Published in final edited form as:
Neuroimage. 2013 November 15; 82: 146–153. doi:10.1016/j.neuroimage.2013.05.095.

Relation between variants in the neurotrophin receptor gene, *NTRK3*, and white matter integrity in healthy young adults

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Abstract

The *NTRK3* gene (also known as *TRKC*) encodes a high affinity receptor for the neurotrophin 3′-nucleotidase (NT3), which is implicated in oligodendrocyte and myelin development. We previously found that white matter integrity in young adults related to genetic variants in genes encoding neurotrophins and their receptors. This underscores the importance of neurotrophins for white matter development. *NTRK3* variants are putative risk factors for schizophrenia, bipolar disorder, and obsessive-compulsive disorder hoarding, suggesting that some *NTRK3* var*i*ant*s* may affect the brain.

To test this, we scanned 392 healthy adult twins and their siblings (mean age, 23.6 ± 2.2 years; range: 20-29 years) with 105-gradient 4-Tesla diffusion tensor imaging (DTI). We identified 18 single nucleotide polymorphisms (SNPs) in the *NTRK3* gene that have been associated with neuropsychiatric disorders. We used a multi-SNP model, adjusting for family relatedness, age, and sex, to relate these variants to voxelwise fractional anisotropy (FA) – a DTI measure of white matter integrity.

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Conflict of interest: The authors declare actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

FA was optimally predicted (based on the highest false discovery rate critical *p*), by five SNPs (rs1017412, rs2114252, rs16941261, rs3784406, and rs7176429; overall FDR critical $p = 0.028$). Gene effects were widespread and included the corpus callosum genu and inferior longitudinal fasciculus - regions implicated in several neuropsychiatric disorders and previously associated with other neurotrophin-related genetic variants in an overlapping sample of subjects. *NTRK3* genetic variants, and neurotrophins more generally, may influence white matter integrity in brain regions implicated in neuropsychiatric disorders.

Keywords

Fractional anisotropy; diffusion tensor imaging; single nucleotide polymorphism; schizophrenia; obsessive compulsive disorder; bipolar disorder

Introduction

White matter integrity is impaired in many neuropsychiatric disorders (Thomason and Thompson, 2011) and much of its variability is due to genetic factors (Chiang et al., 2011b; Kochunov et al., 2011). Variants in genes encoding neurotrophins and their receptors have been associated with brain white matter integrity measures in young healthy adults (Braskie et al., 2012a; Braskie et al., 2012b; Chiang et al., 2011a), suggesting that neurotrophin-regulated signaling pathways may be important for white matter development.

The neurotrophic tyrosine kinase, receptor, type 3 gene (*NTRK3* also known as *TRKC*) encodes the high affinity receptor (TrkC) for the neurotrophin 3′-nucleotidase (NT3). NT3 and TrkC have been implicated in oligodendrocyte and brain myelin development (Barres et al., 1994; Hashimoto et al., 2011; Kumar and de Vellis, 1996), suggesting that *NTRK3* is a good candidate for affecting white matter integrity.

Some structurally connected networks of brain regions are known to modulate higher-order brain functions, such as executive function and complex behaviors (Fuster, 2001). Diminished integrity of these networks, through damage or a failure to develop normally, may disrupt these functions, resulting in profiles of symptoms that may involve mood, learning, perception, or memory, and may be diagnosed as mental illness (Kumar and Cook, 2002). Genetic factors may also affect brain connectivity and white matter integrity in these networks (Jahanshad et al., 2013; Thompson et al., 2010; Tost et al., 2012), possibly increasing the risk for carriers of certain genetic variants to develop mental illness.

NTRK3 genetic variants are implicated in schizophrenia, bipolar disorder (BPD), and obsessive-compulsive disorder (OCD) hoarding (Alonso et al., 2008; Athanasiu et al., 2011; Otnaess et al., 2009). These disorders tend to aggregate in families and also show some degree of comorbidity within the same individuals (Bramon and Sham, 2001; Joshi et al., 2010; LaSalle-Ricci et al., 2006; Potash et al., 2001). This evidence suggests that typical functioning of this gene may be important to mental health and atypical variants may make deviations from normal mental health more likely, regardless of which symptoms are demonstrated. This is in keeping with some prior work – including very recent work by the Psychiatric Genomics Consortium – suggesting that gene effects may extend beyond standard diagnostic boundaries (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Moskvina et al., 2009). All of these disorders have been associated with differences in white matter microstructure (measured as diffusion tensor imaging fractional anisotropy; DTI FA) in patients versus controls (Ellison-Wright and Bullmore, 2009; Vederine et al., 2011; White et al., 2008).

Most DTI studies of these disorders have found regionally reduced FA in patients versus controls e.g. (Friedman et al., 2008; Menzies et al., 2008; Sprooten et al., 2011), but others found regionally increased FA associated with disorder symptoms e.g. (Buchsbaum et al., 2006; den Braber et al., 2011; Hubl et al., 2004; Li et al., 2011; Mahon et al., 2009). Several voxelwise studies also found lower regional FA in those at high risk for these disorders (Bloemen et al., 2010; Chaddock et al., 2009; Clark et al., 2011; Hao et al., 2009; Hoptman et al., 2008; Menzies et al., 2008; Sprooten et al., 2011), suggesting that white matter deficits begin before overt symptoms, and may relate to genetic risk.

Some mental illness risk variants are also associated with FA variability in healthy subjects without other known risk for psychiatric conditions (Braskie et al., 2012a; Chiang et al., 2011a; Konrad et al., 2009; McIntosh et al., 2008; Winterer et al., 2008; Zuliani et al., 2011), suggesting one way that genetics may contribute to mental illness vulnerability. We identified 18 SNPs within the *NTRK3* gene that also have been associated with schizophrenia, BPD, or OCD. Our subjects were young and healthy, without a known predisposition for mental illness, so we are unable to study the specific risk for mental illness. Furthermore, the variants we chose were not top risk factors for these disorders. However, as the selected SNPs were common variants that were previously related to psychiatric disorder risk, they are likely to physically affect the brain in ways we can detect, in our normally developing population. We evaluated the relationship between FA and these genetic variants using a multi-SNP model. This model evaluates the predictive effect of each SNP while adjusting for the effects of all other SNPs in the model; it also builds an aggregate model of the overall effect of a set of SNPs. In this way, the SNPs with the strongest associations can be identified, and the aggregate effect of all the SNPs considered can be evaluated. A multi-SNP model also reduces the number of individual statistical tests made, thus increasing the power to detect effects. In this model, we did not fit interactions among SNPs, as the power to pick up interactions (modulatory effects of one SNP on the effects of another) is very low. In other work, we have begun to assess some of the challenges in picking up SNP interactions (Hibar et al., 2013).

We performed our analyses in a large sample of monozygotic and dizygotic young adults twins and their singleton siblings. Twin samples (or any family-based sample, such as a pedigree) are useful for establishing the heritability of traits to determine whether they might be good candidates for examining genetic control. More precisely, the traits that are not heritable are unlikely to be good targets for more in-depth genetic analysis at the SNP level. We had already demonstrated with these twin data that DTI FA in many parts of the brain white matter is moderately to highly heritable (Chiang et al., 2011b). In the current study, we expanded on our prior work to evaluate the effect of SNPs in the *NTRK3* gene on white matter FA, while controlling for age, sex, and accounting for kinship to avoid confounding effects of these covariates.

Neurotrophins and their receptors have been associated previously with white matter integrity in healthy young adults (Braskie et al., 2012a; Chiang et al., 2011a). As our selected *NTRK3* SNPs were also associated with neuropsychiatric disorders, we hypothesized that they were likely to influence white matter integrity, manifested as higher or lower FA.

Materials and Methods

Subjects

High-angular resolution diffusion-weighted MRI scans, collected as part of the Queensland Twin Imaging (QTIM) Study, were performed on 468 right-handed healthy young adults of European ancestry recruited to examine genetic influences on the brain. Genome-wide

genotyping was performed for all subjects, although our results here are not part of a genome-wide analysis; they arose from a candidate gene design focusing on *NTRK3*. From the overall available sample, we excluded 76 subjects: 8 were ancestry outliers (identified using principal components analysis of all genotyped variants to isolate those at least 6 standard deviations from HapMap CEU and TSI and the GenomeEUTWIN populations for each principal component axis), 58 had technically inadequate scans (spiking artifact, truncated field of view, or signal drop-out or distortion that prevented adequate co-registration), 3 had large ventricles that were inconsistent with good health in a young person, and 7 did not have a measured genotype for at least one of the SNPs of interest. The remaining 392 subjects largely overlapped with those in two previous studies that related DTI FA to variants in both the *CLU* gene (399 subjects) (Braskie et al., 2011) and the *NTRK1* gene (391 subjects) (Braskie et al., 2012a). Differences in subject exclusion related entirely to different availability of genotyping for the SNPs in question. The 392 subjects included here (mean age 23.6 ± 2.2 years; range: 20-29 years) were 94 monozygotic (MZ) twins, 141 dizygotic (DZ) twins or triplets, 41 singleton siblings of other participants included in this study, and 116 individuals who had no twin or singleton sibling included in this study (total of 247 families). We used the Human610-Quad BeadChip (Illumina) to analyze DNA according to the manufacturer's protocols (Infinium HD Assay; Super Protocol Guide; Rev. A, May 2008).

All subjects provided written, informed consent. The study conformed to the National Statement on Ethical Conduct in Human Research (2007) issued by the National Health and Medical Research Council (NHMRC) of Australia. It was approved by the QIMR Human Research Ethics Committee, and by the UCLA Medical Institutional Review Board (MIRB).

Image Acquisition

Magnetic resonance imaging (MRI: on a 4-Tesla; Bruker Medspec scanner) for each subject included T1-weighted inversion recovery rapid gradient echo scans (TI/TR/TE = 700/1500/3.35 ms; flip angle = 8°; slice thickness = 0.9 mm, 256 × 256 × 256), and DTI scans (single-shot echo planar imaging with a twice-refocused spin echo sequence to reduce eddy-current induced distortions (TR/TE 6090/91.7 ms, 23 cm FOV, 128×128; 55 2-mm axial slices/0 mm gap; 1.79 mm ×1.79 mm in-plane resolution). DTI scans each had 94 diffusion-weighted images (b=1149 s/mm^2) and 11 b_0 images (i.e., no diffusion sensitization). Gradient directions were evenly distributed on the hemisphere.

DTI Preprocessing

As described previously (Braskie et al., 2012a), non-brain matter was automatically then manually removed from the images. The raw diffusion-weighted images were adjusted for eddy current distortions (FSL “eddy_correct”), then linearly aligned and resampled to the associated T1-weighted image (FSL's linear image registration tool -FLIRT (Jenkinson et al., 2002)). B_0 images for each subject were elastically registered (using a mutual information cost function (Leow et al., 2005)) to the same subject's T1-weighted scan in common space. This registration method reduces the effects of echo planar imaging-induced susceptibility artifacts. We created FA maps using FSL software.

Template Creation and Registration

We used FA maps from 32 randomly selected, unrelated subjects (matched for sex) to create a template brain, or mean deformation target as described in detail previously (Braskie et al., 2012a; Jahanshad et al., 2010). All susceptibility-corrected FA maps were registered to the template brain using a 3D elastic warping technique with a mutual information cost function (Leow et al., 2005). Both the FA maps and the template brain were thresholded at 0.25 to exclude non-white matter. To further refine the coregistration of white matter between

subject scans and the template brain, we aligned the thresholded FA maps to the thresholded template brain. We used a Gaussian kernel (7 mm full-width-half-maximum; FWHM) to spatially smooth images.

SNP and genotype information

We performed one statistical analysis that considered the association of FA with 18 SNPs in the *NTRK3* gene simultaneously. These 18 SNPs included all of those in the *NTRK3* gene that were both available in our genotype file and also reported previously (as of November 2012) as being associated with schizophrenia (rs999905, rs4887348) (Otnaess et al., 2009), bipolar disorder (rs991728, rs994068, rs16941261, rs2114252, rs3784405, rs3784406, rs3784410, rs4887364, rs7165979, rs9806762, rs10163123, rs11630338, rs11631112, rs12911150) (Athanasiu et al., 2011), or obsessive-compulsive hoarding (rs1017412, rs7176429) (Alonso et al., 2008).

Table 1 shows the genotype summary for each SNP we included. When only one MZ twin from each MZ twin pair was assessed, the percentage of subjects with a given genotype for any SNP was similar to when all subjects were included (≤ 1% difference). Hardy-Weinberg equilibrium (HWE) and minor allele frequencies (MAF) for each SNP are listed in Table 2. The MAF for each SNP studied in our sample was similar to that previously identified for these same SNPs in the HapMap CEPH population (i.e., Utah residents with ancestry from northern and western Europe) (Altshuler et al., 2010). The genotype distribution in our sample showed no detectable deviation from HWE. None of the SNPs of interest are in regions of DNA that code for proteins. All are intronic with the exception of rs1017412 and rs7176429, which are in the 3′ downstream region of *NTRK3* (Sherry et al., 2001). The SNPs in intron 5 to 12 that were associated with bipolar disorder are also predicted to be binding sites for transcription factors Nkx2.2 and Pax6, which are involved in neural system development (Athanasiu et al., 2011). The effect on gene expression of each variant is not well characterized.

To get a sense of how many subjects in the sample carry different risk genotypes, one could consider what proportion of subjects in the sample carried (1) a specific number of minor alleles, or (2) a specific number of risk alleles implicated in mental illness, or (3) a specific number of alleles that might be expected to decrease FA (although each SNP's direction of effect is not typically known in advance). The three may not coincide (i.e., sometimes the major, or more prevalent, allele is the risk allele, and mental illness risk alleles do not always result in decreased FA). However, we assessed how many subjects in our sample carried one or two mental illness risk alleles in multiple SNPs. Subjects carried between 6 and 35 “risk” alleles (mean 15.56). Each “risk” allele may or may not be associated with lower FA.

For more information about how SNPs were selected for inclusion in our model, please see the ***Multi-SNP Model Reduction*** section below.

Statistical analyses

To limit partial volume effects, statistical analyses were performed only in voxels having FA > 0.25 in the template brain, i.e., likely white matter (Figure 1). We used linear mixed-model regression at each voxel to estimate how white matter FA differed by the individual and joint associations of genotypes while controlling for age, sex, and family relatedness as described previously in detail (Kohannim et al., 2012). Family relatedness between each subject and all others was adjusted for using a symmetric $N \times N$ kinship matrix. In this matrix, a coefficient of 1 between subjects indicated that they were MZ twins; a value of 0.5 indicated that they were DZ twins or siblings; and a 0 indicated that they were not closely

related. Ancestry outliers, identified using principal component analysis across genetic markers, were excluded.

Genotype effects were determined using an additive model. The additive model determines whether having more than one copy of a certain allele in a given SNP has additional effect over having only one copy of that allele. All the SNPs are treated as if they are in a multiple regression model, but with step-wise elimination of SNPs that are not improving the fit. This model is essentially ordinary least squares, so the fit of the overall model will treat the presence of multiple SNPs in a unique individual in the same way as additive effects of SNPs across individuals. The effect of each SNP is also controlled for in evaluating the effect of all other SNPs considered, by fitting all of them at once. The statistical interactions between SNPs are not included in the model, because in general the power is extremely low to pick up modulatory effects of one SNP on another. For efforts to boost power for detecting interactions, please see (Hibar et al., 2013). The analyses were performed using Efficient Mixed-Model Association (Kang et al., 2008) (EMMA; http://mouse.cs.ucla.edu/emma/) within the R statistical package (version 2.9.2; http://www.r-project.org/). *P* values for the significance of individual and joint SNP associations with FA were assessed using an *F*-test as described previously in detail (Kohannim et al., 2012).

We employed the widely-used false discovery rate (FDR) method with a threshold of 5% to control for multiple comparisons across all voxels considered (Benjamini and Hochberg, 1995). FDR controls the expected proportion of null results that are falsely identified as significant to a set rate (in our case this rate, $q = 5\%$, which is intended to be similar to the standard $p = 0.05$ frequently used to define significance). Using FDR, comparisons (voxels) having significance that is below a *critical p* value are considered significantly associated with the variable in question, even given the multiple comparisons. Thus, a ***higher*** critical *p* (closer to 0.05) may allow more voxels to be considered significant after adjustment for multiple comparisons. Actual *p* values in many of the significant voxels are much lower than the critical *p* reported.

Multi-SNP Model Reduction

We included all 18 identified SNPs in our initial model, successively removing the SNP with the weakest association to FA. This method gained us FDR-corrected significant results after removing the weakest SNP. Later statistical models were fitted to help identify which SNPs contributed most to the fit. A single multi-SNP analysis provides two main results (as with any regression having multiple predictors). First, we learn whether the included SNPs as a whole are significantly associated with FA after FDR correction across voxels, and if so, what the overall critical *p* value is. Second, we learn the significance for the partial contribution of each SNP in the model to FA. In other words, we learn whether each individual SNP within the model is significantly associated with FA after adjusting for the effects of every other SNP in the model and correcting for FDR across voxels. As with all multiple regressions, the significance values for each SNP in the model are only relevant if the overall model fits.

To identify the SNPs in order of their likely contribution to the model fit, we proceeded as follows. For each SNP in the model, we first identified only those voxels that, for that SNP, had a nominal association (i.e. $p < 0.05$) with FA. Next we obtained the mean *p* value across nominally associated voxels for each individual SNP in the model. SNPs with a higher proportion of voxels in which strong relationships existed between genotype and FA would be considered to have stronger associations with FA than those with mean voxelwise *p* values closer to 0.05. The single SNP with the highest mean *p* value across voxels in each multi-SNP analysis was considered the weakest association and was removed from the model before re-running. This procedure was repeated until only two SNPs were included.

We then examined the ***critical*** *p* values for each model. The overall model with the greatest number of voxels that pass FDR correction for multiple comparisons was considered the optimal model. For a given statistical map of *p* values, we can threshold it to only show voxels with *p* values lower than a given threshold, so the higher the threshold, more voxels will be shown. In the end, a cumulative distribution function is compiled of all the *p* values in the map, the threshold is chosen so as to be the highest *p* value (i.e. the critical *p* value) for which the false discovery rate is controlled. We do tend to choose models with higher critical *p* values but it is worth noting that other criteria could be used to define the best fitting model for the anatomical data, such as the one with the lowest *p* value in a region of interest.

We assessed the strength of the association only in nominally significant voxels to identify those SNPs having voxels that were strongly associated with FA, even in fairly small regions. In this way, we identified relationships in which genotype strongly predicts FA in voxels of interest without preferentially selecting relationships that are more widespread throughout the brain. If all 42,804 voxels in our white matter regions were considered in our analysis, voxels with *p* values close to one would likely diminish small regions of high significance. However, using this threshold does weight small regions of strong significance more than larger regions with voxels having *p* values close to 0.05. Arguably, both very significant results and wide spread nominal results could be considered “strong.” We therefore also performed the same step-wise elimination analysis without thresholding voxels to $p < 0.05$ (i.e., we obtained the mean p value across all voxels in our FA > 0.25 region). We note that there are many ways to sort the nested submodels, and for trading off modeling complexity versus goodness of fit, but this step-wise method had the advantage of eliminating SNPs that contributed least to the prediction, leading to a more efficient and parsimonious model.

To evaluate whether the association between genotypes in our original optimal five SNP model and FA were robust across the entire white matter mask (i.e., FA>0.25), we additionally ran this optimal model on mean FA within that mask. We again controlled for age, sex, and kinship.

In order to determine whether our optimal multi-SNP model provided additional information over the comparison of individual SNPs with FA (outside of a multi-SNP model), we then evaluated the individual association of each SNP from the optimal model in five single SNP analyses (one separate analysis for each SNP in our optimal model). These analyses may provide different results from the partial contributions of SNPs to FA within a multi-SNP model because when evaluated individually, the relationships between SNPs are not considered. We controlled for age, sex, and family relatedness as with the multiple SNP association tool. For these tests, we used the same parameters as with the multiple SNP association. Namely, FA maps were thresholded at 0.25 to exclude non-white matter and we used an isotropic 3D Gaussian kernel (7 mm FWHM) to smooth the images spatially.

Linkage disequilibrium

Linkage disequilibrium (LD) is the non-random association of alleles at two loci – there tends to be a statistical correlation between any given SNP and other SNPs that are close to it on the genome. Therefore, a strong association between a given allele in one SNP and certain brain differences would increase the likelihood that another SNP in moderate linkage disequilibrium with the first would show a similar effect. The multiple SNP association tool used here adjusts the effect of a given SNP for the effects of all others. Thus, a SNP showing a strong association with a brain characteristic if examined alone, may manifest a weaker association if examined in concert with other SNPs in LD with the first. Figure 2 displays the LD between SNPs examined here.

Association of FA with top schizophrenia SNPs

To evaluate whether our results would apply to SNPs implicated in neuropsychiatric disorders in general or were more specific to our SNPs in question, we used the same sample of 392 subjects to compare DTI FA with genotypes for the top five SNPs associated with schizophrenia in November 2011 (http://www.szgene.org/TopResults.asp) (Allen et al., 2008). We included all five SNPs in the multi-SNP model, using an additive model, a smoothing kernel of 7 mm FWHM, and an FA threshold of >0.25 as we used for the *NTRK3* analyses. The SNPs we included were *PRSS16* rs6932590, *PGBD1* rs13211507, *NRGN* rs12807809, *NOTCH4* rs3131296, and *HIST1H2BJ* rs6913660.

Results

We examined the joint effect of the set of candidate SNPs on voxelwise FA in the white matter, using a partial *F*-test and a linear mixed-effects model to compute *p* values. In a stepwise fashion, we removed SNPs in the order of their effects (weakest to strongest). After removing the first SNP, rs11631112, the full model achieved significance after FDR correction. Each subsequent model also remained significant as a whole. The five-SNP model that included rs1017412, rs2114252, rs16941261, rs3784406, and rs7176429 had the most widespread association with FA in the white matter, with a FDR critical $p = 0.028$. Approximately 55.6% of white matter voxels (i.e., 23,806 out of the 42,804 voxels considered) survived this multiple comparison corrected threshold, showing that the model was a relatively good predictor of white matter integrity across widespread regions of the brain. In fact, when the SNPs were considered together, they were also significantly associated with mean FA across the entire white matter mask ($R^2 = 0.06$; joint $p = 0.0004$). Two of the SNPs in our optimal model, rs1017412 and rs7176429, are found in the 3′ downstream region of *NTRK3* (Sherry et al., 2001). Our strongest local effects (uncorrected $p < 0.0001$) were in the corticospinal tract, superior and inferior longitudinal fasciculus (SLF and ILF), inferior fronto-occipital fasciculus (IFO), cingulum, anterior thalamic radiation, and forceps major and minor. We also found significant effects in the posterior thalamic radiations, corpus callosum (*genu*, body, and *splenium*), anterior, posterior, and superior corona radiata, interior and exterior capsule, fornix (column and body), fornix/*stria terminalis*, and uncinate fasciculus. All effects were bilateral (Figure 3). The optimal model explained a mean of 5.2% of the variance in FA in those voxels that survived FDR correction.

In the optimal multiple SNP model, the combined SNPs within the model as well as the individual contributions of two of the five SNPs (rs1017412 and rs2114252) were significantly related to FA after FDR correction. For rs1017412, which was previously associated nominally with OCD hoarding (Alonso et al., 2008), the minor allele, G, was associated in our study with lower DTI FA (critical $p = 0.024$). Strongest effects ($p < 0.0001$) were found in the corticospinal tract, SLF and ILF, IFO, cingulum, anterior thalamic radiation, *forceps major* and *minor*, internal and external capsules, uncinate fasciculus, and the *genu* of the corpus callosum. We also found significant effects in the posterior thalamic radiation, corpus callosum (body, and *splenium*), anterior, posterior, and superior *corona radiata*, and fornix/*stria terminalis*. Again, all effects were bilateral. Additionally, the minor allele, A, of rs2114252, which was previously associated nominally with bipolar disorder (Athanasiu et al., 2011), was associated with lower FA in our sample (critical $p = 0.00026$). Effects with $p < 0.0001$ were in the right corticospinal tract and ILF, left cingulum and anterior thalamic radiation, and bilateral SLF, IFO, and forceps major. There were no other regions in which FA was significantly associated with the genotype at rs2114252.

To assess whether our thresholding of voxels to $p < 0.05$ emphasized small regions of significance to the exclusion of broader regions of nominal significance in other SNPs, we

also performed the same step-wise analysis, evaluating the mean *p* value of all voxels included in our white matter mask (FA > 0.25). Again, the optimal model included five SNPs (critical $p = 0.032$), including rs1017412, rs2114252, and rs7176429 from the thresholded optimal model. The new model also included schizophrenia risk variant, rs4887348, and bipolar disorder risk variant, rs3784405, which were not previously included. Again, rs1017412 (critical $p = 0.028$) and rs2114252 (critical $p = 0.017$) were significantly related to FA after FDR correction as part of the optimal model. The pattern of significance was similar to that in the optimal model obtained through thresholded step-wise elimination of SNPs except was slightly more extensive (particularly in the *genu* of the corpus callosum, bilateral anterior thalamic radiation, and left posterior thalamic radiation) when the non-thresholded method was used.

In an additional test, we considered the individual association of each of the five SNPs from the optimal model using a single SNP analysis (i.e., in this analysis, we no longer considered the effects of other SNPs from the optimal model), controlling for age, sex, and family relatedness. In these analyses, none of the SNPs had alleles significantly associated with DTI FA on a voxelwise basis after FDR correction.

Using the same parameters, multi-SNP tool, and subject set as used for the *NTKR3* SNPs, the top five schizophrenia risk SNPs (*PRSS16* rs6932590, *PGBD1* rs13211507, *NRGN* rs12807809, *NOTCH4* rs3131296, and *HIST1H2BJ* rs6913660) were not significantly associated with DTI FA.

Discussion

Identifying genetic variants that affect white matter integrity is of great interest for personal prediction of brain structure, to better understand disease mechanisms, and to identify therapeutic targets for psychiatric disorders involving aberrant brain connectivity. Eighteen variants in the *NTRK3* gene have been implicated in neuropsychiatric disorders (Alonso et al., 2008; Athanasiu et al., 2011; Otnaess et al., 2009), suggesting that this gene may be important for neuropsychological function, as well as its known role in brain development. When evaluating the effect of *NTRK3* variants on white matter integrity, we found that when considered together, five SNPs (rs1017412, rs2114252, rs16941261, rs3784406, and rs7176429) were most strongly related to voxelwise and mean DTI FA in the brain white matter of young healthy adults. None of those SNPs showed a significant effect on FA when evaluated individually, without considering the effect of the accompanying SNPs. In line with many other studies that have advocated multi-SNP or gene-based testing (Hibar et al., 2011), our results demonstrate the advantage of considering the joint effect of numerous risk SNPs when evaluating their effect on the brain. Three of the SNPs in our optimal model (rs1017412, rs2114252, and rs7176429) were also included in our optimal model when all voxels under our FA > 0.25 mask were considered (rather than only examining voxels with $p < 0.05$), suggesting that these SNPs had fairly broad regions of strong significance.

FA differences in neuropsychiatric patients or those at risk may differ in location from study to study, partly because some studies examine only specific regions rather than performing voxelwise analysis that allow for brain-wide comparison of results across studies. Data analysis methods may also vary, and many studies have small sample sizes. When only the largest (100 or more total subjects) voxelwise studies of adult onset schizophrenia, BPD, or OCD are considered (Buchsbaum et al., 2006; Clark et al., 2011; den Braber et al., 2011; Hao et al., 2009; Kanaan et al., 2009; Perez-Iglesias et al., 2010; Sprooten et al., 2011; Sussmann et al., 2009; Wang et al., 2011), FA was lower in affected individuals across diagnoses in the SLF (56% of studies), and the genu and ILF (44% of studies for each), suggesting that lower white matter integrity in these regions may increase the vulnerability

for mental illness. In our study, two of these regions, the genu and ILF, were among the regions showing the strongest association between lower FA and *NTRK3* variants. The *genu*, or anterior part of the corpus callosum connects the left and right brain hemispheres, particularly carrying fibers that innervate the prefrontal cortex. Lower FA in the anterior part of the corpus callosum has been associated with avolition, or lack of drive (Nakamura et al., 2012) and with poorer executive function ability (Zheng et al., 2013). The ILF connects the occipital and temporal cortex. It is an important part of the visual–limbic pathway that promotes vision-specific emotions, learning and memory. Lower FA in the ILF has been associated with decreased working memory ability (Gu et al., 2013), object recognition (Ortibus et al., 2012) and processing speed (Choi et al., 2012) and with greater depression and anxiety (Choi et al., 2012).

Genetic variation in neurotrophins and their receptors may influence white matter development in regions implicated in a range of neuropsychiatric disorders. Deeper study of genes that encode neurotrophins and their receptors may offer insights into increased vulnerability for developing these disorders. When we used the multi-SNP tool to compare DTI FA with genotypes in the top five variants currently associated with schizophrenia (Allen et al., 2008), none of those SNPs was significantly associated with FA, demonstrating that our effect may be related more to variations within neurotrophin systems than to neuropsychiatric risk *per se*.

Our optimal model found that *NTRK3* variants significantly related to DTI FA broadly throughout the brain, including strongly in the SLF and ILF, and also in the *genu* of the corpus callosum, all regions in which FA is most consistently affected in those with schizophrenia, BPD, and OCD in prior large, voxelwise DTI studies of white matter integrity (Buchsbaum et al., 2006; Clark et al., 2011; den Braber et al., 2011; Hao et al., 2009; Kanaan et al., 2009; Perez-Iglesias et al., 2010; Sprooten et al., 2011; Sussmann et al., 2009; Wang et al., 2011). *NTRK3* variants may modulate white matter integrity in regions implicated in a variety neuropsychiatric disorders. We examined *NTRK3* SNPs that previously increased the risk for developing mental illness in order to increase the likelihood that the SNPs would affect white matter. However, our subjects were young and healthy, and not specifically selected for symptoms or family history that increase their risk for mental illness. Additionally, for our SNP with the strongest relationship to FA (rs1017412), the OCD hoarding risk allele, A, was actually associated with *higher* FA in our sample. For our SNP with the next strongest relationship to FA (rs2114252), the A allele, previously associated with BPD (Athanasiu et al., 2011), was associated with lower FA in our sample. It is therefore unlikely that our results herald a progression toward mental illness in this sample, and are more likely to be a result of normal development. Developmental effects still may increase the risk of mental illness in people having other factors that predispose them to the disorders (Insel, 2010; Weinberger, 1987). Our study may direct future research toward examining *NTRK3* gene effects on white matter integrity in those afflicted by or at increased risk for schizophrenia, BPD, and OCD. Gaining a better understanding of which gene variants help to control brain development, particularly in regions vulnerable to disease, is an important step in understanding those maladies.

Acknowledgments

We thank the twins and siblings for their participation. In Brisbane we thank Marlene Grace and Ann Eldridge for twin recruitment, Aiman Al Najjar and other radiographers for scanning, Kerrie McAloney and Daniel Park for research support, and staff in the Molecular Epidemiology Laboratory for DNA sample processing and preparation. This study was supported by the U.S. National Institutes of Health (R01 HD050735, EB008432, EB008281, EB007813, and AG040060 to PT), and the National Health and Medical Research Council, Australia (NHMRC 486682 and 389875). MNB was funded, in part, by the NIH (P50 AG16570) and by the UCLA Easton Consortium for Biomarker and Drug Discovery in Alzheimer's Disease. OK was funded, in part, by a UCLA Dissertation Year

Fellowship and by an NIH NRSA Award (F30 AG041681). GIdZ was supported by an ARC Future Fellowship (FT0991634).

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Abbreviations

NTRK3* aka *TRKC	neurotrophic tyrosine kinase, receptor, type 3 gene

NT3	neurotrophin 3′-nucleotidase
BPD	bipolar disorder
OCD	obsessive compulsive disorder
DTI	diffusion tensor imaging
FA	fractional anisotropy
SNP	single nucleotide polymorphism
FLIRT	FSL's linear image registration tool
FWHM	full-width at half maximum
LD	linkage disequilibrium
MZ	monozygotic
DZ	dizygotic
HWE	Hardy-Weinberg equilibrium
MAF	minor allele frequencies
FDR	false discovery rate
ILF and SLF	inferior and superior longitudinal fasciculus
IFO	inferior fronto-occipital fasciculus
BDNF	brain-derived neurotrophic factor
NTRK1	neurotrophic tyrosine kinase, receptor, type 1

Highlights

- A multi-SNP analysis compared DTI FA with genotypes in 18 SNPs in the NTRK3 gene.
- SNPs were selected for being previously associated with mental illness.
- FA was best predicted by 5 SNPs (rs1017412, rs2114252, rs16941261, rs3784406, and rs7176429).
- Gene effects included regions implicated in mental illness.
- Effects overlapped with those of other neurotrophin-related genetic variants.

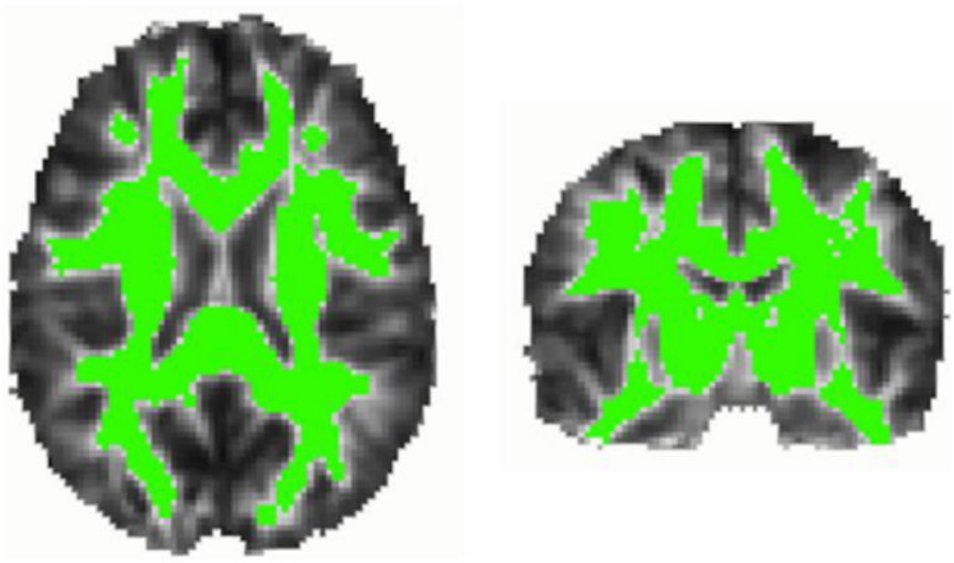

Figure 1. Voxels in which statistical analyses were performed

Regions highlighted in green represent voxels in which FA exceeded 0.25 in the MDT (i.e., likely white matter).

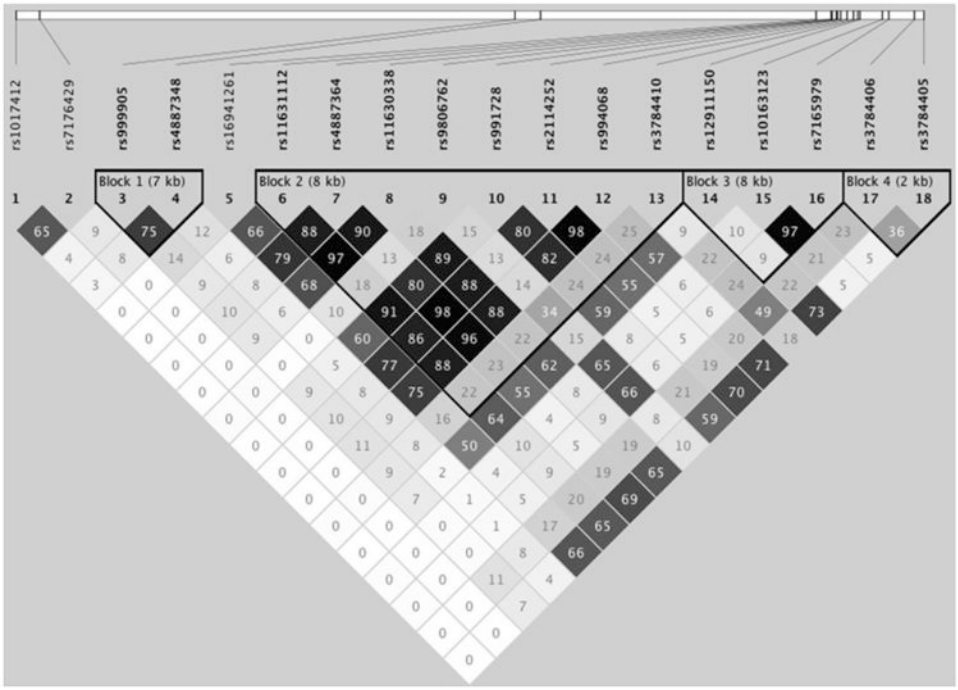

Figure 2.

Linkage disequilibrium plot. The plot below, created in Haploview (Barrett et al., 2005), shows R^2 values representing the degree of linkage disequilibrium among all SNPs considered in this study. One subject per family was selected at random and included in the analysis to avoid any confounding effects of kinship. SNPs that were significantly associated with FA in the optimal model were rs1017412 and rs2114252. Also included in the optimal model were rs16941261, rs3784406, and rs7176429.

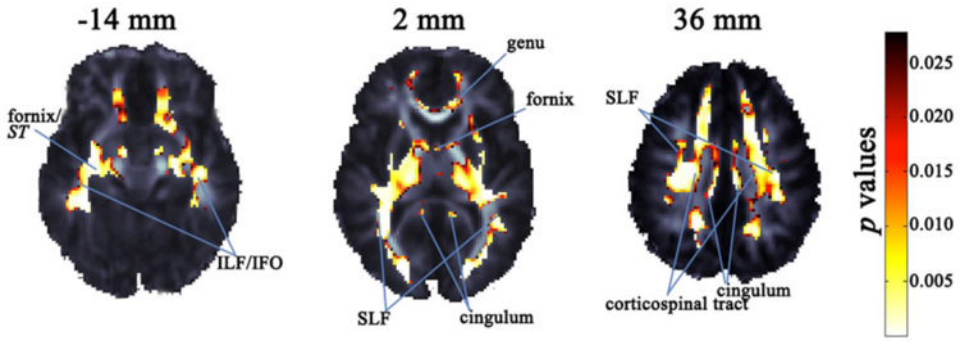

Figure 3. FA association with the optimal five-SNP model of the*NTRK3*gene, displayed on a study-specific FA template

Highlighted areas are the *p* values indicating voxels in which the *NTRK3* SNPs (rs1017412, rs2114252, rs16941261, rs3784406, and rs7176429) in our optimal model are associated with FA after adjusting for age and sex (FDR critical *p* value = 0.028). The left brain hemisphere is displayed on the right. Coordinates listed are for the Z direction in ICBM space. SLF and ILF denote the superior and inferior longitudinal fasciculi, IFO is the inferior fronto-occipital fasciculus, and *ST* is the *stria terminalis*.

Table 1

Variants of interest and genotypes in all included subjects.

SNP	Minor allele	Major allele	Subjects with 0 minor alleles	Subjects with 1 minor allele	Subjects with 2 minor alleles
rs991728	G	C	210	160	22
rs994068	C	G	209	159	24
rs999905	C	G	186	158	48
rs1017412	G	A	197	163	32
rs2114252	A	C	206	161	25
rs3784405	C	T	243	132	17
rs3784406	T	C	128	193	71
rs3784410	T	C	130	202	60
rs4887348	G	A	211	149	32
rs4887364	C	T	208	158	26
rs7165979	C	G	200	161	31
rs7176429	T	G	181	183	28
rs9806762	G	A	161	187	44
rs10163123	G	C	200	160	32
rs11630338	C	T	218	154	20
rs11631112	T	G	222	149	21
rs12911150	G	T	236	142	14
rs16941261	C	G	218	153	21

Table 2

Minor allele frequency (MAF) and evidence for Hardy-Weinberg equilibrium: statistics here are compiled based on using one subject per family to avoid any confounding effects of kinship.

SNP	MAF: our sample	MAF: in CEPH*	HWE χ^2	HWE *p*
rs991728	0.27	0.24	0.31	0.58
rs994068	0.28	0.25	0.33	0.57
rs999905	0.32	0.32	0.59	0.44
rs1017412	0.30	0.28	0.69	0.41
rs2114252	0.28	0.26	0.18	0.67
rs3784405	0.22	0.20	0.29	0.59
rs3784406	0.43	0.42	0.21	0.65
rs3784410	0.40	0.42	1.16	0.28
rs4887348	0.26	0.26	0.00	1.00
rs4887364	0.28	0.25	0.06	0.81
rs7165979	0.28	0.26	0.18	0.67
rs7176429	0.32	0.28	1.02	0.31
rs9806762	0.35	0.33	0.60	0.44
rs10163123	0.28	0.29	0.42	0.52
rs11630338	0.26	0.25	0.14	0.71
rs11631112	0.25	0.24	0.04	0.84
rs12911150	0.22	0.22	0.59	0.44
rs16941261	0.25	0.23	0.40	0.52

*CEPH is a reference population of Utah residents with ancestry from northern and western Europe (Altshuler et al., 2010).