroglial cell maturation and myelinogenesis. Therefore, we hypothesized that hypomyelination should be present in the brain in individuals with RLS. To test the hypothesis, we investigated the expression of myelin-related proteins, including myelin basic protein (MBP), 3'5'-cyclic nucleotide phosphohydrolase (CNPase), and proteolipid protein (PLP). In addition, to expand the postmortem findings to an *in vivo* evaluation, we analyzed the brain imaging of RLS patients and controls using voxel-based morphometry (VBM).

METHODS:

Myelin Analysis in Autopsy brains: Frozen tissues from the frontal (Brodmann area (BA) 9, 10) and from temporal cortices (BA 22) were obtained at autopsy from the brains of 11 RLS (53-91 yrs) and age- matched 11 controls (58-87 yrs) who lacked any significant neurological history. Myelin was extracted from frozen brain tissue [4] with minor modifications. Equal amounts of total protein (20ug) were analyzed by western blot for quantification of MBP, CNPase and PLP.

MRI Data Processing: For imaging studies, 23 RLS patients (55.59 ± 13.16 yrs, 8 male, 15 female) and 23 age- and gender-matched controls (55.97 ± 13.07 yrs, 8 male, 15 female) were recruited. RLS disease was clinically assessed by the International Restless Legs Syndrome Study Group (IRLSSG) rating scale. All of patients stopped taking RLS medication for at least one week in order to eliminate drug confounding effect on change in brain structure. For VBM analysis, a whole brain T1-weighted image (MPRAGE, TR/TE/TI=9.9ms/4.6ms/600ms, matrix size=256×256, slice thickness=1mm) was acquired on a 3.0T system. The T1 images were segmented GM/WM/CSF with tissue probability maps using VBM5 toolbox [5]. After segmentation, the final tissue map of WM was modulated with the Jacobian matrix to analyze volume difference between study populations.

Statistics: Mann Whitney U test was used for the myelin analysis in postmortem brain tissue. Paired *t*-test was applied for the mean comparison between RLS and control group using SPM5. Statistical significance was set at a $p < 0.05$.

Results and Discussion: Figure 1 shows the results three protein involving myelination from RLS brain autopsy sample. A significant decrease in the expression of all three proteins was found in RLS compared with the controls ($p < 0.05$). The *in vivo* imaging analysis in Figure 2 revealed a significant decrease in the white matter volume in RLS compared to controls in the corpus callosum, anterior cingulum and precentral gyrus (*t*-test, $p < 0.05$ corrected). In this study, the frontal and temporal cortices were chosen because it has no known clinical relevance to RLS and thus can be considered a representative sample of subcortical white matter. Thus the decrease in myelin and oligodendrocytes specific protein in this sample is suggestive of a wide-spread alterations in myelin composition and oligodendrocyte function in the RLS. It is noteworthy that observation of smaller volume of RLS group in the medial collosal region may have direct functional significance to the RLS symptoms. The corpus callosum is highly organized with fibers connecting specific cortical areas traveling through distinct callosal regions. In particular, the medial corpus callosum consists of fibers connecting primary motor and somatosensory motor areas which contain highly myelinated fibers [6]. Hence, the reduced volume in the medial corpus callosum may suggest a decrease in the myelination of fibers and thus abnormal sensory-motor processing in the motor areas, which may contribute to the motor restlessness of RLS symptoms. Clinically the impact of hypomyelination on disease such RLS and ADHD has not been addressed from a treatment standpoint because the lack of diagnostic tools and the lack of therapeutic intervention strategies. Our study suggest a diagnostic tool is forthcoming for hypomyelination and that to treat underlying disease. The data reported herein suggest a global brain dysfunction in RLS and strongly support the continued development of iron therapeutic strategies in RLS

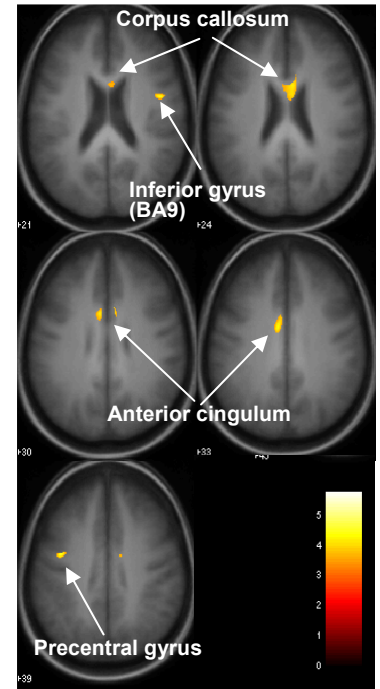


Fig 2. Brain white matter areas (clusters) showed significant volume reduction detected by VBM in RLS group compared to control group ($p < 0.001$, uncorrected) overlaid on customized axial T1-weighted images. T-score of clusters are color-coded in the image.

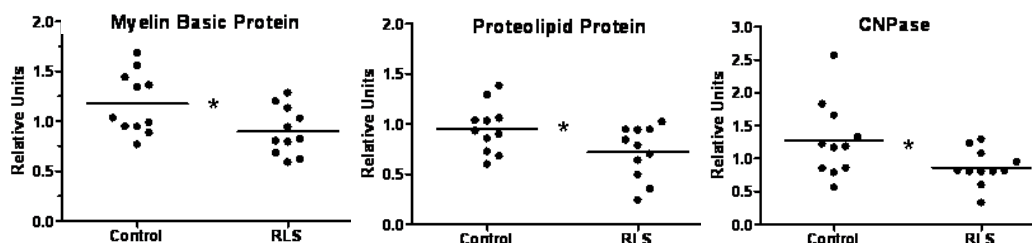


Fig 1. Western blot analyses were performed to determine the relative amount of myelin-related proteins in the crude myelin extracted from BA 9, 10, and 22. MBP, CNPase and PLP are significantly decreased in RLS. The bars represent mean value of for each group.

References:

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