A large proportion of familial frontotemporal dementia is caused by TAR DNA-binding protein 43 (transactive response DNA-binding protein 43 kDa) proteinopathies. Accordingly, carriers of autosomal dominant mutations in the genes associated with TAR DNA-binding protein 43 aggregation, such as Chromosome 9 open reading frame 72 (C9orf72) or progranulin (GRN), are at risk of later developing frontotemporal dementia. Brain imaging abnormalities that develop before dementia onset in mutation carriers may serve as proxies for the presymptomatic stages of familial frontotemporal dementia due to a genetic cause. Our study objective was to investigate brain MRI-based white-matter changes in predementia participants carrying mutations in C9orf72 or GRN genes. We analysed mutation carriers and their family member controls (noncarriers) from the University of British Columbia familial frontotemporal dementia study. First, a total of 42 participants (8 GRN carriers; 11 C9orf72 carriers; 23 noncarriers) had longitudinal T1-weighted MRI over ∼2 years. White-matter signal hypointensities were segmented and volumes were calculated for each participant. General linear models were applied to compare the baseline burden and the annualized rate of accumulation of signal abnormalities among mutation carriers and noncarriers. Second, a total of 60 participants (9 GRN carriers; 17 C9orf72 carriers; 34 noncarriers) had cross-sectional diffusion tensor MRI available. For each participant, we calculated the average fractional anisotropy and mean, radial and axial diffusivity parameter values within the normal-appearing white-matter tissues. General linear models were applied to compare whether mutation carriers and noncarriers had different trends in diffusion tensor imaging parameter values as they neared the expected age of onset. Baseline volumes of white-matter signal abnormalities were not significantly different among mutation carriers and noncarriers. Longitudinally, GRN carriers had significantly higher annualized rates of accumulation (estimated mean: 15.87%/year) compared with C9orf72 carriers (3.69%/year) or noncarriers (2.64%/year). A significant relationship between diffusion tensor imaging parameter values and increasing expected age of onset was found in the periventricular normal-appearing white-matter region. Specifically, GRN carriers had a tendency of a faster increase of mean and radial diffusivity values and C9orf72 carriers had a tendency of a faster decline of fractional anisotropy values as they reached closer to the expected age of dementia onset. These findings suggest that white-matter changes may represent early markers of familial frontotemporal dementia due to genetic causes. However, GRN and C9orf72 mutation carriers may have different mechanisms leading to tissue abnormalities.
Keywords: frontotemporal dementia; MRI; white matter

Abbreviations: AxD = axial diffusivity; C9orf72 = chromosome 9 open reading frame 72; DTI = diffusion tensor imaging; EYO = expected years to symptom onset; FA = fractional anisotropy; FAB = frontal assessment battery; FLAIR = fluid-attenuated inversion recovery; FOV = field of view; GENFI = the genetic frontotemporal dementia initiative; GLM = general linear model; GM = grey matter; GRN = progranulin; MAPT = microtubule-associated protein tau; MD = mean diffusivity; MMSE = mini-mental state examination; NAWM = normal-appearing white matter; RaD = radial diffusivity; ROI = region of interest; TDP-43 = TAR DNA-binding protein 43; TE = echo time; TIV = total intracranial volume; TR = repetition time; Tukey’s HSD test = Tukey’s honest significance test; T1-HypoWMSA = white-matter hypointensities on gradient-echo T1-weighted MRI; T2-HyperWMSA = white-matter hyperintensities on fluid-attenuated inversion recovery or T2-weighted MRI; WM = white matter; WMSA = white-matter signal abnormality; WMSAf = white-matter signal abnormality fraction; WMVf = white-matter volume fraction

Graphical Abstract

White matter abnormalities in presymptomatic frontotemporal dementia mutation carriers compared to non-carrier family controls

Towards Expected Age of Symptoms Onset

- GRN Mutation Carriers
  - Accumulating White Matter Signal Abnormalities
  - Increasing Mean and Radial Diffusivity in Periventricular Regions

- C9orf72 Mutation Carriers
  - Decreasing Fractional Anisotropy in Periventricular Regions

Introduction

Frontotemporal dementia is a common cause of presenile dementia, clinically presented with a heterogeneous range of behavioural and personality changes (behavioural variant frontotemporal dementia) or speech and language difficulties (primary progressive aphasia). Around 30% of all frontotemporal dementia cases are known to be familial, often caused by tauopathies or TAR DNA-binding protein 43 (TDP-43) proteinopathies. Therefore, individuals who carry autosomal dominant mutations in the genes associated with tauopathies [e.g. carriers of mutations in microtubule-associated protein tau (MAPT+)] or TDP-43 proteinopathies [e.g. progranulin (GRN+)] or chromosome 9 open reading frame 72 (C9orf72 +)] have a predictable risk for developing frontotemporal dementia in the future. Accordingly, abnormal brain imaging changes observed in relatively young and asymptomatic mutation carriers may be proxies for the presymptomatic stages of familial frontotemporal dementia due to a genetic cause.

MRI studies have demonstrated that structural imaging abnormalities are apparent decades prior to expected symptom onset, with changes in the white-matter (WM) preceding those in the grey matter (GM). WM signal abnormalities (WMSAs) are a common MRI marker of WM tissue status, which denotes the region of altered fat/water ratio due to a variety of causes, including small vessel disease or an inflammatory environment. WMSAs are typically measured in terms of hyperintensities (T2-HyperWMSA) on fluid-attenuated inversion recovery (FLAIR) or T2-weighted MRI, although similar regions may also appear as hypointensities (T1-HypoWMSA).
on gradient-echo T1-weighted MRI. T2-HyperWMSAs are prominent in symptomatic GRN+ carriers compared with C9orf72+ or MAPT+ carriers, largely affecting the frontal and occipital regions as well as parts of the temporal and parietal lobes. Therefore, it may be hypothesized that in GRN+ carriers, the development of WMSAs begins at some point during the presymptomatic stage and progresses throughout the disease course at a rate higher than that in other mutation subtypes or in normal aging. This was investigated in a previous multi-centre study (The Genetic Frontotemporal Dementia Initiative, GENFI), but the authors did not find evidence of higher T2-HyperWMSA accrual rate in presymptomatic GRN+ carriers relative to those who do not carry the specific mutation (‘noncarriers’). Furthermore, diffusion tensor imaging (DTI), which provides measures of WM microstructural integrity based on the magnitude and direction of tissue water diffusion, have shown different patterns of presymptomatic WM integrity decline across the mutation carrier subgroups. The locations of the altered fibres generally coincide with those affected in symptomatic carriers, suggesting that the deviation from the normal WM structure begins at the presymptomatic phase of familial frontotemporal dementia.

In this study, we assessed the accumulation of WMSAs and the alterations of DTI parameters in predementia carriers of GRN and C9orf72 mutations, leveraging MRI data from the University of British Columbia (UBC) familial frontotemporal dementia study. We hypothesized that (i) GRN+ carriers would have a higher rate of WMSA accrual compared with C9orf72+ carriers, or family members who do not carry the mutations (C9orf72− and GRN−; noncarriers) and that (ii) the level of DTI parameter alterations would be higher in GRN+ and C9orf72+ carriers compared with the noncarriers. It is recognized that WMSAs have significantly altered DTI parameters [e.g. lower fractional anisotropy (FA) and/or higher mean diffusivity (MD)] compared with normal-appearing WM tissues (NAWM; i.e. WM areas not affected by signal abnormalities) and that they can affect the interpretation of DTI findings if not adjusted for. Therefore, we also specifically assessed whether the DTI parameters within the NAWMs become altered in the mutation carriers versus the noncarriers.

Materials and methods

Participants

Participants were recruited from the UBC familial frontotemporal dementia study, which is an ongoing single-centre longitudinal observational study of the Canadian cohort of familial frontotemporal dementia associated with TDP-43 pathology. Study inclusion and exclusion criteria were described previously. The study was approved by the UBC Clinical Ethics Review Board. All participants provided written, informed consent. Genetic testing and the recruitment protocol for this cohort have been described previously. Study recruitment began in January 2006 and a total of 131 participants were enrolled as of February 2022. Of those, a total of 112 participants had at least 1.5T MRIs done as of February 2022. However, because of staggered entry into the study, not every participant had completed the full study protocol at the time of this analysis. Accordingly, we analysed two subsets of the participants selected based on the availability of appropriate data: (i) for the longitudinal accumulation of WMSA, N = 42 participants with both baseline and 2 years of longitudinal follow-up T1-weighted MRIs [two scans per participant; average interval of 28 months ± standard deviation (SD) = 8] and (ii) for the cross-sectional analysis of DTI-based WM abnormalities, N = 60 participants with DTI scans (one scan per participant). For this analysis, we only included the participants who were not classified into the dementia category, as determined by a consensus meeting involving the study neurologist and neuropsychologist.

Genetic status

Study participants were screened for mutations related to familial frontotemporal dementia and then stratified into mutation carriers (C9orf72+ or GRN+) or noncarriers (C9orf72− or GRN−) based on DNA extracted from peripheral blood, according to the previously described protocols. Every C9orf72+ participant had expansions that were at least 100 repeats in length. Throughout the study, the participants and researchers remained blind to the genetic mutation status.

MRI methods

MRI acquisition

All MRI data were acquired on a 1.5T GE Signa scanner at the UBC Hospital MRI Research Centre, using the following imaging parameters: (i) localizers (0:25 min): sagittal/coronal and axial; fast gradient repetition time (TR): 5.4, echo time (TE): 1.6, one average, the field of view (FOV): 22 cm, 256 × 128 matrices; (ii) 3D T1-fast spoiled gradient-echo IR prep (8:35 min): TR/TE (ms) = 10.3/4.8, 8° flip angle, 166 × 256 × 256, 1.0 × 0.98 × 0.98 mm3, FOV: 166; (iii) proton density/T2 dual (4:00 min), axial, TR = 2800, TE = 30/90, 90° flip angle, 256 × 256 × 35, 0.86 × 0.86 × 5.0 mm3, FOV 220; (iv) DTI (11:42 min): axial, spin-echo planar imaging, TR = 13 000 ms TE = 85 ms, asset: 21 000 db, tensor: 25 diff directions, two averages, freq directions (DIR): R/L, phase encoding (PE) DIR: posterior-anterior (PA), number of excitations (NEX) = 2, 256 × 256 × 48, 1.25 × 1.25 × 2.30 mm3, FOV: 320.

MRI processing

T1-weighted images were visually checked for quality and then processed using the FreeSurfer 5.3 pipeline, which provides cortical reconstruction and volumetric segmentation. We utilized a version implemented in the ‘Cloud Engine Resource for Accelerated Medical Image Computing for Clinical Applications’ portal (CERAMICCA; https://ceramicca.ensc.sfu.ca). All FreeSurfer outputs were manually...
examined and corrected for errors. Total intracranial volumes (TIVs) for subsequent statistical analyses were calculated using a multi-atlas label fusion technique, described in detail elsewhere.\textsuperscript{30}

While WMSAs are conventionally represented by T\textsubscript{2}-Hyper WMSAs, the limited resolution of our T\textsubscript{2}-weighted images (with a slice thickness of 5 mm) rendered them less suitable for the segmentation of hyperintense WM voxels. As an alternative indicator of WMSA, we employed T\textsubscript{1}-HypoWMSAs that were segmented as part of the FreeSurfer volumetric outputs. Previously, a study using a similar MRI platform (3D-fast spoiled gradient-echo IR prepapped sequence on a 1.5T GE scanner) has shown that the volumes of FreeSurfer-derived WM hypointensities were strongly correlated with the volumes of hyperintensities on FLAIR images.\textsuperscript{31}

In that context, we considered the T\textsubscript{1}-HypoWMSAs as a non-specific marker of abnormal WM tissues.

DTI images were processed using the FMRIB Software Library Diffusion Toolbox.\textsuperscript{32,33} Briefly, the steps included: (i) manual checking of the diffusion data, (ii) brain extraction on the nondiffusion weighted b0 images using ‘bet’, (iii) eddy currents and subject motion correction using ‘eddy’ and (iv) voxel-wise fitting of the eddy-corrected diffusion tensor data using ‘dtifit’. These steps produced the maps of FA (the degree of anisotropic diffusion within WM fibre tracts) and MD (the magnitude of water diffusion within tissue), as well as radial diffusivity (RaD; a measure of myelin change) and axial diffusivity (AxD; a measure of axonal damage) maps for each participant.

Regional variations in WM tissue abnormalities were assessed in terms of predefined region-of-interest (ROI) comparisons. Specifically, we subdivided the FreeSurfer-generated cerebral WM volumes into lobes and radial layers, similar to the approach used in previous studies.\textsuperscript{6,7} The lobes (frontal, temporal, parietal and occipital) were defined by combining the corresponding Desikan–Killiany atlas labels through FreeSurfer’s ‘mri_annotation2label’ and ‘mri_aparc2aseg’ functions.\textsuperscript{34} Figure 1 provides an example of the lobar ROIs. The radial layers were obtained by determining the normalized distance between the ventricular edge and the cortex-WM boundary and then dividing the WM into four equidistant layers. As in Sudre et al.,\textsuperscript{7} we combined the two middle layers to obtain a total of three layers (i.e. Layer 1: periventricular; Layers 2 + 3: medial; Layer 4: peripheral). Figure 2 provides an example of the radial layers.

### Statistical analysis

#### Participants characteristics

All analyses were conducted using SAS PROC GLM and JMP software (SAS, Cary, NC, USA). The one-way ANOVA was used to compare the mean age, education years, mean age of symptoms onset in family and expected years to symptom onset (EYO) proxy term for how close a participant is to developing frontotemporal dementia symptoms,\textsuperscript{35} calculated by subtracting the participant’s age at scan from the mean age of symptom onset in their respective family) among GRN+, C9orf72+ and noncarriers. Differences in the sex ratio were assessed using the likelihood-ratio $\chi^2$ test. The mini-mental state examination (MMSE) and the frontal assessment battery (FAB) test scores were compared using the Kruskal–Wallis test as they were not normally distributed.

### Baseline differences in T\textsubscript{1}-HypoWMSA burden (cross sectional)

A general linear model (GLM) was used to compare the baseline burden of whole-brain WMSA among GRN+, C9orf72+ and noncarriers. For each participant, we calculated the baseline burden of T\textsubscript{1}-HypoWMSAs as the fraction of the TIV [WMSA fraction (WMSAf)] to account for the differences in head sizes using the ‘proportion’ method.\textsuperscript{36} The calculated whole-brain WMSA fraction (WMSAf) were used as the dependent variable in the following GLM,

$$\text{WMSAf} \sim \text{CarrierSubgroup} + \text{Age} + \text{Sex} + \text{EYO} \quad (1)$$

The CarrierSubgroup term included GRN+, C9orf72+ and noncarriers (pooled GRN− and C9orf72−). Age is a significant risk factor for cerebral small vessel disease,\textsuperscript{37} which appears as WMSAs on MRI. EYO was included to examine whether the rate of WMSA accumulation would be higher in participants getting closer to the family mean age of onset. Additionally, we calculated the WMSAf within each lobe (bilateral frontal, temporal, parietal and occipital) to explore the regional distribution of T\textsubscript{1}-HypoWMSAs. We used a subject-specific random-effects model to compare the baseline WMSA burden among the lobes.

### Differences in the annualized rates of the total T\textsubscript{1}-HypoWMSA accumulation (longitudinal)

For each participant, the longitudinal rates of the total T\textsubscript{1}-HypoWMSA accumulation were expressed in terms of the annualized percentage change relative to the baseline volume. The annualization factor was obtained by dividing 12 months by the months between the baseline and the follow-up MRI visits,

$$\frac{\text{WMSA}_\text{volBaseline} - \text{WMSA}_\text{volFollowup}}{\text{WMSA}_\text{volBaseline} \times \text{Annualization factor}} \times 100 \quad (2)$$

The following GLM was used to compare the annualized percentage change rates of the whole-brain T\textsubscript{1}-Hypo WMSA accumulation among GRN+, C9orf72+ and noncarriers,

$$% \text{Change in WMSA} \sim \text{CarrierSubgroup} + \text{Age} + \text{Sex} + \text{EYO} + \text{TIV} + \text{WMSAf} \quad (3)$$

Definitions of the CarrierSubgroup, age, sex and EYO terms were identical to the abovementioned cross-sectional WMSA.
For this model, we have adjusted for the impact of head size variability by including TIVs as a covariate (i.e. ‘GLM’ approach). Additionally, we have included the baseline burden of WMSAs as they were expected to be predictive of the longitudinal WMSA progression; we have used WMSAf as the covariate as it led to reduced multicollinearity among the covariates as well as more normally distributed GLM model residuals.

For both WMSA models, we first conducted an omnibus F-test to examine whether the model outperformed the null model. After confirming the significance of the model, we assessed an effect test of the CarrierSubgroup term followed by post hoc pairwise tests (three total: GRN+ versus noncarriers, C9orf72+ versus noncarriers and GRN+ versus C9orf72+) using the Tukey’s HSD method to account for multiple comparisons. P<0.05 was considered statistically significant.

Differences in the alterations of NAWM DTI parameters (cross sectional)

For each participant, we calculated the average FA, MD, RaD and AxD parameter values within the periventricular, medial and peripheral layers. Specifically, we focused on the NAWM regions to investigate the differences in WM microstructure that are not captured by the T1-HypoWMSAs. This was done by masking out the T1-HypoWMSA regions during the calculation of DTI parameters.

The following GLM was fitted for the FA and MD values within each radial layer,

\[
\text{DTI Parameter} \sim \text{CarrierSubgroup} + \text{EYO} \\
+ \text{CarrierSubgroup} \times \text{EYO} + \text{Age} \\
+ \text{Sex} + \text{WMVf} + \text{WMSAf} \quad (4)
\]

The definitions of the CarrierSubgroup, EYO, age and sex terms were identical to the WMSA GLM models. The CarrierSubgroup × EYO interaction term was included to assess whether the mutation carriers had different ‘slopes’ of DTI parameter change over the EYO range. WMSA fractions at the time of the DTI scan were also included, as it is possible that the NAWM penumbra regions surrounding the focal T1-HypoWMSA have altered DTI parameters due to more widespread WM tissue injury. WM volume fraction (WMVf; a measure of WM atrophy) was calculated by dividing the total cerebral WM volume by the TIV; this was included as WM atrophy was expected to influence the DTI parameters.

Like the WMSA models, we first examined whether the omnibus F-test was significant and then tested whether the slopes were significantly different between the GRN+ or C9orf72+ carriers versus the noncarriers. Additionally, to investigate the rates of diffusion along the longitudinal and perpendicular axes, we conducted similar GLM-based group comparisons of the RaD and AxD parameters in the WM...
layers that exhibited significant group differences in the FA or MD parameters.

### Results

#### Participant characteristics

The participants’ characteristics are outlined in Table 1 (those who were included in the WMSA analysis) and Table 2 (those who were included in the DTI analysis). Years of education, mean age of onset in families, MMSE and FAB scores were not significantly different among the subgroups. Although ANOVA did not reveal significant subgroup differences in age and EYO, the C9orf72+ carriers in our cohort tended to be younger and therefore further away from the expected symptoms onset. The sex ratio was imbalanced, with more female GRN+ carriers in the study and also more female C9orf72+ carriers who contributed to the DTI analysis.

#### Baseline differences in T1-HypoWMSA burden (cross sectional)

Although not statistically significant, it should be noted that our GRN+ group had overall smaller T1-HypoWMSA volumes at baseline, likely due to the female predominance in the group. Adjusting for age, sex and EYO, baseline WMSAf were not significantly different among the subgroups after correcting for multiple comparisons using Tukey’s HSD method (model-adjusted group comparisons are reported in Table 3).

WM lobar-wise analysis suggested that the frontal lobe had significantly higher T1-HypoWMSA burden compared with the parietal, temporal or occipital lobes ($P < 0.0001$) across the subgroups. Also, the parietal lobe had a significantly higher T1-HypoWMSA burden compared with the temporal or occipital lobes ($P < 0.0001$). The baseline burden was not significantly different between the temporal and occipital lobes. Figure 3 summarizes the findings.

#### Differences in the annualized rates of the total T1-HypoWMSA accumulation (longitudinal)

We found significant differences in the annualized rates of T1-HypoWMSA accumulation among the subgroups; the mean estimated rates were highest in GRN+ (15.87%/year), followed by C9orf72+ (3.69%/year) and noncarriers (2.64%/year). Post hoc pairwise comparisons indicated that the rates of WMSA accumulation in GRN+ were significantly higher compared with those in C9orf72+ ($P = 0.0038$) or noncarriers ($P = 0.02$), with ‘very large’ (Cohen’s $d > 1.4$) effect sizes.

The rates were not significantly different between C9orf72+ and noncarriers. Overall, the rates were not significantly associated with age, sex, EYO and TIV terms. As expected, a lower WMSAf at baseline was associated with higher percentage changes in T1-HypoWMSAs. The estimated rates are summarized in Table 4.

#### Differences in the alterations of NAWM DTI parameters (cross sectional)

We observed significant group effects in the comparison of FA and MD parameters in the periventricular NAWM layer, but not in the medial and peripheral layers. In particular, the effect was driven by the interaction between the mutation subgroup term and the EYO term, suggesting that increasing EYO differentially affected periventricular FA and MD indices among mutation carriers and noncarriers. We also noted a significant group-by-EYO interaction term in the periventricular RD parameter, but not in the AxD parameter. These findings are summarized in Table 5.

Periventricular FA: We found a significant interaction between the CarrierSubgroup and the EYO terms ($P = 0.004$). Specifically, the interaction ‘slopes’ were significantly more

### Table 1: Participant characteristics for those included in the WMSA analysis: demographics, neuropsychological scores and family information

<table>
<thead>
<tr>
<th>Analysis: WMSA Mutation subgroup</th>
<th>Participants included in the WMSA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GRN+</td>
</tr>
<tr>
<td># of included participants</td>
<td>8</td>
</tr>
<tr>
<td>Baseline age in years, mean ± SD</td>
<td>49.5 ± 9.1</td>
</tr>
<tr>
<td>Sex ratio, M:F</td>
<td>1.7</td>
</tr>
<tr>
<td>Years of education, mean ± SD</td>
<td>12.3 ± 1.4</td>
</tr>
<tr>
<td>MMSE at baseline, mean ± SD</td>
<td>29.3 ± 1.0</td>
</tr>
<tr>
<td>FAB at baseline, mean ± SD</td>
<td>16.8 ± 1.0</td>
</tr>
<tr>
<td>Mean age of onset in family years, mean ± SD</td>
<td>57.9 ± 3.2</td>
</tr>
<tr>
<td>EYO, median, [IQR], (range)</td>
<td>−6.5</td>
</tr>
<tr>
<td></td>
<td>[−18 to −1.25]</td>
</tr>
<tr>
<td></td>
<td>(−23 to 4)</td>
</tr>
</tbody>
</table>

Age, MMSE and FAB indicate the average values at baseline. IQR = interquartile range.
negative in C9orf72+ compared with noncarriers, suggesting a faster decline of FA values with increasing EYO. This was not observed between GRN+ and noncarriers. Age, sex and baseline WMSAf terms were not associated with FA values. A smaller WMVf was associated with a lower FA value.

Periventricular MD: We found a significant interaction between the CarrierSubgroup and the EYO terms (P = 0.02). Specifically, the interaction ‘slopes’ were significantly more positive in GRN+ compared with noncarriers, suggesting a faster increase of MD values with increasing EYO. This was not observed between C9orf72+ and noncarriers. Sex and baseline WMSAf terms were not associated with MD values while increasing age was significantly associated with increasing MD values. A smaller WMVf was associated with a higher MD value.

Periventricular RaD: A significant interaction was found between the CarrierSubgroup and the EYO terms (P = 0.02), where the interaction ‘slopes’ were significantly more positive in GRN+ compared with noncarriers. This suggested a faster increase of RaD values with increasing EYO in GRN+ compared with noncarriers, but not in C9orf72+. Sex and baseline WMSAf terms were not associated with RaD values, but increasing age was significantly associated with increasing RaD values. A smaller WMVf was associated with a higher RaD value.

### Section: Discussion

In this study, we compared carriers of GRN or C9orf72 mutations and their non-carrier family members in terms of the longitudinal accumulation of T1-HypoWMSAs and the cross-sectional alterations of DTI parameters. Baseline burden of T1-HypoWMSAs was not significantly different among GRN+, C9orf72+ and noncarriers. Longitudinally, the annualized rates of T1-HypoWMSA accumulation were significantly higher in GRN+ compared with C9orf72+ and noncarriers, indicating more rapid progression of visible WM abnormalities in GRN+. Also, DTI analysis revealed that GRN+ carriers with a higher EYO (i.e. closer to expected symptom onset) were associated with higher MD and RaD values. On the other hand, C9orf72+ carriers with higher EYO were associated with lower FA values. These findings suggest that WM abnormalities progress differently in GRN+ and C9orf72+ carriers during the pre dementia stages of pathogenesis.

To explore the spatial distribution of WM abnormalities at baseline, we first cross sectionally compared the volumes of T1-HypoWMSAs within the WM lobar regions. For all subgroups, the vast majority of the T1-HypoWMSAs were found in the frontal and parietal lobes, while distributions within the occipital and temporal lobes were relatively less. Overall, there were no significant differences in the baseline volumes of T1-HypoWMSAs among the subgroups, which is in line with findings from the GENFI study, where the authors reported nonsignificant differences in T2-HyperWMSA volumes among presymptomatic GRN+, C9orf72+, MAPT+ and noncarriers. However, as the burden of WMSAs is more extensive in symptomatic GRN+ carriers, it can be expected that the average predementia rate of WMSA progression would be higher in GRN+.

As expected, our GRN+ carriers had significantly higher rates of total T1-HypoWMSA accumulation compared with C9orf72+ or noncarriers. Interestingly, our longitudinal finding was unlike that from the GENFI study, where the

### Table 2 Participant characteristics for those included in the DTI analysis: demographics, neuropsychological scores and family information

<table>
<thead>
<tr>
<th>Analysis: DTI Mutation subgroup</th>
<th>Participants included in the DTI analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GRN+</td>
</tr>
<tr>
<td># of included participants</td>
<td>9</td>
</tr>
<tr>
<td>Baseline age in years, mean ± SD</td>
<td>53.8 ± 6.8</td>
</tr>
<tr>
<td>Sex ratio, M:F</td>
<td>1.8</td>
</tr>
<tr>
<td>Years of education, mean ± SD</td>
<td>13.0 ± 3.0</td>
</tr>
<tr>
<td>MMSE at baseline, mean ± SD</td>
<td>29.0 ± 1.0</td>
</tr>
<tr>
<td>FAB at baseline, mean ± SD</td>
<td>17.2 ± 1.2</td>
</tr>
<tr>
<td>Mean age of onset in family in years, mean ± SD</td>
<td>58.4 ± 3.4</td>
</tr>
<tr>
<td>EYO, median, [IQR], (range)</td>
<td>−5</td>
</tr>
<tr>
<td></td>
<td>[−8.3 to 1]</td>
</tr>
<tr>
<td></td>
<td>(−18 to 6)</td>
</tr>
</tbody>
</table>

Overlapping participants: 7 GRN+, 11 C9orf72+ and 21 noncarriers contributed to both WMSA and DTI analyses. Age, MMSE and FAB indicate the average values at the time of DTI. IQR = interquartile range.

### Table 3 Comparison of T1-HypoWMSA burden at baseline, corrected for age, sex and estimated years to onset

<table>
<thead>
<tr>
<th>Region</th>
<th>Mutation subgroup</th>
<th>Baseline T1-HypoWMSA volume in fraction of TIV, marginal mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>GRN+</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>brain</td>
<td>C9orf72+</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Noncarriers</td>
<td></td>
<td>0.12 ± 0.008</td>
</tr>
</tbody>
</table>

T1-HypoWMSA burden was calculated as the fraction of the TIV. GRN+, C9orf72+ and noncarriers were not significantly different. SE = standard error.
authors reported nonsignificant differences in the rates of T2-HyperWMSA accumulation between presymptomatic GRN+ and noncarriers.7 We propose potential reasons for the discrepancy. First, the two studies utilized different WMSA measures: T1-HypoWMSAs versus T2-HyperWMSAs. Even though these two measures tend to be strongly correlated, T2-weighted sequences are relatively more sensitive to WM alterations and can detect higher volumes of WMSAs.31 Yet, T1-HypoWMSAs may represent more focused areas of severe WM injury in which progression is associated with cognitive decline.42 Second, our GRN+ cohort was potentially closer to symptom onset compared with the GENFI cohort, as implied by the median EYO of −6.5 years and a higher average baseline burden of WMSAs [1342.9 mm³ (T1-Hypo) versus 925.9 mm³ (T2-Hyper); the difference would be even greater if the sequences are matched].

Figure 3 WM lobar-wise distribution of the T1-HypoWMSA burden at baseline, calculated as the fraction of the TIV. Boxplots indicate quantiles. Individual dots indicate individual participant data. A subject-specific random-effects model was used to compare the baseline WMSA burden among the lobes. The frontal lobe had significantly higher T1-HypoWMSA burden compared with the parietal, temporal, or occipital lobes (P < 0.0001). The parietal lobe had a significantly higher T1-HypoWMSA burden compared with the temporal or occipital lobes (P < 0.0001). Baseline burden was not significantly different between the temporal and occipital lobes.
Table 4 Whole-brain T1-HypoWMSA progression: estimated annualized rates of total cerebral T1-HypoWMSA accumulation by mutation subgroup, adjusted for age, sex, EYO, TIV and baseline WMSAf

<table>
<thead>
<tr>
<th>Region</th>
<th>Mutation subgroup</th>
<th>Estimated annualized % change from the baseline, marginal mean ± SE, [95% CI]</th>
<th>Post hoc Tukey’s HSD, mean difference, [95% CI], (Cohen’s D), [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>GRN+</td>
<td>15.87 ± 3.25 [9.26, 22.48]</td>
<td>GRN+ versus noncarriers P = 0.0038 [13.22 [3.93, 22.53] (1.59) [0.61, 2.54]</td>
</tr>
<tr>
<td></td>
<td>C9orf72+</td>
<td>3.69 ± 2.65 [-1.69, 9.08]</td>
<td>GRN+ versus C9orf72+ P = 0.020 [12.18 [1.67, 22.68] (1.46) [0.38, 2.51]</td>
</tr>
<tr>
<td>Noncarriers</td>
<td></td>
<td>2.64 ± 1.82 [-1.06, 6.34]</td>
<td>C9orf72+ versus noncarriers P = 0.94 [1.05 [-6.93, 9.04] (0.26) [0.04, 4.92]</td>
</tr>
</tbody>
</table>

Post hoc group-wise comparisons were conducted using the Tukey’s HSD method and significant differences are marked by an asterisk. CI, confidence interval; SE, standard error.

Table 5 Periventricular DTI parameters at baseline: group-wise comparison of the [subgroup: EYO] interaction terms estimated from GLM tested whether the association between periventricular DTI indices and increasing EYO differed between mutation carriers and noncarriers

<table>
<thead>
<tr>
<th>DTI index</th>
<th>Differences in the interaction ‘slopes’ between carriers and noncarriers, estimated mean ± SE, [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA (periventricular)</td>
<td>GRN+ versus noncarriers P = 0.39</td>
</tr>
<tr>
<td></td>
<td>−0.0006 ± 0.0007 -0.00073 [−0.0021, 0.00084]</td>
</tr>
<tr>
<td></td>
<td>C9orf72+versus noncarriers P = 0.0040</td>
</tr>
<tr>
<td></td>
<td>[−0.0011 ± 0.00038] [−0.0019, −0.00038]</td>
</tr>
<tr>
<td>GRN+ versus noncarriers</td>
<td>P = 0.02†</td>
</tr>
<tr>
<td></td>
<td>6.06E-06 ± 2.44E-06 [1.15E-06, 1.08E-05]</td>
</tr>
<tr>
<td></td>
<td>C9orf72+ versus noncarriers P = 0.26</td>
</tr>
<tr>
<td></td>
<td>1.44E-06 ± 2.16E-06 [−1.10E-06, 3.97E-06]</td>
</tr>
<tr>
<td>Mean Diffusivity</td>
<td>GRN+ versus noncarriers P = 0.02†</td>
</tr>
<tr>
<td>(Periventricular)</td>
<td>5.69E-06 ± 2.30E-06 [1.06E-06, 1.03E-05]</td>
</tr>
<tr>
<td></td>
<td>C9orf72+ versus noncarriers P = 0.09</td>
</tr>
<tr>
<td></td>
<td>2.08E-06 ± 1.19E-06 [−3.13E-07, 4.47E-06]</td>
</tr>
</tbody>
</table>

Significant differences are marked by an asterisk.

Third, our study utilized a single 1.5T MRI scanner, whereas the GENFI study utilized 3T MRI scanners. As 3T scanners are generally more sensitive to WMSAs, the GENFI study protocol likely had the ability to detect more WMSA volumes compared with our protocol. For these reasons, it is difficult to directly compare our findings to the GENFI findings, although both studies demonstrate the vulnerability of WM in predementia GRN+ carriers.

The causes of WMSAs in our predementia GRN+ carriers remain to be elucidated due to the unavailability of pathological correlates. Nevertheless, previous histopathological studies have shown significant microglial activation and dystrophy in frontotemporal dementia patients with GRN mutation, with a higher proportion of the amoeboid shape within more severely abnormal areas. In particular, more severely affected T2-HyperWMSA areas exhibited a greater degree of myelin loss and astroglialosis with minimal infarcts or haemorrhages, suggesting that the WMSAs may be inflammatory mediated rather than secondary to vascular events. Whether this explanation also applies to our GRN+ carriers requires further verification, although anecdotal, our cohort was relatively young with an average age of around 50 years. Also, we found that the vast majority of the T1-HypoWMSA accumulation occurred within the periventricular layer, which, compared with the deep WM regions, may be more susceptible to inflammatory responses following blood–brain barrier disruption.

A higher burden of WMSAs is associated with GM volume loss in symptomatic GRN+ carriers, suggesting a potential relationship between WM abnormalities and tissue atrophy. Yet, the temporal relationship between WMSAs and brain atrophy in frontotemporal dementia remains less understood. Cortical and subcortical GM volume loss is relatively more pronounced in C9orf72+ during predementia stages, although changes in GRN+ are also noted in the frontal and the parietal lobes. But once in the symptomatic stage, GRN+ carriers suffer the fastest rates of brain volume loss, led by accelerated decline in the frontal, temporal and parietal lobe. A possible hypothesis is that the rapidly accumulated WMSAs during the predementia stages lead to subsequent secondary degenerations (e.g. Wallerian or retrograde degeneration) of the affiliated tissues, which may contribute to the future accelerated rates of lobar atrophy in GRN+ carriers. For example, Wallerian degeneration in the central nervous system progresses for months to years,
suggesting that the consequences of WMSA accumulations during predementia on atrophy may be delayed and not manifested immediately. Furthermore, an asymmetric pattern of GM atrophy, which variably affects the left or right hemisphere, has been reported in symptomatic GRN+ carriers as well as asymptomatic GRN+ carriers nearing symptomatic onset.\textsuperscript{35,51,53} A previous GENFI study noted that in symptomatic mutation carriers, asymmetry in the frontal lobar GM atrophy is associated with the asymmetric distribution of frontal lobar T2-HyperWMSA; however, the authors did not find any association in asymptomatic mutation carriers.\textsuperscript{7} This suggests that an uneven lateral distribution of WM abnormalities may partly explain asymmetric GM atrophy, although their sequential relationship remains unclear. A longitudinal analysis of the individuals who convert from asymptomatic to symptomatic frontotemporal disorders is warranted to answer whether an early asymmetric progression of WMSA leads to future brain atrophy preferentially affecting the same hemisphere. Another point to consider is the conceivable impact of the increased inflammatory responses (e.g. build-up of extracellular water and/or increase in the number of inflammatory cells) on the brain volume of GRN+ carriers. If significant, this effect may confound the interpretation of brain volume changes upon the initiation of treatments that resolve the inflammatory environment, in terms of 'pseudoatrophy', which refers to transient brain volume loss not due to tissue atrophy, but due to resolution of oedema and gliosis.\textsuperscript{54} Monitoring of brain tissue water change, for example using water-sensitive MRI sequences, may help elucidate this possibility.

In addition to the visibly abnormal tissues, we explored microstructural properties within the NAWM regions using conventional DTI parameters. The tested GLM showed significant effects within the periventricular WM layer (where most of the WMSA accumulation occurred), specifically a relationship between increasing EYO (i.e. getting closer to the expected symptom onset age) and increasing MD and RaD in GRN+ but not in C9orf72+ carriers. This indirectly reflects the progressive increase in free diffusion over the periventricular WM disease course, potentially related to the exacerbation of inflammatory-mediated gliosis and demyelination described in GRN+.\textsuperscript{47} Furthermore, the peri-WMSA NAWM may represent the 'penumbra' that is perturbed by the severely abnormal foci,\textsuperscript{38} and predisposed to conversion to WMSAs in the future.\textsuperscript{40,55} Longitudinal monitoring of the DTI parameters would be required to answer this question.

Intriguingly, there was no evidence of decreasing FA with increasing EYO in GRN+ compared with noncarriers, which is similar to a previous longitudinal finding.\textsuperscript{56} As FA is more influenced by axonal health than by myelin,\textsuperscript{57} our finding suggests relative sparing of axons within the NAWM, despite presumed demyelination, over the predementia disease course. This is in line with the pathological description of mild axonal loss in GRN+\textsuperscript{47} and may explain the absence of volume reduction at baseline in our GRN+ carriers.\textsuperscript{23}

In contrast, our C9orf72+ carriers were characterized by a relationship between increasing EYO and decreasing FA. At the same time, there was no evidence of increasing MD over the predementia course, which, along with the longitudinal stability of T1-HypoWMSAs, suggests that WM abnormalities in our C9orf72+ carriers are less likely to be due to cellularity and oedema-related changes but more likely to be due to declining axonal density and/or membrane integrity. Indeed, the baseline analysis of our C9orf72+ carriers found cortical and subcortical volume reductions compared with GRN+,\textsuperscript{23} potentially reflecting atrophy associated with compromised axons.

Our study had several limitations that laid the foundation for future work. First, being a single-centre study, our sample size was unbalanced, especially with a smaller number of GRN+ carriers with a female preponderance. In that sense, our findings involving the GRN+ group may have been confounded by sex-related differences on WMSA and DTI measures. Second, our longitudinal analysis was based on two time points, restricting us to the use of a linear model. Although our scan interval was relatively short, WM abnormalities over the long term may progress in a non-linear pattern, particularly approaching the time of symptoms onset.\textsuperscript{35} A longer follow-up study, including symptomatic converters, is necessary to determine whether the courses of WMSAs differ between lobes as they approach the time of symptom onset. Similarly, our DTI analysis was cross-sectional, allowing us to capture only a snapshot of the predementia course and assess the progression of DTI indices only indirectly in terms of EYO, not actual time. Additional follow-up of our cohort will alleviate these two issues in the future. Third, a direct comparison of our T1-HypoWMSA and more conventionally used T2-HyperWMSA measures must be done carefully. While hypointensities on T1-weighted 3D gradient-echo images generally correspond to hyperintensities on T2-weighted images, it likely detects relatively smaller volumes of WMSAs (e.g. average 10% difference in non-demented elderly).\textsuperscript{31} In that sense, our definition of NAWM may have encompassed the areas that may have been classified as hyperintensities on T2-weighted images. Although T1-HypoWMSAs and T2-HyperWMSAs tend to be highly correlated, their exact relationships remain less understood. In particular, it needs to be verified in the future whether the two measures follow similar or different trajectories in mutation carriers approaching symptomatic conversion. Fourth, our diffusion images had a relatively lower resolution, which is prone to partial volume effects. It is possible that our DTI findings, especially those from the periventricular regions, may have been influenced by cerebrospinal fluid contamination of brain voxels. Moreover, our findings based on predefined ROIs may have been relatively less sensitive to WM abnormalities due to averaging the signal over multiple regions. Future analyses are warranted to investigate more specific locations of the WM abnormalities. Fifth, our conventional DTI parameters need to be interpreted with caution, as the results could have been confounded by the presence of extracellular water. This may be addressed in the future by using post-processing techniques, such as free-water imaging,\textsuperscript{58} that can yield separate signals from ‘free-water’ (associated with freely diffusing water in the extracellular compartment) and ‘tissue compartment’ (associated with tissue-constrained water, i.e. free-water corrected DTI indices).
Despite these limitations, our study indicates the ongoing presence of WM alterations in predementia GRN and C9orf72 mutation carriers. Notably, our GRN+ carriers had significantly higher rates of T1-HypoWMSA accumulation as well as an increasing tendency of MD and RD along with increasing EYO. These may reflect the initial phases of the inflammatory environment and the vulnerability to demyelination and gliosis described in frontotemporal dementia patients with the GRN mutation. Even though there was no evidence of WMSA or diffusivity changes in our C9orf72 + carriers, a decreasing tendency of FA along with increasing EYO may reflect axonal vulnerability, potentially associated with a higher degree of volume loss observed in these carriers compared with GRN. Accordingly, our findings suggest that WM changes could represent early neuroimaging markers of familial frontotemporal dementia due to a genetic cause and that different mutation carriers may require different targeted preventative measures or treatments.

Acknowledgements

The authors thank all the participants of the research study.

Funding

The study is supported by funding from the Canadian Institutes of Health Research operating grant #179009 and #74580, the Pacific Alzheimer’s Research Foundation (Pacific Alzheimer’s Research Foundation centre grant C06-01), and the National Institutes of Health UH3/UG3 NS103870 to R.R.. H.L. acknowledges support from a Canadian Institutes of Health Research Postdoctoral Fellowship. G.Y.R.H. is supported by funding through the Ralph Fisher Professorship in Alzheimer’s Research (Alzheimer Society of British Columbia, Canada) and by a Canadian Institutes of Health Research Clinical Genetics Investigatorship award. M.F.B. and K.P. are supported by funding from Natural Sciences and Engineering Research Council of Canada (NSERC), Alzheimer’s Society Research program, Pacific Alzheimer’s Research Foundation, Genome British Columbia and Brain Canada.

Competing interests

R.R. is a member of the Scientific Advisory Board or Arkuda Therapeutics and receives royalties from a GRN patent. She also obtains funding from the National Institutes of Health, the Department of Defense, the Koning Boudewijn Stichting, Flanders Institute for biotechnology (VIB) and University of Antwerp. G.R.H. has received research support as a clinical trial site investigator from Anavax, Biogen, Eli Lilly and Roche, and has received research grants from the CIHR, Alzheimer Society of Canada, and NIA/NIH. G.Y.R.H. is supported by the Ralph Fisher Professorship in dementia research from the Alzheimer Society of British Columbia. The other authors report no competing interests.

Data availability

De-identified clinical, imaging and molecular data will be made available at the Canadian Association of Research Library (http://www.carl-abrc.ca/).

References


