

Apparent Fibre Density (AFD) Analysis Reveals Decreases in Axonal Density in the White Matter Pathways of Patients with Grey Matter Heterotopia

Shawna Farquharson^{1,2}, David Raffelt¹, Farnoosh Sadeghian¹, J-Donald Tournier^{1,3}, Simone Mandelstam^{1,4}, Michal Schneider-Kolsky², Samuel F Berkovic⁵, Ingrid Scheffer^{1,5}, Graeme Jackson^{1,6}, and Alan Connelly^{1,5}

¹The Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia, ²Department of Medical Imaging & Radiation Science, Monash University, Melbourne, Victoria, Australia, ³Department of Medicine, Austin Health, University of Melbourne, Victoria, Australia, ⁴Medical Imaging, Royal Children's Hospital, Melbourne, Victoria, Australia, ⁵Department of Medicine, Austin Health, University of Melbourne, Victoria, Australia, ⁶Department of Medicine, Austin Health, University of Melbourne, Victoria, Australia

Target Audience: Clinicians investigating epilepsy patients with neuronal migration disorders such as heterotopia, and researchers performing diffusion weighted imaging analysis.

Purpose: Individuals with neuronal migration disorders such as neuronal heterotopia often present with epilepsy that is refractory to medical and surgical treatment^[1]. In some cases there is a known genetic association e.g. the Filamin A (FLNA) gene; however, in many cases there is no known cause. Current radiological classification is limited to the extent and location of heterotopic regions, despite there being some evidence to suggest that there are functional differences between nodules in individual patients^[2]. Recent fibre tractography studies of patients with Periventricular Nodular Heterotopia (PVNH) have reported abnormal fibre connections associated with some nodules^[3]. Given these findings and the variability in outcome in patients with neuronal migration disorders, it is likely that there may be structural differences beyond regions of heterotopia that are not visible using conventional imaging techniques. It is now possible to perform voxel-based analysis of the whole brain to measure tract specific differences in tissue microstructure using the Apparent Fibre Density (AFD) approach^[4]. AFD is sensitive to both micro-structural axonal loss and macroscopic white matter volume changes. In this study, we apply AFD to investigate whether there are associated differences in fibre density in white matter tracts in patients with PVNH.

Methods: A consecutive series of 13 patients with neuro-imaging confirmed diagnosis of PVNH (4 male: 9 female) and 13 normal volunteers matched for age and sex were investigated. All participants provided informed written consent in accordance with ethical approval from the local Human Research Ethics Committee. MRI data were acquired on a 3T Siemens TIM Trio. Whole-brain axial DWI data were obtained using a twice-refocused single-shot echo-planar imaging sequence, with 60 directions with $b=3000 \text{ s/mm}^2$. High-resolution 3D T1-weighted MPRAGE data were also acquired for anatomical reference. **AFD analysis:** Pre-processing of DWI data involved motion and bias field correction, intensity normalisation and up-sampling by a factor of 2^[4]. Fibre Orientation Distributions (FOD) were calculated using Robust Constrained Spherical Deconvolution^[5,6]. Individual FOD images were non-linearly registered to a population specific template, and final warps were applied with FOD modulation^[4]. The AFD was computed for each population of *fibres* within a *voxel* ('fixel') by integrating the FOD lobe corresponding to the fixel. The data for PVNH patients and normal volunteers were compared at every fixel using t-tests with connectivity-based fixel enhancement and significance was assigned to each fixel using corrected p-values using permutation testing^[8]. Significant fixels ($p < 0.05$) were displayed using tractography streamlines, and colour coded by direction or by effect size (Figure 1). AFD analysis was performed on controls versus: (1) all PVNH patients, (2) a subset of 6 patients with 'moderate' PVNH (e.g. <5 clusters of bilateral nodules in the temporal, occipital and/or parietal regions), and (3) a subset of 7 patients with severe PVNH (e.g. extensive banks of bilateral nodules). (See arrows in Fig 1A & B for example nodules in individual patients).

Results: We observed a significant decrease [$p < 0.05$] in AFD in white matter (WM) tracts beyond regions of heterotopia in PVNH patients compared to healthy control participants. Analysis of the subgroup of PVNH patients classified with a 'moderate' lesion loading identified a significant decrease in AFD (relative to controls) in posterior WM pathways, which included the splenium of the corpus callosum and WM tracts extending into the occipital cortex. (See Figure 1 C, E & G). Analysis of the PVNH subgroup with 'severe' lesion loading identified widespread decreases in AFD (relative to controls) in WM pathways throughout the brain, including (but not limited to) the genu, body & splenium of the corpus callosum, the cingulum, the superior longitudinal fasciculus and the corticospinal pathways. (See Figure 1 D, F & H). In many of the affected WM structures the observed effect size of the AFD decrease relative to controls was in the order of 50-90%. (See Figure 1 E & F). In many regions where there was a significant reduction in AFD, a reduction in the average fibre orientation distributions (FOD) was detectable on visual inspection (see example magnified region of interests shown in Figure 1 G & H).

Discussion & Conclusion: In this study we observe extensive reductions in AFD in patients with PVNH that can be attributed to decreases in axon density in white matter pathways^[4]. The magnitude of AFD decreases in regions beyond the location of the heterotopic nodules suggest that neuronal migration disorders may be associated with a more diffuse process affecting specific white matter pathways, and not disorders solely involving the failure of grey matter neurons to migrate to the cortex *in utero*. These findings are important given the prevalence of neurological disorders (eg epilepsy) in patients with neuronal heterotopia. The work presented here represents important radiological advancement as the findings may provide a structural basis for the variations in functional consequences observed in patients with neuronal migration disorders.

References: [1] Barkovich AJ. & Kjos BO., (1992) *Radiology* 182:493-9 [2] Archer J. et al., (2010) *Epilepsy & Behavior* 19:4:631-634 [3] Farquharson S. et al., (2013) *Proc. SMRT-ISMRRM* 22 [4] Raffelt D. et al., (2012) *NeuroImage* 59, 3976-3994 [5] Tournier J-D. et al., (2013) *Proc. ISMRM* 21, 0773 [6] Tournier J-D. et al., (2012) *International Journal of Imaging Systems and Technology* 2:53-66 [7] Smith R. et al., *NeuroImage* (2013) 67:298-312 [8] Raffelt D. et al., (2013) *Proc. ISMRM* 21, 841

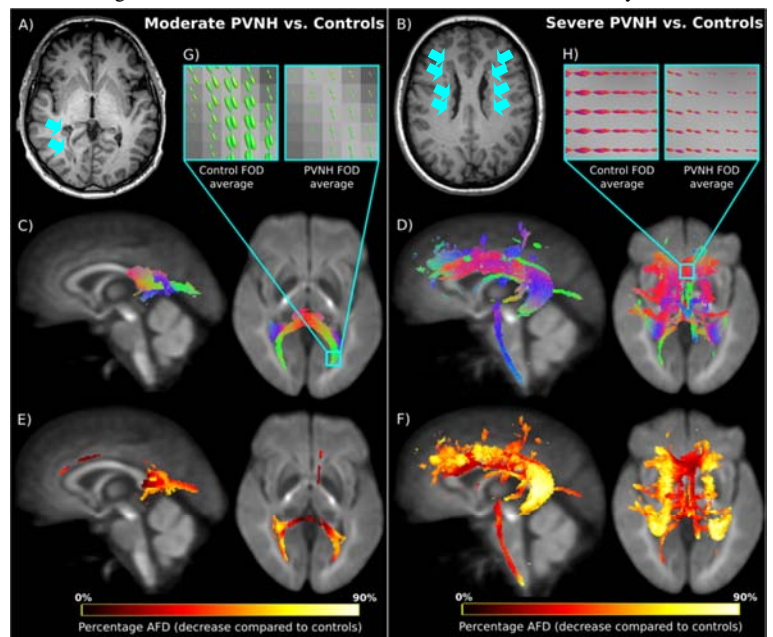


Figure 1: Images A-B: Axial T1 images of example patients with moderate PVNH [A] and severe PVNH [B]. Images C-F: Apparent fibre density (AFD) group results of patients with moderate PVNH [C&E], and severe PVNH [D&F], demonstrating regions of significant decrease in AFD ($p > 0.05$ corrected) compared to controls displayed by colour coded direction [C & D]; and the effect size displayed as a percentage AFD decrease relative to the control subjects [E&F]. Note the visible reduction in the average patient fibre orientation distributions (FOD) in the magnified regions of significant fixels compared to controls [G&H].