REPORT

Adult-onset genetic leukoencephalopathies: A MRI pattern-based approach in a comprehensive study of 154 patients

Inherited white matter diseases are rare and heterogeneous disorders usually encountered in infancy. Adult-onset forms are increasingly recognized. Our objectives were to determine relative frequencies of genetic leukoencephalopathies in a cohort of adult-onset patients and to evaluate the effectiveness of a systematic diagnostic approach. Inclusion criteria of this retrospective study were: (i) symmetrical involvement of white matter on the first available brain MRI; (ii) age of onset above 16 years. Patients with acquired diseases were excluded. Magnetic resonance imaging analysis identified three groups (vascular, cavitary and non-vascular/non-cavitary) in which distinct genetic and/or biochemical testing were realized. One hundred and fifty-four patients (male/female = 60/94) with adult-onset leukoencephalopathies were identified. Mean age of onset was 38.6 years. In the vascular group, 41/55 patients (75%) finally had a diagnosis [including CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, n = 32) and COL4A1 mutation, n = 7]. In the cavitary group, 13/17 (76%) patients had a diagnosis of EIF2B-related disorder. In the third group (n = 82), a systematic biological screening allowed a diagnosis in 23 patients (28%) and oriented direct genetic screening identified 21 additional diseases (25.6%). Adult-onset genetic leukoencephalopathies are a rare but probably underestimated entity. Our study confirms the use of a magnetic resonance imaging-based classification with a final diagnosis rate of 64% (98/154) cases.

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Abbreviation: CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Introduction
Inherited white matter diseases are heterogeneous neurodegenerative diseases. They include hypomyelinating (abnormal myelin development) and demyelinating (myelin degeneration) leukodystrophies.

In a recent paediatric study, their overall estimated incidence was 1/7603 live births, with a definitive diagnosis in half of them (Bonkowsky et al., 2010). Main causes were metachromatic leukodystrophy (8.2%), Pelizaeus-Merzbacher disease (7.4%), mitochondrial diseases (4.9%), and X-linked adrenoleukodystrophy (4.1%).

Adult-onset inherited white matter diseases are considered as rare. Therefore, a comprehensive study has not been previously published. The objectives of our study were to identify patients with adult-onset inherited white matter diseases in a large nationwide study and to evaluate the interest of a systematic diagnostic approach based on MRI pattern (Labauge et al., 2014).

Materials and methods
Patients
This multicentric retrospective study included all the multiple sclerosis centres in France between January 2007 and
July 2012. All the medical records of patients referred for a possible genetic leukencephalopathy were reviewed. Inclusion criteria were: (i) symmetrical and confluent white matter hyperintensities on T2-weighted MRI on the first available brain MRI; and (ii) clinical age of onset above 16 years. Patients with evidence of an acquired disease were excluded. Required MRI investigation included T1, T2, FLAIR and gradient echo sequences.

Clinical data were reviewed by three neurologists (C.C.D., X.A., P.L.) and MRI analysed by a neuroradiologist (C.C.D., X.A., O.B.T.). According to MRI findings, patients were classified in three groups:

Group 1: Vascular leukencephalopathy based on occurrence of hyperintensities involving the deep grey matter, the pons and the external capsules, lacunes on T2/FLAIR sequences. Presence of microbleeds on gradient echo sequence confirmed the vascular disease (Pantoni et al., 2010).

Group 2: Cavitary leukencephalopathy, defined by extensive hypointensities within large areas of hyperintensities on FLAIR sequences (van der Knaap et al., 2006; Labauge et al., 2009).

Group 3: This heterogeneous group included patients with white matter hyperintensities, without any argument for a vascular mechanism or a cavitary leukencephalopathy.

**Molecular and biochemical screening**

In Group 1, the NOTCH3 gene [involved in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy (CADASIL)] was systematically sequenced. Patients with MRI and/or clinical phenotype suggestive of COL4A1-related disorders (Vahedi and Alamowitch, 2011) underwent sequencing of the entire COL4A1 gene (Vahedi et al., 2003). Occurrence of progressive cysts, calciﬁcations and gadolinium enhancement on MRI (Kleinschmidt-DeMasters et al., 2009) led to a diagnosis of leukencephalopathy with calciﬁcations and cysts, without any known mutated gene.

In Group 2, screening of the EIF2B1–5 genes causing the childhood ataxia with CNS h ypomyelination disease was systematic (Fogli et al., 2004).

In Group 3, investigations were conducted in two steps. First, an inborn error of metabolism was searched in all of the cases. The systematic screening included the research of lysosomal disorders (beta-hexosaminidase, betagalactosidase, beta-glucocerebrosidase, beta-galactocerebrosidase, alpha-galactosidase, arylsulfatase A, alpha-l-fucosidase, alpha-mannosidase, beta-mannosidase, arylsulfatase B, beta-glucuronidase), research of peroxysomal disorders (very long chain fatty acid levels), cholestanol levels, homocysteine and urinary glycosphingolipids. Enzymatic deﬁciency was conﬁrmed by genetic analysis.

In patients without any detected enzyme deﬁciency, clinical and MRI ﬁndings were carefully reviewed. Clinical analysis focused on transmission (recessive, dominant, X-linked), neurological and extraneurological ﬁndings (e.g. syndactyly). MRI pattern analysis focused on speciﬁc features (Ahmed et al., 2014) to directly sequence pathogenic genes involved in speciﬁc disease.

**Standard protocol approvals, registrations and patient consents**

Written informed consent was obtained from patients (or their guardians) participating in the study. Protocol approvals was obtained from ethical committee of the ‘centre de protection des personnes Sud-Est VI’, France.

**Results**

Among 311 submitted ﬁles, 154 (male/female = 60/94) fulﬁlled the inclusion criteria (Fig. 1). Clinical ﬁndings were: age of onset of 38.6 years (range 16–75); initial symptoms: spastic paraparesis (n = 47), cerebellar ataxia (n = 33), cognitive decline (n = 29), psychiatric symptoms (n = 22: psychotic n = 9; depressive, n = 13) and stroke (n = 23). Based on MRI ﬁndings, patients were classiﬁed as follows: Group 1 (55/154), Group 2 (17/154), Group 3 (82/154) (Fig. 1). Familial history was noted in 43 patients (27.7%), suggestive of a dominant pat tern of inheritance in 22. The exact transmission was unavailable in 21 cases.

**Group 1: Vascular disease**

This group includes 55 patients [male/female: 22/33; mean age at onset: 41.5 (range: 17–72)]. Forty-one patients (75%) had a diagnosis: 32 CADASIL, seven COL4A1-related disorder and two leukencephalopathy with calcifications and cysts. Fourteen patients remained without diagnosis.

Of the 32 (58%) patients (male/female: 15/17) with a pathogenic NOTCH3 gene mutation (Fig. 2A), a positive familial history was found in 11/32, suggestive of dominant inheritance in 10. Clinical ﬁndings were: mean age of onset: 38.6 years (range: 18–59); initial symptoms: stroke (n = 13), cognitive impairment (n = 3) and depression (n = 5). In 12 patients, MRI was done for non-speciﬁc symptoms (including migraine with or without aura in eight). Most of the patients had a brain MRI suggestive of CADASIL with frequent involvement of brainstem, external capsules and temporal lobe (Fig. 2A).

COL4A1 gene mutations were found in seven cases (male/female: 4/3). Clinical ﬁndings were: mean age of onset: 37.4 (range: 17–72); initial symptoms: intracerebral haemorrhage (n = 2), ischaemic stroke (n = 1), cognitive impairment (n = 1) and fortuitous diagnosis in three. Only two patients had extraneurological features (cataract, retinal arteriolar tortuosities) suggestive of COL4A1 mutation. MRI patterns consisted of: vascular leukencephalopathy (100%), porencephalic cavities (28%) (Fig. 2B), deep calciﬁcations (83%) and microbleeds (85%). All had internal capsules involvement without any temporal involvement. Dominant inheritance was found in 2/7 cases.

Two patients (male/female: 1/1) had a leukencephalopathy with calcifications and cysts diagnosis based on the presence of extensive cysts, with calcifications and
Figure 1 Study design and classification of the adult-onset leukoencephalopathies. CACH/VWM = childhood ataxia with central nervous system hypomyelination syndrome/vanishing white matter disease; LCC = leukoencephalopathy with calcifications and cysts; WM = white matter.

Figure 2 Cerebral MRI illustrating the main results. (A–B and D–H) Axial FLAIR images; (C) enhanced T₁-weighted sequence. (A) CADASIL; (B) COL4A1 mutation; (C) leukoencephalopathy with calcifications and cysts; (D) vascular leukoencephalopathy without diagnosis; (E) childhood ataxia with central nervous system hypomyelination syndrome/vanishing white matter disease; (F) X-linked adrenoleukodystrophy; (G) cerebro-tendinous xanthomatosis; and (H) Krabbe disease.
gadolinium enhancement in all the patients (Fig. 2C). Mean age of onset was 30.5 years (range 30–31). Initial symptoms were stroke in one patient and non-specific in one.

Finally, 14 patients with a vascular leukoencephalopathy remained without diagnosis. Clinical and MRI findings in these 14 patients (Fig. 2D) and in those with NOTCH3 gene pathogenic mutations (n = 32) were not different, except of a more frequent temporal involvement in the CADASIL group (87% versus 62%). Interestingly, a positive familial history, suggestive of an autosomal dominant inheritance, was found in 2 of these 14 patients.

**Group 2: Cavitary disorder**

Thirteen patients (76%) had a pathogenic EIF2B mutation, establishing the diagnosis of adult-onset EIF2B-related disorders (childhood ataxia with central nervous system hypomyelination syndrome). Clinical findings were: sex ratio imbalance (male/female: 3/14), age of onset: 39 years (range 16–57), initial symptoms: cognitive impairment (n = 8), pyramidal symptoms (n = 7), psychiatric disorder (n = 5), including depression in three and psychotic disorder in two and cerebellar ataxia (n = 5). MRI consisted of extensive white matter T2/FLAIR hyperintensities with hypointensities on T1/FLAIR sequences (Fig. 2E). MRI features were similar in mutated and non-mutated patients. Seven patients had the homozygous mutation c.338G>A mutation (p.R113H) in the EIF2B5 gene.

**Group 3: Magnetic resonance without vascular or cavitary aspects**

This group includes 82 patients (male/female: 34/48) with a mean age of onset of 35.9 years (range: 16–75). Initial symptoms were spastic paraparesis (n = 35), cerebellar ataxia (n = 25), cognitive decline (n = 16), psychiatric symptoms (n = 11: five depression / six psychotic), movement disorder (n = 5), seizures (n = 2) and non-specific in six.

Our extensive metabolic investigation led to identify the aetiological diagnosis in 23 cases including X-linked adrenoleukodystrophy (n = 11; Fig. 2F), cerebrotendinous xanthomatosis (n = 5; Fig. 2G), Krabbe disease (n = 3; Fig. 2H), metachromatic leukodystrophy (n = 2), α-mannosidosis (n = 1) and methionine synthetase deficiency (n = 1).

A detailed analysis of MRI and clinical features allowed us to identify a definitive diagnosis in 21 patients (Table 1): fragile X-associated tremor ataxia syndrome (n = 4; Fig. 3A), hereditary spastic paraplegia (SPG10, n = 1, SPG11: n = 2; Fig. 3B), oculodentodigital syndrome (n = 2; Fig. 3C), leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate (n = 2; Fig. 3D), autosomal dominant leukodystrophy (n = 2; Fig. 3E), mitochondrial disease (n = 3; Fig. 3F), hereditary diffuse leukoencephalopathy with spheroids (n = 2; Fig. 3G), X-linked Charcot–Marie–Tooth disease (n = 1; Fig. 3H), Pelizaeus–Merzbacher disease (n = 1) and adult onset polyglucosan body disease (n = 1). Finally, 38 patients of this group remained without diagnosis.

**Discussion**

This series is the first focusing on adult-onset genetic leukoencephalopathies. The high rate of confirmed diagnosis (64%) in the whole cohort (98/154), reaching 75% and 76% in the vascular and cavitary groups, respectively confirms the usefulness of our MRI classification, based on three MRI pattern groups with separate diagnostic work-up.

Indeed, adult-onset leukoencephalopathy aetiologies are quite various, with time-consuming and expensive investigations (Ahmed et al., 2014). MRI-based diagnostic algorithms have been mainly proposed in children with genetic leukodystrophies (Schiffmann and van der Knaap, 2009).

The major discriminant was the presence of hypomyelinating leukoencephalopathy, which is rarely encountered in adult-onset leukoencephalopathies (Steenweg et al., 2010). On the contrary, large series of patients with vascular (CADASIL, COL4A1-related disorders, etc) or cavitary disorders (childhood ataxia with central nervous system hypomyelination/vanishing white matter disease) have been described (Dichgans et al., 1998; Labauge et al., 2009).

Therefore, we focused on the presence of vascular or cavitary findings to simplify the further diagnostic work-up in each group. Family history is usually considered as a major discriminant in the diagnostic work-up of inherited disorders. Familial occurrence was observed in only 42 probands, suggesting autosomal dominant inheritance in 22. Most (n = 15/22) were in the vascular group. The final seven cases were from the third group, with a definitive diagnosis in three: autosomal dominant leukodystrophy, hereditary diffuse leukoencephalopathy with spheroids and oculodentodigital syndrome. No specific mode of inheritance could be defined in 16 patients. The relative low rate (28%) of positive familial history can be explained by recessive inheritance, incomplete penetrance, censoring effect or de novo mutation. At least, acquired diseases cannot be formally excluded.

The first group included vascular diseases, based on involvement of the grey matter, lacunar infarcts and microbleeds. Such aspects strongly suggest a diagnosis of CADASIL. Systematic screening of the NOTCH3 gene found a pathogenic mutation in 69.5% (32/46). Clinical and MRI features (except temporal lobe involvement) are not so different between our mutated and non-mutated patients (Pantoni et al., 2010; Pescini et al., 2012). These results imply that the NOTCH3 gene should be sequenced in all patients under 50 years of age with vascular white matter involvement and in older patients in the absence of atherosclerosis.

We found seven patients with COL4A1 gene mutation (13%). Diagnosis was first suggested by the presence of
<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>Sex ratio (M/F)</th>
<th>Familial history</th>
<th>Age at onset (range)</th>
<th>Main neurological findings</th>
<th>Specific MRI finding</th>
<th>Other clinical findings</th>
<th>Mutated gene(s)</th>
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<tr>
<td>FXTAS</td>
<td>4</td>
<td>4/0</td>
<td>2/4</td>
<td>64.5 (52–75)</td>
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<td>MCP HI (4), cerebellar HI (4) and atrophy (4), CC involvement (3)</td>
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<td>FMRI</td>
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<td>4</td>
<td>3/1</td>
<td>1/4 (AR)</td>
<td>26 (17–39)</td>
<td>Spastic paraparesis (4), cognitive (2),</td>
<td>Hypomyelination (4), corpus callosum atrophy (2)</td>
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<td>Spatacin (SPG1)</td>
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<td>ADLD</td>
<td>2</td>
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<td>2/2</td>
<td>46.5 (45–48)</td>
<td>Cerebellar ataxia and tremor (2), cognitive (2), pyramidal (1)</td>
<td>MCP (2), pons (2), pyramidal tract (2),</td>
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<td>LMNB1</td>
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<td>1/2 (AR)</td>
<td>21 (20–22)</td>
<td>Pyramidal (2), cerebellar (1)</td>
<td>MCP (2), cerebellar atrophy (2), pons and spinal cord HI (2), Hypomyelination (2)</td>
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<tr>
<td>Oculo-dentodigital syndrome</td>
<td>2</td>
<td>0/2</td>
<td>1/2 (AD)</td>
<td>24 (16–32)</td>
<td>Symptomatic peripheral neuropathy</td>
<td>Syndactilia (2)</td>
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<td>GJA1</td>
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<td>no</td>
<td>16</td>
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<td>Cerebellar atrophy (3)</td>
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<td>Connexin 32 (GJA1)</td>
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<td>2/3 (mat)</td>
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<td>1/2 (AD)</td>
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<td>Cognitive (2), dystonia (1)</td>
<td>Pons and medullary HI, vermis, medullary and spinal cord atrophy</td>
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<td>no</td>
<td>46</td>
<td>Pyramidal and cognitive</td>
<td>–</td>
<td>–</td>
<td>GBE1</td>
</tr>
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AD = autosomal dominant; ADLD = adult-onset autosomal dominant leukodystrophy; APBD = adult-onset polyglucosan body disease; AR = autosomal recessive; CC = Corpus Callosum; CMTX = X-linked Charcot-Marie-Tooth disease; FXTAS = fragile X-associated tremor ataxia syndrome; HDLS = hereditary diffuse leukoencephalopathy with axonal spheroids; HI = Hyperintensities; mat = maternal; MCP = middle cerebellar peduncle hyperintensities.
classic phenotypes including porencephalic cavities (but inconstant), calcifications and microbleeds (Vahedi and Alamowitch, 2011). Contrary to CADASIL patients, temporal lobe was always spared. Familial history and extra neurological signs were inconstant.

Cavitary leukoencephalopathies are radiologically defined by the association of a diffuse leukoencephalopathy and hypointensities within large areas of demyelination on FLAIR sequences. This aspect evokes first the diagnosis of childhood ataxia with CNS hypomyelination (Labauge et al., 2009), which was asserted in 76% (13/17) of these patients. This high rate of diagnosis is significant in spite of similar MRI in EIF2B mutated and non-mutated patients. Consequently, sequencing of EIF2B genes is justified in every patient with cavitary leukoencephalopathy. Importantly, the recurrent R113H mutation in the EIF2B5 gene is found in most of the cases (Labauge et al., 2009; Carra-Dalliere et al., 2011).

In Group 3, the systematic metabolic screening allowed a diagnosis of inborn error of metabolism in 28% of the patients (23/82), confirmed by molecular analysis. Similar to Müller vom Haen et al. (2014), we found only five diseases: X-linked adrenoleukodystrophy, cerebrotendinous xanthomatosis, Krabbe disease, metachromatic leukodystrophy and α-mannosidosis. In a series of 122 infantile leukodystrophies, the most common diagnoses were metachromatic leukodystrophy and X-linked adrenoleukodystrophy (Bonkowsky et al., 2010). In our series, X-linked adrenoleukodystrophy was by far the most frequent metabolic diagnosis (n = 11/82, 13.5%). Brain MRI shows various patterns from subtle T2-weighted hyperintensities of the corticospinal tracts to more extensive white matter lesions, sometimes with gadolinium enhancement (Carra-Dalliere et al., 2013). Cerebrotendinous xanthomatosis (with characteristic cerebellar white matter and dentate nuclei hyperintensities) and Krabbe disease were found in five and three patients, respectively. These two diseases have been mainly described as paediatric diseases, with increasing publications of adult-onset patients (Debs et al., 2013; Lionnet et al., 2014). Our results suggest that every patient with an adult-onset leukoencephalopathy without vascular or cavitary pattern should have a systematic metabolic screening to search at least four diseases: X-linked adrenoleukodystrophy, cerebrotendinous xanthomatosis, Krabbe disease and metachromatic leukodystrophy. In case of negative screening, all other inborn errors of metabolism should then be searched.

In Group 3, 21 patients had a diagnosis confirmed by direct molecular screening or pathological analysis (biochemical screening was normal in all). The great number of possible diagnoses requires a careful analysis of brain MRI (Schiffmann and van der Knaap, 2009) (Table 1).
This analysis allowed us to identify specific disorders (Fig. 3):

(i) Middle cerebellar peduncle hyperintensities (12 cases) suggest fragile X-associated tremor ataxia syndrome (Aparisit et al., 2012), cerebrotendinous xanthomatosis, autosomal dominant leukodystrophy and mitochondrial disorders (Finsterer and Zarrouk Mahjoub, 2012). Eight patients with middle cerebellar peduncle hyperintensities had a definitive diagnosis (Table 1).

(ii) The identification of subtle T2-weighted white matter abnormalities with normal T1-weighted MRI (Fig. 3B) suggestive of hypomyelination is of particular importance. Indeed, it has been described, notably in patients with hereditary spastic paraplegia (Hourani et al., 2009), as we found in three cases (cf. Table 1). Two additional patients with similar MRI pattern had a final diagnosis of oculodentodigital syndrome (Ganos et al., 2012).

(iii) Frontal lobe predominance of white matter hyperintensities was found in 13 patients. This pattern has been mainly described in the frontal variant of X-linked adrenoleukodystrophy, metachromatic leukodystrophy and hereditary diffuse leukoencephalopathy with spheroids (Guerreiro et al., 2013). None of our patients had the frontal variant of X-linked adrenoleukodystrophy or metachromatic leukodystrophy, whereas two had pathological confirmation of hereditary diffuse leukoencephalopathy with spheroids, recently linked to mutations in the CSF1R gene. Unfortunately, the genetic analysis of the CSF1R gene was not carried out because the gene was unknown at the time of the data collection.

(iv) Finally, leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate, autosomal dominant leukodystrophy and adult onset polyglucosan body disease; each have singular MRI findings, mainly in the posterior fossa, that can orient the genetic screening (Linnankivi et al., 2004; Melberg et al., 2006; Mochel et al., 2012).

Despite the usefulness of this MRI analysis, some patients from Group 3 have no diagnosis. A cautious analysis of clinical data is of particular importance (Table 1). Indeed, one patient with a symptomatic peripheral neuropathy had a mutation in the GJB1 gene (responsible for X-linked Charcot–Marie–Tooth disease) (Isoardo et al., 2005), whereas two other patients with syndactilia had oculodentodigital syndrome confirmed by amutation in the GJA1 gene (Ganos et al., 2012).

## Conclusion

In conclusion, our series represents the first adult-onset leukoencephalopathy cohort including 154 patients with symmetrical white matter hyperintensities and age of onset >16 years, of whom 64% finally have a diagnosis. In spite of the retrospective analysis and the possible inclusion of some patients with acquired diseases, this series gives an overview of the global burden of inherited adult-onset white matter diseases. Finally, our classification, based on an easy-to-use MRI pattern analysis, proposes a new decision algorithm in the management of adult-onset leukoencephalopathies.

## References


