

How a common variant in the growth factor receptor gene, *NTRK1*, affects white matter

Meredith N. Braskie,¹ Neda Jahanshad,¹ Arthur W. Toga,¹ Katie L. McMahon,² Greig I. de Zubicaray,³ Nicholas G. Martin,⁴ Margaret J. Wright⁴ and Paul M. Thompson^{1,*}

¹Imaging Genetics Center at the Laboratory of Neuro Imaging; Department of Neurology; UCLA School of Medicine; Los Angeles, CA USA; ²Centre for Advanced Imaging; University of Queensland; Brisbane, QLD Australia; ³School of Psychology; University of Queensland; Brisbane, QLD Australia; ⁴Queensland Institute of Medical Research; Brisbane, QLD Australia

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Abbreviations: FA, fractional anisotropy; DTI, diffusion tensor imaging; D_{rad} , radial diffusivity; D_{ax} , axial diffusivity; *NTRK1*, neurotrophic tyrosine kinase receptor 1; TrkA, tropomyosin-related kinase receptor A; *BDNF*, brain derived neurotrophic factor; NT3, neurotrophin 3; *NTRK1-T*, allele T at *NTRK1* variant rs6336; ODFs, orientation distribution functions; DRG, dorsal root ganglion; OPC, oligodendrocyte progenitor cell; CNPase, 2',3'-cyclic nucleotide 3'-phosphodiesterase; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; LINGO-1, LRR (leucine-rich repeat) and Ig domain-containing Nogo receptor-interacting protein; PI3K, phosphatidylinositol-3'-kinase; Akt, protein kinase B

Growth factors and their receptors are important for cellular migration as well as axonal guidance and myelination in the brain. They also play a key role in programmed cell death, and are implicated in a number of mental illnesses. Recently, we reported that healthy young adults who carry the T allele variant in the growth factor gene, *NTRK1* (at location rs6336), had lower white matter integrity than non-carriers on diffusion images of the brain. Diffusion tensor imaging (DTI) revealed how this single nucleotide polymorphism affects white matter microstructure in human populations; DTI is also used to identify characteristic features of brain connectivity in typically developing children and in patients. Newly discovered links between neuroimaging measures and growth factors whose molecular neuroscience is well known offer an important step in understanding mechanisms that contribute to brain connectivity. Altered fiber connectivity may mediate the relationship between some genetic risk factors and a variety of mental illnesses.

Neurotrophins are a family of proteins that influence the migration, development and survival of neurons; in the central nervous system, these growth factors are mainly produced by neurons. They are taken up by other neurons that express the appropriate tropomyosin-related kinase (Trk) receptors. Three of the better-known neurotrophins are nerve growth factor (NGF), which binds with high affinity to the neurotrophic tyrosine kinase receptor 1 (*NTRK1*; also known as TrkA); brain derived neurotrophic factor (BDNF), which binds to *NTRK2* (also known as TrkB), and neurotrophin 3 (NT3), which binds to both *NTRK2* and *NTRK3* (also known as TrkC). Our laboratory discovered that certain mental illness-associated genetic variants in *BDNF*, *NTRK1*, and *NTRK3* are all related to diffusion tensor imaging (DTI) measures of white matter integrity in young healthy adults (aged 20–30).^{1–3} DTI scans are a special type of magnetic resonance image (MRI), sensitive to how water diffuses in the brain, rather than to the hydrogen content of tissues (which is the principle underlying standard anatomical MRI; Fig. 1). The genetic variants we studied are single nucleotide polymorphisms—or SNPs—that are commonly carried, even in healthy

human populations. SNPs are considered to be associated with mental illness if one of the alleles, or genetic forms, at that SNP is over-represented in patients based on large-scale genome-wide association studies. Several genetic variants in neurotrophins and their receptors are associated with mental illnesses including schizophrenia, bipolar disorder, and obsessive-compulsive disorder.^{4–7} These disorders frequently aggregate in families or co-occur within individuals.^{8,9} In addition, each of these disorders has previously been associated, at the group level, with identifiable differences in white matter microstructure (measured using DTI).^{10–12} When “risk genes” for mental illness are identified, their mechanism is not always clear, and it is critical to discover how the variants may combine to impact disease development. Knowledge of how a risk gene operates in the body, or the brain specifically, may improve personal risk assessment in high-risk individuals, encourage tailored treatment and prevention,¹³ and boost the power in disease studies through cohort stratification by relevant genetic differences.

We recently discovered a relationship between white matter integrity and an *NTRK1* genetic variant (rs6336), previously

*Correspondence to: Paul Thompson; Email: thompson@loni.ucla.edu
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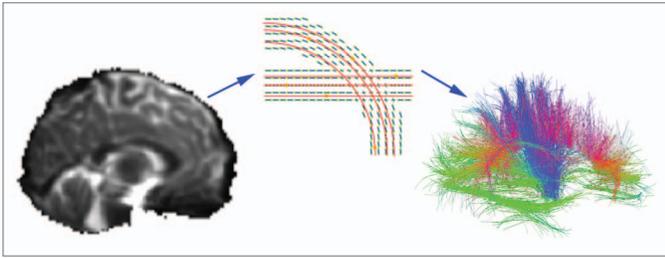


Figure 1. Diffusion tensor imaging and tract tracing. In whole brain tractography, diffusion weighted images (left panel) show the diffusion of water in specific directions (94 directions in the current study). A diffusion function (middle panel) can be reconstructed based on the sampling of diffusion in each direction, to identify axons and major tracts, as water tends to diffuse preferentially along tracts. Tract tracing algorithms can assemble the data into curves and bundles based on diffusion properties. In a three-dimensional map of the recovered fibers (right panel), a color code indicates the fiber directions. These fibers may be grouped into bundles and their integrity and connectivity can be evaluated. This image is adapted from ref. 29.

associated with schizophrenia.^{6,14} This link is promising as it connects measures of fiber integrity on a brain scan to decades of molecular and neurodevelopmental work on growth factors—specifically, nerve growth factor (NGF) and its receptor. The *NTRK1* gene encodes a high affinity receptor for NGF, but approximately 8% of Caucasians from Northern and Western European ancestry¹⁵ carry a different allele T (at locus rs6336 in the *NTRK1* gene), which also may be associated with schizophrenia risk. Intriguingly, the reported T allele frequency was zero in Han Chinese in Beijing, Japanese in Tokyo, and in Yoruban in Ibadan, Nigeria.¹⁵ By scanning the brain with diffusion MRI, we were able to identify one specific way in which this allele disrupts the living human brain—one step toward a more mechanistic understanding of this gene variant.

We found that the T allele of rs6336 (*NTRK1-T*) was associated with decreased fractional anisotropy (FA) and increased radial diffusivity (D_{rad}) as determined using DTI.³ FA is a widely used and widely accepted measure of white matter integrity. It measures the extent to which water diffusion is directionally constrained. Higher values usually, but not always, indicate greater brain fiber myelination.¹⁶ More constrained diffusion indicates greater myelination as the myelin sheaths act as barriers to water diffusion, making the water molecules diffuse preferentially along axons rather than across them. D_{rad} represents diffusion perpendicular to the axon fiber. Higher D_{rad} may indicate less dense axonal packing, a lesser degree of myelination, a greater mean axon diameter, higher permeability of axonal fibers, or a combination of these.¹⁷ There are at least two possible explanations for why the carriers of this genetic variant on average have less directional diffusion in their white matter.

The lower FA and higher D_{rad} we found in T-allele carriers may relate to programmed cell death during development. Early in brain development, neurotrophins are transported in a retrograde fashion along the axon to the soma of neurons. Only those neurons that bind and transport the neurotrophins survive programmed cell death (as reviewed in ref. 18).

Neurons that express the NTRK1 or NTRK3 receptors in both the central and peripheral nervous system die within days in vitro unless exposed to their ligands, NGF and NT3, respectively.¹⁹ Because of this, genetic polymorphisms that increase the ratio of NTRK1 or NTRK3 receptors to available NGF or NT3 may increase neuronal death developmentally, resulting in a decrease in the density of fibers connecting specific brain regions. *NTRK1-T* is a missense mutation in other words the resulting codon in the genome codes for a different amino acid. The minor allele, or “risk allele,” T codes for tyrosine, rather than the histidine encoded by the more common C allele at position 598 within the kinase domain.⁶ It is unclear whether the mutation increases or decreases expression of the NTRK1 receptor in the brain. It is possible though that the decreased fractional anisotropy and increased radial diffusivity we found in *NTRK1-T* carriers previously³ may reflect such a decrease, as lower fiber packing allows for more diffusion perpendicular to the length of an axon.

Since our previous *NTRK1* report was published,³ we have been studying how the same variant affects patterns of brain connectivity, so here we are taking the opportunity to report an update. In a subset of 359 of the 391 subjects (330 C/C, 27 C/T; 2 T/T) from our prior study,³ we examined cortical connectivity by *NTRK1* genotype across the brain’s white matter using the analysis methods detailed previously.²⁰ Participant information is available in our earlier publication.³ Cortical connectivity was defined as the estimated proportion of fibers in the brain that connect one cortical region to another—compared with total number of detected fibers in a given subject. Briefly, cortical regions were delineated on each subject’s anatomical brain scans (T1-weighted) in a common template space. Using a method called whole-brain tractography, we were able to build a map of fiber trajectories in the brain by following the paths of maximum water diffusion. The connections between pairs of cortical and subcortical regions were identified and counted. To ensure that only consistently present connections were evaluated, the only connections compared by genotype were those found in at least 95% of the study participants.

Connectivity between each pair of cortical regions was compared by genotype group. In this kind of analysis, it is possible to define a useful measure of anatomical connectivity in terms of the density of recovered fibers connecting any pair of brain regions, which is what we did here.

There were 32 subjects excluded from the current analysis but included in our previous study of *NTRK1-T* differences in DTI FA.³ For 21 of them, the cortical segmentation was inadequate, for 9, the whole-brain tractography was too sparse and failed to adequately cover the full brain, and for 2, other technical difficulties made the connectivity data unusable.

In this new analysis, *NTRK1-T* allele carriers had a higher relative density of fibers in the anatomical pathway between the left superior parietal cortex and supramarginal gyrus. This was not a region with FA differences between genotypes.³ However, our connectivity analysis lacked significant support for lower relative fiber density in our *NTRK1-T* carriers. This suggests that either differences in fiber density are not the main driving component

of our FA differences, or that genotype differences were associated with fairly uniform changes in fiber density across the brain, in which case our relative connectivity analysis would not detect differences. NTRK2, the high affinity receptor for BDNF, is not implicated in cell death,¹⁹ so differences in programmed cell death processes are unlikely to explain the relationship our laboratory found between the *Val* allele at BDNF rs6265 and lower DTI FA.¹ Cortical thickness and volume may not be directly correlated with neuronal count,^{21,22} so neuronal loss related to programmed cell death may or may not be reflected in measures of cortical thickness or volume.

NTRK1-T may cause the FA differences we saw by influencing axon myelination. Neurotrophins and their receptors have a well-established role in peripheral nervous system myelination.²³ Additionally, oligodendrocytes, a major component of myelin in the central nervous system express and secrete neurotrophins. The presence of NGF in dorsal root ganglion (DRG)-oligodendrocyte progenitor cell (OPC) co-culture decreases myelination. Its presence reduces the expression of the oligodendrocyte marker: 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), and markers of oligodendrocyte maturation: myelin-associated glycoprotein (MAG) and myelin basic protein (MBP). NGF also prevents a large proportion of oligodendrocytes from extending processes and myelinating axons. These prior findings indicate that NGF reduces oligodendrocyte generation and maturation and also impairs myelination by those oligodendrocytes, once the oligodendrocytes are mature.²⁴ The effect is mediated by activating NTRK1 receptors in neuronal (rather than glial) cells. This suggests that NGF inhibits myelination by regulating neuronal signaling rather than acting directly on the glia.²⁴ NGF and NTRK1 exert their effect on myelination in part by helping to regulate LRR (leucine-rich repeat) and Ig domain-containing Nogo receptor-interacting protein (LINGO-1), which is a strong axonal inhibitor of oligodendrocyte differentiation and myelination.²⁵ If the *NTRK1-T* variant were therefore to increase expression of the neuronal NTRK1 receptors in the presence of excess ligand (such as NGF), it could reduce brain myelination, which could explain our lower FA in *NTRK1-T* carriers. To elucidate the mechanisms behind our findings, more information is needed on how the variant relates to NTRK1 receptor expression in the brain.

Finally, *NTRK1-T* could increase axonal diameter (caliber). When stimulated by neurotrophins, Trk kinases such as NTRK1 activate intracellular pathways. One such pathway includes phosphatidylinositol-3'-kinase (PI3K) and the protein kinase Akt, both of which have been shown to increase axonal diameter in culture.²⁶ Such an increase in diameter could explain the greater

D_{rad} we found in *NTRK1-T* carriers if the mutation resulted in greater NTRK1 signaling. However, increased axonal diameter would likely also be associated with increased D_{ax} (ref. 27) (diffusion parallel to the axonal fiber), which we did not find in our previous study.³ Therefore, an increase in axonal diameter related to increased NTRK1 signaling is a less likely explanation for our FA results.

In summary, we previously found that a missense mutation in *NTRK1* at rs6336 related to lower brain white matter integrity in carriers of the T allele. Carriers of this allele may, on average, exhibit differences in programmed neuronal death during development or differences in central nervous system myelination, both of which are influenced by activation of NTRK1 receptors by NGF. The next step in this work will be to compare the mean number of regional axonal fibers in a larger sample of *NTRK1-T* carriers to non-carriers, and to determine how the variant affects NTRK1 expression in central nervous system neurons. Once we understand how variants in growth factor genes affect a neuronal pathway, we may also evaluate the composite effect on white matter integrity of numerous polymorphisms in the same pathway using a tool designed to evaluate multiple variants together while correcting for the effects of each variant on all the others.²⁸ Such information may help predict individual risk for brain disease, and may inform treatment efforts by stratifying those receiving treatment into genetic strata with different white matter architecture.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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