Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2009) **8:** 275–282

Genotype differences in cognitive functioning in Noonan syndrome

E. I. Pierpont*,†, M. E. Pierpont‡,§, N. J. Mendelsohn‡,§, A. E. Roberts¶,,††, E. Tworog-Dube¶ and M. S. Seidenberg†**

†*Department of Psychology, University of Wisconsin-Madison, Madison, WI, USA,* ‡*Department of Medical Genetics, Children's Hospitals and Clinics of Minnesota, St. Paul-Minneapolis, MN, USA,* §*Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA,* ¶*Harvard Medical School Partners Health Care Center for Genetics and Genomics, Boston, MA, USA,* **Department of Cardiology and* ††*Division of Genetics, Children's Hospital Boston, Boston, MA, USA*
**Corresponding author: E. I. Pierpont, Department of Psychology, University of Wisconsin, 1202 W. Johnson Street, Madison, WI 53706, USA. E-mail: eipierpont@wisc.edu*

Noonan syndrome (NS) is an autosomal-dominant genetic disorder associated with highly variable features, including heart disease, short stature, minor facial anomalies and learning disabilities. Recent gene discoveries have laid the groundwork for exploring whether variability in the NS phenotype is related to differences at the genetic level. In this study, we examine the influence of both genotype and nongenotypic factors on cognitive functioning. Data are presented from 65 individuals with NS (ages 4–18) who were evaluated using standardized measures of intellectual functioning. The cohort included 33 individuals with *PTPN11* mutations, 6 individuals with *SOS1* mutations, 1 individual with a *BRAF* mutation and 25 participants with negative, incomplete or no genetic testing. Results indicate that genotype differences may account for some of the variation in cognitive ability in NS. Whereas cognitive impairments were common among individuals with *PTPN11* mutations and those with unknown mutations, all of the individuals with *SOS1* mutations exhibited verbal and nonverbal cognitive skills in the average range or higher. Participants with N308D and N308S mutations in *PTPN11* also showed no (or mild) cognitive delays. Additional influences such as hearing loss, motor dexterity and parental education levels accounted for significant variability in cognitive outcomes. Severity of cardiac disease was not related to cognitive functioning. Our results suggest that some NS-causing mutations have a more marked impact on cognitive skills than others.

Keywords: *BRAF*, cognition, genotype-phenotype correlations, *PTPN11*, *SOS1*, Noonan syndrome

Received 26 September 2008, revised 3 November 2008, 19 November 2008, accepted for publication 20 November 2008

Introduction

Noonan syndrome (NS) is a multiple congenital anomaly syndrome characterized by short stature, facial anomalies, heart disease and learning disabilities. Incidence is estimated to be between 1:1000 and 1:2500 live births (Mendez *et al.* 1985). Germline gain-of-function mutations in several RAS-MAP kinase pathway genes have recently been found to cause NS. Missense mutations in the *PTPN11* gene are the most common cause of NS and account for approximately 50% of cases (Tartaglia *et al.* 2001). Mutations in *SOS1*, *RAF1* and *KRAS* genes account for an additional 10–15%, 3–17% and <5% of cases, respectively (Pandit *et al.* 2007; Razzaque *et al.* 2007; Roberts *et al.* 2007; Schubbert *et al.* 2006; Tartaglia *et al.* 2007). Although typically associated with cardiofaciocutaneous (CFC) syndrome, recent studies have found that *BRAF* mutations can also result in a NS phenotype (Nystrom *et al.* 2008; Razzaque *et al.* 2007). The genetic etiology remains unknown in roughly 30% of NS patients.

Learning disabilities are commonly cited as a key characteristic of NS; yet, the causes of these impairments are poorly understood. Average IQ scores among affected individuals are lower than expected based on normative data (Lee *et al.* 2005). However, cognitive abilities of NS patients may range from moderate mental retardation to superior abilities. Van der Burgt *et al.* (1999) noted that individuals who displayed more severe physical features of NS performed more poorly on some cognitive tests than those with moderate features. One possible explanation for this finding is that one or more of the medical sequelae of NS could interfere with cognitive functioning. For example, research indicates that children with severe congenital heart disease tend to exhibit overall lower cognitive abilities than those with less severe heart disease (Karsdorp *et al.* 2007). Congenital heart defects are a primary characteristic of NS and are present in roughly 85% of patients with *PTPN11* mutations (Sznajer *et al.* 2007). Hearing loss and motor incoordination are two additional features commonly seen in NS (Lee *et al.* 2005; Qiu *et al.* 1998). These medical characteristics could affect cognitive development in NS, but their influence has not been explored to date.

An alternative explanation for the association between severity of NS expression and cognitive ability is that certain NS mutations may have a generally more deleterious effect, resulting in abnormal physical *and* mental development. The signal transduction pathway in which the known NS genes act, RAS-MAP kinase, plays a role in numerous biological processes including embryologic development (Schubbert *et al.* 2007). Some research suggests that dysregulation of this pathway can affect brain development. Altered activation of protein tyrosine phosphatase SHP-2, the *PTPN11* gene

doi: 10.1111/j.1601-183X.2008.00469.x

product, can interfere with neural cell-fate decisions (Gauthier *et al.* 2007). Whether the molecular changes resulting from other NS mutations have similar effects on central nervous system development is not currently known.

The purpose of the present study was to examine whether some of the variability in cognitive functioning in NS could be explained by genotype, and to explore additional medical, developmental and environmental influences on these skills.

Materials and methods

Participants

Sixty-five individuals with NS completed this study. Participants were part of a larger investigation of behavior and learning in individuals with NS. Families were recruited through clinics at Children's Hospitals and Clinics of Minnesota ($n = 20$), Children's Hospital Boston ($n = 25$), the Waisman Center at the University of Wisconsin-Madison ($n = 7$), and at the annual meeting of the Noonan Syndrome Support Group ($n = 13$). The study was approved by the Internal Review Board at each of the participating institutions. Participants and their primary caregivers signed written informed consents before enrollment in the study.

Participants were recruited for the study if they had received a clinical diagnosis of NS from a clinical geneticist. Relevant medical and genetic information was obtained from hospital case notes requested from the child's primary geneticist or cardiologist using Health Insurance Portability and Accountability Act authorizations signed by the families. Review of these records was used to determine whether participants fit inclusion criteria for the study. Criteria for inclusion, based on a scoring system developed by van der Burgt *et al.* (1994), were identical to those used in previous studies (Roberts *et al.* 2007).

Procedures

Cognitive evaluation

Intellectual abilities were evaluated using the Differential Ability Scales (DAS). This measure includes norms for individuals aged 2.5–18 years and provides a verbal and nonverbal cluster for all ages (Elliott 1990). In older children (>6 years), the Special Nonverbal Composite, which is used in our analyses as the main measure of nonverbal ability, can be further divided into spatial and nonverbal reasoning scales. All assessments were administered in a quiet room by the same examiner (E.I.P.).

Hearing screening

A pure-tone hearing screening was performed at the time of the assessment using a portable Beltone audiometer. Pass/fail data were collected for both ears at 20 dB for frequencies of 1000-, 2000- and 4000-Hz. A screening score (ranging from 0 to 6) was assigned based on the number of frequencies in which the participant was able to detect the tone.

Motor dexterity

Manual motor dexterity was evaluated using the Purdue Pegboard Test (Tiffen 1968). This task requires the examinee to place small metal pegs into a series of slots as quickly as possible during a limited period of time (30 seconds). Three conditions were administered: preferred hand, nonpreferred hand and both hand conditions. A composite score was obtained by averaging participants' standard scores for the three conditions. Standard scores for each trial were calculated using appropriate age norms (Gardner & Broman 1979; Yeudall *et al.* 1986).

Rating of cardiac disease severity

Based on a review of each participant's medical record by a pediatric cardiologist (M.E.P.), individuals were assigned a rating of medical severity of cardiac disease. The score was based on the Cardiologist's Perception of Medical Severity scale (DeMaso *et al.*, 1991). This scale indexes cardiac severity as follows: (1) no or insignificant disorder – disorder has no impact on child's health; (2) mild disorder – lesion requires no operative intervention, only long-term follow-up (e.g. small ventricular septal defect); (3) moderate disorder – child is asymptomatic, but has had or will require operation, easy repair (e.g. atrial septal defect); (4) marked disorder – child quite symptomatic, has had or will require major difficult repair (e.g. tetralogy of Fallot, transposition of great arteries); (5) severe disorder – uncorrectable cardiac lesions or only complex palliative repair possible (e.g. pulmonary vascular obstruction, Fontan repair and valve replacement).

Family socioeconomic status

Parents were asked to report their highest level of formal education. The average of the paternal and maternal years of education was calculated to index socioeconomic status. Because parental education levels have been shown to have significant impact on intellectual development, especially verbal IQ (Rowe *et al.* 1999), this measure was included as an additional factor in our analyses.

Genotyping

Gene testing reports were available for 53 participants. The remaining individuals in the sample (12 participants) had not completed any genetic testing. Of the individuals tested, 33 (62%) tested positive for a *PTPN11* gene mutation and 6 (11%) tested positive for an *SOS1* mutation. Thirteen patients tested negative for mutations in *PTPN11*; of these participants, 11 had not completed testing for the remaining NS genes, and 2 had undergone *SOS1* and *KRAS* testing with negative results. One participant tested positive for the *BRAF* mutation. Although *BRAF* mutations are typically associated with CFC syndrome, this individual met diagnostic criteria for the NS phenotype and therefore was not excluded from the study. Specific information about the genotypes of participants is included in Table 1.

Results

Demographics

The cohort included 35 males and 30 females between the ages of 4 and 18 years (mean = 10.0, SD = 4.1). Family characteristics and developmental history were obtained through a review of medical reports and parent accounts. For 54 of the individuals assessed, the parents were married and living together. The remaining 11 participants were from single-parent families or were living with one biological parent and one step-parent. Parental education levels ranged from some high school to advanced graduate degrees (mean = 15.6 years, SD = 1.9). The cohort included one set of monozygotic twins, two families with two affected siblings and one family with three affected siblings. In 13 patients (including the 9 participants with affected siblings in the cohort), the NS mutation was known to be inherited from an affected parent. In four additional participants, a diagnosis of NS was suspected but not confirmed in at least one other first-degree family member. In the remaining 48 cases, NS was thought to be sporadic (nonfamilial). Thirty-seven of the participants (57%) had received a cognitive, learning or behavioral disability diagnosis at some point in development. The most common specific diagnoses in the cohort were Attention Deficit/Hyperactivity Disorder (29%), Reading Disability (11%), speech/language impairment (9%), Math Disability (9%), mental retardation (8%) and autism spectrum disorders (8%). One child (aged 4 years, 6 months) was nonverbal at the time of assessment.

Table 1: Gene mutations in 40 individuals with NS

n	Gene	Exon	Nucleotide substitution	Amino acid change
1	PTPN11	3	A>G	N58D
1	*PTPN11*	3	A>G	D61G
1	*PTPN11*	3	G>A	D61N
1	*PTPN11*	3	T>G	Y62D
3	*PTPN11*	3	A>G	Y63C
1	*PTPN11*	3	A>T	E69V
1	*PTPN11*	3	G>T	E76D
2	*PTPN11*	3	A>G	Q79R
1	*PTPN11*	3	A>C	D106A
1	*PTPN11*	3	A>C	N106A
2	*PTPN11*	4	G>C	E139D
3	*PTPN11*	7	A>G	I282V
1	*PTPN11*	8	T>C	F285S
4	*PTPN11*	8	A>G	N308D
2	*PTPN11*	8	A>G	N308S
1	*PTPN11*	8	A>C	N308T
2	*PTPN11*	13	C>T	P491S
1	*PTPN11*	13	G>C	G503R
3	*PTPN11*	13	A>G	M504V
1	*PTPN11*	13	C>G	Q510E
1	*SOS1*	6	C>A	T266K
2	*SOS1*	6	T>G	M269R
1	*SOS1*	11	G>A	C441Y
1	*SOS1*	11	A>C	S548R
1	*SOS1*	16	G>A	E846K
1	*BRAF*	15	C>G	L597V

Cognitive ability in NS

Intellectual skills varied widely among participants, ranging from Very Low (> 2 SDs below the mean) to High (> 1.5 SD above the mean) levels of functioning. As a group, our NS cohort scored significantly lower on the DAS than expected based on normative data (mean = 100, SD = 15), $t(64) = -6.05$, $P < 0.001$. Mean scores for males and females were not significantly different, $t(63) = 0.28$, $P = 0.78$. Performance on the DAS was not significantly correlated with the chronological age of participants ($r = -0.02$, $P = 0.90$). The distribution of scores among NS patients spanned a wide range but was shifted downward compared with the normative population (Fig. 1). The group mean of 86.2 (SD = 18.4; range: 44–123) was approximately 1 SD below the general population average.

Individuals without an established cognitive or learning disability diagnosis at the time of assessment scored significantly higher on the full-scale assessment than those with an established diagnosis, $t(63) = 2.16$, $P < 0.05$. However, learning/cognitive disabilities may be somewhat underidentified in this population. Eight of the 23 individuals who scored in the 'Low' or 'Very Low' range on the DAS (>1.5 SD below the mean) had not previously been diagnosed as learning disabled. Eleven participants in the cohort (17%) obtained a score below 70, in the range of mental retardation. This rate

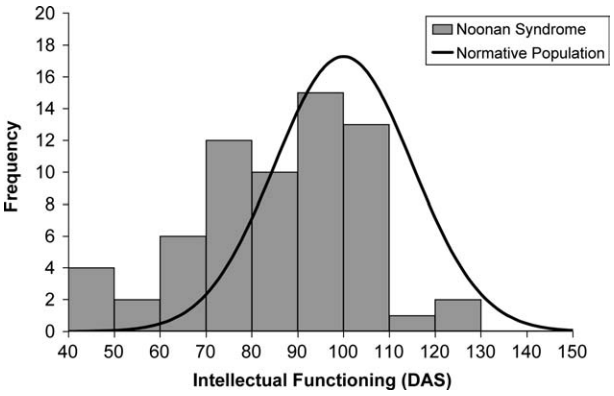

Figure 1: Distribution of intelligence scores for individuals with NS (*n* = 65) and normative sample.

in NS is higher than the incidence within the general population (2%), $\chi^2 = 73.9$, df = 1, $P < 0.001$. To examine the possibility of ascertainment bias based on site of recruitment, we compared performance for groups tested at each of our four research sites. Cognitive scores did not vary significantly as a function of the recruitment/testing location, $F_{3,61} = 0.85$, $P = .47$. This suggests that a similar range of abilities was seen in patients identified through medical clinics, research studies, and through the NS Support Group.

Patterns of discrepancy across domains of intellectual skill were also examined. On average, verbal skills were significantly higher than nonverbal skills, $t(64) = 2.84$, $P < 0.01$. To determine the direction of discrepancies for individuals in the sample, differences between verbal and nonverbal abilities were compared with critical values for statistical significance at the $P = 0.05$ level. Fourteen participants (22%) had a verbal score that was significantly higher than their nonverbal score. Only six participants (9%) had the opposite pattern, with significantly higher nonverbal abilities. Among school-aged children (> 6 years), nonverbal skills could be further broken down into two clusters: spatial skills and nonverbal reasoning. Differences between spatial and nonverbal reasoning skills did not reach levels of significance, $t(50) = 1.1$, $P = .28$.

Genotype–phenotype analysis

Several analyses were conducted to explore whether genetic differences could account for variability in cognitive functioning in NS. The cohort was first examined based on the gene in which a mutation was found. The single participant with a *BRAF* mutation was not included in these analyses. Figure 2 depicts the distribution of full-scale DAS scores for individuals with *PTPN11* mutations, *SOS1* mutations and unknown mutations. The 'unknown' mutation group was expected to be heterogeneous with respect to the disease-causing gene.

All six participants with *SOS1* mutations scored within the average range or higher on the cognitive assessment (range: 91–123). The average full-scale DAS performance for the *SOS1* group did not differ from the normative population (100), $t(5) = 0.74$, $P > 0.40$. In contrast, more than half of individuals with *PTPN11* mutations ($n = 20$; 61%) scored

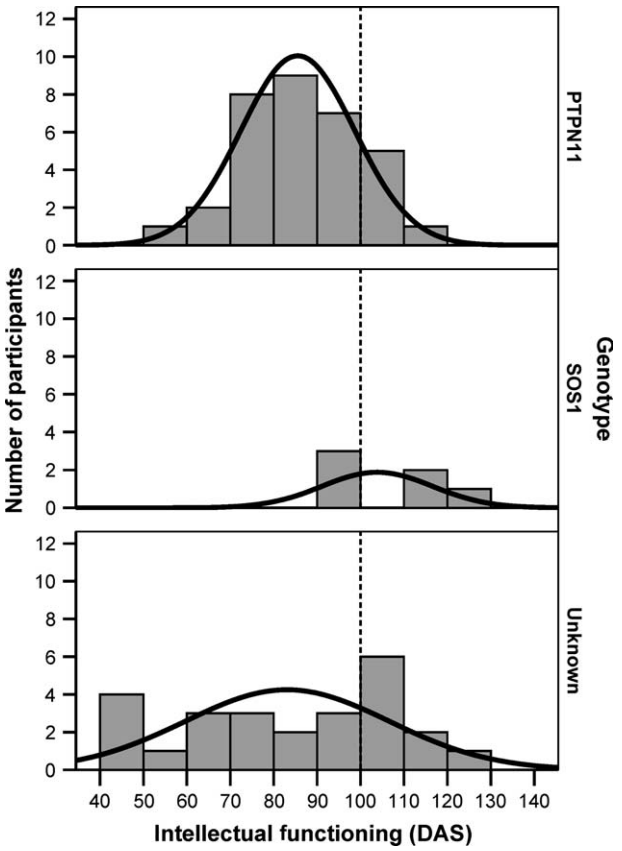

Figure 2: Distribution of intelligence scores for individuals with *PTPN11, SOS1* and unknown mutations, with normal curves for each group displayed. Dotted lines indicate the normative sample mean.

below the average range (<90). Scores in this group ranged from 59 to 110. The mean DAS performance for the *PTPN11* group was significantly lower than expected based on the normative population mean, $t(32) = -6.3$, $P < 0.001$. Similar to the *PTPN11* group, the group of participants with unknown mutation status scored lower on the DAS than expected based on the population mean, $t(24) = -3.59$, $P < 0.001$. Scores for the unknown mutation group ranged from 44 to 123. The distribution of scores for the unknown group was less similar to a normal curve than the distributions for the *PTPN11* genotype. Descriptive statistics (e.g. range and standard deviation) for this group were also larger, suggesting that this group had greater variability with respect to cognitive skills. Note that it is expected that approximately 50% of the 12 untested individuals in the unknown mutation group have a *PTPN11* mutation and 10% an *SOS1* mutation.

Comparisons were conducted to determine whether cognitive functioning in NS patients differed based on the presence or absence of specific mutations. Because 12 participants in the unknown mutation group had not been tested for any NS genes, these individuals were excluded from the following analyses. Three groups remained: a *PTPN11*-positive group, an *SOS1*-positive group and a group of *PTPN11*-negative individuals whose genotype is unknown (two of whom were also known to be *SOS1* negative). A one-way ANOVA was conducted to examine whether there were reliable differences in intellectual ability among the three genotype groups. This analysis indicated a significant difference in full-scale DAS scores between the groups, $F_{2,49} = 3.50$, $P < 0.05$.

Several planned comparisons were conducted to examine these genotype differences in cognitive ability more closely. The first analysis compared the performance of the *SOS1* group and the *PTPN11* group. The *SOS1* group scored significantly higher on the DAS than the *PTPN11* group, $t(37) = 3.16$, $P < 0.01$. To examine whether the observed genotype difference was consistent across different domains of intellectual functioning, the *PTPN11* and *SOS1* groups were compared separately on the verbal cluster and the nonverbal cluster. Results were identical to the full-scale test. The *SOS1* group performed significantly better than the *PTPN11* group on both verbal, $t(37) = 2.80$, $P < 0.01$, and nonverbal, $t(37) = 2.90$, $P < 0.01$ cognitive scales (Fig. 3).

The *SOS1* and *PTPN11* mutation groups were then compared with individuals without mutations in each of those genes, respectively. An analysis was performed to contrast individuals with *SOS1* mutations to an *SOS1* mutation-negative group. The latter group included both *PTPN11*-positive individuals (with the assumption that these patients would not also be *SOS1* positive) as well as individuals with unknown mutations who tested negative for *SOS1* mutations. *SOS1*-positive individuals scored higher than *SOS1*-negative individuals on the DAS, $t(39) = 2.73$, $P < 0.01$. The *PTPN11*-positive group was compared with a *PTPN11*-negative group (including both the *PTPN11*-negative individuals of unknown genotype and the *SOS1*-positive participants). *PTPN11*-positive individuals scored significantly lower than the *PTPN11*-negative individuals on the DAS, $t(50) = -2.09$, $P < 0.05$. When the six participants with identified *SOS1* mutations were removed from this analysis; however, the difference between the *PTPN11*-positive and *PTPN11*-negative groups was no longer significant, $t(44) = -1.08$, $P = 0.29$.

Additional analyses examined the *PTPN11*-positive group in greater depth. Table 2 displays DAS scores grouped by exon

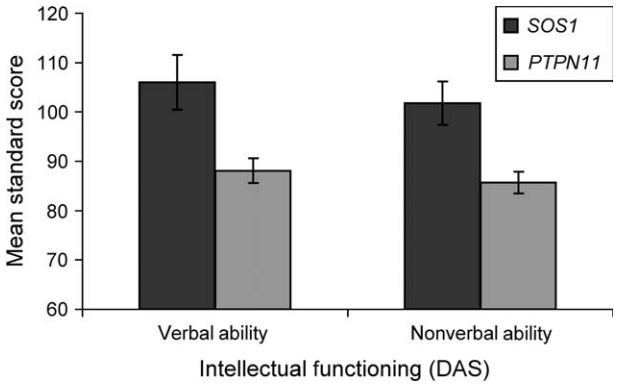

Figure 3: Mean verbal and nonverbal cluster scores for individuals with *PTPN11* and *SOS1* mutations.

Table 2: Descriptive statistics for *PTPN11*-positive individuals on DAS clusters*, grouped by exon in which the mutation is located

Measure	Exon 3 ($n = 13$)	Exon 4 ($n = 2$)	Exon 7 ($n = 3$)	Exon 8 ($n = 8$)	Exon 13 ($n = 7$)
Verbal ability					
Mean	88.1	89.5	76.0	95.8	84.0
SD	12.0	13.4	17.3	17.2	13.9
Range	68–105	80–99	56–87	54–106	59–100
Nonverbal ability					
Mean	80.2	88.0	78.7	91.9	90.0
SD	10.0	19.8	11.0	15.3	12.1
Range	68–97	74–102	66–86	68–111	81–115

*Normative mean = 100; SD = 15.

in which a mutation was detected. Group sizes were not sufficient to detect differences among these groups. However, a wide range of abilities was seen for each exon group; at least one participant in each group had low-borderline functioning, and at least one participant in each group scored in the average range or higher for a given cluster. Hence, it appears that *PTPN11* mutations across the whole gene have the potential to interfere with cognitive development. However, *PTPN11* mutations in all exons are compatible with normal cognitive development.

For individuals with mutations in exon 8, the group mean was in the average range for both verbal and nonverbal skills. In a previous study (Tartaglia *et al.* 2002), 17 patients with an N308D mutation in exon 8 were all found to attend regular education classrooms. The four individuals with this mutation in our sample fit this profile of having no (or mild) cognitive delays. Three had full-scale DAS scores in the average range and one scored in the low average range (mean = 92.5, SD = 6.3). To determine whether this finding could extend to all individuals with N308 mutations, we also examined the scores of individuals in our cohort with N308S and N308T mutations. The two patients with N308S mutations both received a full-scale score in the average range [standard score (SS) = 104 and 101]. The single individual with an N308T mutation scored in the range of mild mental retardation (SS = 61). Hence, even within N308 mutations, variability was evident for different amino acid substitutions. Nevertheless, the absence of marked cognitive deficits among any individuals with N308D and N308S mutations in our sample suggests that some N308 mutations are likely to be associated with mild cognitive effects.

The individual with a *BRAF* mutation in our sample (age 14 years, 4 months) achieved an overall DAS score in the Low range (SS = 76; 5th percentile), with a verbal score in the Low Average range (SS = 85; 15th percentile) and a nonverbal score in the Low range (SS = 74; 4th percentile). Although scores in all domains were below the average range, mental retardation was not present. This participant scored higher than 24% of the individuals with *PTPN11* mutations, and 32% of individuals with unknown mutations on the DAS. Thus, observed scores for this *BRAF*-positive individual were within the range of scores seen in other NS patients.

Additional influences in cognitive skills

To further probe potential influences on cognitive abilities in NS, four additional factors were also explored. These factors included two medical features associated with NS (severity of cardiac disease and hearing screening scores), a developmental factor (motor co-ordination) and a measure of socioeconomic status (years of parental education). Descriptive statistics for these factors are reported in Table 3. Multiple regression analyses were conducted to investigate whether these variables had significant influence on verbal skills and nonverbal skills. Three variables accounted for 34% of the variability in verbal intellectual functioning: hearing screening scores, manual motor dexterity and years of parental education (Table 4). Two variables, motor dexterity and parental education, were significantly predictive of nonverbal skills, accounting for 40% of the variability in DAS nonverbal scores. Severity of heart disease was not predictive of any of the cognitive outcomes.

To examine whether the observed genotype difference between the *PTPN11* and *SOS1* groups could be explained by group differences on these additional (nongenotypic) factors, we compared the scores of these two genotype groups on each factor. Mean scores for individuals with *SOS1* and *PTPN11* mutations on each factor are included in Table 3. Scores were not significantly different between the two groups for severity of cardiac disease, $t(37) = 1.15$, $P = 0.26$, hearing screening scores, $t(37) = 0.11$, $P = 0.91$, motor dexterity, $t(28) = 0.02$, $P = 0.98$, or parental education levels, $t(37) = .43$, $P = .67$. Thus, the *SOS1* group and *PTPN11* group did not differ reliably on scores for any of the additional predictors of cognitive functioning that were measured.

Additional analyses were conducted to determine whether individuals in our sample who also had a parent with NS (who may have had learning disabilities that impacted educational attainment) were driving the association between parental education and cognitive ability. The parental education levels of participants with an affected parent and those without an affected parent were compared. Parent education levels of the 17 participants with likely inherited NS mutations (mean = 15.56 years, SD = 1.2) did not differ significantly from the

Table 3: Descriptive statistics for nongenotypic predictor variables

	Full sample ($n = 65$)	*PTPN11* only ($n = 33$)	*SOS1* only ($n = 6$)
Cardiac disease severity (CSEV rating)	2.7 (1.0)	2.7 (1.0)	3.3 (1.1)
Total passes, hearing screening	5.2 (1.9)	5.1 (1.9)	5.2 (1.3)
Motor dexterity (Purdue Pegboard Test)*	72.9 (22.2)	78.0 (17.4)	78.2 (13.1)
Years of parental education	15.6 (1.9)	15.5 (1.8)	15.8 (1.5)

Values are given as mean (SD). CSEV, Cardiologists Perception of Medical Severity Scale.

*Normative mean = 100, SD = 15.

Table 4: Medical, developmental, and environmental predictors of cognitive skills in 65 individuals with NS: multiple regression analysis*

	DAS clusters	
Predictor variables	Verbal ability	Nonverbal ability
Cardiac disease severity (CSEV rating)	0.09	0.03
Total passes, hearing screening	0.31†	0.11
Motor dexterity (Purdue Pegboard Test)	0.30†	0.51‡
Years of parental education	0.27†	0.25†
Total R-square	0.34‡	0.40‡

CSEV, Cardiologists Perception of Medical Severity Scale.

*Data are standardized regression coefficients.

†$P < 0.05$.

‡$P < 0.01$.

48 patients with sporadic mutations (mean = 15.63 years, SD = 2.1), $t(62) = 0.13$, $P = 0.90$. Individuals with familial NS also did not differ in levels of cognitive ability from those with sporadic mutations, $t(63) = -0.55$, $P = 0.58$. To control for mutation type, we also examined differences between familial vs. sporadic cases among only those individuals with *PTPN11* mutations. Differences in parental education level, $t(31) = -0.92$, $P = 0.36$, and cognitive ability, $t(31) = 0.67$, $P = 0.51$, did not reach levels of significance. Hence, parental education levels have a significant impact on their child's cognitive ability independent of whether a parent also has a mutation; in addition, having a familial form of NS does not appear to pose additional cognitive risk.

Discussion

Although multiple studies have established that cognitive impairments are more common in NS than in the general population, little is known about the causal pathways that lead to these outcomes. The current study is the first to explore whether genetic differences can explain some of the wide variation in cognitive functioning in individuals with NS.

As a group, the pattern of performance on cognitive assessments among NS individuals in this study was similar to previous reports (van der Burgt *et al.* 1999; Lee *et al.* 2005). Cognitive disabilities were present with greater frequency in NS than in the general population, and the overall distribution of cognitive test scores was shifted significantly downward. Nevertheless, a large proportion of individuals in this sample (~50%) showed intellectual skills in the average range or higher.

Cognitive scores in our cohort did not vary significantly as a function of the chronological age or gender of participants, or based on whether the mutation was sporadic vs. familial. On average, individuals in our sample had significantly better verbal abilities than nonverbal abilities, a finding that is consistent with one study (van der Burgt *et al.*, 1999), but opposite of the pattern found in another (Lee *et al.* 2005). This inconsistency in patterns could reflect differences in the measurement tools used. For example, the school-age nonverbal scale in the DAS contains a test that requires participants to draw complex spatial figures from memory (Recall of Designs). Individuals with NS in our study achieved lower scores on this subtest than any other, perhaps because this subtest relies somewhat on fine motor skills. Manual fine motor skills were severely impaired (>2 SD below the mean) in 34% of those tested on the Purdue Pegboard Task, and below average (>1 SD below the mean) in 72% of the sample. Hence the greater reliance on motor skills for nonverbal tests could potentially account for the verbal advantage in our sample. This explanation does not account for the discrepant results in the previous studies, which were conducted using two versions of the same (Wechsler Intelligence, WISC) scale, the WISC-R and the WISC-RN. An alternative explanation for the inconsistency in patterns of verbal–nonverbal ability is that the sample sizes available for each study were relatively small, which may lead to some instability in outcomes. Nevertheless, taken together, studies of cognitive functioning in NS suggest that a consistent pattern of cognitive strengths and weaknesses is unlikely to emerge in NS.

Our genotype–phenotype analyses indicate that some of the variation in cognitive skills in NS is attributable to differences in genotype. Individuals with *SOS1* mutations performed significantly higher on both verbal and nonverbal cognitive tests than individuals with *PTPN11* mutations and *SOS1*-negative individuals with unknown mutations. Although limited research has been conducted to examine individuals with *SOS1* mutations, this finding is consistent with reports that these individuals are more likely to be placed in regular education classrooms than those with *PTPN11* mutations (Tartaglia *et al.* 2007). Our results also support previous reports (Tartaglia *et al.* 2002) that individuals with the N308D mutation in *PTPN11* are likely to have no or mild cognitive disabilities.

The cognitive differences between individuals with *SOS1* and *PTPN11* mutations in this sample were seen in both verbal and nonverbal domains, suggesting that the group difference is not because of a specific domain of strength or weakness caused by mutations in a particular gene. Age differences also cannot account for the discrepancy, as the two groups did not differ significantly in chronological age, $t(37) = 1.15$, $P = .26$, and age was uncorrelated with performance on the cognitive tests. Note that this sample did not include infants and toddlers under age 4, for whom developmental delays have been reported in *SOS1*-positive (Narumi *et al.* 2008) as well as *PTPN11*-positive individuals. The difference between the groups was also not because of differences in rates of heart disease, hearing loss, motor incoordination or socioeconomic level, suggesting that some other factor is responsible for the differences in cognitive performance.

One possible explanation for the group differences in cognitive performance is that alterations in *SOS1* gene expression have less impact on central nervous system development than alterations in other RAS-MAP kinase genes. Another possibility is that other unidentified medical or developmental factors vary across the genotypes, and one or more of these factors are affecting cognitive outcomes.

A third possibility is that some individuals with *SOS1* mutations do have cognitive disabilities, but these individuals may carry a diagnosis other than NS and therefore would not have been recruited for the current study. It has been recognized that many *SOS1* patients have unusual ectodermal features similar to those seen in CFC syndrome (Tartaglia *et al.* 2007). Given the substantial overlap in physical features of NS patients with *SOS1* mutations and CFC syndrome patients (Narumi *et al.* 2008), this possibility should be further explored. Indeed, replication of our genotype–phenotype results with a larger sample of *SOS1* individuals is necessary for distinguishing among these possibilities.

Our sample included only one individual with a *BRAF* mutation, presumably because people with this genotype typically carry a CFC syndrome diagnosis rather than NS (Rodriguez-Viciana *et al.* 2006). This case is notable because of the fact that the individual we assessed did not have mental retardation, although mental retardation has been reported to be universally present in CFC syndrome (Armour & Allanson 2008; Yoon *et al.* 2007). Our *BRAF*-positive participant also lacked the ectodermal features that are present among most individuals with an CFC diagnosis. She did display below average cognitive skills; however, her verbal ability was near the average range. In addition, this person achieved scores well within the range seen in other individuals with NS in our sample. This individual provides further evidence for an overlap in the NS and CFC phenotypes, even among individuals with known mutations (Nystrom *et al.* 2008). In addition, although mental retardation is common among *BRAF*-positive individuals, our study suggests that mental retardation is not a necessary consequence of all *BRAF* mutations. Further research is needed to delineate whether differences in cognitive phenotype can be linked to specific mutations in *BRAF*.

In addition to the genetic differences observed in this study, we also investigated whether additional medical, developmental or environmental variables accounted for variation in cognitive ability. Severity of cardiac disease was not associated with cognitive functioning in this population. However, failure to pass a hearing screening was significantly associated with lower performance on verbal tests. In addition, verbal and nonverbal cognitive abilities were significantly predicted by the socioeconomic status and fine motor abilities of the children in our study. These analyses indicate that not only genetic factors but also developmental and social factors play a significant role in cognitive development in NS.

Studies of genotype–phenotype correlations in the neuropsychological realm, which have been enabled by important advances in molecular genetics, have significant implications for clinical care. If the genotype is known for a given patient, specific avenues for early intervention and other educational planning may be indicated. Our research suggests that individuals with *PTPN11* mutations and unknown mutations are at risk for cognitive disabilities, although a wide range of abilities was observed. Comprehensive neuropsychological testing can help to identify areas that require special attention. NS patients with *SOS1* mutations and N308D/N308S mutations in *PTPN11* appear likely to develop normal range cognitive skills, although functioning in other areas (e.g. motor development) may be delayed.

It is important to note that the substantial overlap in the distributions of cognitive scores between all NS genotypes suggests that having a specific genetic anomaly does not indicate reliably what the cognitive outcome will be for any given individual. Indeed, the significant influence of several nongenotypic factors suggests that a number of steps can be taken to foster development in NS regardless of genotype. For all patients, physical or occupational therapies for motor impairments may improve achievement on cognitive as well as physical tests. Adaptations for individuals with motor difficulties can be implemented in educational settings so that these disabilities do not affect achievement in other areas. In addition, identification of hearing impairments and subsequent intervention are of great importance to enhance the development of verbal skills. It is critical that clinicians and school professionals are made aware of the range of issues associated with NS so that all proper evaluations and modifications can be administered.

This study represents a first step toward examining the differential effects of RAS-MAP kinase pathway gene mutations on measurable cognitive behaviors. Further research is needed to examine other aspects of learning and behavior in NS, and to determine how these characteristics relate to aspects of the medical and genetic history of affected individuals. Establishing genotype–phenotype relations in the neuropsychological realm may help to improve our knowledge of the impact of specific genes on the developing nervous system. However, this line of research has only begun to get underway. Identification of new NS genes and their roles in the RAS-MAP kinase pathway is occurring at a rapid pace, and these advances will continue to add crucial pieces to the puzzle.

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Acknowledgments

The authors are grateful to all of the participating families who made this research possible. Special thanks go to Wanda Robinson and The Noonan Syndrome Support Group (TNSSG) for their support. We thank Dr Susan Ellis Weismer and Dr Robert Nellis for their invaluable advice and suggestions, and Dr Richard Pauli, Dr David Wargowski and Jody Haun at the Waisman Center for their assistance in recruiting participants. The work was supported by NIH grant T32 HD049899-01 and a Royalty Foundation Research Fellowship.