Pelizaeus–Merzbacher–Like Disease Presentation of *MCT8* Mutated Male Subjects

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Pelizaeus–Merzbacher Disease is an X-linked hypomyelinatiing leukodystrophy. We report mutations in the thyroid hormone transporter gene *MCT8* in 11% of 53 families affected by hypomyelinating leukodystrophies of unknown aetiology. The 12 *MCT8* mutated patients express initially a Pelizaeus–Merzbacher-Like disease phenotype with a latter unusual improvement of magnetic resonance imaging white matter signal despite absence of clinical progression. This observation underlines the interest of determining both free T3 and free T4 serum concentrations to screen for *MCT8* mutations in young patients (<3 y) with a severe Pelizaeus–Merzbacher-Like disease presentation or older severe mentally retarded male patients with "hypomyelinated" regions.

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Pelizaeus–Merzbacher disease (PMD; MIM 312080) is an X-linked leukodystrophy caused by an inborn error of myelin formation.[1] Clinical features of the classic form of the disease associate in male patients: nystagmus and impaired motor development within the first months of life followed by ataxia, dystonia, dysarthria, and progressive spasticity. Intracerebral nerve conduction is severely impaired, and brain magnetic resonance imaging (MRI) shows a diffuse "hypomyelinated" pattern. PMD involves the *PLP1* gene (Xq22), which encodes the main proteins of the central nervous system (CNS) myelin, the proteolipid proteins. Gene identification demonstrated a wide clinical spectrum of PLP-related disorders in affected male individuals, ranging from severe PMD, characterized by no developmental progress and death during the first decade, to childhood-onset spastic paraplegia (spastic paraplegia type 2 [SPG2]). The level of motor performance correlates with cognitive abilities, with most PMD patients failing to achieve walking and to develop speech.[2,3] About 20% of individuals with a typical PMD phenotype do not have *PLP1* molecular defects, a condition referred as Pelizaeus–Merzbacher–like disease (PMLD). Genetic heterogeneity of PMD was recently demonstrated by the identification of mutations in the gap junction α12 *(GJA12)* gene (PMLD1, MIM 608804). However, *GJA12* mutation screening in our cohort of 114 PMLD patients identified mutations in only 8% of patients, suggesting a larger genetic heterogeneity.[3]

The *monocarboxylate transporter 8* gene (*MCT8,* or *SLC16A2,* MIM 300095), encoding a thyroid hormone transporter, has been implicated in syndromic X-linked mental retardation with a wide spectrum of clinical presentation. The most severe forms are characterized by severe, early-onset hypotonia, nystagmus, dystonic movements, spastic quadriplegia, and virtually no motor or speech acquisition.[4,5] The mildest forms, reported both in male and female patients,[6] have been described as Allan–Herndon–Dudley syndrome (AHDS, MIM 309600). They are characterized by a delay in developmental milestones related to marked hypotonia, leading later to spasticity associated with dysarthria, ataxia, choreoathetoid movements, and facial/neck weakness.[7] MRI in *MCT8* mutated patients has been initially reported as normal,[4,5] but additional observations suggest at least a cortical atrophy[8,9] or even an "hypomyelinated" pattern in young patients.[10–13] Abnormal thyroid hormone transporter function is reflected by increased free triiodothyronine (T3), low free thyroxine (T4), and normal thyroid-stimulating hormone (TSH) levels in the serum. This profile of thyroid parameters is now considered as the best marker for *MCT8* mutation screening in mentally retarded patients. Here we describe the implication of *MCT8* mutations in patients presenting initially with clinical, electrophysiological,

and MRI features characteristic of PMLD, and demonstrate the usefulness of thyroid hormone dosages to select patients for *MCT8* mutation screening.

Patients and Methods

Patients

A peculiar thyroid hormone profile (slightly increased free T3, low free T4, and normal TSH levels) was identified in the serum of an 8-year-old male patient (632) reevaluated after an initial diagnosis of PMLD (see the Supplementary Table). He had severe congenital hypotonia without head control, multidirectional nystagmus with bobbling movements of the head and trunk with subsequent decrease in intensity after the first year of life, dystonic movements of the upper limbs, slow improvement in communication skills, no behavioral problems, and lack of myelination signal on brain MRI performed at 1 year of age (Fig 1A). *PLP1* gene analysis performed at 34 months was normal. Follow-up evaluation at respectively 42 months and 5 years of age showed improved white matter signal on T1 and T2 sequence brain MRI (see Figs 1B, C), and dissociated slowing of CNS conduction on evoked potentials, contrasting with the absence of motor or cognitive improvement and worsening of axial dystonic posture and epileptic features. The abnormal thyroid profile associated with this unusual clinical and MRI evolution for PMLD led us to analyze the *MCT8* gene in this patient and allowed to identify a new *MCT8* mutation (see Results).

This observation and the similarities of the symptoms reported in *PLP1* and *MCT8* mutated patients[4,5] led us to further screen *PLP1*-negative patients from our cohort of undetermined X-linked "hypomyelinated" leukoencephalopathies and SPG for *MCT8* mutation. We selected 52 families with 1 or more affected boys with no *PLP1* mutation. Forty-four were PMLD families selected according to these criteria: (1) our published criteria for PMD,[1] that is, families including only affected boys with an early impairment of motor development (<6 months of life), neurological signs that were gradually modified by the maturing nervous system (nystagmus, choreoathetotic movements, ataxia and progressive spasticity), severe decrease in CNS nerve conductions on multimodal-evoked potentials (brainstem auditory or somatosensory-evoked potentials, or both), diffuse hypomyelination pattern on T1 and T2 brain MRI imaging after 1 year; and (2) absence of mutations in the *PLP1* and *GJA12* genes. We selected PMLD patients with a severe form (no motor acquisition or acquisition of only head control) in accordance with the phenotype of the *MCT8*-mutated PMLD patient described earlier. Eight additional families were also included: one family with a sporadic form of SPG with a diffuse "hypomyelinated" supratentorial white matter on brain MRI, four X-linked SPG families with an early onset (<10 years), and three families with predominant X-linked mental retardation and immature "hypomyelinated" regions on brain MRI. In the latter two groups of seven X-linked families, the *MCT8* genomic region was not excluded by haplotype analysis.

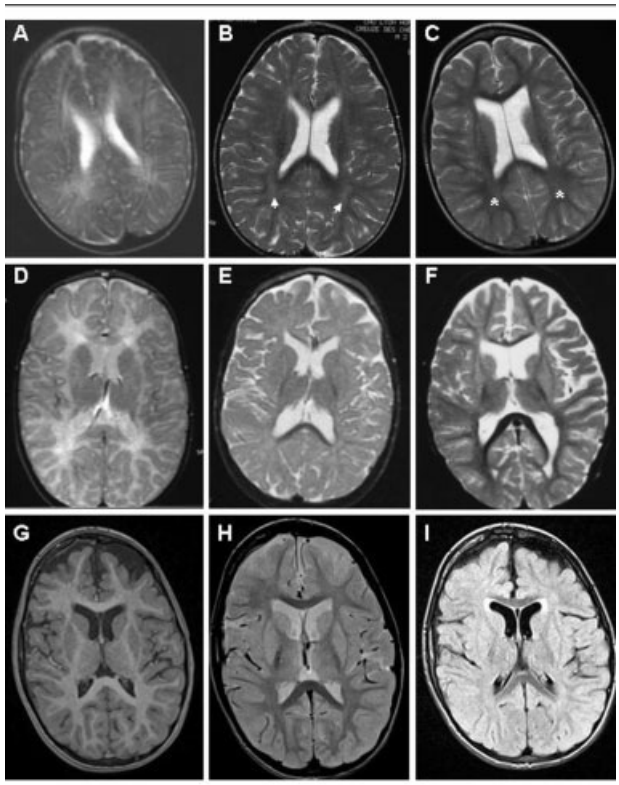

Fig 1. Brain magnetic resonance imaging (MRI) of MCT8-mutated patients. (A–C) Follow-up of T2-weighted imaging in Patient 362 at 1 (A), 3 (B), and 5 (C) years of age. MRI demonstrates a progressive improvement of the white matter (WM) signal between 1 and 5 years of age, reflecting a myelination delay rather than a persistent hypomyelination. The corpus callosum appears similarly atrophic at the different ages. (D) T2-weighted imaging demonstrating in Patient 719 at 7 months of age an abnormal diffuse hypersignal of the WM. (E) The abnormal WM signal is restricted to the periventricular regions in Patient 17.2 at 4 years of age. (F) For Patient 17.1, at 10 years of age, the WM is atrophic with a ventricular dilatation but a normal signal. Atrophy of the corpus callosum is clearly present in the three patients (D–F). (G–I) MR images for Patient 664 (T1 [G], T2 [H], and fluid-attenuated inversion recovery [FLAIR] [I]) at 10 years old showing only a mild hypersignal of the periventricular WM on the FLAIR sequence associated with a mild frontal cortical atrophy.

MCT8 Gene Analysis

The six exons of the *MCT8* gene were analyzed after polymerase chain reaction (PCR) amplification by Denaturating High Performance Liquid Chromatography (DHPLC) (WAVE System; Transgenomic, Coultaboeuf, France). Primer sequences, PCR, and DHPLC conditions are available on request. Sequences of the PCR products demonstrating abnormal retention times on DHPLC were performed using the GenomeLab DTCS kit (Beckman Coulter, Fullertone, CA) and analyzed on a capillary CEQ 2000XL DNA Analysis System (Beckman Coulter) with the CEQ 8000 software. Segregation of the mutations in the families and

analysis of 300 control chromosomes were performed to exclude common polymorphisms.

Results

Molecular Analysis

Direct sequencing of the PCR product demonstrating an abnormal retention time on DHPLC for Patient 632 identified a novel nonsense *MCT8* mutation, c.1558C>T, predicted to lead to a truncated protein, p.Gln520X, missing the 93 last amino acids of the MCT8 11th and 12th transmembrane domains. Analysis of the *MCT8* coding sequences in the cohort of 52 patients identified 5 mutations (see Fig 2). Among those, 4 were new and not found in 300 male healthy white control subjects, including 1 frameshift mutation (c.1826delC [p.Pro609LeufsX70]), 1 deletion (encompassing exons 2–4), and 2 missense mutations (c.661G>A [p.Gly221Arg], c.962C>T [p.Pro321Leu]). Amino acids affected by those new missense mutations are localized respectively in transmembrane domains 2 and 5, and are conserved in the five species evaluated (see Fig 2). In addition, both PolyPhen (http://coot.embl.de/PolyPhen/) and SNPs3D (http://www.snps3d.org) software predict damaging functional consequences of the p.Pro321Leu and p.Gly221Arg transitions. The last mutation (c.1003C>T [p.Gln335X]) was a previously reported nonsense mutation.[14]

Clinical Characteristics of MCT8 Mutated Patients

The clinical characteristics of the 12 affected patients from the 6 *MCT8* mutated families are summarized in the Supplementary Table. All patients presented early (<6 months) symptoms related to poor motor acquisitions associated with early nystagmus in four and choreoathetosis or ataxia in seven. All experienced development of severe spastic quadriplegia or paraplegia associated in eight cases with generalized or axial dystonia. Motor and cognitive acquisitions were limited with no or poor head control and absence of words in all affected patients except in Family 1371 with affected boys who acquired with delay the sitting position or walking with help. In the latter family, survival was prolonged, whereas in Families 17 and 210, patients died in the first decade from severe neurovegetative dysfunction (achalasia, apneas, swallowing and feeding difficulties, sudden death). A myelin defect affecting the first myelinated areas (brainstem, internal capsule, and corpus callosum) with a thin corpus callosum was observed on brain MRI performed before 2 years of age (see Figs 1A, D), whereas at an older age, only periventricular regions appeared poorly myelinated (see Figs 1E, F, I), contrasting with an absence of clinical improvement. Dissociated alterations of CNS conductions without peripheral involvement were noticed. Thyroid parameters in the three patients available for serum dosages demonstrated increased T3, decreased T4, and normal TSH levels.

Neither mental retardation nor neuromotor abnormal presentation was reported for the carrier mothers, and only behavioral problems were noticed in all mothers from Family 17. Brain MRI of the mother of Patient 632 was normal, but molecular analysis has demonstrated that she was not carrying the mutation that may therefore correspond to a neo mutation (see Fig 2).

Discussion

MCT8 mutations were found in 11% of our 53 families with male members affected with a severe form of undetermined "hypomyelinated" leukodystrophies. The six index cases had clinical presentations suggestive of severe forms of PMLD with early signs of myelination impairment (hypotonia, nystagmus, ataxia/choreoathetotic movements, abnormal evoked potentials, and hypomyelinated pattern of the brain MRI) and severe axonal dysfunction (progressive severe spastic paraplegia, dystonia, and central neurovegetative signs). However, the supratentorial myelinated areas and the mild CNS conduction abnormalities noted before 2 years of age in our *MCT8* mutated patients are in favor of a less severe myelin defect, as usually observed in the mildest forms of *PLP1* or *GJA12* mutated patients. The favorable evolution of MRI during patients' follow-up and the mildly hypomyelinated pattern observed in older *MCT8* mutated patients confirmed that *MCT8* mutations are responsible for a severe myelination delay rather than a permanent myelin defect, as observed in *PLP1* or *GJA12* hypomyelinating disorders. Abnormal serum thyroid hormone profiles, with increased free T3, decreased free T4, and normal TSH concentrations, appear to be a useful marker to screen for *MCT8* mutations in patients with myelination impairment.[15]

MCT8 mutations appear to induce a deficiency in T3 cell entry despite high circulating T3 concentrations.[15] Absence of neurological improvement observed in *MCT8*-mutated patients even with a T4 substitutive therapy confirmed the specific need for T3 in the brain. Based on specific neuronal *Mct8* expression reported in mice,[16] a defect in T3 hormone in neurons is considered responsible for the severe mental and motor deficiencies observed in *MCT8*-deficient patients.[15] The similar clinical characteristics found in *MCT8*- and *PLP1*-mutated patients, despite an apparent myelination delay rather than a permanent myelin defect on MRI of *MCT8*-mutated patients, suggest a link between MCT8 and oligodendrocyte maturation. Whether *MCT8* loss of function affects myelination directly via T3 transport impairment in oligodendrocytes or indirectly via alterations of myelin-axon interactions,

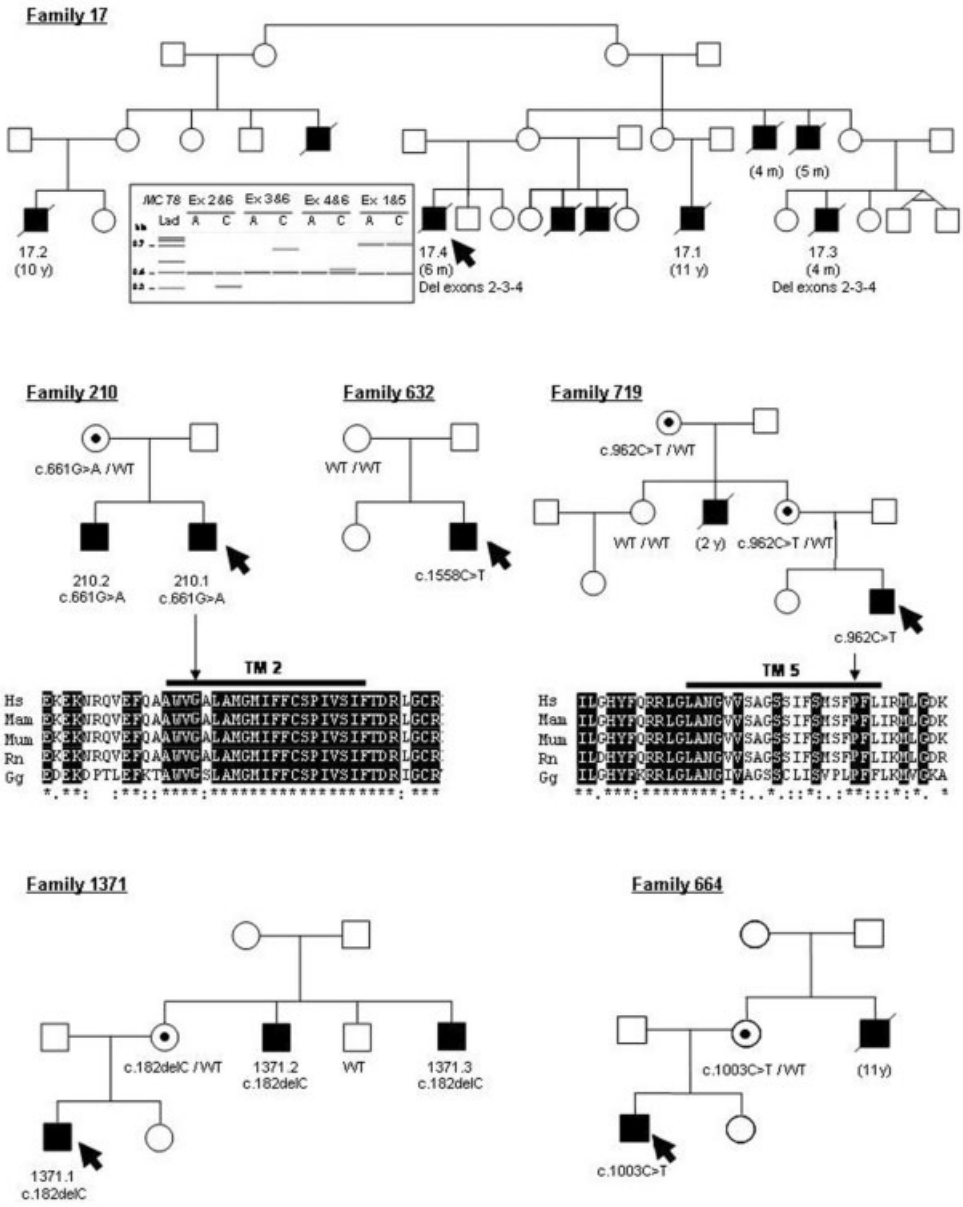

Fig 2. Pedigree of hypomyelinated families with MCT8 mutation. Arrows *indicate the index case of each family that was first analyzed for MCT8 mutation.* Small black circles *indicate mothers with an established carrier status. Ages of death of the affected boys are shown in parentheses. Mutation segregation in Families 210, 664, 719, and 1371 is in accordance with an X-linked mode of inheritance. For Families 17 and 1371, suggestive logarithm of odds score values (Z_{max} = 2.59 and 1.2, respectively) have been previously obtained by linkage analysis for regions encompassing the MCT8 gene. Exon 2 to 4 deletion in Family 17 was characterized after migration on a 2100 Bioanalyzer (Agilent Palo Alto, CA.) of duplex polymerase chain reaction (PCR) products. After coamplification of exon 6 with either exon 2, 3, or 4, it appears that exons 2, 3, and 4 never amplify from the affected patient's DNA (A), whereas exon 6 does, as well as exons 1 and 5. All expected coamplifications were obtained with control DNA (C). Similar results were obtained for Patient 17.3, confirming in this family the deletion of the genomic sequence encoding exons 2 to 4. For amino acids (AAs) affected by new missense mutations, that is, Gly221 (Family 719) and Pro321 (Family 210) localized respectively in transmembrane (TM) domains 2 and 5, analysis of AA conservation between human and other species (macaque:* Macaca mulatta *[Mam], mouse:* Mus musculus *[Mum], rat:* Rattus norvegicus *[Rn], and chicken:* Gallus gallus *[Gg]), demonstrates that both AAs are highly conserved.*

or both, remain to be addressed by further experiments.

In conclusion, our report underlines the interest of determining serum thyroid parameters including both T3 and T4 concentrations to screen for *MCT8* mutations in either young patients with a severe PMLD presentation or older male patients with severe mental and motor retardation with "hypomyelinated" regions on

MRI. The 11% of *MCT8*-mutated patients found in our group of severe PMLD suggest a role of MCT8 and/or free T3 in myelin production and axon-glial interactions.

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