



Neuroradiological, clinical and genetic characterization of new forms of hereditary leukoencephalopathies

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Background

White matter abnormalities in adult patients often evoke the diagnosis of inflammatory-demyelinating diseases, in other words the multiple sclerosis spectrum. However, the advance in neuroimaging techniques, especially in Magnetic Resonance, has revealed a considerable variety of brain white matter abnormalities that can hardly be included in the nosography of multiple sclerosis. As a result, neuroradiologists are more and more confronted with a number of undiagnosed leukoencephalopathies which have become an emerging problem in neurosciences. While multiple sclerosis and vascular causes are often considered in the differential diagnosis of white matter abnormalities in adult patients, an underlying genetic cause is rarely considered unless there is a clear family history or rapid progressive deterioration.

Many observations and genetic studies support that leukodystrophies can manifest themselves in adulthood. Some leukodystrophies due to inherited disorders of metabolism may have a late onset in adolescence or adulthood; these forms may present with clinical and neuroradiological pictures that differ a lot from the classical paediatric-onset forms. Due to the low prevalence compared to that in the paediatric population, neurologists and adult neuroradiologists are usually poorly familiar with these forms of leukodystrophies and, as a consequence, a correct diagnosis can be delayed in these patients.

Given that new forms of late-onset leukodystrophies are now reported, some authors believe that the distinction between late-onset and childhood-onset leukodystrophies as distinct identities should be reconsidered (Sedel et al, J Inherit Metab Dis 2008).

Other well documented leukodystrophies are specific of adulthood and more often present an autosomal dominant modality of inheritance. Among these, the most common are Adult-onset autosomal dominant leukodystrophy (ADLD), and Cerebral Autosomal Dominant Arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). An interesting example of clinical spectrum between paediatric and adult leukoencephalopathies is represented by *TREX1*-related disorders. While autosomal recessive *TREX1* mutations give rise to Aicardi-Goutières Syndrome, a paediatric onset leukodystrophy, dominant *TREX1* mutations cause adult-onset Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL). Further evidence for a clinical spectrum caused by mutations in the same gene is observed in carriers of dominant *COL4A1* mutations which can present in both children and adults. The variable age of onset of vanishing white matter disease cases caused by mutations in any of the five *EIF2B1-5* genes, as documented also by us recently (La Piana et al, Arch Neurol 2012), further underlines the concept of spectrum proposed above.

It is estimated that at least 30% to 40% of patients with leukodystrophies remain without a precise diagnosis despite extensive investigations (Schiffmann, Neurology 2009). The absence of a precise diagnosis and the impossibility to formulate a prognosis represent an extra burden for patients affected with undiagnosed forms of white matter diseases. Leukoencephalopathies of unknown cause represent an even greater diagnostic challenge for neuroradiologists and clinicians. The diagnosis of leukodystrophies and leukoencephalopathies rely heavily on their clinical presentation combined to their MRI pattern recognition of white matter involvement (Lyon et al, Top Magn Reson Imaging 2006). Increasingly, however, genetics is contributing to the diagnosis of new distinct forms of leukoencephalopathies that are then incorporated in the list of leukodystrophies.

Much of the success of the last three decades in the identification of new diseases characterized by white matter involvement has relied on the study of large or consanguineous families and cases that are ethnically closely related. The French-Canadian and aboriginal populations of Quebec are particularly suited for the characterization of new forms of hereditary leukoencephalopathies due to regional founder effects. The first original Quebec specific subtypes identified was the recessive Cree leukoencephalopathy a severe form of Vanishing White Matter disease caused by mutations in *EIF2B5* gene.

An approach based on regional clustering combined with homogeneous clinical and MRI patterns has been successfully used in the last years by Dr Brais research team. A new form of spastic ataxia with frequent leukoencephalopathy (ARSAL) was characterized in a French-Canadian cohort by Dr Brais group and mapped to chromosome 2 (Thiffault et al, Brain 2006).

The Brais lab also characterized, mapped and identified the genes responsible for a new form of recessive childhood-onset leukodystrophy, now referred to as Pol-III related leukodystrophy. This was achieved by combining clinical and neuroradiological patterns with a geographical clustering approach that led to the discovery of mutations in the *POLR3A* gene (Bernard et al, AJHG 2011) and subsequently in the *POLR3B* gene (Tetreault et al, AJHG 2011).

These examples demonstrate that the proposed methodology is effective at identifying new forms of leukodystrophies by combining neuroradiological data with clinical phenotypes and regional clustering.

Hypothesis

We hypothesize that new forms of hereditary leukoencephalopathies with onset in adolescence and onward can be identified by MRI pattern recognition in combination with clinical phenotypes and genetic analyses in particular in cohorts of familial cases and cases with shared Quebec regional origins.

Goals

The present project is part of a more extensive research study aiming at defining MRI and clinical criteria and mutated genes responsible for new forms of adult-onset leukodystrophies. The neuroradiological characterization of white matter involvement in patients with undiagnosed leukoencephalopathy represents the preliminary step to address the subsequent genetic analysis.

This project proposal focuses on the neuroradiological characterization and pattern recognition and has three main objectives:

- 1) Recruitment and collection of neuroradiological data of families with cases of undiagnosed leukoencephalopathies of probable hereditary origin;
- 2) MRI characterization of new forms of hereditary leukoencephalopathies;
- 3) Identification of clusters of patients with a shared pattern of white matter involvement.

Methods

Participants

We have already identified 25 cases with undiagnosed leukoencephalopathy among the patients either evaluated at the Department of Neuroradiology of the Montreal Neurological Hospital or referred to Dr Brais' Neurogenetics clinic. Figures 1 to 3 provide some examples of familiar cases already identified at our institution.

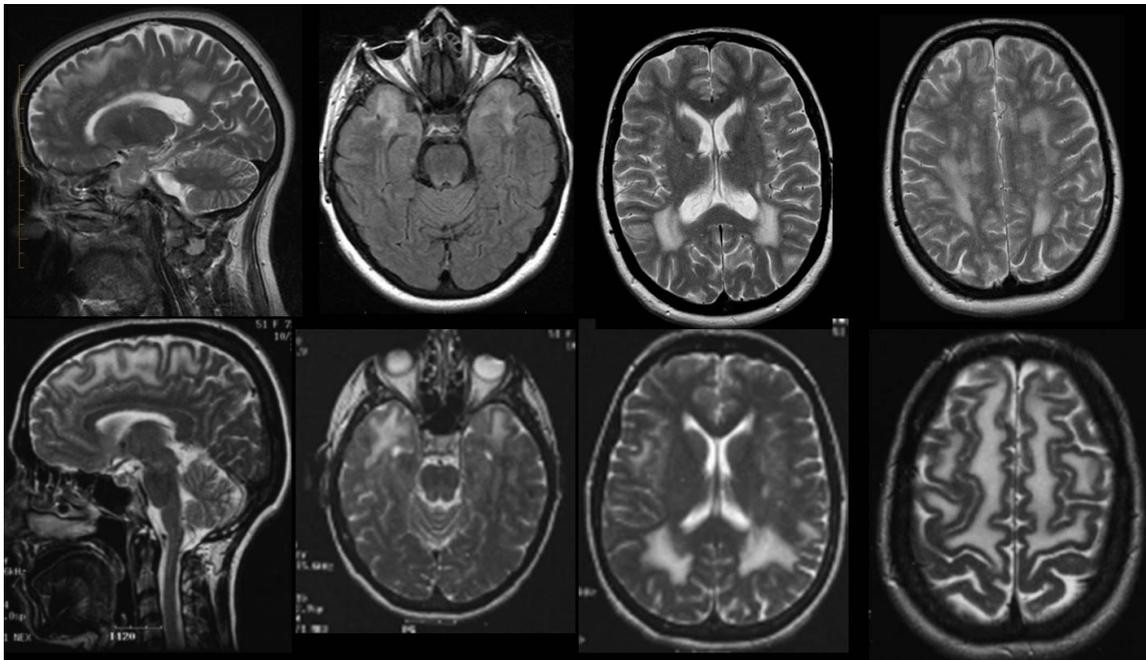


Figure 1: sagittal and axial T2- and FLAIR weighted images of a 44 year-old woman (top) and of her 52 year-old sister (bottom). A diffuse and symmetric white matter involvement is seen extending to the arcuate fibres and to the temporal poles.

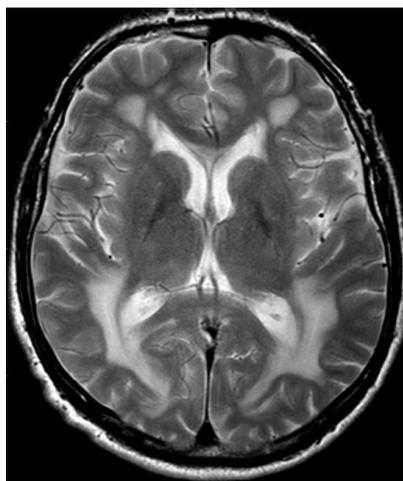


Figure 2: axial T2- weighted images of a 57 year-old male patient, showing a diffuse and symmetric leukoencephalopathy involving the deep white matter and sparing the arcuate fibres. A brother of the subject has similar clinical symptoms (headache, vertigo, neurosensorial hearing loss); MRI is pending.

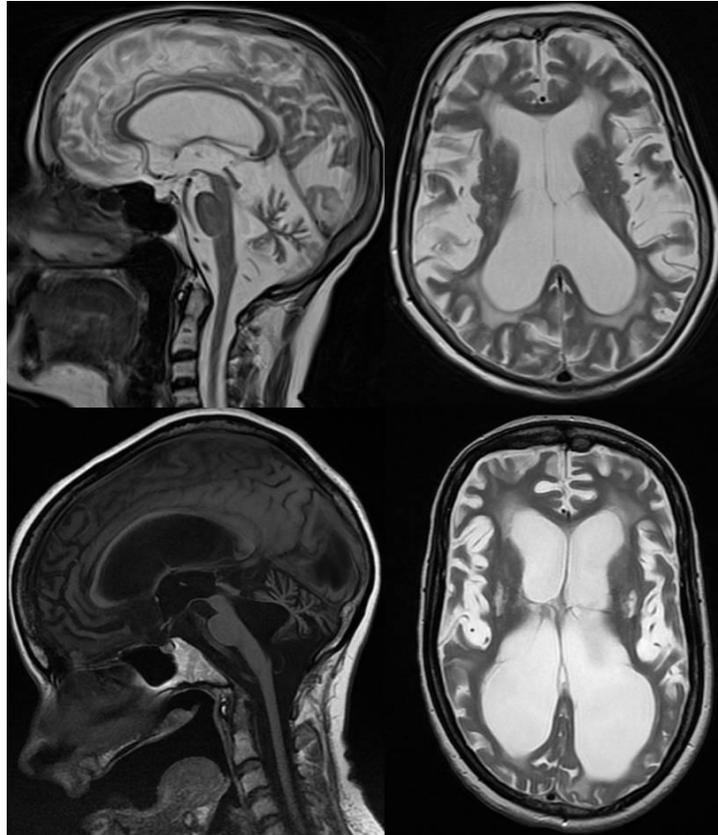


Figure 3: sagittal and axial T2-weighted images of a 63 year old woman (top) and sagittal T1- and axial T2-weighted images of her 41 year old daughter. A severe leukoencephalopathy with important loss of white matter is shown; cerebellar atrophy is an associated feature in both subjects.

In order to clarify the modality of transmission of the disease in the affected families, we will ask first degree relatives of patients to participate in the study and to undergo the MRI protocol (see below).

A group of healthy control subjects matched for age and sex to patients will be also recruited.

All the participants, their relevant first degree relatives and the controls will undergo MRI examination with the Magnetic Resonance (MR) protocol specifically designed (see below).

A copy of previous neuroradiological exams (i.e. CDs of brain MRI or CT scans) performed elsewhere will be obtained with the patient's permission. This will allow us to better evaluate the evolution of the white matter involvement for each patient.

Scanning protocol

Given the central role of MRI as a diagnostic tool in white matter diseases, a **Magnetic Resonance (MR) protocol** will be specifically designed and implemented to study white matter alterations. It will include the following:

- axial and sagittal T1-weighted images for signal characteristics and for cortical thickness analysis;
- axial T2- and Proton Density (PD)- weighted images;
- axial T2 Fluid-Attenuated Inversion Recovery (FLAIR) images;
- axial T2 Gradient Echo images;
- axial T1-weighted images post gadolinium injection
- diffusion weighted imaging;
- quantitative magnetization transfer imaging (QMTI) data;
- MR spectroscopy.

MR imaging will be performed on a Siemens 3T Trio MRI system (Siemens Medical Systems, Erlanger, Germany) at the McConnell Brain Imaging Centre of the Montreal Neurological Institute.

Data analysis

All acquired images will be reviewed by our team. A **systematic approach** will be used to analyze the data and will include qualitative parameters and regional analysis:

- **Qualitative parameters:** symmetry (y/n); pattern of involvement (isolated / multifocal / diffuse); contrast enhancement (y / n); nature of the pathological process (demyelination / dysmyelination / lack of myelination / cystic degeneration); associated features (involvement of cortex / basal ganglia / vascular anomalies).
- **Regional analysis:** extension (supratentorial / infratentorial / both); involved fibers (arcuate fibers / associative fibers / capsules / long tracts); location (frontal / parietal / temporal / occipital / brainstem / cerebellar white matter).

This systematic analysis aiming to pattern recognition will allow us to identify clusters of patients sharing a similar white matter involvement. Pattern recognition has been successfully used in white matter diseases by the major experts in the fields (van der Knaap MS, Valk J. Magnetic resonance of myelination and myelin disorders. 3rd Ed. 2005).

QMTI data will be processed and analysed according to the methodology developed by Pike and colleagues at our Institution (Levesque et al, Magn Reson Med 2010).

Duration of the study

This project is part of a more extensive and longer study that is planned to last five years.

We plan to achieve a neuroradiological characterization of the already identified subjects with undiagnosed leukoencephalopathy in the first year of the study. Based on the number of subjects already identified and their access to our Institution, we expect to recruit approximately 20 cases, 20 family members and 20 controls in the first year.

In the following years we plan to extend the recruitment of subjects with undiagnosed leukoencephalopathy through the collaboration with neuroradiologists and neurologists all over the province of Quebec who could refer patients to our team for undiagnosed leukoencephalopathy of probable genetic origin.

Research Ethics approval

A request for the approval of the study by the Research Ethics Board of the Montreal Neurological Institute and Hospital will be submitted in the next month.