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Microstructural Alteration of White Matter Tracts due to 16p11.2 Chromosomal Duplication and Deletion



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# Microstructural Alteration of White Matter Tracts due to 16p11.2 Chromosomal Duplication and Deletion

by

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THESIS

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# DEDICATION AND ACKNOWLEDGEMENTS

I would like to acknowledge the support of my colleagues and professors from the Master of Science in Biomedical Imaging program at the University of California, San Francisco.

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# Microstructural Alteration of White Matter Tracts due to 16p11.2 Chromosomal Duplication and Deletion

Seoiyoung Ahn

## Abstract

This study aims to characterize microstructural white matter alterations and inter-hemispheric asymmetry that are induced by deletion and duplication at the chromosomal locus 16p11.2, a genetic locus closely associated with neuropsychiatric disorders such as autism spectrum disorder, bipolar disorder, schizophrenia and language disorders (Chang et al., 2016). In order to examine structural alterations of white matter tracts due to copy number variations at the chromosomal locus 16p11.2, diffusion tensors maps, including fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD), and tract density maps were generated from diffusion-weighted MR images. Our findings indicate that 16p11.2 chromosomal deletion induces systemic increase in AD and FA as well as tract densities in white matter tracts while the duplication at the chromosomal locus 16p11.2 increases RD while decreasing both FA and track density. Interestingly, white matter tracts that were severely affected by 16p11.2 chromosomal deletion and duplication were splenium of corpus callosum (SCC), genu of corpus callosum (GCC) and body of corpus callosum (BCC), all of which are crucial to inter-hemispheric communication of the human brain. These alterations in the microstructures of corpus callosum are thought to significantly contribute to inter-hemispheric asymmetry, which is often an apparent biomarker of many neuropsychiatric and neurodevelopmental disorders. Overall, this

study demonstrates the ramification of 16p11.2 chromosomal deletion and duplication on white matter microstructures, and we believe that this relationship between the copy number variations at the chromosomal locus 16p11.2 and the brain structure is a step towards devising and implementing a more efficient and effective interventions and therapies for currently-untreatable neuropsychiatric and neurodevelopmental disorders.

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# Introduction

Recent advancements in non-invasive brain imaging and gene sequencing technologies has rendered the process of investigating the interplay among genes, brain structures and behaviors much more efficient and effective (Owen et al., 2014). With such technological innovations, scientists across multiple scientific disciplines, such as neuroscientists, neurologists, neurosurgeons and neuroradiologists, were able to identify specific genetic mutations that are intimately associated with abnormal brain structures and that lead to behavioral and cognitive deficits of neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder. For example, it was recently shown that deletion at the chromosomal locus 15q11.2 BP1-BP2 induces systemic increase in fractional anisotropy of the posterior limb of internal capsule while its duplication induces significantly reduced FA (Silva et al., 2018).

Our study examines the reciprocal 600 kb deletion and duplication at the chromosomal locus 16p11.2, which is a genetic locus that is widely known to be closely associated with several neuropsychiatric and neurodevelopmental disorders, such as autism spectrum disorder, schizophrenia, bipolar disorder, attention-deficit hyperactivity disorder and speech and language disorders (Chang et al., 2016). Indeed, the pioneering study by Chang et al. (2016) has provided insights into how the copy number variants of the chromosomal locus 16p11.2 influence microstructural integrity of white matters, demonstrating that 16p11.2 chromosomal deletion increases fractional anisotropy (FA) throughout supratentorial white matter and that duplication of the same genetic locus displays an extensive and systemic decrease in FA. Similarly, children with 16p11.2 chromosomal deletion display alterations to the microstructural organization of white matter tracts involved in auditory and language processing, a piece of information that

partially explains why language ability impairment is a common feature of 16p11.2 deletion carriers (Berman et al., 2015). These pediatric carriers also display increased axial diffusivity (AD) in major central white matter tracts, including the anterior corpus callosum as well as bilateral internal and external capsules (Owen et al., 2014). This higher AD was strongly correlated with lower non-verbal IQ, corroborating the linkage between 16p11.2 deletion and language function impairment (Owen et al., 2014). Besides the behavioral manifestation of 16p11.2 deletion and duplication, copy number variants of the chromosomal locus 16p11.2 also affect morphological characteristics of an individual: while 16p11.2 deletion carriers tend to have higher body mass index and head circumferences, 16p11.2 duplication carriers have lower body mass index and head circumferences (Chang et al., 2018). Although scientists have delved into how the copy number variants of 16p11.2 affect the white matter structures, the ramification of these copy number variations on the white matter tract density and inter-hemispheric symmetry, both of which may also play an important role in affecting behaviors and cognitions, has not yet been fully explored.

Here, we aim to provide a more comprehensive explanation of the impacts of 16p11.2 chromosomal deletion and duplication on the diffusion tensor and tract density of white matter structures and also to investigate how those microstructural perturbations influence the inter-hemispheric asymmetry of white matter tracts. We hypothesized that both diffusion tensor, tract density and lateralization of white matter will be increased by 16p11.2 deletion systemically, leading to diffuse and nonspecific microstructural alterations to white matter structures.

# Materials and methods

### *Study subjects*

As a retrospective study, diffusion-weighted MR data of ninety-four subjects (50 males and 44 females) were previously recruited, tested and imaged from the University of California, San Francisco (UCSF) and the Children's Hospital of Philadelphia (CHOP). 14 deletion carriers and 14 duplication carriers were from UCSF, while the rest — 16 deletion carriers, 16 duplications carriers and 34 controls — were provided by CHOP. Moreover, all the subjects were age- and gender-matched as well as administered the Differential Ability Scales—Early Years & School Age (DAS- II) Intelligence Test for Children (WISC) to measure and assess their full-scale verbal IQ and non-verbal IQ sub-scores.

*Table 1. Subject Characteristics*

| | Controls (n = 34) | Deletion (n = 30) | Duplication (n = 30) |
|---|---|---|---|
| Age | 18.1 (Mean) ± 12.4 (SD) | 18.7 (Mean) ± 13.3 (SD) | 18.4 (Mean) ± 13.9 (SD) |
| Gender | 18 M, 16 F | 16 F, 14 M | 16 F, 14 M |
| Handedness | 18 Right, 16 Left | 16 Right, 14 Left | 17 Left, 13 Right |
| Verbal IQ | 101.4 (Mean) ± 14.5 (SD) | 87.92 (Mean) ± 14.41 (SD) | 89.64 (Mean) ± 17.2 (SD) |
| Non-verbal IQ | 103.5 (Mean) ± 13.3 (SD) | 91.63 (Mean) ± 14.72 (SD) | 88.4 (Mean) ±16.1 (SD) |
| Center | 34 CHOP | 14 UCSF, 16 CHOP | 14 UCSF, 16 CHOP |

### Neuroimaging pre- and post-processing analysis

*Diffusion tensor imaging data preprocessing*

The motion and eddy currents in the acquired diffusion-weighted images were corrected by Functional Magnetic Resonance Imaging of the Brain's (FMRIB's) Linear Image Registration

Tool with 12-parameter linear image registration (Jenkinson et al., 2002). Then, all diffusion-weighted images were registered to the reference image (b-value = 0 s/mm2 volume). In order to evaluate movements of each subject, a scalar parameter quantifying the transformation of each diffusion volume to the reference was calculated. Moreover, the non-brain tissue was removed using the Brain Extraction Tool of FMRIB's Software Library. Then, DTIFit of FMRIB's Software Library were performed to generate Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) maps (Fig. 1).

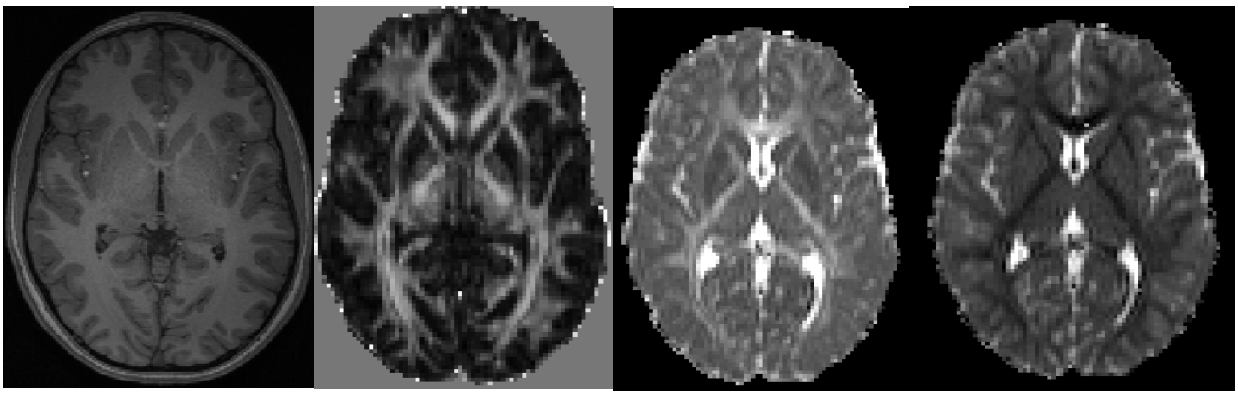

**Figure 1.** T1-Weighted images and diffusion tensor maps 1) Example axial slice of the T1-weighted images 2) Example axial slice of FA maps 3) Example axial slice of AD maps 4) Example axial slice of RD maps.

*Tract density map construction*

After parameter FA, MD, AD and RD maps were constructed through FMRIB's Software Library (FSL), FreeSurfer Software (http://surfer.nmr.mgh.harvard.edu/) was performed to segment out white matter from gray matter (Dale et al., 1999). In the process, FreeSurfer generated a white matter mask, which displays only white matter tracts in the brain anatomical T1-weighted images, as well as a termination mask, which only displays cortical gray matter of

the brain, for each subject (Dale et al., 1999). PROBTRACKX2 of FSL was then performed to construct tract density maps, in which each voxel represents the density of tracts in each brain region. In order to generate tract density maps, the white matter mask and the gray matter mask were fed into the GUI as a seed mask and a termination mask, respectively. With the information, PROBTRACKX2 of FSL computed the density of tracts that stem from each voxel of 3D brain imaging volume, thereby producing tract density maps (Fig. 2).

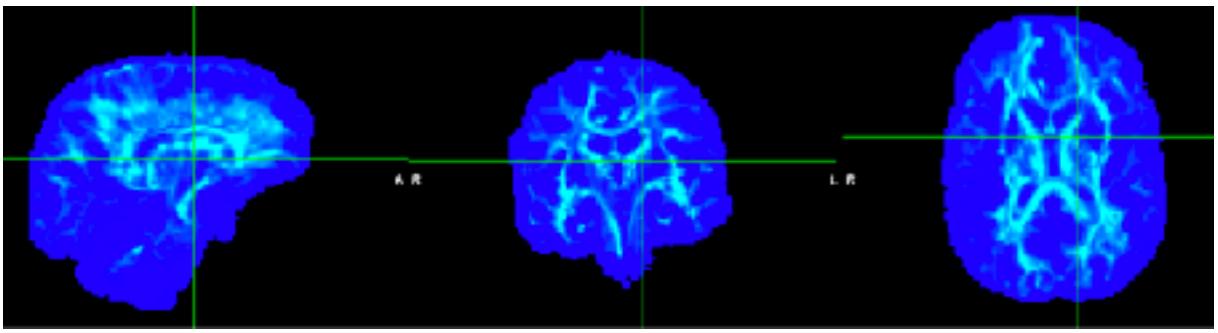

**Figure 2.** Tract Density Maps. The brightness of each voxel/region in sagittal, coronal and axial slices of the tract density maps represents the density of tracts.

*Inter-hemispheric asymmetry analysis*

While the majority of white matter tracts in healthy brains tend to maintain inter-hemispheric symmetry, it has also been shown that some tracts in healthy brains are

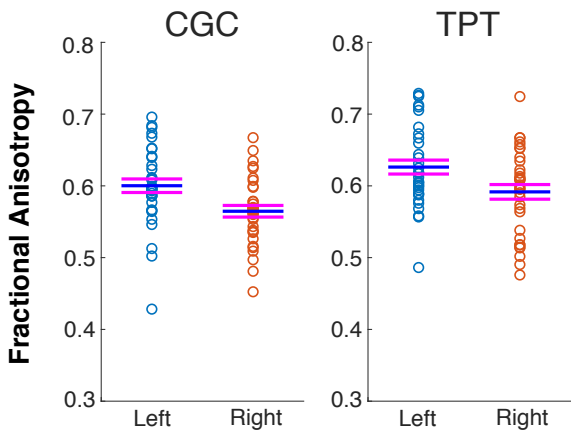


**Figure 3.** Example inter-hemispherically asymmetric white matter tracts. The difference between mean of left and right cingulum in cingulate gyrus (CGC) and left and right tapetum (TPT) are statistically significant ($p < 0.5$)

asymmetrically lateralized. For example, tapetum (TPT) and cingulum in cingulate gyrus (CGC) in healthy brains displayed statistically significant inter-hemispheric asymmetry (Fig. 3).

In order to quantify the degree of lateralization of parameters that characterize white matter microstructure, we used a common lateralization index, which is calculated as shown below:

*Lateralization Index: [Left - Right] / [Left + Right]*

Furthermore, throughout the analysis, we utilized lateralization index to examine the how inter-hemispheric asymmetry of each white matter tract is affected by either deletion or duplication at the chromosomal locus 16p11.2.

***Statistical Analysis***

*Tract-based spatial statistics analysis*

Using tract-based spatial statistics (TBSS) in FSL (Smith et al., 2006), fractional anisotropy maps from all subjects were aligned to the FA map of the FMRIB58_FA standard-space image that is provided by FSL. Once all subjects were registered to the standard, the fractional anisotropy, axial diffusivity, radial diffusivity and mean diffusivity maps were thinned using the FA threshold of 0.2 to skeletonize the white matter. Afterwards, the skeletonized AD, RD, MD and tract density maps were then created and registered to the most representative subject found with the FA maps. In order to identify the regions of white matter whose change in microstructural parameters were statistically significant, the “randomize” function of FSL was performed. The “randomize” function conducts nonparametric permutation testing, thereby

allowing for cluster-level inference when the threshold-free cluster enhancement (TFCE) approach is used (Smith and Nichols, 2009). The resulting maps for each comparison were corrected for multiple voxel-wise comparisons with TFCE using a significance threshold of p-value 0.05 (Owen et al., 2014). Then, the precise anatomic locations of white matter regions corresponding to statistically significant voxels were determined from the Johns Hopkins University (JHU) ICBM-DTI-81 White-Matter Labeled Atlas and the JHU White-Matter Tractography Atlas (Mori et al., 2005; Wakana et al., 2007; Hua et al., 2008).

# Results

### *Microstructural alteration of white matter diffusion tensor parameters*

*Voxel-wise analysis between 30 deletion and 30 duplication carriers*

We used voxel-wise comparison and analysis of TBSS to globally identify and assess extensive regions of white matter tracts whose fractional anisotropy, radial diffusivity and axial diffusivity values were significantly influenced by the copy number variants at locus 16p11.2 through voxel-wise comparison. Indeed, carriers of deletion at the chromosomal locus 16p11.2 displayed significantly-increased fractional anisotropy and axial diffusivity systemically, as opposed to the duplication carriers that displayed significantly reduced fractional anisotropy and increased radial diffusivity throughout the brain (Fig. 4). This finding demonstrated that the significant increase in FA among the deletion carriers was primarily AD-driven and that the significantly reduced FA among the duplication carriers was primarily RD-driven.

*JHU white matter track atlas analysis*

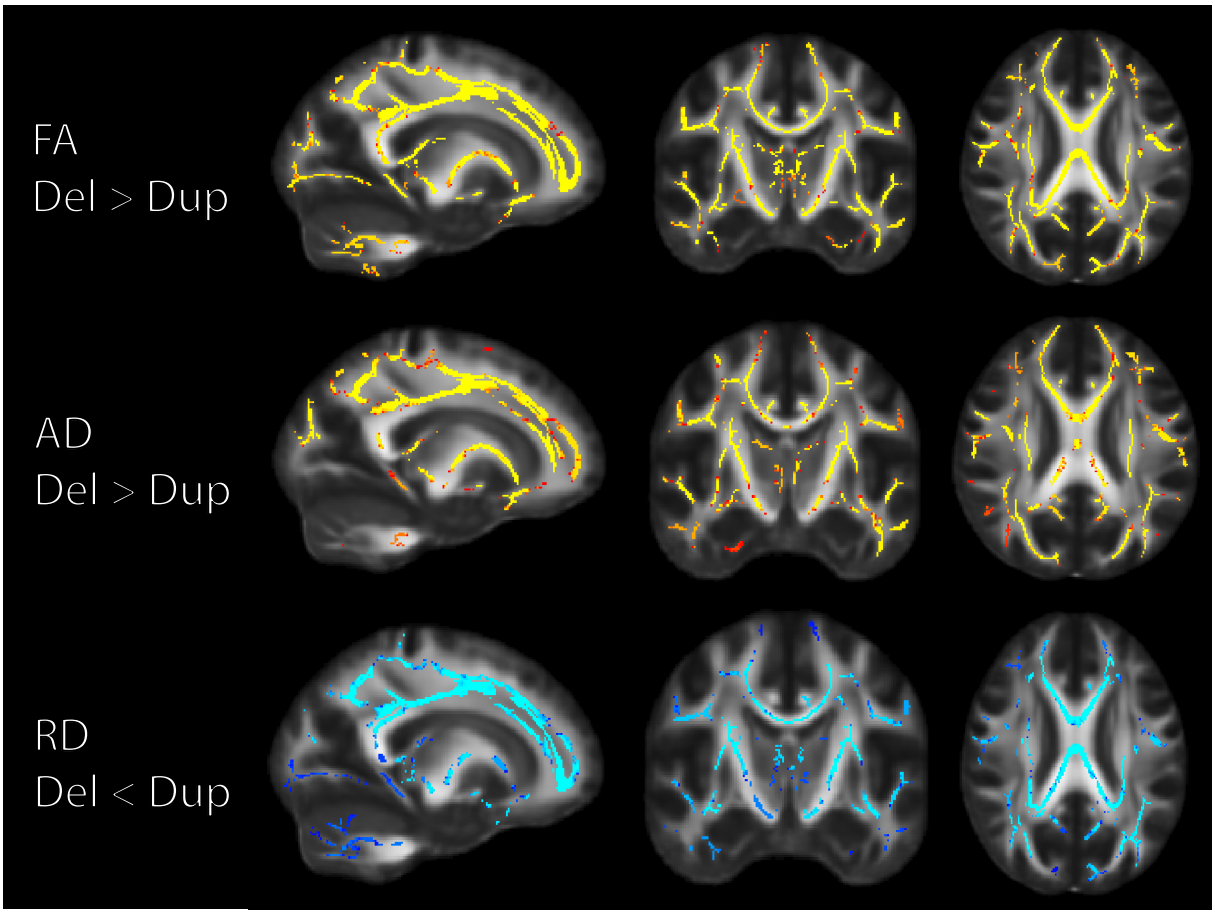


**Figure 4.** DTI TBSS voxel-wise comparison. DTI TBSS results in the 16p11.2 duplication carriers (n = 30) compared to the 16p11.2 deletion carriers (n = 30). Results show white matter regions with significantly increased FA and AD displayed in yellow and significantly reduced RD displayed in blue ($p < 0.05$).

Then, using JHU white matter atlas, we found that the 16p11.2 chromosomal deletion systemically increased FA values of many white matter tracts while chromosomal duplication of 16p11.2 locus displayed widespread significant decrease in FA. Specifically, cingulum in the cingulate gyrus (CGC), cingulum adjoining the hippocampus (CGH), posterior corona radiata (PCR), superior corona radiata (SCR), posterior limb of internal capsule (PLIC) and retrolenticular part of the internal capsule (RLIC) displayed significantly elevated FA values as a result of deletion at the chromosomal locus 16p11.2 (Fig. 5). However, 16p11.2 chromosomal

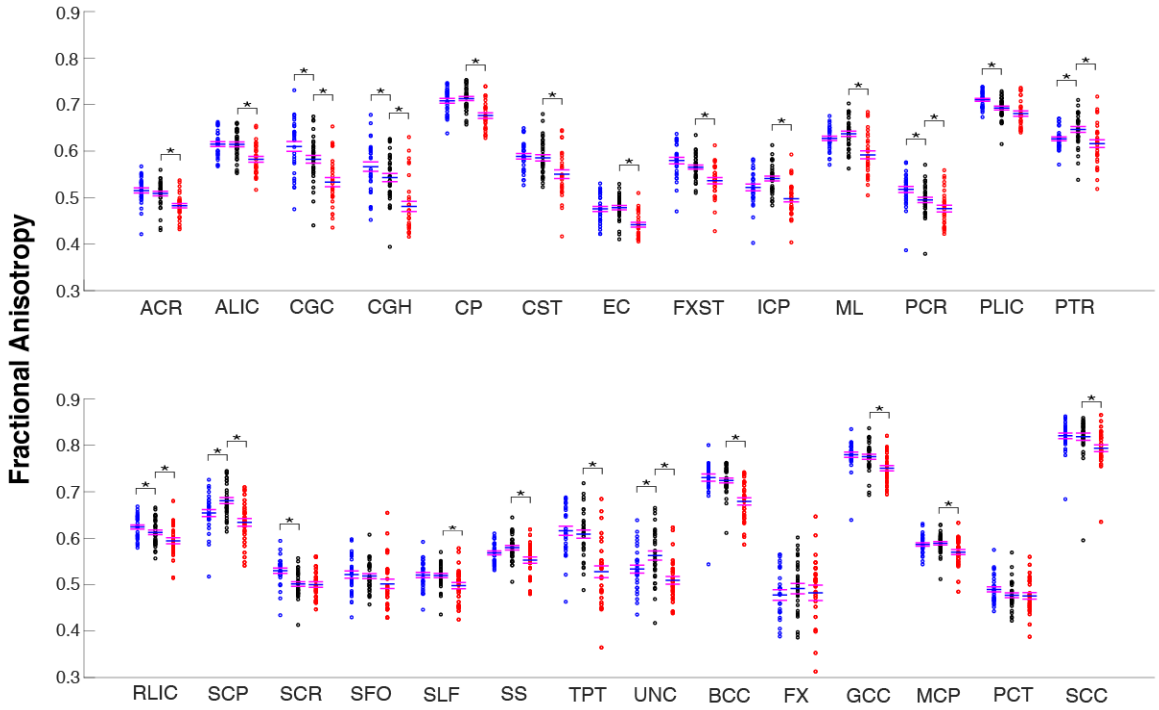


**Figure 5.** Fractional anisotropy of white matter tracts. Blue, black and red dots represent FA of white matter voxels of deletion, control and duplication cohorts, respectively. Star indicates that the difference in the mean FA is statistically significant ($p < 0.05$).

deletion also spawned significantly reduced FA in some white matter tracts, such as posterior thalamic radiation (PTR), superior cerebellar peduncle (SCP) and uncinate fasciculus (UNC) (Fig. 5). As opposed to 16p11.2 deletion, 16p11.2 chromosomal duplication caused anterior corona radiata (ACR), anterior limb of internal capsule (ALIC), cingulum in the cingulate gyrus (CGC), cingulum adjoining the hippocampus (CGH), cerebral peduncle (CP), corticospinal tract (CST), external capsules (EC), Fornix Stria Terminalis (FXST), inferior cerebellar peduncle (ICP), medical lemniscus (ML), posterior corona radiata (PCR), posterior thalamic radiation (PTR), retrolenticular pat of the internal capsule (RLIC), superior cerebellar peduncle (SCP), superior longitudinal fasciculus (SLF), sagittal stratum (SS), tapetum (TPT), uncinate fasciculus

(UNC), medium cerebellar peduncle (MCP), body of corpus callosum (BCC), genu of corpus callosum (GCC) and splenium of corpus callosum (SCC) to have significantly reduced FA values (Fig. 5). This finding clearly illustrates that deletion and duplication at the locus 16p11.2 have reciprocal effects on the fractional anisotropy of white matter tracts: while 16p11.2 chromosomal deletion primarily increases FA of white matter tracts, the duplication reduces FA values of the majority of white matter tracts.

Furthermore, 16p11.2 chromosomal deletion was also shown to significantly increase axial diffusivity (AD) while 16p11.2 chromosomal duplication did not affect AD values of white matter tracts. The 16p11.2 deletion-induced increase in AD were displayed in anterior corona radiata (ACR), anterior limb of internal capsule (ALIC), cingulum in the cingulate gyrus (CGC), cingulum adjoining the hippocampus (CGH), external capsule (EC), fornix stria terminalis (FXST), inferior cerebellar peduncle (ICP), posterior corona radiata (PCR), posterior limb of internal capsule (PLIC), posterior thalamic radiation (PTR), retrolenticular part of the internal capsule (RLIC), superior corona radiata (SCR), superior frontal occipital fasciculus (SFO), superior longitudinal fasciculus (SLF), sagittal stratum (SS), body of corpus callosum (BCC), genu of corpus callosum (GCC) and septum of corpus callosum (SCC) (Fig. 6). On the other hand, the duplication at the chromosomal locus 16p11.2 increased AD merely in superior corona radiata (SCR) and posterior corona radiata (PCR) while decreasing AD in medical lemniscus (ML) (Fig. 6). This finding clearly reveals that AD of the white matter tracts are more effectively and significantly affected by deletion at the chromosomal locus 16p11.2 chromosomal than the duplication.

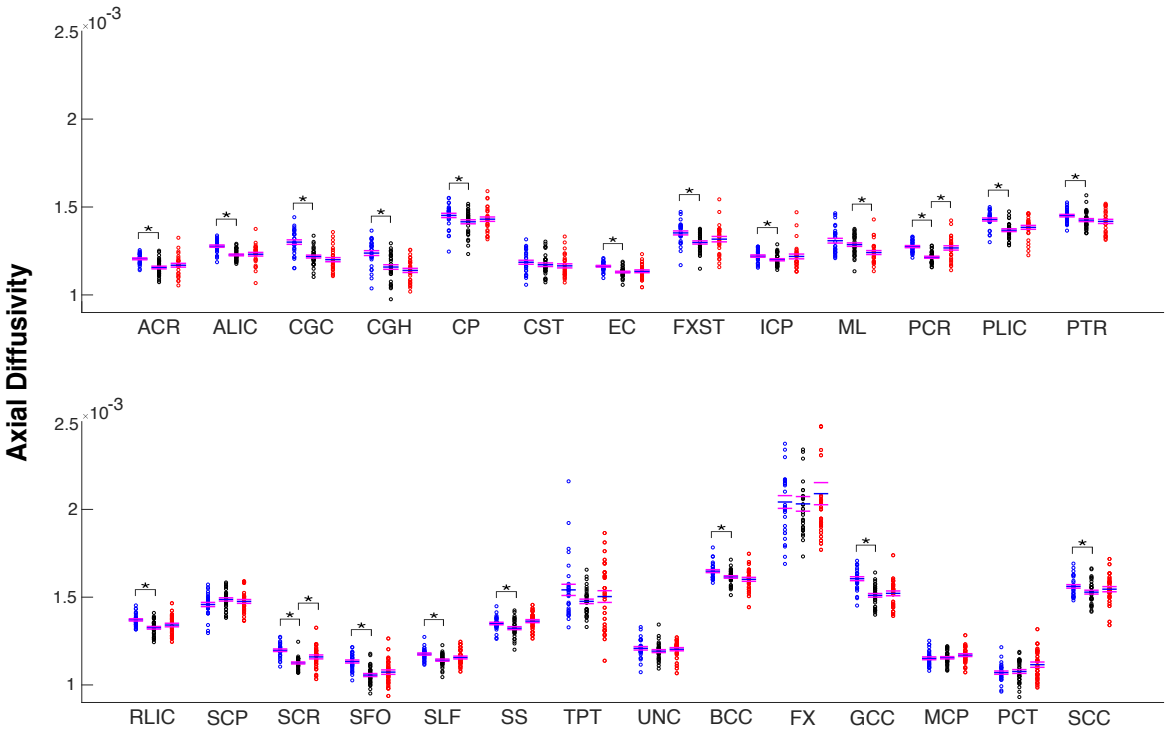


**Figure 6.** Axial diffusivity of white matter tracts. Blue, black and red dots represent AD of white matter voxels of deletion, control and duplication cohorts, respectively. Star indicates that the difference in the mean AD is statistically significant ($p < 0.05$).

While 16p11.2 chromosomal duplication did not have much impacts on axial diffusivity of white matter tracts, the duplication rather led to widespread increase in radial diffusivity in anterior corona radiata (ACR), anterior limb of internal capsule (ALIC), cingulum in cingulate gyrus (CGC), cingulum adjoining hippocampus (CGH), cerebral peduncle (CP), corticospinal tract (CST), external capsule (EC), fornix stria terminalis (FXST), inferior cerebellar peduncle (ICP), medial lemniscus (ML), posterior corona radiata (PCR), posterior limb of internal capsule (PLIC), posterior thalamic radiation (PTR), retrolenticular prat of internal capsule (RLIC), superior frontal occipital fasciculus (SFO), superior longitudinal fasciculus (SLF), sagittal stratum (SS), tapetum (TPT), uncinate fasciculus (UNC), body of corpus callosum (BCC), genu

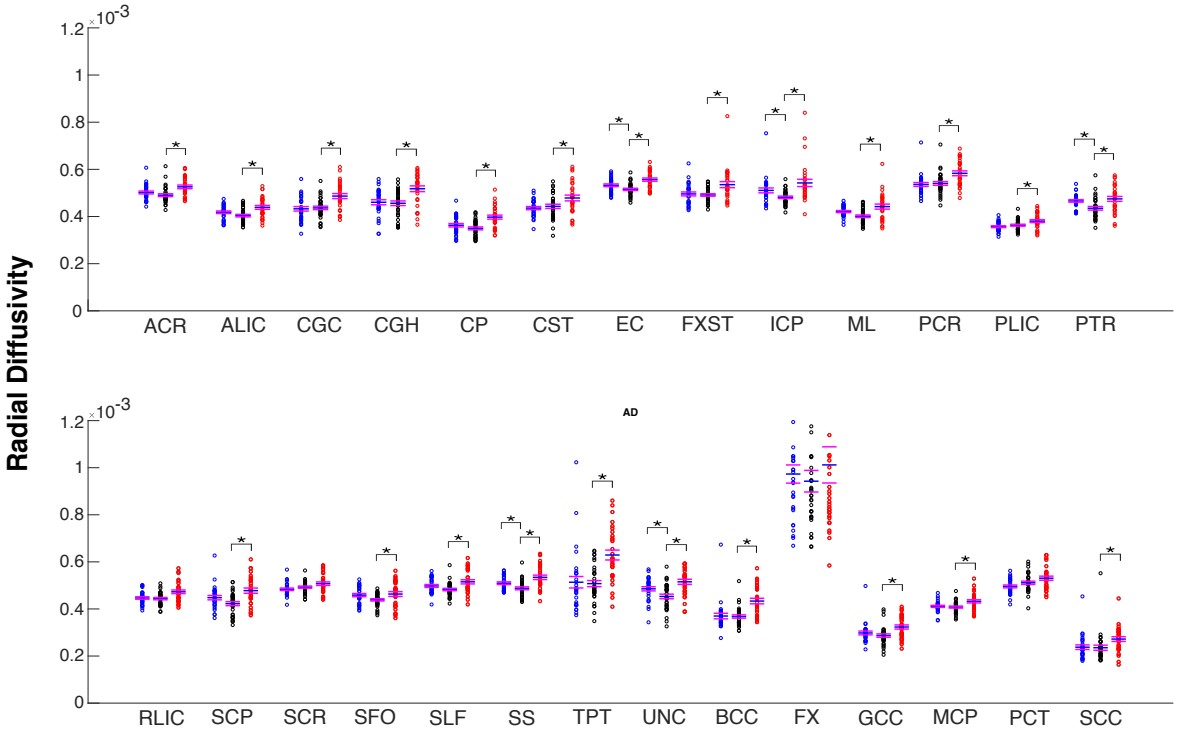


**Figure 7.** Radial diffusivity of white matter tracts. Blue, black and red dots represent RD of white matters of deletion, control and duplication cohorts, respectively. Star marks that difference in mean RD of two groups is statistically significant ($p < 0.05$).

of corpus callosum (GCC), medial cerebellar peduncle (MCP) and splenium of corpus callosum (SCC) (Fig. 7). On the other hand, 16p11.2 deletion only significantly affected the RD values of external capsule (EC), inferior cerebellar peduncle (ICP), posterior thalamic radiation (PTR), sagittal stratum (SS) and uncinate fasciculus (UNC) (Fig. 7).

Overall, these findings indicate that 16p11.2 deletion primarily increases axial diffusivity of the white matter tracts while 16p11.2 duplication mostly increase in radial diffusivity. Thus, these pieces of information combined account for the fact that deletion at the chromosomal locus 16p11.2 causes widespread increase in FA while 16p11.2 chromosomal duplication triggers FA of white matter tracts to decrease.

***Inter-hemispheric asymmetry of white matter diffusion tensor parameters***

It was shown that deletion and duplication at the chromosomal locus 16p11.2 could induce lateralization of diffusion tensor parameters. Using the lateralization index, we found that cingulum adjoining hippocampus (CGC), anterior limb of internal capsules (ALIC), cingulum adjoining the hippocampus (CGH), corticospinal tract (CST), external capsule (EC), fornix stria terminalis (FXST), retrolenticular limb of internal capsule (RLIC), superior cerebellar peduncle (SCP), superior corona radiata (SCR), superior longitudinal fasciculus (SLF) and tapetum (TPT) also displayed inter-hemispheric asymmetry in the absence of copy number variants of the locus 16p11.2 (Fig. 8).

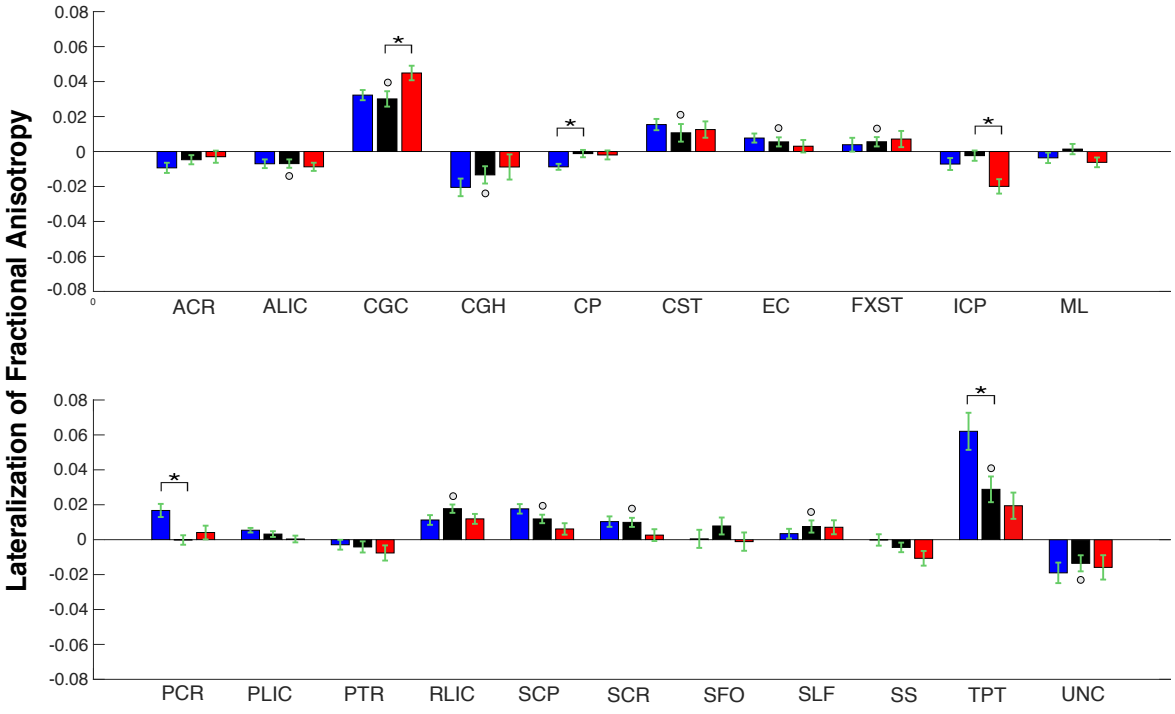


**Figure 8.** Lateralization of fractional anisotropy. Microstructural lateralization index of white matter tracts in deletion (blue), control (black) and duplication (red) cohorts. Star indicates that the difference in mean LI is statistically significant ($p < 0.05$).

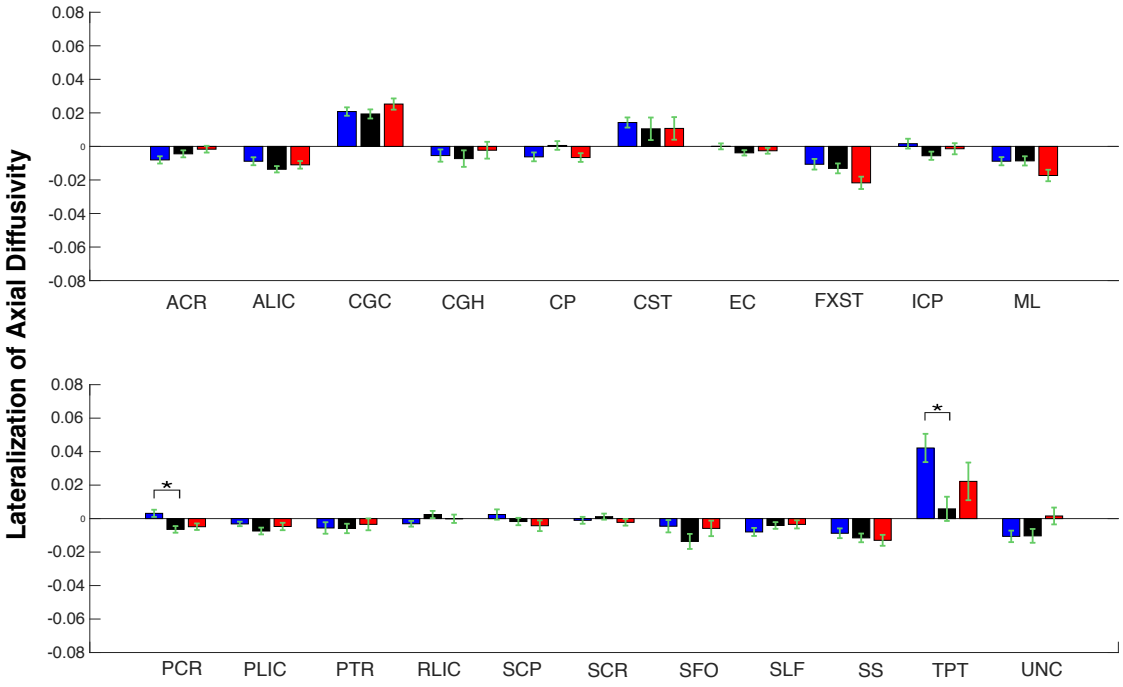


**Figure 9.** Lateralization of axial diffusivity. Microstructural lateralization index of white matter tracts in deletion (blue), control (black) and duplication (red) cohorts. Star indicates that the difference in the mean is statistically significant ($p < 0.05$).

Furthermore, we were intrigued by how the copy number variants of the chromosomal locus 16p11.2 would further polarize the pre-existing inter-hemispheric asymmetry or even introduce inter-hemispheric FA asymmetry in the white matter tracts that were originally symmetric. Figure 8 display a complete list of FA lateralization index of each tract in deletion, control and duplication carriers. This finding demonstrates that duplication of the chromosomal locus 16p11.2 significantly promotes inter-hemispheric asymmetry in cingulum the cingulate gyrus (CGC) and inferior cerebellar peduncle (ICP), while 16p11.2 deletion significantly affected the lateralization index of cerebral peduncle (CP), posterior corona radiata (PCR) and tapetum (TPT) (Fig. 8). Similarly, it was shown that 16p11.2 chromosomal deletion significantly

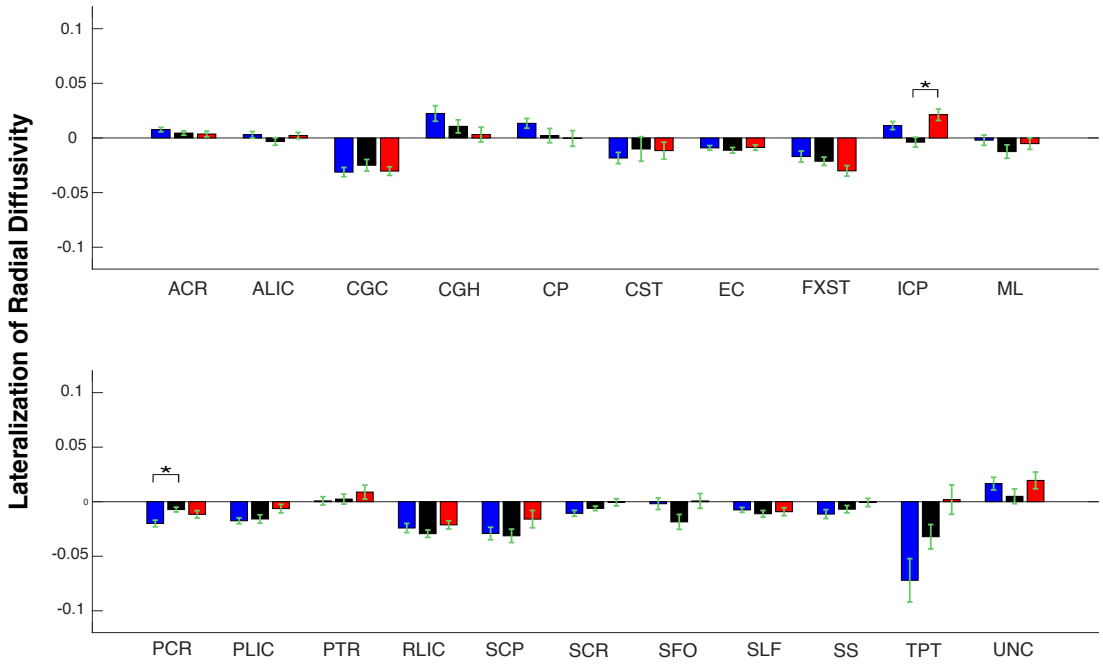


**Figure 10.** Lateralization of radial diffusivity. Microstructural lateralization index of white matter tracts in deletion (blue), control (black) and duplication (red) cohorts. Star indicates that the difference in the mean is statistically significant ($p < 0.05$).

increases inter-hemispheric AD asymmetry in tapetum (TPT) (Fig. 9) and that 16p11.2 chromosomal deletion does not affect inter-hemispheric RD asymmetry (Fig. 10), corroborating that FA increase in tapetum (TPT) of 16p11.2 deletion carriers were primarily driven by increase in AD.

### *Microstructural alteration of white matter tract density*

*Voxel-wise analysis between 14 deletion and 12 duplication carriers*

We performed voxel-wise comparison and analysis of TBSS to globally identify and assess white matter regions whose tract density were significantly affected by the copy number

variants at the chromosomal locus 16p11.2. However, increase in global tract density among 16p11.2 deletion carriers compared the duplication carriers reached statistical significance.

*JHU white matter tract atlas analysis*

In order to white matter tract-specific alteration induced by the copy number variants of 16p11.2, we performed JHU white matter tract analysis. Then, deletion and duplication at the chromosomal locus 16p11.2 were shown to have significant ramifications on tract density. Individuals with deletion at the chromosomal locus 16p11.2 had several white matter tract regions whose tract density was significantly higher than the density of white matter tracts in the same region of 16p11.2 duplication carriers; those white matter tracts include cingulum adjoining the hippocampus (CGH), corticospinal tract (CST), fornix (FX), fornix stria terminalis (FXST), medical lemniscus (ML), superior frontal orbital fasciculus (SFO) and sagittal stratum (SS) (Fig. 10). However, 16p11.2 chromosomal deletion was also found to reduce tract density in posterior corona radiata (PCR) and splenium of corpus callosum (SCC) (Fig. 10). Although the effects of 16p11.2 chromosomal deletion and duplication on white matter macrostructures are not as straightforward as its impacts on the white matter microstructures, this finding indicates that 16p11.2 chromosomal deletion carriers tend to contain higher tract density than 16p11.2 duplication carriers.

***Inter-hemispheric asymmetry of white matter tract density***

Consistent with how 16p11.2 deletion and duplication affected inter-hemispheric asymmetry of white matter DTI parameters, it was also shown that 16p11.2 deletion and

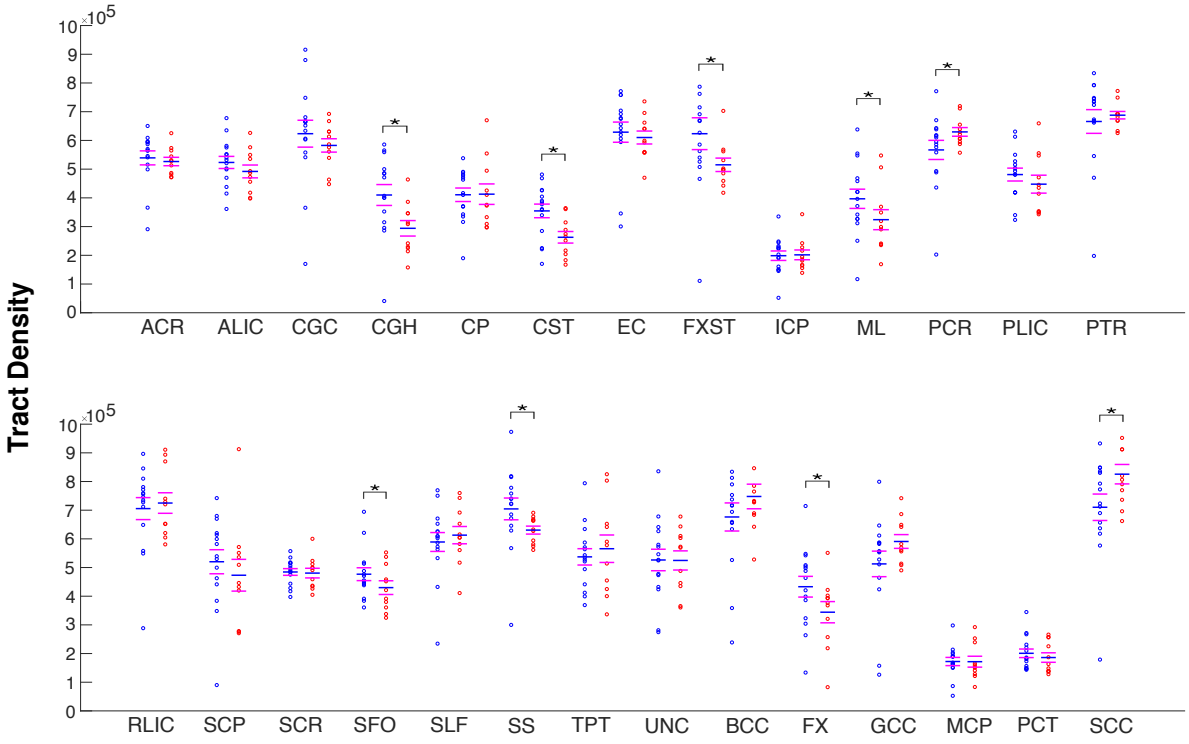


**Figure 11.** Tract density of white matter. Blue represents deletion carriers, black control group and red duplication carriers. Star indicates that the difference in the mean is statistically significant ($p < 0.05$).

duplication influence inter-hemispheric asymmetry of white matter tract density. Interestingly, it was found that in sagittal stratum (SS) and tapetum (TPT), 16p11.2 chromosomal deletion induced an increase tract density of right hemisphere while the duplication increased the density of the same tract in the left hemisphere. This attests that 16p11.2 chromosomal deletion and duplication have reciprocal ramifications on the microstructure of white matter.

## Discussion

This study demonstrates that deletion and duplication at the chromosomal locus 16p11.2 have reciprocal impacts on the microstructure of white matter tracts in human

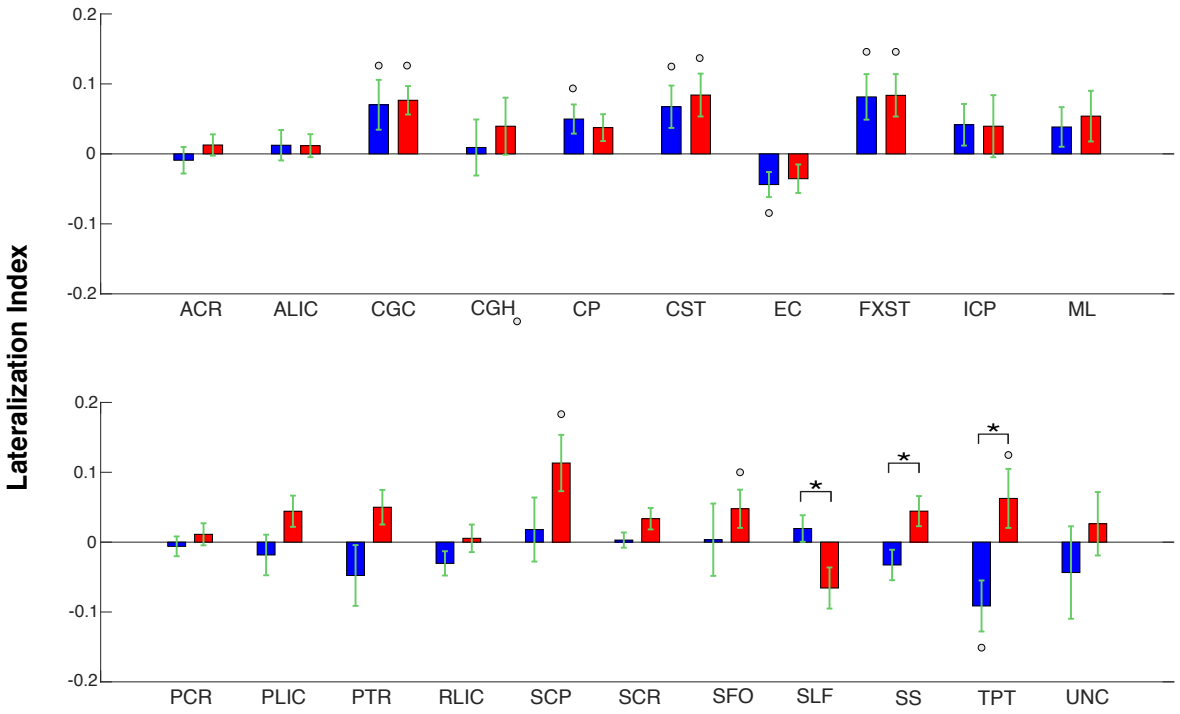


**Figure 12.** Lateralization of tract density. Macrostructural lateralization index of white matter tracts in deletion (blue), control (black) and duplication (red) cohorts. Star indicates that the difference in the mean is statistically significant ($p < 0.05$).

brains. The deletion at the chromosomal locus 16p11.2 induced widespread and non-specific increase in fractional anisotropy and axial diffusivity, as opposed to 16p11.2 chromosomal duplication that introduced systemic increase in radial diffusivity and decrease in fractional anisotropy (Fig. 1). This finding of AD-driven increase in FA among 16p11.2 deletion carriers and RD-driven decrease in FA among the duplication corroborates a previous study by Chang et al (2016), which demonstrates that the 16p11.2 chromosomal deletion induces significant increase in FA throughout supratentorial white matter while its duplication exhibits global decrease in FA. Moreover, this study also relates to a recent study on another neuropsychiatric disorder-related gene clusters

15q11.2 BP1-BP2. This study illustrates that chromosomal deletion at 15q11.2 BP1-BP2 induces systemic increase in FA of the posterior limb of internal capsule while its duplication induces significantly reduced FA (Silva et al., 2018). As the copy number variants of chromosomal loci both 15q11.2 and 16p11.2 increased fractional anisotropy of different white matter tracts, it indicates that specific chromosomal locus is associated with different white matter in the brain.

On a similar note, it was shown that 16p11.2 chromosomal deletion was shown to elevate tract density. Although TBSS Voxel-wise analysis of tract density showed no significant difference between deletion and duplication carriers, we were able to identify some specific white matter structures whose tract density and lateralization was affected (Fig. 11 & Fig. 12). This finding demonstrates a point that while global tract density may not be affected, a specific white matter can be particularly susceptible to the copy number variants at the chromosomal locus 16p11.2. For example, progressive supranuclear palsy (PSP) a type of neurodevelopmental disorders, is associated with an increase in tract density in supra- and infratentorial brain structures (Nigro et al., 2018). Thus, a possible association between density of various white matter tracts and neurological disorders could be the first step in our journey of understanding those mysterious diseases.

Lastly, it was very interesting to note how the copy number variants at the chromosomal locus 16p11.2 could either further polarize the pre-existing inter-hemispheric asymmetry or even introduce asymmetry to white matter tracts that are inter-hemispherically symmetric in healthy brains. While the precise causes of such induced or

promoted inter-hemispheric asymmetry was not fully addressed in this study, this can be attributed to the fact that the copy number variants of the chromosomal locus 16p11.2 affects the microstructure of corpus callosum, such as splenium of corpus callosum (SCC), genu of corpus callosum (GCC) and body of corpus callosum (BCC), all of which are crucial to the inter-hemispheric communication. Thus. the disturbed microstructure of corpus callosum could cause the inter-hemispheric symmetry among the deletion and duplication carriers. As many neurological disorders, such as depressive disorders, autism spectrum disorder and schizophrenia, are known to be closely associated with abnormal brain lateralization (Bruder et al., 2017), lateralization of white matter microstructures could also be another cause of the behavioral and cognitive characteristics of individuals with neurological disorders.

***Future directions***

Previous findings have demonstrated that individuals with 16p11.2 copy number variations induced changes in diffusion tensor parameters is strongly correlated with cognitive measures, such as FSIQ, NVIQ, VIG, SRS and SCQ. (Stefansson et al., 2014; Chang et al., 2016). Hence, future research could look into how CNV-induced lateralization correlates with cognitive and behavioral characteristics of affected individuals. We expect that the more lateralized the brain is, the more severe cognitive and behavioral symptoms will be. This study would also endow us with a more comprehensive understanding of how genes affect micro- and macro-structures of the brain that ultimately is closely associated with behavior and cognition.

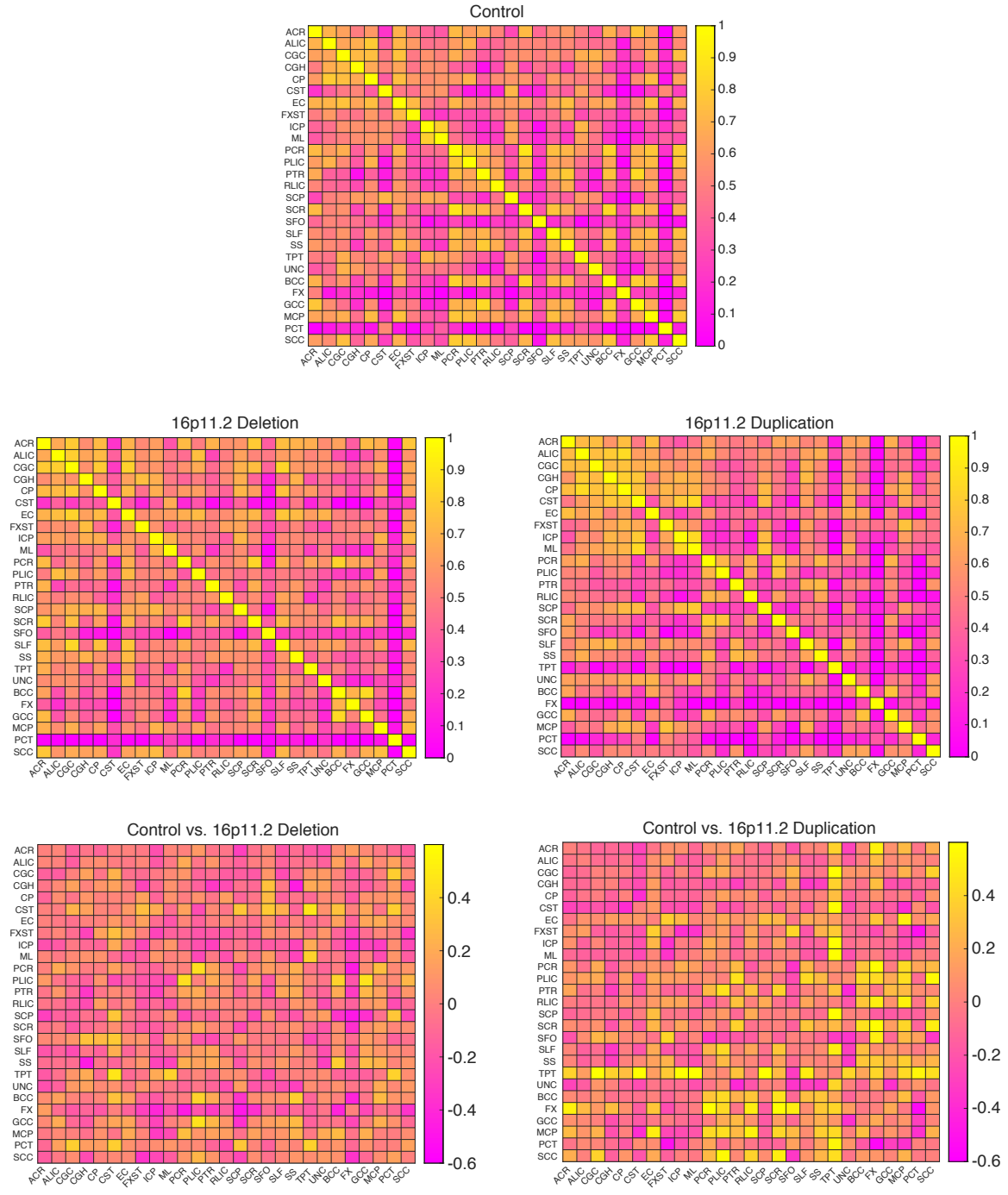


**Figure 13.** Example heat maps of inter-tract correlation. (Top) Heat-map of inter-tract correlation in healthy brain. (Middle) Heat-map of inter-tract correlation in 16p11.2 deletion and duplication carriers. (Bottom) The difference in above heat-maps, thereby showing how either deletion and duplication of the chromosomal 16p11.2 disturbs inter-tract correlation

Moreover, as this study primarily focused on how the copy number variants of the chromosomal locus 16p11.2 induced lateralization in each white matter tract, a future study can also examine how such copy number variants of 16p11.2 affect inter-tract correlation (Fig. 13). This study will provide us insight into how deletion and duplication at locus 16p11.2 influence the overall network topology of the brain. Moreover, while we investigate the macro-structural alteration of white matter tracts, the causal relationship between micro- and macro-structural white matter alterations induced by 16p11.2 chromosomal deletion and duplication could also be explored.

***Limitations***

Due to time limitation when constructing tract-density maps via using FSL, we were only able to construct tract density images only for 26 subjects (14 deletion and 12 duplication carriers). Since this is not enough data to draw a concrete conclusion with a strong statistical power, we strongly recommend adding more subjects into the study for a more convincing finding.

Another limitation is that subjects analyzed in this study involves wide age span. Although all the subjects and age- and gender-matched, mixing young children and adults could wash away some meaningful findings. Since the biggest structural difference is apparent in young subjects, we also recommend examining perturbations of micro- and macro-structural differences in children and adults. Such study could also indicate how ages affect the brain structures differently despite the same genetic mutation.

# References

Behrens, T., Berg, H. J., Jbabdi, S., Rushworth, M., & Woolrich, M. (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? NeuroImage, 34(1), 144-155. doi:10.1016/j.neuroimage.2006.09.018

Behrmann, M. (2004). Faculty of 1000 evaluation for White matter structure in autism: Preliminary evidence from diffusion tensor imaging. F1000 - Post-publication Peer Review of the Biomedical Literature. doi:10.3410/f.1018153.207621

Bertero, A., Liska, A., Pagani, M., Parolisi, R., Masferrer, M. E., Gritti, M., . . . Gozzi, A. (2018). Autism-associated 16p11.2 microdeletion impairs prefrontal functional connectivity in mouse and human. Brain, 141(7), 2055-2065. doi:10.1093/brain/awy111

Bruder, G. E., Alvarenga, J., Abraham, K., Skipper, J., Warner, V., Voyer, D., . . . Weissman, M. M. (2015). Brain laterality, depression and anxiety disorders: New findings for emotional and verbal dichotic listening in individuals at risk for depression. Laterality: Asymmetries of Body, Brain and Cognition, 21(4-6), 525-548. doi:10.1080/1357650x.2015.1105247

Chang, Y. S., Owen, J. P., Pojman, N. J., Thieu, T., Bukshpun, P., Wakahiro, M. L., . . . Mukherjee, P. (2016). Reciprocal white matter alterations due to 16p11.2 chromosomal deletions versus duplications. Human Brain Mapping, 37(8), 2833-2848. doi:10.1002/hbm.23211

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., . . . Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage, 31(3), 968-980. doi:10.1016/j.neuroimage.2006.01.021

Greene, C., Cieslak, M., & Grafton, S. T. (2018). Effect of different spatial normalization approaches on tractography and structural brain networks. Network Neuroscience, 2(3), 362-380. doi:10.1162/netn_a_00035

Jenkinson, M. (2002). Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. NeuroImage, 17(2), 825-841. doi:10.1016/s1053-8119(02)91132-8

Kumar, V. J., Grissom, N. M., Mckee, S. E., Schoch, H., Bowman, N., Havekes, R., . . . Nickl-Jockschat, T. (2018). Linking spatial gene expression patterns to sex-specific brain structural changes on a mouse model of 16p11.2 hemideletion. Translational Psychiatry, 8(1). doi:10.1038/s41398-018-0157-z

Li, Y., Yang, F. G., Nguyen, C. T., Cooper, S. R., Lahue, S. C., Venugopal, S., & Mukherjee, P. (2011). Independent component analysis of DTI reveals multivariate microstructural correlations of white matter in the human brain. Human Brain Mapping, 33(6), 1431-1451. doi:10.1002/hbm.21292

Mukherjee, P., Chung, S., Berman, J., Hess, C., & Henry, R. (2008). Diffusion Tensor MR Imaging and Fiber Tractography: Technical Considerations. American Journal of Neuroradiology, 29(5), 843-852. doi:10.3174/ajnr.a1052

Mukherjee, P., Berman, J., Chung, S., Hess, C., & Henry, R. (2008). Diffusion Tensor MR Imaging and Fiber Tractography: Theoretic Underpinnings. American Journal of Neuroradiology, 29(4), 632-641. doi:10.3174/ajnr.a1051

Owen, J. P., Chang, Y. S., Pojman, N. J., Bukshpun, P., Wakahiro, M. L., Marco, E. J., . . . Mukherjee, P. (2014). Aberrant White Matter Microstructure in Children with 16p11.2

Deletions. Journal of Neuroscience, 34(18), 6214-6223. doi:10.1523/jneurosci.4495-13.2014

Owen, J. P., Marco, E. J., Desai, S., Fourie, E., Harris, J., Hill, S. S., . . . Mukherjee, P. (2013). Abnormal white matter microstructure in children with sensory processing disorders. NeuroImage: Clinical, 2, 844-853. doi:10.1016/j.nicl.2013.06.009

Owen, J. P., Chang, Y. S., & Mukherjee, P. (2015). Edge density imaging: Mapping the anatomic embedding of the structural connectome within the white matter of the human brain. NeuroImage, 109, 402-417. doi:10.1016/j.neuroimage.2015.01.007

Owen, J. P., Bukshpun, P., Pojman, N., Thieu, T., Chen, Q., Lee, J., . . . Sherr, E. H. (2018). Brain MR Imaging Findings and Associated Outcomes in Carriers of the Reciprocal Copy Number Variation at 16p11.2. Radiology, 286(1), 217-226. doi:10.1148/radiol.2017162934

Pustina, D., Doucet, G., Sperling, M., Sharan, A., & Tracy, J. (2014). Increased microstructural white matter correlations in left, but not right, temporal lobe epilepsy. Human Brain Mapping, 36(1), 85-98. doi:10.1002/hbm.22614

Qureshi, A. Y., Mueller, S., Snyder, A. Z., Mukherjee, P., Berman, J. I., Roberts, T. P., . . . Buckner, R. L. (2014). Opposing Brain Differences in 16p11.2 Deletion and Duplication Carriers. Journal of Neuroscience, 34(34), 11199-11211. doi:10.1523/jneurosci.1366-14.2014

Silva, A. I., Ulfarsson, M. O., Stefansson, H., Gustafsson, O., Walters, G. B., Linden, D. E., . . . Stefansson, K. (2018). Reciprocal white matter changes associated with copy number

variation at 15q11.2 BP1-BP2 – A diffusion tensor imaging study. Biological Psychiatry. doi:10.1016/j.biopsych.2018.11.004

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