Commonly-carried variants in the visual pathway gene, *Raftlin*, are associated with reduced white matter integrity in 451 young twins imaged with DTI

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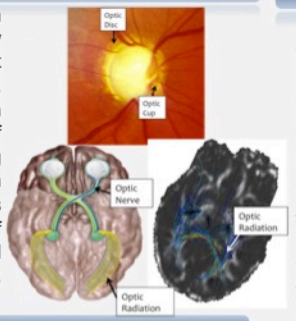
Introduction

One of the most extensively studied neural circuitries involves the human visual system. While several disorders in visual development and functionality have been found to be under genetic control, specific genetic variants that impact visual pathways have yet to be traced inside the healthy human brain. Recently, a common variant in the raft-linking gene *Raftlin* was associated with anatomic variations in the optic nerve head [1], a location where initial signs of visual impairment are recognized. In one of the largest diffusion imaging studies ever performed, we examined how a variant near the *Raftlin* gene (on chr3) influenced fractional anisotropy (FA), a measure of myelination levels and fiber coherence. *Raftlin*, a raft-linking protein, is important for trafficking of cholesterol - a major component of white matter. Common *Raftlin* variants and other raft-linking proteins have been associated with the visual system, suggesting that this candidate may influence visual pathway integrity.



Diffusion tensor imaging

DTI is a variant of standard brain MRI sensitive to fiber integrity and white matter microstructure. The most widely accepted DTI measure, fractional anisotropy (FA), evaluates the extent to which water diffusion is directionally constrained. FA ranges from 0 to 1.

•Higher FA generally reflects preferential diffusion along more intact, heavily myelinated axons.

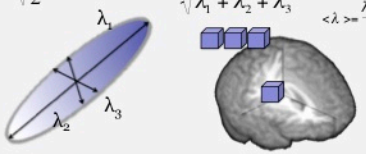
•Demyelination, neurological disease symptoms, and slowed nerve conduction have been associated with lower FA in white matter [2], suggesting that it may reflect reduced white matter integrity.

White matter integrity is highly heritable in twin studies [3], making DTI-derived parameters promising phenotypes for genetic association.

Distortion corrected diffusion weighted images were used to calculate anisotropy measures for each subject. Under a single-tensor model [4] diffusion attenuates the MR signal in the given direction.

Diffusion tensors were computed using FSL software (http://fsl.fmrib.ox.ac.uk/fsl/). FA was computed from the tensor eigenvalues (λ_1, λ_2, λ_3) at each voxel.

$$FA = \sqrt{\frac{3}{2}} \frac{\sqrt{(\lambda_1 - <\lambda>)^2 + (\lambda_2 - <\lambda>)^2 + (\lambda_3 - <\lambda>)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \qquad <\lambda> = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$



Genotyping and Image Acquisition

We scanned 451 healthy young adult twins and siblings (mean age: 23.8+/-2.2 yrs.; 60% female) with a series of 30 images (including 27 diffusion weighted images with b=1132) on a 4 Tesla MRI scanner. We assessed white matter fiber integrity using 3D maps of fractional anisotropy (FA). Genotyping for the Raftlin variant was performed on an Human610-Quad BeadChip (Illumina) with DNA extracted from blood.

Image Registration and Statistical Analysis

•**Registration** of all the images to the template was performed using elastic warping to improve tract alignment across subjects.

•The template was a study specific FA based mean deformation template (MDT) created from a subset of individuals to geometrically center the anatomies.

• To further ensure alignment of the white matter tracts, both the MDT and all the individual registered images were thresholded to only contain regions of highly probable white matter defined in this case by FA >0.25. Elastic registration was then performed on the thresholded images.

•**Association** was conducted at every voxel using an additive model controlling for age and sex with a mixed-model regression to account for familial relationships.

This model was according to the formula: $\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e}$.

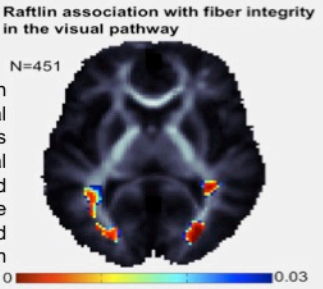
$\mathbf{y}$ is a vector representing the FA at a voxel across subjects; $\mathbf{X}$ is a matrix of fixed effects containing the additive genetic effect of the SNP, sex, and age; $\boldsymbol{\beta}$ is a vector representing the fixed effect regression coefficients; $\mathbf{Z}$ is an identity matrix; $\mathbf{u}$ is the random effect with $Var(\mathbf{u}) = \sigma^2_g\mathbf{K}$, where $\mathbf{K}$ is the kinship matrix; and $\mathbf{e}$ is a matrix of residual effects with $Var(\mathbf{e}) = \sigma^2_e\mathbf{I}$. This analysis was performed using EMMA; http://mouse.cs.ucla.edu/emma/

•Computing thousands of tests of associations on a voxelwise level can introduce a high type 1 (false positive) error rate in neuroimaging studies.

•We used a regional search for false discovery rate (FDR) correction [5] to control for multiple comparisons

Results: Association of *Raftlin* with FA

Remarkably, variations in the *Raftlin* gene were associated with systematically reduced fiber integrity in the posterior visual pathway, including Meyer's loop, bilaterally. This association was not anatomically uniform, but targeted thalamocortical visual pathways. In our recent DTI studies, these same regions had lower integrity (FA) in blind versus sighted subjects. When the sample was split in half, the pattern of association was replicated in the independent split samples, suggesting that the association is robust.



Conclusions

This system-specific association suggests that downstream effects of a candidate gene for visual impairment can be mapped in the posterior visual pathways of young healthy adults. The potential for DTI studies to find system and pathway specific genetic association is highlighted in this study.

References & Acknowledgements

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