Restricted Diffusion in Vanishing White Matter

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Objective: To investigate the occurrence of restricted diffusion in vanishing white matter, the affected structures, the time of occurrence in the disease course, and the histopathologic correlate.

Design: Retrospective observational study.

Patients: Forty-six patients with vanishing white matter.

Setting: VU University Medical Center.

Main Outcome Measures: We evaluated all available diffusion-weighted imaging studies in our database and recorded the areas that displayed restricted diffusion in 1 or more patients. We measured the mean apparent diffusion coefficients of these areas in all patients and used the putamen for internal quality control. We recorded age and disease duration during magnetic resonance imaging, and we obtained a magnetic resonance image of a postmortem vanishing white matter brain slice and subsequently performed histopathologic stainings.

Results: Areas with decreased apparent diffusion coefficient values were found in the U fibers (n = 21 patients), cerebellar white matter (n = 18), middle cerebellar peduncle (n = 8), pyramids (n = 8), genu (n = 8) or splenium (n = 9) of the corpus callosum, and posterior limb of the internal capsule (n = 10). Overall, patients showing restricted diffusion (n = 32) were younger and had shorter disease duration. Histopathologic analysis of the brain slice revealed that regions with restricted diffusion had a higher cell density.

Conclusion: In vanishing white matter, restricted diffusion can be found in relatively spared regions with high cellularity particularly in young patients with short disease duration.


EUKOENCEPHALOPATHY WITH vanishing white matter (VWM) (OMIM 603896), also called childhood ataxia with diffuse central nervous system hypomyelination, 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 it may affect people of all ages. The disease is caused by mutations in the genes encoding the eukaryotic translation initiation factor eIF2B. Magnetic resonance imaging (MRI) typically shows a diffuse and symmetrical involvement of the cerebral white matter, which becomes progressively rarefied and eventually replaced by fluid. Relatively spared regions are the U fibers, corpus callosum, internal capsule, anterior commissure, brainstem, and cerebellar white matter.

Only a few studies mention the results of diffusion-weighted imaging (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 2 patients with DNA-confirmed VWM 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 patients with VWM, the affected structures, and the time of occurrence during the disease course. We obtained an MRI of a postmortem brain slice of a patient with VWM and investigated its histopathology to correlate the DWI findings with histopathology.

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METHODS

STUDY DESIGN

We performed a retrospective observational study and included all available digital diffusion-weighted MRI studies in our database up to January 1, 2010. The database contains all patients with VWM referred to our center for DNA analysis and their MRIs. If a patient underwent more than 1 DWI study, the first was used for primary analysis.
we reviewed both DWI and apparent diffusion coefficient (ADC) volunteers. A scanner, without structural abnormalities and 6 MRIs of healthy control group comprised 31 diagnostic MRIs, obtained with a 1.5-T age range, 0.1-24.1 years) was used to establish reference values (eFigure 1 and eFigure 2, http://www.archneurol.com). The con- 

tations in 1 of the genes encoding eIF2B (EIF2B1-5). We used the mean ADC of a structure that was not affected in VWM for in-

ternal quality control. We chose the putamen because of its size.14 If the mean ADC of the putamen in a patient was less than 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 patients with VWM for areas of restricted diffusion. All regions that displayed restricted diffusion in at least 1 patient with VWM were then systematically analyzed in all patients with VWM and control subjects. We noted the signal behavior of the selected areas on fluid-attenuated inversion recovery (FLAIR) images.

**POSTMORTEM BRAIN TISSUE: MRI AND HISTOPATHOLOGIC ANALYSIS**

An MRI of a formalin-fixed brain slice of 1 of the deceased pa-

**STANDARD PROTOCOL APPROVALS, REGISTRATIONS, AND PATIENT CONSENTS**

Approval from the ethical standards committee at VU University Medical Center 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 2 mu-

tinations in 1 of the genes encoding eIF2B (EIF2B1-5). We ex-

cluded those lacking clinical information and those affected by an additional neurologic disease. We used age and disease du-

ration at MRI as clinical parameters.

A data set of DWI studies of control subjects (n = 37; male to female ratio, 18:19; mean [median] age, 5.3 [2.6] years, range, 0.1-24.1 years) was used to establish reference values (eFig-

ure 1 and eFigure 2, http://www.archneurol.com). The control group comprised 31 diagnostic MRIs, obtained with a 1.5-T scanner, without structural abnormalities and 6 MRIs of healthy volunteers.

**MRI EVALUATION**

All available MRIs of patients with VWM and control subjects were scored by consensus of 2 investigators (H.D.W.vdL. and M.E.S.). For the identification of studies with restricted diffusion, we reviewed both DWI and apparent diffusion coefficient (ADC) maps. For the definitive assessment of diffusion, we only used ADC maps to avoid the problem of T2 shine-through. Regions of interest 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 region of interest was adapted to the size of the structure. Only structures clearly visible and large enough to draw a region of interest within the structure boundaries on axial images were analyzed. Region of interest sizes varied between 6 mm² (pyramids) and 70 mm² (putamen).

For each structure investigated, a scatterplot of the ADC values of the control subjects was created and a fitted 5% prediction line was determined to use as the lower level of normal per age (eFigure 1 and eFigure 2). A mean ADC of a structure less than the reference ADC for that age scored by both investi-

gators was used as criterion for restricted diffusion.

The MRIs were collected from many different centers and consequently, different MRI scanners and DWI pulse se-

quences 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 was not affected in VWM for in-

ternal quality control. We chose the putamen because of its size.14 If the mean ADC of the putamen in a patient was less than the reference ADC for that age, the DWI study was excluded from the analysis. We also excluded all poor-quality DWI studies.

After imaging, the brain slice was cut at the level of the MRI study and embedded in paraffin. Eight-µm-thick sections were obtained and stained with hematoxylin-eosin using standard techniques.
Table. Restricted Proton Diffusion per Structure

<table>
<thead>
<tr>
<th>Structure</th>
<th>All Patients</th>
<th>Patients With Restricted Diffusion</th>
<th>Patients Without Restricted Diffusion</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Patients</td>
<td>Age at MRI, y</td>
<td>Disease Duration, y</td>
<td>No. of Patients</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>18</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 structuresb</td>
<td>46</td>
<td>13.2 (13.5)</td>
<td>5.5 (8.4)</td>
<td>32</td>
</tr>
</tbody>
</table>

Abbreviation: MRI, magnetic resonance imaging.
aTotal number of scans in which the structure could be evaluated.
bP values are of comparison of patients with and without restricted diffusion.
cComparison of all patients with 1 or more structures with restricted diffusion vs patients without any restricted diffusion.

RESULTS

RESTRICTED DIFFUSION IN PATIENTS WITH VWM

The database contained 72 DWI studies of 56 patients. One patient (1 DWI study) was excluded because of co-morbidity (encephalocele, abnormal gyration, and neural 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 less than 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 and 3 had 2 follow-up MRIs) to see what happened with restricted diffusion over time.

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

Decreased ADC values were found on the first available MRI in 32 of the 46 patients and included the U fibers (n=21 patients), cerebellar white matter (n=18), middle cerebellar peduncles (n=8), pyramids (n=8), genu (n=8) or splenium (n=9) of the corpus callosum, and posterior limb of the internal capsule (n=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 the Table. Apparent diffusion coefficient values of patients and control subjects for each structure can be found in eFigure 1, eFigure 2, and the eTable. 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 younger age and shorter disease duration was visible for patients with restricted diffusion in the middle cerebellar peduncles, splenium of the corpus callosum, or posterior limb of the internal capsule.

Of the patients who underwent multiple DWI studies, 2 showed no restricted diffusion at all; in 1, restricted diffusion arose on the second MRI; in 1, it was initially present and disappeared; in 2, it partially disappeared; and in 1, it was initially present, disappeared, and arose again.

DWI OF POSTMORTEM BRAIN TISSUE AND HISTOPATHOLOGIC CORRELATION

The scanned postmortem coronal brain slice was of a girl who died at age 5.6 years. Magnetic resonance imaging when the girl was aged 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. When the girl was aged 2.1 years, diffusion restriction was limited to the U fibers and posterior limb of the 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 VWM disease with a high cell density of oligodendrocytes and oligodendrocyte precursor cells.1,14,20-24

**COMMENT**

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,25,26 which is associated with cell swelling and compression of the extracellular spaces. However, decreased diffusion is 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.25-29

We observed decreased ADC values in specific white matter structures in VWM: U fibers, the corpus callosum, the internal capsule, cerebellar white matter, middle cerebellar peduncles, and pyramids. These are regions known to be relatively spared in VWM.1,14,20-24 In all patients with areas of restricted diffusion, 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 numbers than in control brain tissue.4,21,23,24 In particular, high numbers of oligodendrocytes21-24 and oligodendrocyte precursor cells30 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 2 patients with VWM in whom restricted diffusion was reported before had the Cree en-
cephalopathy variant of VWM, which occurs in infants and young children. However, not all patients with short disease duration 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, restricted diffusion is seen in relatively spared regions with a high cell density in VWM.

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REFERENCES

15. van der Knaap MS, Schüffmann R, Schepker GC. Conversion of a normal MRI showing an MRI showing a cystic leukencephalopathy is not a known feature of vanishing white matter. Neuropediatrics. 2007;38(5):264.