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PTPN11 Mutations in the Ras-MAPK Signaling Pathway Affect Human White Matter Microstructure

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Abstract

We examined whether *PTPN11* mutations affect the white matter connectivity of the developing human brain. Germline activating mutations to the *PTPN11* gene cause overactivation of the Ras-Mitogen-Activated Protein Kinase pathway. Activating mutations cause Noonan syndrome (NS), a developmental disorder associated with hyperactivity and cognitive weakness in attention, executive function, and memory. In mouse models of NS, *PTPN11* mutations cause reduced axon myelination and white matter formation, while the effects of *PTPN11* mutations on human white matter are largely unknown. For the first time, we assessed 17 children with NS (9 females, mean age, 8.68 ± 2.39) and 17 age- and sex-matched controls (9 female, mean age, 8.71 ± 2.40) using diffusion brain imaging for white matter connectivity and structural magnetic resonance imaging to characterize brain morphology. Children with NS showed widespread reductions in fractional anisotropy (FA; 82 613 voxels, $t = 1.49$, $P < 0.05$) and increases in radial diffusivity (RD; 94 044 voxels, $t = 1.22$, $P < 0.05$), denoting decreased white matter connectivity. In NS, the FA of the posterior thalamic radiation correlated positively with inhibition performance, whereas connectivity in the genu of the corpus callosum was inversely associated with auditory attention performance. Additionally, we observed negative and positive correlations, respectively, between memory and the cingulum hippocampus, and memory and the cingulum cingulate gyrus. These findings elucidate the neural mechanism underpinning the NS cognitive phenotype, and may serve as a brain-based biomarker.

Key words: diffusion tensor imaging, executive function, genetics, noonan syndrome, sensorimotor

Introduction

Noonan syndrome (NS) is a genetic disease that impacts 1 in 2000 live births in the United States of America (Tartaglia et al. 2011). NS, which is caused by mutations to the Ras-MAPK pathway, is associated with multisystem phenotypic characteristics including abnormal facial structure, heart anomalies, short stature, ADHD symptomatology, memory impairment, and learning disabilities (Verhoeven et al. 2008; Gelb and Tartaglia 2011; Pierpont et al. 2013).

Germline mutations to the Ras-MAPK signaling pathway have been implicated in brain development aberrations in mouse models and in the development of NS in humans

(Aoki et al. 2016). However, the effects of germline mutations on human brain development have received far less attention than those of somatic mutations to the Ras-MAPK pathway, which have long been implicated in the development of cancer and are well-documented in the literature (Santarpia et al. 2012).

Among the germline mutations that lead to NS, mutations to the *PTPN11* gene, which encodes for the protein SHP2 in the pathway, are responsible for nearly half of all cases of NS (Aoki et al. 2016). SHP2 over-activation has been associated with reductions in axon myelination, hippocampal hyperexcitability, and deficient long-term potentiation in mouse models (Lee et al. 2014), and has additionally been found to promote

neurogenesis at the expense of astrocyte formation. By directing neural precursors to develop into neurons rather than astrocytes, over-activated SHP2 has been linked with substantial reductions in astrocyte formation in the forebrain and hippocampus of NS mouse models (Gauthier et al. 2007). Notably, astrocytes have been implicated as mediators of myelination by secreting factors that promote the activity of oligodendrocytes (Barnett and Linington 2013).

Mutations downstream of SHP2 have also been linked to NS pathology in animal models. For instance, deletion of the Map2k1/Mek1 and Map2k2/Mek2 kinases leads to the inactivation of the Ras-MAPK pathway and disrupts the elongation of axons along the corticospinal tract in mice (Xing et al. 2016).

These findings in mouse models point to a possible robust effect of PTPN11 gain-of-function mutations on white matter microstructure in the human brain. Here, we sought to identify the nature of these microstructural changes, if any were apparent. To answer this question, we used diffusion tensor imaging (DTI) to investigate differences in white matter microstructure between 17 children with PTPN11 NS (9 female; ages 4.43–11.93, mean: 8.68 ± 2.39) and 17 individually age- and sex-matched control participants (9 female; ages 4.05–11.89, mean: 8.71 ± 2.40). Additionally, we gathered morphometric structural magnetic resonance imaging MRI data and behavioral scores from our participants, in an effort to identify associations with our DTI findings.

DTI outputs several metrics of diffusivity, including fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD), which can each be linked to white matter microstructure.

FA is a proxy measure of white matter tract directionality and is found by measuring the flow of water molecules. Pure isotropy (i.e., low FA) describes a random distribution of water molecule movement, while pure anisotropy (i.e., high FA) describes movement along a single axis. Thus, increased FA is thought to represent a highly coherent white matter tract structure, presumably indicating increased myelination, insofar as myelination acts as an insulating material for neurons. Accordingly, decreased FA is thought to represent a “leaky” tract (i.e., a tract that is structurally incoherent and is presumably less myelinated), or flow through crossing fibers. Along similar lines, AD measures the degree to which water molecules diffuse along the primary axis of diffusion, while RD represents the degree to which water molecules diffuse perpendicular to the main axis of diffusion.

Based on the available literature from animal models of NS (Lee et al. 2014), we expected to find reduced FA, increased RD, and reduced AD in the NS group relative to the control group, indicating reduced white matter microstructural connectivity. Moreover, based on our prior structural findings in children with NS (Johnson et al. 2019), we expected to find reduced volume in the striatum and reduced cortical thickness in the parahippocampal structures among children with NS. Finally, we expected to observe tracts connecting to these regions to be correlated with reduced performance in behavioral measures of executive function and memory. Prior work (Xing et al. 2016) also suggested that corticospinal tracts would be significantly different between the two groups.

Materials and Methods

A total of 17 (9 female and 8 male) children with NS (ages 4.43–11.93, mean: 8.68 ± 2.39) and 17 (9 female and 8 male) individually age- and sex-matched typically developing controls (ages 4.05–11.89, mean: 8.71 ± 2.40) were scanned and assessed

during this study. Initially, 44 participants were included: 22 NS and 22 individually age- and sex-matched controls that were chosen from a larger cohort). One participant with NS (and the corresponding age- and sex-matched control participant) was removed from the analysis due to poor scan quality resulting from lack of cooperation in the scanner. Four children with NS (along with the corresponding control participants) were removed from the final analyses due to a scanner upgrade that was applied to the GE Discovery MR750 used for data collection and a change in the DTI scanning parameters. Specifically, these scans were acquired with a different slice thickness relative to that of the control group, despite the in-plane resolution remaining unchanged. NS participants were recruited via the Stanford University School of Medicine's website and through the National Noonan Syndrome Foundation physician network. For inclusion in the study, NS participants were required to provide proof of genetic testing to demonstrate the presence of PTPN11 mutations (Supplementary Table 2 for detailed genetic information). Selecting this specific mutation enabled us to achieve more informative results, in that homogeneity in the subject group allowed for more precision in attributing the results to the subjects' genetic profiles. Moreover, PTPN11 is the most common mutation associated with NS, making it a logical choice when evaluating white matter tracts in NS for the first time. Typically developing participants were recruited through parent networks and local print media for a broader study on Turner Syndrome also conducted at Stanford University School of Medicine, which used the same behavioral assessment and scan protocols.

Both children with NS and typically developing control participants were excluded if they met the following criteria: known diagnosis of a major psychiatric or current neurological disorder (including seizures), diagnosed gross structural malformations (e.g., Arnold Chiari malformation), MRI scan contraindications, premature birth (gestational age under 32 weeks), or low birth weight (under 2000 g). Major psychiatric disorders included psychotic disorders, major mood disorders, as well as full scale IQ score lower than 70. $IQ \geq 70$ is estimated to include >90% of individuals with NS according to recent studies (Wingbermühle et al. 2012; Pierpont et al. 2015).

Legal guardians provided informed, written consent for all participants, in addition to written assent provided by participants over the age of 7. The Stanford University School of Medicine Institutional Review Board approved the methods and protocols of this study, which were carried out per the requirements of the Board.

Diffusion Tensor Imaging

DTI is a noninvasive neuroimaging method that characterizes the white matter microstructure of the brain through the motion of water molecules. DTI is built on the principle that the diffusion of water molecules takes place by Brownian motion, the random and erratic movement of fluid molecules colliding with one another. The diffusion of water is influenced by the shape of the tracts through which the water is diffusing, since the water molecules will collide with the edges of the tract and flow differently than when flowing outside of a tract. Diffusion is thus a useful measure of the structure of parts of the brain characterized by long pathways of water diffusion (most frequently, white matter tracts), since it can describe the shape and integrity of these pathways. A number of different scalar measurements are often used in describing these DTI data, such as RD, AD, and FA.

It should be noted that these metrics are all interpretations of DTI data, but may not necessarily reflect the specific underlying biological processes in the brain. RD is understood to be a measure of myelination, as it is calculated by taking the mean of diffusivities perpendicular to the main diffusion axis. RD values would therefore decrease with increased myelination. By contrast, AD measures water diffusivity along the main diffusion axis within a voxel, and is therefore thought to represent fiber connectivity and microstructure of axonal membranes, which are both positively related to AD, and neurofilaments, microtubules, and axonal branching, which are all negatively related to AD.

Increased FA values are understood to represent increases in myelination, intravoxel fiber-tract coherence, and fiber tract density, which all differentially impact the movement of water molecules in the brain. Conversely, decreased FA values are thought to arise from increased intravoxel spacing and extracellular diffusion.

DTI Data Acquisition

Participants were scanned in a research-dedicated 3 Tesla GE Discovery MR750 scanner (GE Healthcare) at the Lucas Center for Neuroimaging, Stanford University, using the parameters outlined in [Supplementary Table 3](#).

Behavioral Data Acquisition

The NEPSY-II ([Korkman et al. 2007](#)) was used to assess each participant's performance in several cognitive domains. Specifically, we concentrated on the results of the inhibition, switching, auditory attention, response set, list memory and list memory delayed, memory for faces delayed, narrative memory free recall, and narrative memory free and cued recall subscores of the NEPSY-II. The inhibition task requires the child to inhibit automatic responses in favor of novel responses, such as by naming an upward-pointing arrow as a downward-pointing arrow. The switching task assesses a child's ability to switch between response types. The auditory attention task assesses a child's ability to exert and sustain selective auditory attention. Response set assesses the ability to inhibit previously learned responses, while correctly responding to matching or contrasting stimuli. The list memory task requires a child to learn a word list, and then recall the list after learning an interference list. The list memory delayed score assesses the child's ability to recall the same list 25–35 min later. The memory for faces delayed task involves showing a child a series of faces and, after 15–25 min, showing them another series of faces and asking which faces were shown in the original set. The narrative memory free recall task involves the child listening to a story and then repeating it. The cued recall component is administered by asking the child about specific portions of the story.

To assess general cognitive abilities, we utilized the Wechsler Preschool and Primary Scale of Intelligence Edition 4 (WPPSI-IV) for participants under the age of 6 and the Wechsler Intelligence Scale for Children Edition 5 (WISC-V) for participants above the age of 6. From the WPPSI-IV, we considered the Full-Scale Intelligence Quotient scores, and composite scaled scores of the Verbal Comprehension Index, Processing Speed Index, and Performance Intelligence Quotient. From the WISC-V, we analyzed the Full-Scale Intelligence Quotient scores, and composite scaled scores of the Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index, and Processing Speed Intelligence scores.

Data Analysis

Tract-Based Spatial Statistics

TBSS ([Smith et al. 2006](#)) is a whole-brain method for aligning and analyzing DTI scans from multiple subjects to assess the average neuroanatomical connectivity of the group of scanned brains. Since no two brains are exactly alike, this method is important for finding significant changes between groups, dampening the noise that inherently comes with any heterogeneous group of brains to allow for the signal to be clearly identified.

Our analysis was conducted using DTI Studio (<http://dsi-studio.labsolver.org/>) to process usable DTI scans, and the Functional MRI Brain (FMRIB) Software Library (FSL, <http://www.fmrib.ox.ac.uk/fsl/>) to create and analyze whole-brain maps of FA, RD, and AD.

TBSS aligns FA data (a metric of local tract structure directionality) across all of the subjects within a grouping to generate a “mean FA skeleton.” This is done by comparing every FA image with every other FA image (across both NS and control scans) to assess which image is most similar to all the rest, and is therefore the “most representative” of all of the images under consideration. After the most representative image is selected, all of the other images are warped to align with this image. The mean of all of these warped images becomes the mean FA skeleton, a representation of the average tract geometry across all of the images. At this point, it is possible to make group comparisons, using the mean FA skeleton as a representation in which the tracts are aligned. The control group and the NS group can each be compared with the skeleton to identify the voxels that vary significantly between the skeleton and each group.

This comparison is made using the FSL “randomize” function, which is a tool for nonparametric permutation inference. Nonparametric permutation testing is used when the data points under consideration are not necessarily assumed to be normally distributed. In such a test, the null hypothesis is that there is no significant difference between the control and experimental groups, meaning that the “control” and “experimental” labels should be interchangeable across every subject and the differences across the two groups (with arbitrarily assigned labels) should remain the same. In other words, randomly selecting two groups in any possible permutation should always lead to approximately the same value across the two groups in the null hypothesis. To reject the null hypothesis, it must be shown that the random assignment of each subject to a group does not yield the same mean value across every distribution of the subjects. To carry out this test, the randomize function assigns every brain, whether NS or control, to an arbitrarily selected group (i.e., controls may be labeled NS or continue to be labeled controls, and vice versa). Then, it runs a comparison across the two groups and computes the statistical differences. It does this on every possible permutation of the brains. If it finds that the case in which the NS brains all happened to be assigned to the NS label and the control brains all happened to be assigned to the control label shows a significant difference relative to the differences found in every other permutation, the null hypothesis can be rejected.

Tract-Based Analysis

We used the Johns Hopkins University (JHU) DTI-based white matter atlas ([Wakana et al. 2004](#)) to define white matter tract masks and calculate each tract's mean FA value based on the voxels that comprised it ([Fig. 2](#), [Supplementary Table 4](#)). We

Figure 1. Statistically significant ($P < 0.05$) voxel differences between the Noonan and control groups, controlling for age and sex. Z labels indicate slice number in the Z direction and correspond to the cross-slice image on the right. (a) Significant between-group differences in FA over nearly the entire brain (dark voxels). (b) Increases in RD between the two groups were likewise widespread (dark voxels).

focused on FA in our analysis, as it is the main measure of diffusivity. To ensure the JHU atlas masks mapped well onto our data, we adjusted the size of the masks to visually match the size of the imaged tracts. Of 48 total masks, we adjusted 21 in this manner.

Statistical Analysis

Statistical analysis was performed using R (<https://www.r-project.org/>) and SPSS (<https://www.ibm.com/products/spss-statistics>). The t-tests were used to identify differences in group demographic characteristics. After masking out individual tracts, we ran an ANCOVA with tract FA value as the dependent variable, group as the independent variable, and age and sex as covariates to identify significant tracts, using an FDR correction to account for multiple comparisons. We also determined Cohen's d for each tract to identify the effect size of the observed FA differences.

Separately, we conducted an analysis of structural MRI data using a Wilcoxon signed-rank test to identify significant differences in volume, area, and thickness, between regions of the NS group's and control group's brains. We used an FDR correction to account for multiple comparisons, and controlled for overall brain volume.

We also ran t-tests to identify differences in performance on the inhibition, fingertip tapping, and hand imitation subscores of the NEPSY-II for each group. We conducted brain-behavior correlations (Pearson correlation) between tract FA values and performance on the aforementioned tasks. Some behavioral data points (Inhibition: controls = 1, NS = 2; fingertip tapping: controls = 3, NS = 7; hand imitation: controls = 2, NS = 7) were missing due to participants' lack of cooperation. Additionally, we used a Fisher's test to examine whether these correlations were different between the groups.

Results

Children with NS Exhibited Widespread Reductions in White Matter Connectivity

We analyzed the data using tract-based spatial statistics (TBSS) (Smith et al. 2006), a whole-brain method enabling the alignment and comparison of DTI data from multiple subjects. Relative to controls, we found widespread reduced FA values in the NS group (82 613 voxels, $t = 1.49$, $P < 0.05$) after controlling for the effects of age and sex. We also found widespread increased RD values in the NS group (94 044 voxels, $t = 1.22$, $P < 0.05$; Fig. 1). However, contrary to our prediction, the AD metric did not exhibit significantly reduced values. Instead, AD showed nominal increases in NS, with two small clusters constituting the significant voxel differences: cluster 1 was comprised of 223 voxels in the medial region ($t = 0.662$, $P < 0.05$); cluster 2 was comprised of 125 voxels in the dorsolateral region ($t = 0.412$, $P < 0.05$). Thus, while AD in these medial and dorsolateral regions increased slightly, FA and RD in the brain were more isotropic than in the control group, suggesting a decrease in white matter connectivity.

Children with NS Exhibited Large Reductions in Corticospinal, Striatal, Thalamic, and Hippocampal Tract Connectivity

Next we were interested in identifying the white matter tracts affected by the PTPN11 mutation. To determine which white matter tracts exhibited changes in the children with NS, we used the Johns Hopkins University (JHU) DTI-based white matter atlas (Wakana et al. 2004) to define white matter tract masks and calculate each tract's mean FA value based on the voxels that comprised it (Supplementary Table 4, Fig. 2). In line with the widespread results of the voxel-based analysis, we found differences between the groups in 40 out of 48 tested tracts

Figure 2. Selected tract ROIs, colored by reduction in FA value, represented as the negative logarithm of the FDR-adjusted P-value. Light blue represents low P-values and dark blue represents high P-values. The top slice is the 77th in the z-axis, the middle slice is the 89th, and the bottom slice is the 100th.

(using ANCOVA with FA value as the dependent variable, group as the independent variable, and age and sex as covariates; FDR-corrected; [Supplementary Table 4](#) for all tracts). Given the pervasive effect of NS on white matter tracts, we used an effect size analysis to determine the clinical significance of our findings. Seventeen tracts exhibited effect sizes for FA values with Cohen's d greater than 1.5, and of those, 9 exhibited effect sizes greater than 2 ([Supplementary Table 4](#) for all tract effect sizes). Although these tracts were distributed throughout the brain, most of the tracts fell into three general groupings: 1) parts of the corticospinal tract, which receives input from the motor cortex, and is chiefly responsible for motor activity ([Van Wittenbergh and Peterson 2020](#)), 2) tracts projecting to the striatum, thalamus, and hippocampus, and 3) tracts of the corpus callosum. Significant components of the corticospinal tract included the bilateral cerebral peduncle (L: $F_{(1,30)} = 90.6$, $P = 3.44 \times 10^{-9}$, Cohen's d (d) = 2.32, R: $F_{(1,30)} = 123.4$, $P = 1.80 \times 10^{-10}$, $d = 2.98$); the bilateral superior cerebellar peduncle (L: $F_{(1,30)} = 69.5$, $P = 4.08 \times 10^{-8}$, $d = 2.66$, R: $F_{(1,30)} = 56.8$, $P = 1.45 \times 10^{-7}$, $d = 2.37$); the bilateral posterior limb (L: $F_{(1,30)} = 67.9$, $P = 4.08 \times 10^{-8}$, $d = 2.79$, R: $F_{(1,30)} = 63.2$, $P = 6.84 \times 10^{-8}$, $d = 2.71$), left anterior limb ($F_{(1,30)} = 31.6$, $P = 1.76 \times 10^{-5}$, $d = 1.58$), and bilateral retrolenticular part (L: $F_{(1,30)} = 29.6$, $P = 2.48 \times 10^{-5}$, $d = 1.79$, R: $F_{(1,30)} = 19.7$, $P = 2.50 \times 10^{-4}$, $d = 1.50$) of the internal capsule. The internal capsule also connects the striatum and thalamus to the cortex ([Chowdhury et al. 2010](#); [Safadi et al. 2018](#)). Other significant tracts interfacing with the striatum and thalamus included the bilateral sagittal striatum tracts (L: $F_{(1,30)} = 8.05$, $P = 1.08 \times 10^{-2}$, $d = 0.984$, R: $F_{(1,30)} = 23.7$, $P = 9.46 \times 10^{-5}$, $d = 1.53$), and the bilateral posterior thalamic radiation (L: $F_{(1,30)} = 58.2$, $P = 1.32 \times 10^{-7}$, $d = 2.15$, R: $F_{(1,30)} = 41.6$, $P = 1.01 \times 10^{-5}$, $d = 1.81$), which connects the thalamus to the occipital lobe. Meanwhile, tracts projecting to the hippocampus included the bilateral cingulum of the cingulate gyrus (L: $F_{(1,30)} = 24.4$, $P = 8.35 \times 10^{-5}$, $d = 1.69$, R: $F_{(1,30)} = 41.9$, $P = 2.11 \times 10^{-6}$, $d = 2.20$) and the cingulum of the hippocampus (L: $F_{(1,30)} = 19.7$, $P = 2.50 \times 10^{-4}$, $d = 1.57$, R: $F_{(1,30)} = 34.3$, $P = 1.01 \times 10^{-5}$, $d = 2.01$). Finally, the genu ($F_{(1,30)} = 5.97$, $P = 0.021$, $d = 0.811$), splenium ($F_{(1,30)} = 14.3$, $P = 7.04 \times 10^{-4}$, $d = 1.17$), and body of the corpus callosum ($F_{(1,30)} = 19.9$, $P = 1.06 \times 10^{-4}$, $d = 1.45$) also differed significantly between the groups.

Decreases in Striatal Volume and Parahippocampal Volume, Surface Area, and Thickness

In order to examine whether the aforementioned white matter changes were associated with changes to structural neuroanatomy, we conducted a complementary, secondary analysis of structural MRI data from the participants ([Supplementary Table 5](#) for all structural measurements). The analysis produced concordant results, showing that the striatum and regions surrounding the hippocampus generally exhibited significant (FDR-corrected) decreases in volume, area, and thickness, even after controlling for overall brain volume. Specifically, the results of our Freesurfer analysis demonstrated significant decreases in volume in the following regions of the striatum: the bilateral putamen (using Wilcoxon signed-rank test, Left: $W = 224$, $P = 0.027$; Right: $W = 215$, $P = 0.044$), right caudate ($W = 234$, $P = 0.018$), and right pallidum ($W = 222$, $P = 0.027$).

Regions surrounding the hippocampus exhibited the following changes: the bilateral entorhinal cortex exhibited significant decreases in surface area (Left: $W = 247.5$, $P = 1.26 \times 10^{-2}$; $W = 235$, Right: $P = 0.023$) and volume (Left: $W = 246$, $P = 0.017$; $W = 231$, Right: $P = 0.039$), as did the right temporal pole surface area

($W = 249.5$, $P = 0.013$) and volume ($W = 232$, $P = 0.039$) and right posterior cingulate cortex surface area ($W = 229$, $P = 0.043$) and volume ($W = 245$, $P = 0.020$). The right parahippocampal gyrus also exhibited a decrease in thickness ($W = 233$, $P = 0.040$).

Cognitive Measures of Executive Function and Memory Skills Were Correlated with Connectivity in Specific White Matter Tracts

To identify associations between white matter variability and behavior, we tested for Pearson correlations between tract FA values and the behavioral scores for each group. We then compared the correlation coefficients between the two groups. We assessed each participant's performance in several cognitive domains using the NEPSY-II ([Korkman et al. 2007](#)), a set of tests assessing neuropsychological abilities. We particularly focused on executive function and memory, which are both reported to be affected in NS ([Pierpont et al. 2013, 2015](#)). Accordingly, we hypothesized that executive function deficits would be linked to FA and RD aberrations in a number of tracts that have been previously shown to exhibit reduced connectivity in children with ADHD ([van Ewijk et al. 2012](#); [Wu et al. 2017](#)), namely the genu, splenium, and body of the corpus callosum; the sagittal striatum; the superior and inferior longitudinal fasciculus; the internal capsule; the corona radiata; the posterior thalamic radiation; and the external capsule. As for tracts related to memory, we investigated the cingulum (cingulum hippocampus and cingulum cingulate gyrus), building on past work identifying it as a marker of cognitive development ([Bathelt et al. 2019](#)) associated with ADHD severity ([Cooper et al. 2015](#)).

To assess executive function, we used two contrast scores from the inhibition test: Inhibition (inhibition vs. naming)—which requires the child to inhibit automatic responses in favor of novel responses, such as naming an upward-pointing arrow as a downward-pointing arrow—and Switching (switching vs. inhibition), which assesses a participant's cognitive flexibility. Additionally, we used two auditory attention scores (auditory attention and response set). To assess memory, we used four other scores from the NEPSY-II: List Memory and List Memory Delayed, Memory for Faces Delayed, Narrative Memory Free Recall, and Narrative Memory Free and Cued Recall.

We found that the NS group performed worse than controls on the inhibition task (Control mean = 10.7 ± 3.5 , NS mean = 7.1 ± 3.7 , t -test: $P = 0.0095$). Additionally, differences between controls and NS on the auditory attention task trended toward significance (control mean = 10.6 ± 2.95 ; NS mean = 8.75 ± 2.72 ; t -test: $P = 0.079$). We did not detect differences between NS and controls on the switching or response set subtests ([Supplementary Table 6](#) for all executive function-related brain-behavior correlations). However, only 11 out of 17 participants with NS and 11 out of 17 control participants completed the switching subtests, which reduced our power to detect differences between the groups. Likewise, 13 out of 17 NS and 12 out of 17 control participants completed the response set subtest, limiting our statistical power.

As shown in [Fig. 3](#), the FA of the genu of the corpus callosum was negatively correlated with performance on the auditory attention task in NS and approached significance in the difference between the groups ($R = -0.58$, $P = 0.019$; Fisher's test, $z = 1.54$, $P = 0.06$; [Fig. 3b](#), bottom, right). This relationship was complementarily reflected in the RD of the genu, which was positively correlated with performance on the same task in NS, and significantly differed between groups ($R = 0.5$, $P = 0.051$;

Figure 3. Brain-behavior correlations. (a) Five tracts showed correlations with behavioral tasks that were significant or approached significance, while also differing from control correlations significantly or near significantly. (b) The genu of the corpus callosum and right posterior thalamic radiation are associated with executive function in NS. The dark grey values in the upper left of each graph correspond to the results of the correlation within the control group, while the light grey values correspond to those within the NS group. The z- and P-values in the lower left of each graph are for the Fisher's test comparing the NS and control correlations between a given tract and measure. The correlation between auditory attention and the RD of the genu of the corpus callosum (lower left) was significant. The correlations between inhibition and the FA of the genu of the corpus callosum (lower right) and the FA of the right posterior thalamic radiation (upper right) approached significance. (c) The left cingulum hippocampus and bilateral cingulum cingulate gyrus are associated with memory in NS. All of the depicted correlations were significant.

Fisher's test, $z = 1.63$, $P = 0.05$; Fig. 3b, bottom, left). Further, we found a strong positive correlation between the FA values of the right posterior thalamic radiation and inhibition scores, which approached a significant difference between the groups ($R = 0.65$, $P = 0.0061$; Fisher's test, $z = 1.57$, $P = 0.06$; Fig. 3b, top, right).

The NS group performed worse than controls in various measures of memory assessed by the NEPSY-II, including memory for faces delayed (control mean = 12.5 ± 2.83 ; NS mean = 7.88 ± 2.87 ; t-test: $P = 7.41 \times 10^{-5}$), narrative memory free recall (control mean = 12 ± 3.24 ; NS mean = 9.76 ± 2.91 ; t-test: $P = 0.04$), and narrative memory free and cued recall (control mean = 12.12 ± 3.92 ; NS mean = 9.24 ± 3.36 ; t-test: $P = 0.03$). Additionally, NS performance reductions in the list memory and list memory delayed task approached significance (control mean = 10.92 ± 3.28 ; NS mean = 8.46 ± 3.23 ; t-test: $P = 0.07$). We detected a strong positive correlation in the NS group, but not in controls, between the mean FA value of the left cingulum cingulate gyrus and narrative memory free recall performance ($R = 0.53$, $P = 0.028$). The correlations of the NS and control group for these two values also differed significantly between the groups (Fisher's test, $z = 1.64$, $P = 0.05$; Fig. 3c, top, right). In the right cingulum cingulate gyrus, we found a near-significant positive correlation in the NS group, but not in controls, between the mean FA value and list memory performance ($R = 0.53$, $P = 0.062$; Fisher's test, $z = 2.30$, $P = 0.01$; Fig. 3c, bottom, right). In contrast, we found a strong negative correlation between the mean FA value of the left cingulum hippocampus and narrative memory free recall scores ($R = -0.5$, $P = 0.041$; Fisher's test, $z = 2.03$, $P = 0.02$; Fig. 3c, top, left). In the same tract, the correlation approached significance for narrative memory free and cued recall scores ($R = -0.46$, $P = 0.063$), yet correlations differed between the groups (Fisher's test, $z = 2.04$, $P = 0.02$; Fig. 3c, bottom, left). See Supplementary Table 7 for all memory-related brain-behavior correlations.

Discussion

In this study, we sought to elucidate the neural mechanisms underpinning the cognitive symptoms manifested in NS using DTI, structural MRI, and behavioral tests. We found widespread reductions in DTI measures of diffusivity, leading us to conclude that NS manifests with reduced connectivity in the white matter microstructure. To better localize this loss of connectivity to specific tracts, we conducted an effect size analysis, which identified corticospinal, parahippocampal, and striatal tracts as being the most affected. We further assessed whether these changes were related to structural changes to the neuroanatomy of our participants, and found that the striatum exhibited a decreased volume, while various parahippocampal structures exhibited decreased volume, surface area, and/or thickness. These results are consistent with our previous morphometric findings in children with PTPN11 NS, in which we observed reduced gray matter volume in the striatum and reduced cortical thickness in limbic structures vital to hippocampal circuitry (Johnson et al. 2019). The results of the present study suggest that altered microstructural connectivity in tracts leading to the striatum, hippocampus, and thalamus may be associated with these decreases in structural volumes.

Children with NS presented with elevated attention problems and hyperactivity compared with controls. To identify the relationship between specific tracts and NS cognitive symptoms, we tested correlations between tract connectivity measures and scores on attention and memory tasks.

In NS, auditory attention scores were negatively correlated with the FA of the genu of the corpus callosum and positively correlated with RD values of the genu of the corpus callosum, indicating that, for NS, a lower tract integrity is associated with better performance in attention tasks. Thus, for attention, we identify inverse brain-behavior correlations in the genu of the corpus callosum, which is responsible for connecting the medial frontal cortices. Prior work has implicated the corpus callosum in disorders of attention, namely ADHD, among nonsyndromic populations. Valera et al. (2007) found in a meta-analysis that corpus callosum volumes tended to be significantly reduced in children with ADHD compared with typically developing controls. Moreover, a number of studies (e.g., Chao et al. 2009; Cao et al. 2010) found that children with ADHD exhibit decreased FA in the genu of the corpus callosum, consistent with the findings described in NS herein. Furthermore, Ercan et al. (2016) found that children with the inattentive subtype of ADHD exhibited increased RD in the corpus callosum, similar to our findings. Our results thus suggest that NS affects the integrity of the genu of the corpus callosum ($P = 0.021$, Cohen's $d = 0.811$), with similar effects as those found in nonsyndromic ADHD.

In contrast, in NS, inhibition scores were positively correlated with the FA values of the right posterior thalamic radiation, indicating that, for NS, a higher tract integrity is associated with better performance in inhibition tasks. We thus detected a strong association between inhibition and tract integrity in the posterior thalamic radiation, which connects the caudal parts of the thalamus with the parietal and occipital lobe and is implicated in children (Peterson et al. 2011) and adults (Cortese et al. 2013) with ADHD. It has been shown in health that the integrity of the posterior thalamic radiation is positively associated with performance in executive function. For instance, Chaddock-Heyman et al. (2013) found that higher FA values in the posterior thalamic radiation were associated with better performance on a cognitive control task in 61 children between 7 and 9 years of age. Likewise, Treit et al. (2014) assessed a group of 49 healthy children aged 5–16 years, and also found a strong association between the FA of the posterior thalamic radiation and performance on the specific NEPSY-II inhibition task we used in this study. It is possible that strong brain-behavior associations in the posterior thalamic radiation may be a compensatory mechanism, given the inverse relationship between the genu FA values and performance on auditory attention tasks.

In addition to executive function deficits, children with NS exhibited diminished performance on memory tasks relative to controls, in a manner consistent with past findings in the literature (Pierpont et al. 2013). Performance on memory tasks was positively correlated with FA value in the bilateral cingulum cingulate gyrus and negatively correlated with FA values in the left cingulum hippocampus in NS. Taken together, our findings in the cingulum cingulate suggest a strong positive correlation between increased connectivity and better memory abilities, a relationship also demonstrated in health (e.g., Kantarci et al. 2011). In contrast, in the cingulum hippocampus we detected a de-coupling of the positive brain-behavior correlation observed in health (e.g., Sasson et al. 2013), with a strong negative correlation between narrative memory and FA values. Studies in other clinical populations implicate reduced tract integrity of the parahippocampal cingulum in negative effects on memory, and specifically in episodic memory. For instance, Bozzali et al. (2012) found that reduced measures of white matter connectivity in the parahippocampal region of the cingulum were

predictive of episodic memory dysfunction in patients with mild cognitive impairment. Likewise, Rémy et al. (2015) found that patients with Alzheimer's disease exhibited FA reductions in the parahippocampal cingulum, concordant with the results described above.

As such, our results not only demonstrate widespread reductions in FA and increases in RD in children with NS (see Fig. 1), but also suggest that these differences are associated with cognitive abilities. The most significant changes to FA values were observed in tracts connecting to the motor cortex, striatum, thalamus, and hippocampus (see Fig. 2), some of which were also correlated with behavioral changes observed in NS (see Fig. 3). Our results recapitulate the findings of the animal model literature, which also report disruptions to myelination and axonal elongation, particularly within the corticospinal tract (Xing et al. 2016), and reinforce the human structural (Johnson et al. 2019) and behavioral (Pierpont et al. 2015) findings reported in the literature.

Given the cross-sectional nature of the data, we cannot directly ascertain from our results whether these differences are associated directly with downstream effects of the mutation or arise over the course of development in response to environmental, behavioral, and hormonal changes. A longitudinal study evaluating the development of white matter in children with NS would offer a causal understanding of the role that genetics and the environment each play in the phenomena observed herein.

It should also be noted that the relative rarity of NS makes it a difficult condition to study. Although our cohort was relatively small, we were able to increase the homogeneity of our sample by ensuring that the participants in our study were all prepubertal (see Tanner Staging, Supplementary Table 1) and all had NS resulting from a PTPN11 mutation. Additionally, we addressed our sample size limitation by making appropriate adjustments to the P-values in our statistical analyses, and by following logical tract-based hypotheses rather than whole-brain analyses to limit the number of comparisons made.

Building on these findings, a promising future direction would be to explore the effects of treatment on DTI measurements in children with NS, which could function as a more precise brain-based biomarker. For instance, previous work using animal models of Neurofibromatosis 1 (NF1, which results from a different set of mutations to the Ras-MAPK pathway) has shown that statins are effective at normalizing deficits in long-term potentiation and learning (Lee et al. 2014). However, when statins were administered to human subjects with NF1, behavioral measures—that is, full-scale intelligence (van der Vaart et al. 2013), nonverbal long-term memory and attention (van der Vaart et al. 2013), and visuospatial learning (Payne et al. 2016)—were not sensitive enough to indicate that statins were effective at mitigating NF1 symptomatology. By contrast, neuroimaging assays were sensitive enough to enable researchers to measure changes to the brain in response to statin use (Stivaros et al. 2018). This kind of research has introduced the possibility of using brain-based measures, such as DTI, to more precisely monitor the effects of treatment. Potentially, such measures could serve as biomarkers to track disease progression over the course of development. However, these relationships must first be validated through large-scale studies, to ensure that the imaged changes are in fact related to functional outcomes. Future exploration should assess the viability of DTI as a brain-based measure of NS.

Finally, these results may be important in deriving insights into idiopathic neurodevelopmental disorders such as ADHD.

Given that nearly one third of children with NS are also diagnosed with ADHD (Pierpont et al. 2013), NS may offer a useful model for the study of increased risk for ADHD and its associated cognitive effects. Supporting the use of such a model is the finding that NS-related mutations that increased basal Ras-MAPK activation (leading to increased glutamatergic excitatory synaptic transmission) were associated with learning deficits in mice (Lee et al. 2014). Moreover, in humans, genes governing the network of metabotropic glutamate receptors, which regulate the Ras-MAPK pathway (Wang et al. 2004), were mutated in 20% of youth with ADHD (Elia et al. 2018).

Building on animal models of ADHD, a human disease model (i.e., NS as a model for ADHD) may offer an even deeper understanding into the specific subtypes of idiopathic ADHD that affect children with NS. Since the genetic underpinnings of NS are well-understood, such research could offer greater genetic understanding of the etiology of ADHD, in addition to potentially translatable findings for NS.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

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Notes

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