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Original Article

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PTPN11 Gain-of-Function Mutations Affect the Developing Human Brain, Memory, and Attention

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Abstract

The Ras-MAPK pathway has an established role in neural development and synaptic signaling. Mutations in this pathway are associated with a collection of neurodevelopmental syndromes, Rasopathies; among these, Noonan syndrome (NS) is the most common (1:2000). Prior research has focused on identifying genetic mutations and cellular mechanisms of the disorder, however, effects of NS on the human brain remain unknown. Here, imaging and cognitive data were collected from 12 children with *PTPN11*-related NS, ages 4.0–11.0 years (8.98 ± 2.33) and 12 age- and sex-matched typically developing controls (8.79 ± 2.17). We observe reduced gray matter volume in bilateral corpus striatum (Cohen's $d = -1.0$:-1.3), reduced surface area in temporal regions ($d = -1.8$:-2.2), increased cortical thickness in frontal regions ($d = 1.2$–1.3), and reduced cortical thickness in limbic regions ($d = -1.6$), including limbic structures integral to the circuitry of the hippocampus. Further, we find high levels of inattention, hyperactivity, and memory deficits in children with NS. Taken together, these results identify effects of NS on specific brain regions associated with ADHD and learning in children. While our research lays the groundwork for elucidating the neural and behavioral mechanisms of NS, it also adds an essential tier to understanding the Ras-MAPK pathway's role in human brain development.

Key words: ADHD, hippocampus, Noonan syndrome, striatum, structural imaging

Introduction

Rasopathies are genetic conditions caused by mutations affecting the Ras-Mitogen-activated protein kinases (MAPK) pathway. The Ras-MAPK pathway plays a crucial role in neural development and synaptic signaling. In humans, this pathway can be affected by genetic mutations and subsequently lead to a collection of neurodevelopmental syndromes (i.e., Rasopathies). Rasopathies consist of several genetic disorders (e.g., Noonan syndrome [NS], Neurofibromatosis 1, Costello syndrome) affecting 1:1000 people. Among them, NS is the most common (1:2000) (Tartaglia et al. 2011). NS is characterized by physical abnormalities such as short stature, craniofacial anomalies, and cardiac defects (Tartaglia et al. 2011; Roberts et al. 2013). Behaviorally, individuals with NS are at risk for cognitive dysfunction, particularly related to attention, executive function, memory, and social cognition (Alfieri et al. 2011; Pierpont et al. 2013, 2015). Behavioral problems among children diagnosed with NS often lead to additional diagnoses such as attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), anxiety disorders, and learning disabilities (Adviento et al. 2014; Pierpont et al. 2015).

Approximately half of all NS cases can be attributed to mutations in the *PTPN11* gene. The affected *PTPN11* gene is responsible for encoding the active form of protein tyrosine phosphatase, Shp2 (Feng 1999; Tonks and Neel 2001; Neel et al. 2003; Tartaglia et al. 2011; Roberts et al. 2013), a major regulator in the Ras-MAPK pathway (Tartaglia et al. 2001; Kusakari et al. 2015). Among genetic mutations, a "gain-of-function" mutation refers to a specific mutation that leads to enhanced protein activity. *PTPN11*-related NS is considered a "gain-of-function" mutation due to this increased Shp2 protein activity (Tartaglia et al. 2001). In mouse models, active Shp2 reduces myelination of axons (Ehrman et al. 2014), subsequently increasing excitatory synaptic function in hippocampal neurons and leading to deficits in long-term potentiation (LTP) (Lee et al. 2014). Active Shp2 also increases neuron cell density and count, while simultaneously decreasing astrocyte cell density and count within the hippocampus and forebrain (Gauthier et al. 2007). Also, Araki et al. (2004) reported craniofacial abnormalities such as shorter skulls and a "triangular" facial appearance, and a broader and blunter snout among NS model mice compared to wild-type mice. These craniofacial features (i.e., a "triangular" shaped face and a shorter, broader nose) might be analogous to facial features observed in humans with NS (Roberts et al. 2013). Behaviorally, mouse models of NS caused by *Ptpn11* mutations present as inattentive and hyperactive (Lee et al. 2014; Kusakari et al. 2015) and show memory and learning deficits (Lee et al. 2014). Taken together, these studies emphasize the significance of mutations in the *PTPN11* gene and their ability to influence the Ras-MAPK pathway. In turn, it is possible that altered brain development due to disruptions in this pathway can result in cognitive-behavioral deficits associated with the syndrome (Gauthier et al. 2007; Kusakari et al. 2015).

Despite the important empirical support that NS affects cognition and behavior in humans (Wingbermühle et al. 2012; Pierpont et al. 2015), a major gap exists in understanding how *PTPN11* variants increase risk for these deficits. Indeed, while effects of NS on the mouse brain are evident, its specific effects on the human brain remain largely unknown. Assessing such effects on the developing human brain is an essential first step to provide a link between *PTPN11* and deficits in attention, executive function, memory, learning, and social interactions in children with NS (Wingbermühle et al. 2012; Pierpont et al. 2015).

To that end, here we examine structural brain differences between children with *PTPN11*-related NS and age- and sex-matched typically developing (TD) controls using high-resolution structural magnetic resonance imaging (MRI) scans. We had 2 overarching goals that can be addressed as hypothesis testing and hypothesis generating. Accordingly, we first aimed to examine whether findings from NS mouse model research and existing behavioral studies in individuals with NS translate into findings in the subcortical regions. Next we aimed to systematically measure brain morphometry in children with NS. Goal 1: Based on findings from NS mouse model research implicating the role of the hippocampus in memory deficits (Lee et al. 2014)—specifically that enhanced Ras-MAPK activation impairs LTP—we hypothesize that children with NS will show aberrant morphometry of the hippocampus. In addition, given the high risk for attention deficits among those with NS (Pierpont et al. 2015) and findings from nonsyndromic populations highlighting the role of the striatum in attention deficits and hyperactivity (Bush 2011), we hypothesize that children with NS will show aberrant morphometry of the striatum. Goal 2: As the first study to systematically measure brain morphometry in children with NS, we report all morphometric findings across the brain (Fig. 2 and Table 2). To estimate clinical significance we used effect sizes, which allow for the identification of clinically significant differences that can be used for future hypothesis formation.

Materials and Methods

Participants

We scanned and assessed 12 children with *PTPN11*-related NS between the ages of 4.0–11.0 (8.98 ± 2.33) and 12 age- and sex-matched TD controls between the ages of 4.0 and 11.0 years (8.79 ± 2.17). Participants with NS were recruited through the National Noonan Syndrome Foundation, a local network of physicians, and advertisements posted on the Stanford University School of Medicine's website. The TD controls for this study were recruited through a larger Turner syndrome (TS) study conducted at Stanford University School of Medicine and were recruited through local print media and parent networks. Both studies used the same scan and behavioral assessment protocols.

Exclusion criteria for both NS and TD participants included premature birth (gestational age under 32 weeks), low birth weight (less than 2000 g), known diagnosis of a major psychiatric disorder and any contraindications for an MRI scan. Potential participants with a history of neurological disorders known to impact cognitive development or brain structure, including seizures, or with diagnosed gross structural malformations (e.g., Arnold Chiari malformation) were also excluded. Furthermore, for the purposes of this study, we focused our efforts on NS participants with proof of the *PTPN11* mutation. All NS participants had previous *PTPN11* mutation documentation and showed proof of genetic testing for inclusion in this study (see Table S1). By limiting our study to this mutation, we were able to further control across an otherwise variable genetic condition.

Informed, written consent was obtained from a legal guardian for all participants, as well as written assent from participants over the age of 7. The Stanford University School of Medicine Institutional Review Board approved this study and all procedures followed the approved protocols.

Tanner Staging and Medication History

A physician with experience in evaluating physical development stages (TG) (Green et al. 2017) examined participants' current puberty status using Tanner stages (Marshall and Tanner 1970). Tanner staging of each participant was assessed across both groups. To control for the effect of pubertal development on the brain, only participants below or equal to Tanner stage 2 (Marshall and Tanner 1970) were included in this study. In addition to Tanner staging, we also noted medication status of all participants. Overall, 5 NS participants were taking growth hormones, 4 NS participants were taking stimulants, and 1 NS participant was on SSRIs for the duration of this study. A complete list of demographic and medical measures recorded are reported in Table 1.

MRI

Participants underwent behavioral training in a mock MRI scanner prior to their actual scan to familiarize them with the appearance and sounds of an MRI and thus reduce motion-related artifacts. Imaging data were acquired at Stanford University Lucas Center for Imaging. All scans were collected on a GE Healthcare Discovery 3.0 T whole-body MR system (GE Medical Systems, Milwaukee, WI) using a standard 8-channel

Table 1 Participant demographic and medical information

	Noonan syndrome	Neurotypical controls	*P* value
Number of participants	12	12	–
Sex (*n*)	Female (6)	Female (6)	ns
	Male (6)	Male (6)	ns
Age range	4.73–11.93	4.05–11.71	–
Tanner stage	≤2	≤2	–
GH	5	0	–
Stimulants	4	0	–
SSRI	1	0	–
Mean age	8.98 ± 2.33	8.79 ± 2.17	ns
FSIQ (WISC/WPPSI)	90.33 ± 11.09	110.25 ± 8.56	$P < 0.001$
PIQ	95.42 ± 9.33	111.67 ± 10.65	$P < 0.005$
VIQ	97.25 ± 12.73	115.75 ± 13.99	$P < 0.01$
PSI[a]	87.4 ± 12.85	97.00 ± 13.45	ns
WMI[a]	80.9 ± 9.98	100.8 ± 4.71	$P < 0.001$
List memory and list memory delayed[a]	9.7 ± 2.9	10.8 ± 2.5	$P < 0.05$
Memory for faces[b]	9.4 ± 3.3	9.3 ± 2.1	ns
Memory for faces delayed[b]	8.2 ± 2.8	12.5 ± 2.6	$P < 0.005$
Narrative memory free and cued recall[a]	9.0 ± 3.2	10.7 ± 3.2	ns
Narrative memory free recall[a]	9.7 ± 3.2	11.3 ± 3.0	ns
Attention problems	62.6 ± 9.9	46.3 ± 6.6	$P < 0.005$
Hyperactivity	66.9 ± 15	48.7 ± 8.3	$P < 0.01$
N (%) Clinical Impairment[c]	5 (41.67%)	0 (0%)	–

All values are reported in mean ± standard deviation; Wicoxon rank sum test was used to assess significance between groups; GH, growth hormones; SSRI, selective serotonin reuptake inhibitor; FSIQ, full-scale intelligence quotient; PIQ, performance intelligence quotient; VIQ, verbal intelligence quotient; PSI, processing speed intelligence; WMI, working memory intelligence; ns, not significant.

[a]*n* of 10 for in each group, PSI, WMI and List Memory and List Memory Delayed given age-restrictions of respective assessments (WISC [PSI, WMI] >6 years; List Memory/List Memory Delayed >7 years).

[b]*n* of 11 in each group, Memory for Faces and Memory for Faces Delayed are not administered to children age <5.

[c]"Clinical impairment" indicated when parent-reported *T*-score of >70 of either the "Attention Problems" or "Hyperactivity" scale on BASC-2.

head coil. Technical details of the pulse sequence and image quality check can be found in the Supplemental Material.

Morphometric Analysis (FreeSurfer)

Image analysis specifically focused on subcortical structures. This focus was derived from available findings in NS mouse model research as well as studies linking NS behavioral and attention differences to possible subcortical aberrations (Greven et al. 2015; Kusakari et al. 2015). Cortical reconstruction and subcortical segmentation were performed using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu/). All scans were preprocessed using bias field correction methods available with SPM8 (http://www.fil.ion.ucl.ac.uk/spm) before using the FreeSurfer pipeline. Details of the FreeSurfer pipeline and procedure can be found in the Supplementary Material.

Once constructed, cortical models of brain surfaces for each hemisphere were parcellated into 34 distinct regions based on gyral and sulcal landmarks (Fischl et al. 2004; Desikan et al. 2006). The gray matter volume (GMV), surface area (SA) of the gray–white boundary, and mean cortical thickness (CT) for each parcellated region were then calculated. For the purposes of this exploratory study, we report our findings for all 34 regions, regardless of significance (Table 2).

Within this study, total cortical volume is referred to as total brain volume (TBV). We controlled for TBV within all of our regional analyses due to the observed trend for smaller TBVs among NS participants. By controlling for this difference in volume, the significant differences reported cannot be attributed to overall smaller brain volume. Furthermore, given the preliminary nature of this study ($n = 12$ in each group), we also calculated effect sizes for all analyses. Effect sizes not only allow us to quantify the differences between NS and control groups, but also provide an anchor for future studies' power calculations given the absence of data on brain development in NS.

Cognitive and Behavioral Assessment

A battery of age-appropriate cognitive and behavioral assessments was administered to all participants. Cognitive and behavioral symptoms were measured using age appropriate Wechsler Scales of Intelligence, A Developmental NEuroPSYchological Assessment (NEPSY-II), and Behavior Assessment System for Children (BASC-II). Details regarding the assessment administration and subtests used can be found in the Supplemental Material.

Statistical Analysis

All statistical analyses were carried out using The R Project for Statistical Computing (R) (http://www.r-project.org). For each brain region, we used a linear regression model to control for TBV variations across groups. Participants were individually age- and sex-matched prior to statistical analysis and therefore we did not need to statistically control for these differences. For both cognitive tests and brain region data, the Mann–Whittney *U* test within R (version 3.4.0) was used to compare across groups. Given our sample size ($n = 24$) we chose this nonparametric test due to its decreased sensitivity to violations of parametric assumptions. Cohen's *d*, effect size (Cohen 1988), was used to provide a measure of clinical significance that enables the evaluation of the effect of NS on brain regions. All

Table 2 Effect sizes for parcellated brain regions

Brain region[a]	LEFT			RIGHT		
	GMV	SA	CT	GMV	SA	CT
Subcortical						
Caudate	**−1.1535065**	–	–	**−1.3110753**	–	–
Putamen	**−1.2438946**	–	–	**−1.1484639**	–	–
Pallidum	**−1.0327863**	–	–	**−1.2348753**	–	–
Hippocampus	−0.4367628	–	–	−0.4156214	–	–
Amygdala	−0.4052514	–	–	−0.060681	–	–
Frontal						
Caudal middle frontal	−1.08590357	−0.68239748	**−1.3192397**	−0.08827384	0.03718254	−1.1613911
Lateral orbitofrontal	0.08565905	−0.13199369	0.1022659	−0.02521572	−0.41660895	0.4990708
Medial orbitofrontal	−0.03899295	−0.7818052	0.6621581	−0.06609507	−0.74073834	0.8167566
Paracentral	0.6792092	0.12123483	−0.2966572	0.09641032	0.07790677	−1.0212688
Parsopercularis	−0.42523633	−0.43857539	**−1.1536462**	0.23108925	0.0926515	−0.4010888
Parsorbitalis	−0.15285857	−0.08029667	−0.5274115	0.14401571	−0.33256548	**1.2972611**
Parstriangularis	0.09808786	−0.23212405	−0.3952327	0.27793989	−0.06530875	0.6060875
Precentral	−0.17121778	−0.28391096	−0.7898264	−0.62559883	−0.45945186	−0.9636591
Rostral middle frontal	−0.59805086	−1.01919569	−0.1505245	−0.53631702	−1.21423363	**1.2844895**
Superior frontal	0.34557202	−0.17289302	−0.4186732	0.5308245	−0.08946872	−0.2381157
Frontal pole	0.57090392	0.11265431	0.687522	−0.02250416	−0.83416671	0.9461986
Parietal						
Inferior parietal	0.1569582	−0.08376827	−0.2196128	−0.49889121	−0.41684044	−0.6954174
Post central	0.5439823	0.34552498	−0.4547243	0.23955142	−0.27141139	−0.1886138
Precuneus	−0.93269	−0.91288546	−0.6540304	−0.38035182	−0.37449668	−0.9664361
Superior parietal	−0.8096576	−0.97652911	−0.3321202	−1.32261275	−1.18641058	−0.8772299
Supramarginal	0.253669	0.0801548	−0.4852971	0.09502154	−0.02841816	−0.521448
Cingulate						
Caudal anterior cingulate	−0.16842941	−0.74576981	0.1618584	−0.4646722	−0.7466461	0.7075931
Isthmus cingulate	−0.31860011	0.02184604	−1.0348617	−0.5039033	−0.1151467	−0.4098115
Posterior cingulate	0.04531489	−0.24371566	−0.0255469	−0.9972955	−0.9307259	0.6924886
Rostral anterior cingulate	0.2593757	−0.40925362	0.6225709	−0.3873266	−0.403505	0.9405566
Temporal						
Banks of the superior temporal sulcus	0.20622314	0.29268667	−0.1975113	−0.46224544	−0.51784952	−0.672970821
Entorhinal	−1.12123552	**−2.22908819****	1.155581	−0.98396148	**−1.84409776***	0.40265557
Fusiform	−0.39245324	−0.64341948	−0.1411044	−0.65887745	−0.95122491	−0.288039999
Inferior temporal	−0.02019813	−0.6115886	−0.1782626	−0.64129791	−0.92414026	−0.550918164
Middle temporal	0.01937989	−0.08910661	−0.8404845	−0.06955522	−0.47029656	−0.02101681
Parahippocampal	−0.43487928	0.34135002	−1.3006035	−0.13498194	0.19903343	**−1.597143105**
Superior temporal	−0.07343425	−0.18565374	−0.6719805	0.65669879	−0.06734104	0.259582323
Temporal pole	−0.49715662	−0.93588649	0.3139539	−1.00265026	−1.05827469	0.085943385
Transverse temporal	0.35640804	−0.06354193	−0.2022507	0.66683486	0.34437735	0.002277579
Insula	−0.26846644	−0.89293038	0.5617668	0.08341528	−0.51984373	0.655007848
Occipital						
Cuneus	−1.1722242	−0.94067261	−0.3708284	−0.5270504	−0.7516818	−0.4180365
Lateral occipital	0.5579369	−0.03935621	0.7910616	0.3001058	−0.1920463	0.01733488
Lingual	−0.7487105	−0.82951917	−0.3873149	−0.8670094	−0.8035236	−0.55746827
Pericalcarine	−0.5607025	−0.97577369	0.1432723	−0.8966565	−1.0635345	−0.78831458

[a]34 Brain regions were parcellated using FreeSurfer software. Children with Noonan syndrome were compared with TD controls, values represent Cohen's *d* effect size; Bolded value with no asterisk has a *P*-value of <0.05, a bolded value with * has a *P*-value of <0.005, and finally, a bolded value with ** has a *P*-value of <0.001.

significance values reported here were adjusted for multiple comparisons using false discovery rate corrections (FDR) (Benjamini and Hochberg 1995). An FDR correction was applied to account for multiple between-group tests focused on the following brain regions: cingulate, bilateral subcortical, frontal, parietal, temporal, and occipital.

Results

As expected from our individual matching design (age and sex), there were no significant differences in age or sex between groups (Table 1). Cognitively, the NS group scored significantly lower for all IQ measures when compared with TD controls ($P < 0.01$). There were no significant differences in TBV between the groups, however, TBV in participants with NS trended toward being smaller than TD controls ($W = 43$, $P = 0.099$, Cohen's $d = -0.885$). Thus, we controlled for TBV to remove the possible effect this trend could have on subsequent regional differences. Total SA and total CT did not differ between groups (total SA: $W = 56$, $P = 0.378$, $d = -0.60$; total CT: $W = 60$, $P = 0.514$, $d = -0.40$). Therefore, we did not control for SA or CT in subsequent regional analyses.

After controlling for TBV and correcting for multiple comparisons (using FDR correction) (Benjamini and Hochberg 1995), significant subcortical volumetric differences emerged in both hemispheres. The caudate (left: $W = 31$, $P = 0.029$, $d = -1.15$; right: $W = 23$, $P = 0.018$, $d = -1.31$), putamen (left: $W = 27$, $P = 0.029$, $d = -1.24$; right: $W = 30$, $P = 0.034$, effect size = −1.15) and pallidum (left: $W = 29$, $P = 0.029$, $d = -1.032$; right: $W = 32$, $P = 0.034$, $d = -1.24$) volumes were significantly smaller in participants with NS compared with TD controls. There were no significant between-group differences in hippocampal or amygdala regional volumes in either hemisphere (hippocampus: left, $W = 52$, P = ns, $d = -0.44$; right, $W = 54$, P = ns, $d = -0.42$; amygdala: left, $W = 56$, P = ns, $d = -0.41$; right, $W = 68$, P = ns, $d = -0.61$) (Fig. 1).

Effect size was assessed using Cohen's d, with main regional volumetric differences emerging in the medial aspect of the occipital and parietal cortexes as well as in the lateral aspect of the frontal cortex. Within these regions, effects of NS were present in GMV, SA, and CT (Fig. 2).

After correcting for multiple comparisons using FDR correction (Benjamini and Hochberg 1995), significant effects on SA in the bilateral entorhinal regions (left: $W = 4$, $P = 0.00018$, $d = -2.23$; right: $W = 13$, $P = 0.0.003$, $d = -1.84$) were present in participants with NS. Additionally, significant between-group CT differences emerged. The left caudal middle frontal region ($W = 26$, $P = 0.047$, $d = -1.32$), left parsopercularis region ($W = 24$, $P = 0.047$, $d = -1.15$), and right parahippocampal region ($W = 19$, $P = 0.049$, $d = -1.60$) were significantly thinner in participants with NS compared with TD controls. In contrast, the right parsorbitalis region ($W = 118.5$, $d = 1.30$, $P = 0.047$) and right rostral middle frontal region ($W = 121$, $d = 1.28$, $P = 0.047$) were thicker in participants with NS compared with TD controls (see Table 2 for full results).

With respect to behavioral and cognitive assessments, we focused on memory and ADHD symptoms. Relative to their TD counterparts, children with NS performed worse on memory measures, specifically tests assessing unstructured auditory memory measured using the List Memory/List Memory Delayed task ($W = 71$, $P = 0.032$, $d = -1.06$) and on delayed visual memory assessed by Memory for Faces Delayed tasks ($W = 106$, $P = 0.003$, $d = -1.6$). Behaviorally, we observed significantly higher scores for attention problems ($W = 9$, $P = 0.0002$, $d = 1.9$) and hyperactivity ($W = 19.5$, $P = 0.002$, $d = 1.5$) in children with NS compared with controls per parent-report (Table 2). In comparison with the TD group, 41.67% of children with NS were reported to have clinically significant symptoms of ADHD (as indicated by a BASC-2 score of >70). Of these children with NS, 60% displayed clinically significant symptoms of both hyperactivity and attention problems, while 40% reported only symptoms of hyperactivity (Table 1). These findings are in line with previous research, with reported rates of 22–34% (Pierpont et al. 2015; Perrino et al. 2018). Perrino et al. (2018) further reported that 48% of children in their sample demonstrated subsyndromal ADHD symptomatology, suggesting that many children with NS may demonstrate symptoms of ADHD while failing to meet stringent diagnostic criteria.

In addition, we correlated attention problems and hyperactivity scores (BASC-2) with the volume of the bilateral corpus striatum. We used the Spearman's rank-order correlation (the nonparametric version of Pearson correlation). These analyses yield a significant correlation between hyperactivity and the left putamen in the NS group ($r = 0.636$, $P = 0.026$) but not in the control group ($r = 0.337$, P = ns). However, these correlation coefficients were not significantly different between the NS and control groups (Fischer test, $z = 0.85$, P = ns).

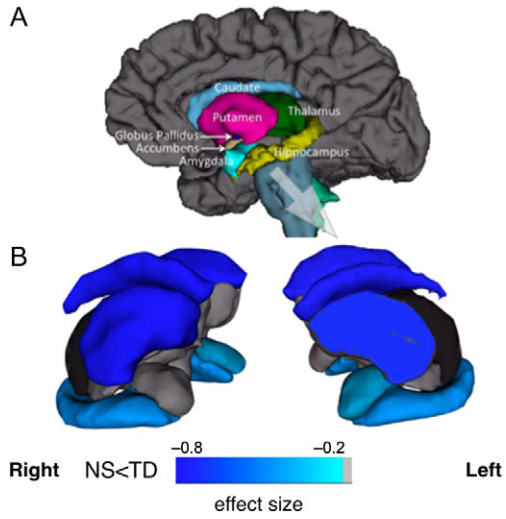

Figure 1. Illustration of subcortical structures hypothesized to be affected by Noonan syndrome. (A) A depiction of the human brain highlights subcortical structures of particular interest in Noonan syndrome during analysis. (B) Effects of Noonan syndrome on extracted subcortical regions are represented by a color bar to denote effect size. As revealed by analyses, bilateral caudate, putamen, and pallidum were all significantly smaller in Noonan syndrome when compared with controls. Hippocampal and amygdala regional volumes were not significantly different between Noonan syndrome and controls.

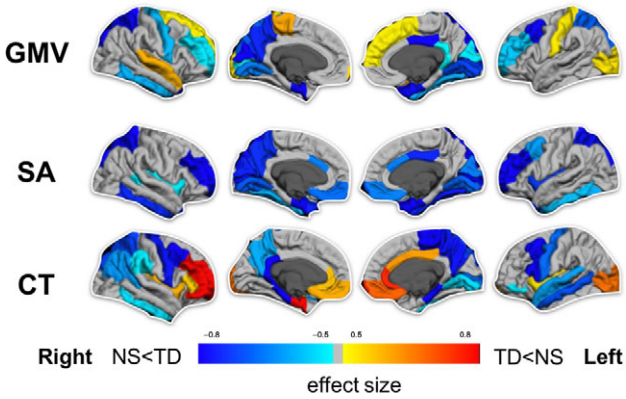

Figure 2. Surface-based analysis of the gray matter volume (GMV), surface area (SA), and cortical thickness (CT). Surface-based analyses of the brain revealed an overall trend of smaller brain morphometry in Noonan syndrome compared with controls. Effects were most prominent in the SA, although significant throughout GMV and CT as well. The medial occipital and parietal regions as well as lateral frontal regions of the brain were most strongly affected by Noonan syndrome.

Discussion

Overall, this study was designed to investigate the effect of *PTPN11* mutations on the developing human brain. Here we report subcortical and cortical GMV, SA, and CT differences between children with NS and TD controls. Most notably, the caudate, putamen, and pallidum that make up the corpus striatum were significantly reduced in size among individuals with NS compared with controls. The corpus striatum is responsible for motor and action planning, motivation, decision-making, reinforcement, and reward perception. Its dysfunction and reduced volume are often linked to inattentiveness and hyperactivity—behavioral traits observed among those with NS (Pierpont et al. 2015)—as well as clinical diagnoses such as

ADHD (Greven et al. 2015; Hoogman et al. 2017) and OCD (van den Heuvel et al. 2005; Cubillo et al. 2012).

Despite relatively intact intellectual functioning (Pierpont et al. 2009), overall, children with NS are at an increased risk for neurodevelopmental disorders such as ADHD (Pierpont et al. 2015), impairments in executive function (Pierpont et al. 2015), oppositional behavior (Alfieri et al. 2014), and deficits in social cognition (Wingbermühle et al. 2012). We found an average IQ in the low average range (FSIQ=90.33) that is consistent with previous findings in children with *PTPN11*-related NS (Pierpont et al. 2009). Our cognitive and behavioral data reflect this, with NS participants displaying significantly higher scores of ADHD symptoms, namely attention problems and hyperactivity (Table 1) compared with their age- and sex-matched controls. In NS, brain morphology alterations in the striatum, specifically to the caudate and putamen, might be associated with ADHD-related behaviors (Faraone et al. 2015; Greven et al. 2015; Pierpont et al. 2015). While brain–behavior correlation did not differ between the groups, we found a significant positive correlation between hyperactivity and the left putamen in the NS group. Indeed, compared with controls, children with NS displayed significantly smaller GMVs in striatal structures as well as decreased CT of left dorsal lateral prefrontal cortex, a region crucial to executive function (Bush 2011). In NS, brain morphology alterations in the striatum, specifically to the caudate and putamen, may be associated with ADHD-related behaviors (Faraone et al. 2015; Greven et al. 2015; Pierpont et al. 2015). In support of this hypothesis, mouse models of NS (caused by gain-of-function mutations in the *Ptpn11* gene) were reported to demonstrate hyperactive/inattentive behaviors (Lee et al. 2014; Kusakari et al. 2015).

By its nature, the study design exploring humans with NS is observational, and we cannot determine a causal effect of *PTPN11* mutations on the brain. For example, it is possible that the aberrations in brain structure observed in the NS group are related to ADHD and learning difficulties. Indeed, nonsyndromic children with ADHD show a similar pattern of reduced GMVs in subcortical regions, specifically within the caudate and putamen (Faraone et al. 2015; Hoogman et al. 2017). However, outcomes from this study in NS and the study of other genetic conditions challenge this assumption. First, in a large sample of individuals with ADHD, Hoogman et al. (2017) reported effect sizes of ADHD on the caudate (left $d = -0.09$, right $d = -0.13$) and putamen (left $d = -0.13$, right $d = -0.14$). These effect sizes are of significantly smaller magnitude than the effect sizes of striatal structures reported in NS for the caudate (left $d = -1.15$, right $= -1.31$) and putamen (left $d = -1.24$, right $d = -1.15$). Second, studies in other genetic conditions that are also associated with ADHD do not show a specific effect on the striatum. For example, girls 4–11 years old with TS, a genetic condition associated with ADHD and executive function deficits (Green et al. 2015), do not show a specific effect on the striatum (Lepage et al. 2013). Thus, this suggests differing neural mechanisms associated with ADHD symptoms between NS and TS. It is possible that in NS, the neural mechanisms of ADHD symptoms are more related to structural striatal aberrations than in TS. This premise supports our understanding of ADHD as possibly related to several different neural mechanisms. With respect to brain morphometry, in mouse models of NS, the expression of a Shp2 mutant protein (encoded by *PTPN11*) promoted neurogenesis and inhibited astrogenesis. Given that genesis of astrocytes, the most abundant cell type in the brain, is inhibited in NS, this observation might be a possible cellular mechanism for the reduced GMVs of subcortical structures in children with NS.

In contrast to our hypothesis, we did not find differences in hippocampal volumes between participants with NS and controls. This hypothesis stemmed from the reported effects of the *Ptpn11* mutation on mouse hippocampal neurons (Lee et al. 2014) and previously reported effects of NS on memory in children (Alfieri et al. 2011; Pierpont et al. 2013). Conversely, we observed a reduction in the GMV in bilateral entorhinal regions, a major source of afferent fibers to the hippocampus, and a reduction in SA in the bilateral corpus striatum, a major target of efferent fibers from the hippocampus. Furthermore, in individuals with NS, we observed reduced CT in the right parahippocampal gyrus, a region largely responsible for memory retrieval and encoding (van Strien et al. 2009). Taken together, these observations indicate a significant effect of NS on brain structures that are part of the hippocampal circuitry (van Strien et al. 2009) and provide preliminary evidence for NS's effect on the brain circuitry involved in memory.

Using effect sizes, we observed an overall pattern of increased CT and decreased SA in frontal and occipital areas in NS compared with controls. One possible explanation for these differential effects is that distinct genetic influences control SA and CT development (Panizzon et al. 2009; Rimol et al. 2010; Winkler et al. 2010). Moreover, this pattern of increased CT and decreased SA has been reported in other neurogenetic conditions such as Williams syndrome (Green et al. 2016), 22q11.2 deletion syndrome (Schmitt et al. 2001), TS (Lepage et al. 2013), and Down syndrome (Lee et al. 2015). Of note, such differences were not reported in Neurofibromatosis 1 (Violante et al. 2013). Conversely, longitudinal studies in TD populations find a continuous CT decrease from 3 years of age onward (Walhovd et al. 2016). The observation of increased CT across these conditions might indicate a developmental delay in these groups compared with TD cohorts. Our data suggest that these effects are more prominent in the frontal and occipital lobe compared with other brain regions in NS.

In line with the findings linking hippocampal circuitry to neurodevelopmental effects of NS, visual/auditory learning as well as memory abilities were considered an area of concern in children with NS. These cognitive findings align with and expand upon previous findings. Pierpont et al. (2013), demonstrated an effect of NS on learning and memory compared with reference data. Within our current study, we observed differences in learning and memory in children with NS compared with age- and sex-matched controls. Overall, we observed deficits in learning and information retrieval in children with NS, noting specific deficits in memory for unstructured verbal information and facial stimuli. Taken together, the pattern of scores for memory of unstructured verbal information and faces stimuli in NS (Table 1) suggests that children with NS will be more prone to difficulties in learning verbal, and possibly visual, information. More specifically, while children with NS can encode facial features, their memory of these features deteriorates quickly. This might indicate a direct effect of NS on memory. In the NS mouse model, Lee et al. (2014) reported significant memory and spatial learning deficits. Future studies could use noninvasive imaging techniques such as resting-state fMRI or diffusion weighted imaging to examine the connectivity of the hippocampus to the aforementioned limbic, striatal, and temporal regions. Such future studies have the potential to further elucidate the effect of *PTPN11* gain-of-function mutations on neural mechanisms of memory.

To the best of our knowledge, this study provides the first examination of the effect of NS caused by *PTPN11* mutations on developing human brain morphology. Such investigations have already been performed in other Rasopathies, specifically Neurofibromatosis type 1 (NF1), a less common genetic condition (~1:3000–3500) (Duarte et al. 2014) than NS. Similar to NS, NF1 is also caused by dysfunction in the Ras-MAPK pathway. Although both are associated with similar behavioral phenotypes (e.g., attention problems), they present with distinct Ras-MAPK signaling associated cellular mechanisms—increases in AMPA receptors in NS and increases in GABA release in NF1 (Lee et al. 2014). Consequently, in hippocampal neurons, NS increases basal excitatory synaptic transmission (Lee et al. 2014), while NF1 increases inhibitory synaptic transmission (Tonks and Neel 2001; Neel et al. 2003).

Compared with the sparse imaging literature in NS, several studies have reported structural and functional differences in individuals with NF1 versus controls (Margariti et al. 2007; Payne et al. 2010; Karlsgodt et al. 2012; Huijbregts et al. 2015; Loitfelder et al. 2015; Tomson et al. 2015). In contrast to NS, children with NF1 tend to exhibit large structural brain volumes (Margariti et al. 2007; Payne et al. 2010; Huijbregts et al. 2015), specifically larger GMV of subcortical structures compared with controls (Payne et al. 2010; Karlsgodt et al. 2012). Thus, when carefully compared with the available imaging literature in NF1, our study suggests that these syndromes, although related through the Ras-MAPK pathway, present with different and potentially opposite effects on human brain morphology. Future work will enable us to delineate the differences between Noonan and NF1 syndromes and provide data on the neural pathways that lead to neurodevelopmental disorders amongst individuals afflicted by each disorder.

This study is mainly limited by its sample size, a common issue when studying relatively rare genetic disorders (Green et al. 2012, 2014, 2015). Despite our limited sample size (n = 12 per group), not only were we able to increase the strength of our sample through genetic homogeneity—including only children with *PTPN11*-related NS—but we were also able to reduce potential confounding factors through diligent age- and sex-matching to control participants. To address our limited sample size and to quantify the difference between participants with NS and controls, we used effect sizes in our analyses. Nevertheless, we report significant and large effects of NS on the morphology of specific brain regions—namely, limbic, temporal, and striatum. We also strengthen and expand on findings from previous studies by observing that NS is associated with cognitive deficits, specifically verbal and visual memory as well as ADHD symptoms, not only when compared with reference data or siblings samples (Pierpont et al. 2015), but also to age- and sex-matched controls. While we and others (Pierpont et al. 2015) found that cognitive function in children with NS is in the low normal range (FSIQ normal range 90–110; NS FSIQ = 90.33 ± 11.09), there were significant differences in IQ scores between NS and controls (Table 1). This confounder limits our ability to determine if brain morphometry differences are attributed to the cognitive abilities rather than to the genetic variant and NS diagnosis. In future studies, it would be important to look at a cohort of NS patients matched on cognitive and behavioral symptoms.

In conclusion, this study is the first step to defining the neural substrates underlying behavioral deficits among the NS population. Such findings not only demonstrate how neural variations associated with NS affect attention and memory in the developing human brain, but also hold promise for providing novel brain-based outcome measures for future clinical interventions in NS. For example, medication decreasing activation in Ras-MAPK pathway signaling (enhanced in NS) has already shown beneficial effects on learning in mouse models of NS (Lee et al. 2014). Taken together, these results identify the effect of NS on the specific brain regions associated with ADHD symptoms and learning in children. While our research has laid the groundwork for elucidating the neural and behavioral mechanisms of NS, combined with additional data collected from Ras-MAPK related conditions (mostly neurofibromatosis 1), it also contributes to a broader understanding of brain development in the Rasopathies.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

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Notes

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