CHAPTER 1

General introduction
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GENERAL INTRODUCTION

Vanishing white matter (VWM; OMIM number 603896) is a genetic leukoencephalopathy linked to mutations in either of the five genes encoding eukaryotic translation initiation factor 2B (eIF2B). It is a disease of all ages. Patients experience slowly progressive neurologic deterioration with additional episodes of rapid clinical decline triggered by physical stress like febrile infections and minor head trauma. The disease is fatal. VWM is one of the most prevalent inherited childhood white matter disorders, although its exact incidence has not been determined. The diagnosis of VWM can be made with confidence in individuals presenting with typical clinical findings, characteristic abnormalities on cranial MRI, and identifiable mutations in one of five genes, encoding the subunits of eIF2B. There is no specific treatment for VWM. Management is at present supportive, based on treatment of symptoms, avoidance of stress situations known to provoke deterioration, prevention of secondary complications and genetic counselling of individuals and families.

HISTORY

The history of VWM is longer than usually assumed. Probably one of the first descriptions of the disease that can be found dates back to 1962 when Eicke described clinical features and autopsy findings characteristic for VWM in a 36-year-old woman who presented at age 31 years with gait difficulties and secondary amenorrhoea. She experienced chronic progressive disease with episodes of rapid deterioration after minor physical trauma. At autopsy a diffuse, cystic destruction of the cerebral white matter was seen with around the cystic areas high numbers of oligodendrocytes. Only mild fibrillar astrocytosis and scant sudanophilic lipids were present. The diagnosis was “atypical diffuse sclerosis”. Similar neuropathological case descriptions by Watanabe, Girard, Anzil, Deisenhammer, Gautier, and Graveleau and their co-workers were published. Cavitative degeneration of the cerebral white matter and the presence of increased numbers of oligodendrocytes were central findings. Some mentioned febrile infections and minor trauma as provoking factors. The disease was not recognised as one disease entity until 1993, when Hanefeld and Schiffmann and colleagues described series of patients with a disease characterised by a childhood-onset, progressive leukoencephalopathy with an autosomal recessive mode of inheritance. Minor head trauma as a provoking factor was recognized and the typical proton magnetic resonance spectroscopy (MRS) findings were described: a decrease of all MRS signals in the affected white matter. Van der Knaap and colleagues described another series of patients with a larger clinical variation in age of onset and rate of progression and recognised both febrile infections and minor head trauma as provoking factors for the disease. MRI and MRS findings were interpreted as indicative of progressive cystic degeneration of the cerebral white matter rather than hypomyelination, which was confirmed by autopsy findings. In line with these observations the name “vanishing white matter” was proposed. Brück and co-workers used the name “myelinopathy centralis diffusa”.

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In 2001 and 2002 it became known that the disease is caused by mutations in any of the five genes, encoding the subunits of eukaryotic translation initiation factor 2B (eIF2B), which has an important role in protein synthesis and in the regulation of protein synthesis rates under different conditions, including cellular stress. The known clinical variation has been expanding ever since. The term “eIF2B-related disorders” was proposed to include all clinical phenotypes related to mutations in eIF2B subunit genes.

**CLINICAL MANIFESTATIONS**

VWM is in its so-called classical form characterized by chronic progressive neurological deterioration with cerebellar ataxia, less prominent spasticity and relatively mild mental decline. In addition, rapid deterioration may occur during febrile illness or following minor head trauma or fright. A slight deterioration may be observed in the early infantile forms up to patients with an onset in adulthood with slowly progressive neurological decline. The brain is the most severely affected organ in all variants. The age of onset is predictive of disease severity. An overview of all reported patients world-wide showed that approximately 20% of the patients have an onset before the age of 2 years, 45% between ages 2 and 5, 20% between ages 6 and 16, and 15% after the age of 16 years. The time course of disease progression varies from individual to individual even within the same family ranging from rapid progression with death occurring within a few months up to very slow progression with death occurring many years after onset. In the literature different clinical phenotypes have been described based on age of onset.

**Severe phenotype: antenatal – infantile onset**

The antenatal/congenital onset form is characterized by a severe encephalopathy. The most severe variants of VWM known, present in the third trimester of pregnancy with decreased fetal movements, contractures, oligohydramnios, growth failure and microcephaly. A rapid decline soon after birth occurs with feeding difficulties, failure to thrive, axial hypotonia, limb hypertonia or hypotonia, cataract and microcephaly. Apathy, irritability, intractable seizures, and finally apneic episodes and coma follow. In addition to signs of a serious encephalopathy and ovarian dysgenesis in females, only the antenatal onset patients may display growth failure, microcephaly, cataracts, hepatosplenomegaly, pancreatic abnormalities, and kidney hypoplasia. Death follows within a few months.

A slightly milder, but also severe and rapidly fatal form of VWM is characterized by an onset in the first year of life with death before the age of two. Francalanci et al. describe two sisters with irritability, stupor, and rapid loss of motor abilities following an intercurrent infection at age 10 to 11 months and death at age of 21 months. “Cree leukoencephalopathy”, described among the native North American Cree and Chippewayan indigenous population, has its onset between 3 and 9 months and death occurs before the age of 2 years.
Classical phenotype: early childhood onset

The most frequent, ‘classical’ variant of VWM has its onset in early childhood, between the ages of 2 and 6 years.\textsuperscript{1,15,16,18} Initially motor and intellectual development is normal or mildly delayed, followed by chronic progressive neurological deterioration, although patients may also be stable for a long period at any stage of the disease. Cerebellar ataxia usually dominates the clinical picture, whereas spasticity is less prominent and intellectual abilities are relatively preserved.\textsuperscript{1,15,16,18} Epilepsy, often mild and well treatable, may occur.\textsuperscript{1,15,16,18} Exceptional cases with more serious epilepsy have been reported.\textsuperscript{27} Optic atrophy may develop with loss of vision at later stages, but not in all patients.\textsuperscript{16} In a few cases peripheral neuropathy has been reported, although in most patients there is no clinical and neurophysiologic evidence of involvement of peripheral nerves.\textsuperscript{38,39} The head circumference is normal in most patients but especially in more severe patients progressive macrocephaly may occur in the context of rapidly progressive cystic degeneration of the cerebral white matter.\textsuperscript{40,41}

Additionally episodes of rapid deterioration may occur, during which patients rapidly lose motor skills and become hypotonic. Irritability, vomiting, and seizures are followed by somnolence and lowering of consciousness.\textsuperscript{1-18} The decline may end in coma and death. If recovery occurs, it is usually incomplete. The episodes are provoked by febrile infections, minor head trauma and, rarely, fright. With head trauma and fright, the deterioration occurs instantaneously, whereas the deterioration occurs in the days after the beginning of febrile infections, independent of the course of the infection and recovery from it. Strikingly, not every provoking incident is followed by deterioration. Most patients die a few years after disease onset, but some do so after only a few months while other patients remain relatively stable for decades.\textsuperscript{1,15,16,18}

Mild phenotype: late-childhood – adult onset

Over time milder variants with an adolescent or adult onset of VWM were recognized.\textsuperscript{6,18,28,42-44} The latest onset of disease that has been reported is 62 years.\textsuperscript{28} The clinical presentation becomes more variable with an onset at later age. Later onset disease generally has a more insidious onset, a slower course and the stress-provoked episodes of rapid deterioration are less common.\textsuperscript{28} In some adults, the disease starts with motor deterioration, similar to the classical phenotype.\textsuperscript{45} However, alteration in intellectual abilities and behavioral changes can be the initial sign in adult onset forms.\textsuperscript{29,31,43,44,46} Occasional seizures\textsuperscript{29}, complicated migraines, psychiatric symptoms\textsuperscript{28,29,46} and presenile dementia\textsuperscript{28,47} have been described as first signs of the disease. Unexpectedly rapid progression and death within a few months has also been published.\textsuperscript{18}

In females with VWM primary or secondary amenorrhea related to ovarian failure is frequently observed.\textsuperscript{32,48} The signs of ovarian failure may precede or follow the neurological deterioration.\textsuperscript{28}

Asymptomatic cases

A- or presymptomatic patients have been described, also with a typically affected sibling.\textsuperscript{2,29,46,49}
Ovarian failure
The juvenile and adult forms are often associated with primary or secondary ovarian failure in females, a syndrome referred to as “ovarioleukodystrophy”. Ovarian dysgenesis, however, may occur in all different disease severities. At autopsy in infantile and childhood cases ovarian dysgenesis has been found. The affected individuals were prepubertal and the ovarian dysgenesis was clinically not manifest. Premature ovarian failure in the absence of leukencephalopathy is not associated with mutations in EIF2B1-5.

Phenotypic spectrum
It is becoming clear that VWM may occur at all ages. Whereas VWM was initially regarded a disease of children, an increasing number of adults has been diagnosed. At present limited information is available on the relative occurrence and phenotypic presentation over all ages.

MAGNETIC RESONANCE
The second step in the diagnosis of VWM is the cranial magnetic resonance imaging (MRI). Validated MRI criteria allow an MRI-based diagnosis of VWM in patients with a typical MRI. MRI is an effective tool for the diagnosis; the correlation between in MRI findings typical of VWM and detection of mutations in the EIF2B1-5 genes is very high.

Figure 1 | Normal axial T2-weighted (a) and FLAIR (b), and sagittal T1-weighted (c) images of a 3-year-old child. On T2-weighted (a) and FLAIR (b) images, cortex, basal ganglia and thalami are gray; myelinated white matter structures are dark-gray. CSF is white on T2-weighted images and black on FLAIR images. On T1-weighted images (c), cortex is gray, myelinated white matter is white and CSF is black.

In healthy persons normal, myelinated white matter has a low signal on T2-weighted, proton density and FLAIR images. The signal is high on T1-weighted images (figure 1). CSF has a high signal on T2-weighted images and a low signal on proton density, fluid-attenuated inversion recovery (FLAIR) and T1-weighted images (figure 1).
In VWM MRI typically shows symmetrically diffuse abnormality of all or almost all the cerebral hemispheric white matter with evidence of progressive white matter rarefaction in a “melting-away” pattern. Well-delineated cysts are rare. The U-fibres may be relatively spared.\textsuperscript{1,18,54} This change is best shown by proton density and FLAIR images. In contrast to MRI in healthy individuals the abnormal white matter has a high signal on proton density, T2-weighted and FLAIR images and a low signal on T1-weighted images (figure 2). Cystic white matter has the signal behaviour of CSF, different from abnormal white matter on proton density and FLAIR images (figure 2). A fine meshwork of remaining tissue strands is usually visible within the areas of CSF-like white matter, with a typical radiating appearance on sagittal and coronal images and
a dot-like pattern in the centrum semiovale on the transverse images (figure 2). Over time, MRI shows evidence of progressive rarefaction and cystic degeneration of the affected white matter, which is replaced by fluid.\textsuperscript{1,3,5,18,54} In the end-stage, all white matter has disappeared between the ependymal lining and the cortex. A fluid-filled space remains, although the cerebral cortex does not collapse (figure 2).\textsuperscript{6} Using genetic analysis as the ‘golden standard’, the proposed MRI criteria have 95\% sensitivity and 94\% specificity.\textsuperscript{1,5,18}

**MRI CRITERIA FOR THE DIAGNOSIS OF VWM\textsuperscript{5}**

**Obligatory criteria**

1. The cerebral white matter exhibits either diffuse or extensive signal abnormalities; only the immediately subcortical white matter may be spared.

2. Part or all of the abnormal white matter has a signal intensity close to or the same as CSF on proton density or FLAIR images, suggestive of white matter rarefaction or cystic destruction.

3. If proton density and FLAIR images suggest that all cerebral white matter has disappeared, there is a fluid-filled distance between ependymal lining and the cortex, and not a total collapse of the white matter.

4. The disappearance of the cerebral white matter occurs in a diffuse “melting away” pattern.

5. The temporal lobes are relatively spared, in the extent of the abnormal signal, degree of cystic destruction, or both.

6. The cerebellar white matter may be abnormal, but does not contain cysts. 7. There is no contrast enhancement.

**Suggestive criteria**

1. Within the abnormal white matter there is a pattern of radiating stripes on sagittal and coronal T1-weighted or FLAIR images; on axial images, dots and stripes are seen within the abnormal white matter as cross-sections of the stripes.

2. Lesions within the central tegmental tracts in the pontine tegmentum.

3. Involvement of the inner blade of the corpus callosum, whereas the outer blade is spared.
Figure 3 | Axial T2- images of a VWM patient, obtained at 6 days (a) and 5 months (b). The initial MRI (a) shows broadening of gyri and a mildly swollen aspect of the cerebral white matter. Its signal intensity is normal for unmyelinated white matter. The follow-up MRI (b) shows an impressive atrophy of the cerebral white matter with highly dilated lateral ventricles. What remains of the white matter has too high a signal intensity, even for unmyelinated white matter.
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Figure 4 | The axial FLAIR image of a 15-year-old boy with recent onset disease (a) shows extensive cerebral white matter abnormalities, sparing the subcortical white matter. The inner blade of the corpus callosum is affected whereas the outer blade is better preserved. There is no evidence of white matter rarefaction. The axial FLAIR image of a 46-year-old woman (b), who has been symptomatic for approximately 10 years, shows the same with additional white matter atrophy. The axial FLAIR image of a 42-year-old man (c), who has been symptomatic for 18 years, shows the same picture as the previous patient, with additional cystic degeneration of the cerebral white matter. The cerebral white matter atrophy is more severe. In contrast, the axial FLAIR image of a 37-year-old woman (d), who has been symptomatic for 2 years, shows the classical MRI picture, comparable to figures 2c and 2d.6

In the most severe, and also in the mildest cases or earliest stages of the disease at any age, MRI findings may be atypical and the MRI criteria may not apply.1,6,29,55 In early infantile VWM the gyral pattern may look immature and the white matter may look swollen preceding the stage of rarefaction. The cerebral white matter may become highly atrophic over time, with the ependymal lining touching the depth of the gyri (figure 3).6,22,32,54

Several presymptomatic and mildly symptomatic patients underwent MRI with initially not necessarily evidence of white matter rarefaction. For example, in an asymptomatic child at the age of 2 a diffuse leukoencephalopathy was seen without cavitation. One year later cystic degeneration was found.1 In addition, absence of any evidence of white matter rarefaction on MRI was found in an 18-year-old woman who only experienced a tonic-clonic seizure.29

On diffusion-weighted images, the rarefied and cystic white matter demonstrates an increased diffusivity.56,57 Areas of restricted diffusion can be found within the non-rarefied white matter.56,57 The histopathologic correlate of the diffusion restriction is unclear.

Proton magnetic resonance spectroscopy

In VWM the findings with proton MRS depend on the stage of white matter rarefaction. The white matter spectrum is relatively preserved when there is little white matter degeneration. Follow-up investigations reveal progressive reduction of all the white matter metabolites. In the end stage, the spectrum is similar to that of CSF with some lactate and glucose and no or minor "normal" signals. This may be seen in any brain disease with cystic degeneration and is not diagnostic for VWM. The cortical, gray matter spectrum stays well preserved throughout the disease course.1,6,15-18,55,58
GENETICS

The diagnosis VWM is completed by demonstrating that both alleles of one of the genes encoding the subunits of eukaryotic translation initiation factor eIF2B contain a pathogenic mutation.

History
The step-wise search for the genetic cause of VWM started in the late nineteen nineties when a genetic linkage study was initiated using exclusively MRI criteria to select patients for this study. The focus on Dutch patients lowered the risk of genetic heterogeneity and two founder effects in The Netherlands were each key to finding disease-causing mutations in a gene. The two genes, EIF2B5 and EIF2B2, are both encoding a subunit of eIF2B. Subsequently, it was shown that VWM could be related to mutations in any of the five genes (EIF2B1-5), encoding the five subunits of eIF2B (eIF2Bα, β, γ, δ and ε).

Mutations
Several reports of the VWM-causing mutations have been published. Almost 170 different mutations have been published, of which approximately 80% are missense mutations. If patients are compound heterozygous for two mutations, the mutations always affect the same gene. Mutations in EIF2B5 are most frequent; two-thirds of the patients with VWM have mutations in EIF2B5. It is the largest subunit, but it also contains a disproportionately high number of mutations.

Frameshifts and nonsense mutations are rare and have been reported only in the compound-heterozygous state. Patients never have two null-mutations. Patients have at most one null-mutation, invariably in combination with a missense mutation. The pathogenic mutation leading to the amino acid change p.Arg113His in the eIF2Bε subunit is by far the most frequently observed mutation. This mutation is found in approximately 40% of the patients. Other more frequent amino acid changes affect Thr91, Arg315 and Arg339 in eIF2Bε and Glu213 in eIF2Bβ. The eIF2B complex is highly conserved in all eukaryotes. The low number of non-synonymous single nucleotide polymorphisms (SNPs) occurring in the EIF2B1-5 genes reflect the importance of sequence conservation.

Genotype-phenotype correlation
A wide variability in severity has been observed among VWM patients, even among patients with the same mutations, and among patients within families. That is why the existence of a genotype-phenotype correlation was questioned and why it was concluded that environmental and/or genetic factors other than the eIF2B mutations determine at least part of the phenotype. However, it is clear that some mutations are consistently associated with a relatively benign phenotype, such as p.Arg113His in eIF2Bε and p.Glu213Gly in eIF2Bβ. A high percentage of patients with adult onset VWM with slow disease progression have
the p.Arg113His mutation in eIF2Be in the homozygous state.\textsuperscript{28,29} This mutation is also most frequently found in women with ovarioleukodystrophy.\textsuperscript{48,65,31} Arg113 is not conserved even among mammals; histidine is the normal amino acid at the equivalent position in mouse and rat, which could explain why p.Arg113His is responsible for a milder phenotype in humans.\textsuperscript{7,48} In the other end of the spectrum of VWM, specific mutations, including p.Arg195His in eIF2Be (the Cree founder mutation), p.Val309Leu in eIF2Be, p.Pro247Leu in eIF2Bδ and p.Gly200Ala in eIF2Bβ are consistently associated with a severe phenotype.\textsuperscript{6,7,22,23,34,35,52} All in all, there is evidence for a genotype-phenotype correlation, but a confirmatory study on the subject is lacking.

**MALE-FEMALE RATIO**

Males and females are equally affected among the patients with infantile and childhood onset of the disease.\textsuperscript{6} Surprisingly, among adult onset VWM patients, a predominance of females has been observed.\textsuperscript{28} The reason for the predominance of females among the older patients is not understood. It has been suggested that with mild mutations, females are more prone to disease presentation, while more males remain asymptomatic.\textsuperscript{28}

**PATHOPHYSIOLOGY OF VWM**

The genes mutated in VWM, \textit{Eif2B1-5}, encode the subunits of a pentameric complex that is involved in protein synthesis, the eukaryotic initiation factor 2B (eIF2B).\textsuperscript{2,21}

**Physiology of eIF2B**

eIF2B is an enzyme that is crucial for the initiation step of the translation of all mRNAs. It activates its substrate eIF2 through the exchange of GDP for GTP (figure 5). Only eIF2-GTP and not eIF2-GDP can form a ternary complex with initiator methionyl-tRNA. This complex binds to the 40S ribosomal subunit, which only then binds the 5' cap structure of an mRNA and starts scanning for an AUG start codon in the 5' untranslated region (5'UTR) of a gene. Upon AUG start codon recognition by the tRNA anti-codon loop, the 60S ribosomal subunit joins the complex and forms a translation-competent 80S ribosome. Simultaneously, eIF2-GTP is hydrolyzed to eIF2-GDP, which subsequently leaves the translation complex. The guanine nucleotide exchange (GEF) activity of eIF2B is indispensable to regenerate active eIF2-GTP to allow new rounds of initiation to occur.\textsuperscript{66,67} The best-studied pathway of regulation of the activity of eIF2B occurs through the phosphorylation of the α-subunit of eIF2. When phosphorylated on its α-subunit, eIF2 binds eIF2B so tightly that it inhibits its activity, leading to a reduction or shut-down of overall protein synthesis.\textsuperscript{68} This makes eIF2B a key regulator of general protein synthesis.
The purpose of the initiation of translation is to position a translation competent ribosome on the start codon of the messenger RNA. This process starts by binding of a ternary complex consisting of eIF2, GTP and charged initiator methionyl-tRNA to the small ribosomal subunit (40S), which leads to formation of the 43S pre-initiation complex. Subsequent binding of the mRNA results in 48S formation. The ribosome will scan the 5’ untranslated region for an AUG start codon. Upon recognition of the start codon the large ribosomal subunit (60S) binds to form an 80S ribosomal complex. Concomitantly, the GTP on eIF2 is hydrolysed to GDP and eIF2 is released from the ribosome. The 80S ribosome will enter the elongation phase of translation. The inactive eIF2-GDP is reactivated by exchanging GDP for GTP. eIF2B is essential in this step by dissociating GDP from eIF2. The main mechanism to regulate the activity of eIF2B is through phosphorylation of eIF2 on the α-subunit. Phosphorylated eIF2 binds tightly to eIF2B and acts as a competitive inhibitor of the GDP-GTP exchange reaction. Several other translation initiation factors that are involved in the initiation process were omitted from this drawing for clarity.6

Down-regulation of eIF2B activity is part of the cellular stress response. Protein synthesis is downregulated under different stress condition, for example heme deficiency, amino acid starvation, misfolded proteins in the endoplasmic reticulum, and during viral infections as part of the interferon response. This response is important to guarantee cell survival under harmful conditions and could link to the clinical observation that VWM patients rapidly deteriorate during systemic infections and head trauma.6,69-73

Altered eIF2B activity
The functional effects of mutations in eIF2B can affect the eIF2B activity in diverse ways: by loss of function of the affected subunit, altering the stability of individual subunits, failure to form complexes with the other subunits, altering its catalytic activity, affecting the interaction with the substrate eIF2, or a combination of these.74-77
At first mutations in eIF2B were reported to decrease eIF2B activity by 20 to 70% as measured in patient-derived lymphoblasts or fibroblasts. The severity of the decrease was reported to correlate with the clinical severity, although later data showed inconsistencies in this correlation. In patients’ lymphoblasts and fibroblasts, the decreased eIF2B activity was not found to affect the rate of global protein synthesis, before, during or after stress (e.g. heat shock or recovery after), or the ability of these cells to proliferate and survive. These observations suggest that basal eIF2B activity by itself may not or not straightforwardly explain the disease. This conclusion warrants further investigations. One reason for this is that assessment of eIF2B activity in patient-derived lymphoblasts or fibroblasts has been proposed as a tool in the diagnosis of VWM and lack of correlation with disease mechanisms raises the question what is actually assessed when eIF2B activity is measured.

Pathology findings

VWM is a cavitating orthochromatic leukoencephalopathy. Characteristic neuropathological findings include tissue rarefaction and cystic degeneration of the white matter with surprisingly meagre reactive gliosis, dysmorphic astrocytes, and paucity of myelin despite a striking increase in oligodendrocytic cellular density. On macroscopic examination the cerebral white matter varies from appearing grayish and gelatinous to more cystic and cavitory (figure 6). The frontoparietal white matter, particularly deep and periventricular, is more commonly involved with relative sparing of the temporal lobe, optic tracts, corpus callosum, anterior commissure, and internal capsule. The cortex and other gray structures are normal. In contrast with children, neonates and infants show brain swelling with flattening of the gyri, while adults display a variable degree of atrophy.
Figure 6. | Gross morphology of VWM, Luxol fast blue staining. A coronal section of the left hemisphere demonstrates myelin loss of the centrum semiovale extending to the gyral white matter but sparing the U-fibers. Note the relative preservation of the striatal and pallidal white matter and of the internal capsule. Cortical and subcortical gray matter appears to be uninvolved.  

Microscopic examination of VWM brain tissue shows that white matter oligodendrocytes and astrocytes bear the brunt of the disease in this disease (figure 7). Increased numbers of oligodendrocytes are present around cystic areas and in less affected white matter. Part of the oligodendrocytes display an abundant foamy cytoplasm and are in that way a distinguishing pathological feature of VWM. The paradoxical coexistence of increased numbers of oligodendrocytes and paucity of myelin in relatively preserved areas prompted a question regarding the functional maturity of oligodendrocytes in VWM.
Astrocytes are dysmorphic with short blunt processes instead of the fine arborisations seen in activated normal astrocytes.\textsuperscript{6,81,82} The abnormal appearance of astrocytes may be explained by abnormality in the cytoskeletal composition, with an abnormal increase in the cytoskeletal protein GFAP-delta.\textsuperscript{84} Recent studies on maturation of macroglia in VWM brains confirmed that the maturation status of astrocytes and oligodendrocytes is affected. Astrocytes proliferate but remain immature, which probably explains the lack of astrogiosis in damaged white matter.\textsuperscript{84} Oligodendrocyte precursor cells are highly increased in numbers. A block in their maturation may explain the striking concurrence of oligodendrocytosis and myelin paucity.\textsuperscript{84} Additionally, high molecular weight hyaluronan, a known inhibitor of oligodendrocyte maturation, and its receptor CD44 were found to be elevated in VWM white matter.\textsuperscript{83,84} Hyaluronan is produced by astrocytes. A correlation was shown between the level of high molecular weight hyaluronan and the degree of white matter damage in VWM.

**eIF2B and involvement of specific tissues**
The reason why the white matter of the central nervous system and, less consistently, the ovaries are selectively vulnerable to mutations in genes coding for eIF2B is as yet not understood.

**Aims/Scope and outline of this thesis**
In the nineties VWM was recognized as disease entity. In 2001 and 2002, before the start of this study, the genetic defect underlying VWM was found. This discovery made it possible to study different aspects of this currently untreatable disorder. This thesis describes the research that has been done to increase our understanding of the phenotypic variation and correlation between genotype and phenotype in VWM.
Large studies on phenotypic variation in VWM are scarce. In chapter 2 a cross-sectional observational study is presented. We investigated the disease course in a cohort of 228 patients. We collected data on prevalence and characteristics of subgroups of patients defined by age of onset and explored male versus female differences. One aim of this study is to increase our knowledge of the clinical phenotype of VWM and in that way increase insight into the disease. A second aim is to collect historical control information, which may be needed for trials on therapies that do not allow blinding, such as cell-based therapies.

In VWM MRI typically shows diffuse and symmetrical abnormalities of the cerebral white matter. Over time the cerebral white matter becomes progressively rarefied and cystic. Before DNA testing was available, the diagnosis of VWM was made by clinical and MRI criteria. Some patients, however, underwent MRI in the presymptomatic or early symptomatic stage and their MRIs may not fulfill the criteria. Insight in early MRI characteristics is lacking. We therefore performed a study on early MRI characteristics in VWM. In chapter 3 the results are presented.

In chapter 4 we focus on diffusion-weighted imaging (DWI). DWI reveals increased diffusion of the rarefied and cystic regions in VWM, but we also observed areas with restricted diffusion in some patients. It is unclear what the underlying histology is in the areas with restricted diffusion. We investigated the occurrence of restricted diffusion in VWM, the affected structures, the time of occurrence in the disease course and the histopathologic correlate.

The disease onset, clinical severity and disease course of VWM patients vary greatly and the influence of genotype and gender on the phenotype is unclear. A study on the genotype-phenotype correlation is hampered by the great number of private mutations, but careful selection of patient groups sharing mutations allowed the study presented in chapter 5.

VWM is caused by mutations of the genes encoding eIF2B, the enzyme that catalyses the exchange of GDP for GTP on eIF2 (GEF activity). It is at present unclear what the correlation between decreased GEF activity measured in patient-derived lymphoblasts and the disease is. In chapter 6 we focus on the functional effects of selected VWM mutations in eIF2B-β, -γ, -δ and -ε by co-expressing mutated and wild-type subunits in human cells and on measurement of the GEF activity in patient derived cells.

The implications/results of these chapters are summarized and discussed in chapter 7.
REFERENCES


Chapter 1

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