CHAPTER 4

Restricted diffusion in vanishing white matter

H. D. W. van der Lei
M. E. Steenweg
M. Bugiani
P. J. W. Pouwels
I. M. Vent
F. Barkhof
W. N. van Wieringen
M. S. van der Knaap

Arch Neurol. 2012;69:723-727
ABSTRACT

Objective
Vanishing white matter (VWM) is a leukoencephalopathy characterized by slowly progressive ataxia, spasticity and stress-provoked episodes of rapid deterioration. MRI shows diffuse involvement of the cerebral white matter, which becomes rarefied and is eventually replaced by fluid. Diffusion-weighted imaging (DWI) reveals increased diffusion of the rarefied and cystic regions. We also observed areas with restricted diffusion in some patients. We investigated the occurrence of restricted diffusion in VWM, the affected structures, the time of occurrence in the disease course and the histopathologic correlate.

Design
In a retrospective observational study we evaluated all available DWI studies in our database and recorded the areas that displayed restricted diffusion in one or more patients. We measured the mean ADC of these areas in all patients and used the putamen for internal quality control. We recorded age and disease duration at MRI. We obtained an MRI of a postmortem VWM brain slice and subsequently performed histopathologic stainings.

Results
Forty-six patients were included. Areas with decreased ADC values were found in the U-fibers (21 patients), cerebellar white matter (18), middle cerebellar peduncle (8), pyramids (8), genu (8) or splenium of the corpus callosum (9) and posterior limb of the internal capsule (10). Patients showing restricted diffusion (32) were overall younger and had shorter disease duration. Histopathology of the brain slice revealed that regions with restricted diffusion had a higher cell density.

Conclusion
In VWM, restricted diffusion can be found in relatively spared regions with high cellularity, particularly in young patients with short disease duration.
INTRODUCTION

Leukoencephalopathy with vanishing white matter (VWM; MIM #603896), also called childhood ataxia with diffuse central nervous system hypomyelination (CACH), is a white matter disorder characterized by ataxia and spasticity with a variable rate of progression and additional episodes of major deterioration provoked by stress. It is one of the most prevalent inherited childhood white matter disorders, but may affect people of all ages. The disease is caused by mutations in the genes encoding the eukaryotic translation initiation factor eIF2B. MRI typically shows a diffuse and symmetrical involvement of the cerebral white matter, which becomes progressively rarefied and is eventually replaced by fluid (figure 1). Relatively spared regions are the U-fibers, corpus callosum, internal capsule, anterior comissure, brain stem and cerebellar white matter.

Only few studies mention the results of diffusion-weighted images (DWI) in VWM. In general, DWI reveals increased diffusion of the rarefied and cystic white matter related to highly expanded extracellular spaces. However, diffusion restriction has recently been reported in two DNA-confirmed VWM patients in the corpus callosum and U-fibers. We decided to perform a systematic study on the subject. We investigated the occurrence of restricted diffusion in a large series of VWM patients, the affected structures and the time of occurrence during the disease course. We obtained an MRI of a postmortem brain slice of a VWM patient and investigated its histopathology to correlate the DWI findings with histopathology.

PATIENTS AND METHODS

Study design

We performed a retrospective observational study and included all available digital diffusion-weighted MR studies in our database up to January 1, 2010. The database contains all VWM patients referred to our center for DNA analysis, and their MRIs. If a patient had more than one DWI study, the first was used for primary analysis.
Figure 1 | MR imaging in VWM disease. 1-year-old VWM patient. Axial T$_2$-weighted image (A) shows diffusely abnormal white matter. On FLAIR (B), abnormal but intact white matter is hyperintense, rarefied white matter is hypointense. Central white matter is rarefied, corpus callosum, internal capsule and U-fibers are not (B). DWI (C) shows low signal in rarefied white matter and high signal in abnormal, non-cystic regions. Low ADC values (D) indicate restricted diffusion in non-rarefied regions.

Standard protocol approvals, registrations, and patient consents
Approval of the ethical standards committee was received for retrospective analysis of clinical and MRI information with waiver of informed consent.

Patients and controls
All patients were diagnosed with VWM on the basis of two mutations in one of the genes encoding eIF2B (EIF2B1-5). We excluded those lacking clinical information and those affected by an additional neurological disease. We used age and disease duration at MRI as clinical parameters. A dataset of DWI studies of control persons (n=37; male/female=18/19; mean age 5.3 years;
Restricted diffusion in vanishing white matter

Median 2.6; range 0.1-24.1) was used to establish reference values (supplementary figure e-1, figure e-2, see page 101-102). The control group comprised 31 diagnostic MRIs, obtained at a 1.5 T scanner, without structural abnormalities and 6 MRIs of healthy volunteers.

MR images evaluation
All available MRIs of VWM patients and controls were scored by consensus of two investigators (HDWvdL and MES).

For the identification of studies with restricted diffusion we reviewed both DWI and ADC maps. For the definitive assessment of diffusion, we only used apparent diffusion coefficient (ADC) maps in order to avoid the problem of T2-shine-through. Regions of interest (ROIs) were drawn manually to measure the mean ADC per structure. Special care was taken to minimize partial volume effects caused by adjacent structures, ventricles and cystic areas. The size of each ROI was adapted to the size of the structure. Only structures clearly visible and large enough to draw a ROI within the structure boundaries on axial images were analyzed. ROI sizes varied between 6 mm² (pyramids) and 70 mm² (putamen).

For each structure investigated, a scatter plot of the ADC values of the controls was created and a fitted 5% prediction line was determined to use as the lower level of normal per age (supplementary figure e-1, figure e-2, page 103-105). A mean ADC of a structure below the reference ADC for that age, scored by both investigators, was used as criterion for restricted diffusion.

The MRIs were collected from many different centers and, consequently, different MRI scanners and DWI pulse sequences had been used, resulting in potentially different ADC values. All MRI scanners were 1.5 T machines. We used the mean ADC of a structure that is not affected in VWM for internal quality control. We chose the putamen because of its size. If the mean ADC of the putamen in a patient was below the reference ADC for that age, the DWI study was excluded from the analysis. We also excluded all poor quality DWI studies.

We evaluated all available ADC maps of VWM patients for areas of restricted diffusion. All regions that displayed restricted diffusion in at least one VWM patient were then systematically analyzed in all VWM patients and controls. We noted the signal behavior of the selected areas on FLAIR images.

Postmortem brain tissue: MRI and histopathology
An MRI of a formalin-fixed brain slice of one of the deceased VWM patients was performed to correlate restricted diffusion to histopathology. The study was conducted on a 1.5 T whole body MR scanner (Siemens, Sonata, Erlangen, Germany). The 1.7 cm thick brain slice was placed in a slice holder, which fits into an 8-channel phased-array head coil. The MR imaging protocol included a dual-echo proton density (PD)/T2-weighted fast spin echo sequence (TR 2500 ms; TE 24/85 ms; 4 measurements; slice thickness 4 mm, in-plane resolution 1 x 1 mm, interpolated to 0.5 x 0.5 mm) and a single-slab 3D-FLAIR sequence17 (TR 6500 ms; TE 355 ms; TI 2200 ms; 1 measurement; slice thickness 1.25 mm, in-plane resolution 1.1 x 1.1 mm). DWI was performed with a single shot STEAM sequence18,19 (TR 5200 ms; TE 48 ms; 80 averages, each consisting of a reference image with b=0 s/mm² and a 3-scan trace-weighted diffusion image with b=750 s/mm²; slice
thickness 5 mm, in-plane resolution 1.17 x 1.17 mm). The PD/T₂ and DWI images were located at the center of the brain slice, which was covered by several thin FLAIR images. After imaging the brain slice was cut at the level of the MRI study and embedded in paraffin. Eight micron thick sections were obtained and stained with Hematoxylin and Eosin using standard techniques.

Statistical analysis
Summary statistics (mean and standard deviation, the latter in brackets) of clinical variables are given in years. Clinical variables of patient subgroups were compared using either the two-sample Student t-test or one way ANOVA. For all brain structures investigated, scatter plots were created from the mean ADC values of the controls by age. By robust regression analysis (to accommodate possible outliers) of the log-transformed variables, the 5% prediction line per structure was determined, and after back transformation to the original scale, used as the lower level of normal. Analyses were performed using SPSS for Windows version 15.

RESULTS
Restricted diffusion in VWM patients
The database contained 72 DWI studies of 56 patients. One patient (1 DWI study) was excluded because of co-morbidity (encephalocele, abnormal gyration and neuronal heterotopias) and 4 patients (4 DWI studies) because of a lack of any clinical information. Five DWI studies (excluding 1 patient) were excluded because of poor image quality and 6 studies (excluding 4 patients) because the ADC value of the putamen was below 5% of the reference. Of the remaining 56 DWI studies obtained in 46 patients, we used the first 46 MRIs for our primary study. We evaluated the 10 follow-up MRIs (4 patients had 1 follow-up MRI; 3 patients had 2 follow-up MRIs) to see what happened with restricted diffusion over time.

The 46 patients included in the study had a male/female ratio of 16/30; mean age of 13.2 years, age range of 0.3-47.6 years; age at onset of 7.7 (range 0.2-37.0) and a disease duration of 5.5 years (range 0-28.8).

Decreased ADC values were found on the first available MRI in 32 of the 46 patients, and included the U-fibers (21 patients), cerebellar white matter (18), middle cerebellar peduncles (8), pyramids (8), genu (8) or splenium of the corpus callosum (9) and posterior limb of the internal capsule (10). All regions with restricted diffusion were hyperintense rather than hypointense on FLAIR images (figure 1), indicative of tissue abnormality without cystic degeneration.

Age and disease duration at the time of MRI of patients with and without restricted diffusion are given in Table 1. ADC values of patients and control persons for each structure can be found in supplementary figure e-1, figure e-2 and table e-1 (see page 89-91). Patients with restricted diffusion had a lower age and shorter disease duration. This effect was most marked for patients with restricted diffusion in the U-fibers, cerebellar white matter, pyramids, or genu of the corpus callosum. To a lesser degree the trend of lower age and shorter disease duration was visible for
Table 1 | Restricted proton diffusion per structure: number of MRIs, age and disease duration at first MRI

<table>
<thead>
<tr>
<th>structure</th>
<th>all patients</th>
<th>patients with restricted diffusion</th>
<th>patients without restricted diffusion</th>
<th>p-value***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>age at MRI, y**</td>
<td>disease duration, y</td>
<td>n</td>
</tr>
<tr>
<td>frontal U fibers</td>
<td>46</td>
<td>13.2 (±13.5)</td>
<td>5.5 (±8.4)</td>
<td>12</td>
</tr>
<tr>
<td>parietal U fibers</td>
<td>46</td>
<td>13.2 (±13.5)</td>
<td>5.5 (±8.4)</td>
<td>14</td>
</tr>
<tr>
<td>occipital U fibers</td>
<td>46</td>
<td>13.2 (±13.5)</td>
<td>5.5 (±8.4)</td>
<td>18</td>
</tr>
<tr>
<td>temporal U fibers</td>
<td>46</td>
<td>13.2 (±13.5)</td>
<td>5.5 (±8.4)</td>
<td>14</td>
</tr>
<tr>
<td>cerebellar white matter</td>
<td>45</td>
<td>13.4 (±13.6)</td>
<td>5.6 (±8.5)</td>
<td>18w</td>
</tr>
<tr>
<td>middle cerebellar peduncle</td>
<td>44</td>
<td>13.6 (±13.7)</td>
<td>5.7 (±8.6)</td>
<td>8</td>
</tr>
<tr>
<td>pyramidal tracts</td>
<td>44</td>
<td>13.6 (±13.7)</td>
<td>5.7 (±8.6)</td>
<td>8</td>
</tr>
<tr>
<td>genu of corpus callosum</td>
<td>39</td>
<td>13.0 (±13.7)</td>
<td>4.7 (±7.8)</td>
<td>8</td>
</tr>
<tr>
<td>splenium of corpus callosum</td>
<td>42</td>
<td>13.0 (±13.7)</td>
<td>4.9 (±8.0)</td>
<td>9</td>
</tr>
<tr>
<td>posterior limb of internal capsule</td>
<td>45</td>
<td>13.5 (±13.6)</td>
<td>5.6 (±8.5)</td>
<td>10</td>
</tr>
<tr>
<td>all structures****</td>
<td>46</td>
<td>13.2 (±13.5)</td>
<td>5.5 (±8.4)</td>
<td>32</td>
</tr>
</tbody>
</table>

*total number of scans in which the structure could be evaluated **values are mean (± SD) in years ***p-value of comparison of patients with and without restricted diffusion ****comparison of all patients with one or more structures with restricted diffusion versus patients without any restricted diffusion.
patients with restricted diffusion in the middle cerebellar peduncles, splenium of the corpus callosum or posterior limb of the internal capsule.

Of the patients with multiple DWI studies, two showed no restricted diffusion at all; in one restricted diffusion arose on the second MRI; in one it was initially present and disappeared; in two it partially disappeared; in one it was initially present, disappeared and arose again.

**DWI of postmortem brain tissue and histopathologic correlation**

The scanned post mortem coronal brain slice was of a girl who died at 5.6 years. MRI at age 1.6 years had shown restricted diffusion in the U-fibers, cerebellar white matter, middle cerebellar peduncles, pyramids, genu and splenium of the corpus callosum, and posterior limb of the internal capsule on both sides. At age 2.1 diffusion restriction was limited to the U-fibers and posterior limb of internal capsule. The postmortem ADC map of the brain slice showed restricted proton diffusion in the U-fibers (figure 2).

Macroscopically, the white matter appeared diffusely grayish and gelatinous to frankly cystic in the periventricular and deep hemispheric regions. Microscopic examination revealed that the regions showing restricted diffusion had a highly increased cellular density with relative myelin preservation. No signs of acute tissue degeneration with cytotoxic edema were detected (figure 3). The areas had the typical characteristics of the relatively spared regions in vanishing white matter disease with a high cell density of oligodendrocytes and oligodendrocyt precursor cells.\(^1,2,4,20,24\)

![Figure 2](image)

**Figure 2** Postmortem diffusion-weighted imaging in a brain slice of a VWM patient. Coronal brain slice of a VWM patient, who died at 5.6 years. FLAIR (A) shows a large cystic area and abnormal, high signal in the non-cystic white matter. DWI (B) shows that part of the subcortical white matter has a relatively high signal (1); the remainder of the white matter has a lower signal (2). The ADC map (C) displays low signal in area 1 and a high signal in the rest of the white matter.
Restricted diffusion in vanishing white matter

Patients with restricted diffusion in the middle cerebellar peduncles, splenium of the corpus callosum or posterior limb of the internal capsule.

Of the patients with multiple DWI studies, two showed no restricted diffusion at all; in one restricted diffusion arose on the second MRI; in one it was initially present and disappeared; in two it partially disappeared; in one it was initially present, disappeared and arose again.

DWI of postmortem brain tissue and histopathologic correlation

The scanned post mortem coronal brain slice was of a girl who died at 5.6 years. MRI at age 1.6 years had shown restricted diffusion in the U-fibers, cerebellar white matter, middle cerebellar peduncles, pyramids, genu and splenium of the corpus callosum, and posterior limb of the internal capsule on both sides. At age 2.1 diffusion restriction was limited to the U-fibers and posterior limb of internal capsule. The postmortem ADC map of the brain slice showed restricted proton diffusion in the U-fibers (figure 2).

Macroscopically, the white matter appeared diffusely grayish and gelatinous to frankly cystic in the periventricular and deep hemispheric regions. Microscopic examination revealed that the regions showing restricted diffusion had a highly increased cellular density with relative myelin preservation. No signs of acute tissue degeneration with cytotoxic edema were detected (figure 3). The areas had the typical characteristics of the relatively spared regions in vanishing white matter disease with a high cell density of oligodendrocytes and oligodendrocyte precursor cells.

DISCUSSION

We focused on restricted diffusion in VWM. Increased diffusion generally reflects increased extracellular spaces, whereas decreased diffusion is seen in conditions of decreased extracellular spaces. In conditions characterized by acute tissue degeneration, decreased diffusion is generally caused by cytotoxic edema. Cytotoxic edema is associated with cell swelling and compression of the extracellular spaces. Decreased diffusion is, however, also seen in conditions of storage of substances, myelin vacuolation and intramyelinic edema, and high cellularity, such as in tumors with a high cell density and abscesses.

We observed decreased ADC values in specific white matter structures in VWM: U-fibers, corpus callosum, internal capsule, cerebellar white matter, middle cerebellar peduncles and pyramids. These are regions known to be relatively spared in VWM. In all patients with areas of restricted diffusion, the FLAIR images confirmed that these areas were affected but not rarefied or cystic. In VWM, less affected regions may have a high cellular density with much higher cell density.
numbers than in control brain tissue. \cite{S21,S22,S23,S24}. Especially high numbers of oligodendrocytes \cite{S21,S24} and oligodendrocyte precursor cells \cite{S30} have been observed in better preserved regions. Our DWI-histopathology correlation confirms that areas of restricted diffusion are relatively spared regions with high cellularity. The morphology of the cells in those areas is compatible with oligodendrocytes and precursor cells.

We found restricted diffusion mainly in younger patients with short disease duration, suggesting it is an early feature of the disease. The two VWM patients in whom restricted diffusion has been mentioned before, have the Cree encephalopathy variant of VWM, which occurs in infants and young children. \cite{S16} Not all patients with short disease duration, however, show areas with restricted diffusion and we also found restricted diffusion in some older patients. At present, we have no explanation for these observations.

In conclusion, restricted diffusion in metabolic disorders is often easily ascribed to tissue necrosis and cytotoxic edema. Strikingly, however, in VWM restricted diffusion is seen in relatively spared regions with a high cell density.
REFERENCES

15. Van der Knaap MS, Schiffmann R, Scheper GC. Conversion of a normal MRI into an MRI showing a cystic leukoencephalopathy is not a known feature of vanishing white matter. Neuropediatrics 2007;38:264.
<table>
<thead>
<tr>
<th>structure</th>
<th>right hemisphere</th>
<th>left hemisphere</th>
<th>patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>n</td>
<td>n**</td>
</tr>
<tr>
<td>frontal U fibers</td>
<td>7</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>parietal U fibers</td>
<td>12</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>occipital U fibers</td>
<td>13</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>temporal U fibers</td>
<td>12</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>cerebellar white matter</td>
<td>6</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>middle cerebellar peduncle</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>pyramidal tracts</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>genu of corpus callosum</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>splenium of corpus callosum</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>posterior limb of internal capsule</td>
<td>6</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

*total number of patients showing restricted diffusion in the structure in this hemisphere **total number of patients showing restriction in the right and/or the left hemisphere
Figure e-1. ADC values of controls and patients

Legend: Marks reflect ADC values (average of both investigators) of patient (red triangles) and control persons (black circles) by age. Fifty (black line) and five percent prediction line (black striped line) of control values. See table e-1 for total numbers of patients with restricted diffusion per structure.
Figure e-2. ADC values of controls and patients

Legend. Marks reflect ADC values (average of both investigators) of patient (red triangles) at first MRI and control persons (black circles) by age. Fifty (black line) and five percent prediction line (black striped line) of control values. See table e-1 for total numbers of patients with restricted diffusion per structure.