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Effects of Germline Mutations in the Ras/MAPK Signaling Pathway on Adaptive Behavior: Cardiofaciocutaneous Syndrome and Noonan Syndrome

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Abstract

Cardiofaciocutaneous syndrome (CFC) and Noonan syndrome (NS) are two phenotypically overlapping genetic disorders whose underlying molecular etiologies affect a common signaling pathway. Mutations in the *BRAF, MEK1 and MEK2* genes cause most cases of CFC and mutations in *PTPN11, SOS1, KRAS and RAF1* typically cause NS. Although both syndromes are associated with developmental delays of varying severity, the extent to which the behavioral profiles differ may shed light on the different roles these respective genes play in development of skills necessary for everyday functioning. In this study, profiles of adaptive behavior of individuals with CFC and NS who had confirmed pathogenic mutations in Ras/MAPK pathway genes were investigated. Patterns of strengths and weaknesses, age-related differences, and risk factors for difficulties in adaptive skills were assessed. Although genes acting more downstream in the Ras/MAPK pathway were associated with more difficulties in adaptive functioning than genes more upstream in the pathway, several inconsistencies highlight the wide spectrum of possible developmental courses in CFC and NS. Along with clinical and genetic factors, variables such as chronological age, gestational age at birth and parental education levels accounted for significant variance in adaptive skills. Results indicate that there is wide heterogeneity in adaptive ability in CFC and NS, but that these abilities are correlated to some extent with the specific disease-causing genes.

Keywords

Noonan syndrome; cardiofaciocutaneous syndrome; *PTPN11*; *SOS1*; *BRAF*; *KRAS*; *RAF1*; *MEK1*; *MEK2*; adaptive behavior

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INTRODUCTION

Cardiofaciocutaneous syndrome (CFC) and Noonan syndrome (NS) are multiple congenital anomaly disorders with similar clinical features including heart disease, dysmorphic facial features and growth delay. Both CFC and NS belong to a group of phenotypically related syndromes that also includes Costello syndrome, LEOPARD syndrome and neurofibromatosis type 1. The recent discovery that the genes causing these syndromes encode proteins which function in a common signal transduction pathway has explained much of the phenotypic overlap. These syndromes are caused by mutations in genes acting within the Ras/mitogen-activating protein kinase (MAPK) signaling cascade, a metabolic pathway whose functions include regulating growth factors and embryological development [Schubbert et al., 2007]. The majority of germline mutations in this pathway result in increased signal transduction [Gelb and Tartaglia, 2006; Tidyman and Rauen, 2008].

Variation across the Ras/MAPK pathway syndromes suggests that different gene mutations affecting this pathway can have markedly different developmental effects. Generally, a NS phenotype results from mutations in the *PTPN11, SOS1* and *RAF1* genes [Pandit et al., 2007; Razzaque et al., 2007; Roberts et al., 2007; Tartaglia et al., 2001; Tartaglia et al., 2007], which encode upstream components of the Ras/MAPK pathway, whereas a CFC phenotype results from mutations in *BRAF, MEK1* and *MEK2* [Niihori et al., 2006; Rodriguez-Viciana et al., 2006], which function more downstream in the cascade. However, there remains some ambiguity in nosological classification of some individuals with mutations in the Ras/MAPK pathway. *BRAF* mutations (which are usually associated with CFC) have been reported among a few patients with a clinical diagnosis of NS or LEOPARD syndrome [Koudova et al., 2009; Nystrom et al., 2008; Razzaque et al., 2007]. Mutations in the *KRAS* gene result in an intermediate phenotype that is highly variable, and individuals with this mutation may be classified as having either CFC or NS [Nystrom et al., 2008]. Further, there remains a significant percentage of patients with these syndromes for whom the molecular cause is as of yet unidentified. In approximately 30% of clinically defined cases of NS and 10–40% of CFC [Armour and Allanson, 2008; Nystrom et al., 2008], no pathogenic mutation in any of the known genes has been found.

Despite the difficulty in classifying some individuals, it is clear that phenotypic variation both within and across the syndromes is related to genetic heterogeneity. Several genotype-phenotype studies have documented that variation in CFC and NS features such as cutaneous anomalies, heart disease, and neurological development can be at least partially accounted for by genotype [Nava et al., 2007; Pandit et al., 2007; Yoon et al., 2007]. Genotype-phenotype correlations in aspects of behavior and cognition have only very recently begun to be explored in these syndromes [Cesarini et al., 2009; Pierpont et al., 2009; Verhoeven et al., 2008]. Such studies may prove to be extremely relevant to classification, prognosis, and ultimately, behavioral and functional therapies for affected individuals.

Some researchers have questioned whether the presence of intellectual disability can help to differentiate the diagnosis of CFC from NS [Nystrom et al., 2008; Wieczorek et al., 1997]. Studies suggest that the rate of intellectual disability among individuals with a CFC diagnosis ranges from 91 to 100% [Armour and Allanson, 2008; Kavamura et al., 2002; Yoon et al., 2007], with higher rates cited in the studies including only participants with molecular genetic confirmation of their diagnosis. In contrast, results of the three most recent studies of cognitive ability in clinically-diagnosed individuals with NS indicate prevalence rates of intellectual impairment (IQ < 70) between 17 and 23% [Lee et al., 2005; Pierpont et al., 2009; van der Burgt et al., 1999]. Two genotype-phenotype correlation studies of cognitive abilities in individuals with Ras/MAPK pathway syndromes provide

further evidence to support the hypothesis that heterogeneity in intellectual functioning in the Ras/MAPK pathway syndromes can be at least partially accounted for by differences in the mutation or gene involved [Cesarini et al., 2009; Pierpont et al., 2009]. Nevertheless, it is unclear the extent to which these findings can be extended to other aspects of behavioral functioning. Cognitive ability is only one component of the definition of intellectual disability as established by the American Association for Intellectual and Developmental Disabilities (AAIDD) [Luckasson et al., 2002]. Another key component is adaptive behavior.

Adaptive behavior is defined as the collection of conceptual, social and practical skills that are necessary for functioning in everyday life [Luckasson et al., 2002]. Examining adaptive functioning has several advantages over other behavioral measurements. First, adaptive behavior provides a broader view of an individual's abilities, since measurements can be obtained for multiple different domains. Within an individual or a group, strengths and weaknesses across areas such as self-care skills, expressive and receptive communication, interpersonal relations and motor functioning can be ascertained. From this information, therapeutic goals can be identified that capitalize on areas of relative strength. Second, adaptive skills can fluctuate over the life span (unlike IQ, which is thought to be a relatively stable measure). Therefore, it is possible to examine how behaviors can change across different ages in these syndromes. Finally, adaptive skills can be accurately measured indirectly, by interviewing a parent or caregiver. This allows researchers to overcome the practical obstacle of assessing individuals with severe motor problems, difficulties sustaining attention and/or inability to communicate verbally-- limitations which are common in CFC and NS [Cesarini et al., 2009; Horiguchi and Takeshita, 2003; Lee et al., 2005; Yoon et al., 2007].

In the present study we investigated adaptive behavior in a large cohort of individuals with clinical and molecular confirmation of CFC or NS. The purposes of the study were: (a) to identify strengths and weaknesses in adaptive behavior in CFC and NS; (b) to investigate potential medical and environmental risk factors for adaptive behavior difficulties in these populations; (c) to examine how adaptive skills vary across individuals of different ages; and (d) to determine whether some of the variation in adaptive behavior among the Ras/MAPK pathway syndromes can be explained by genotype differences.

MATERIALS AND METHODS

Participants

Individuals were recruited for this study if they had received a diagnosis of CFC or Noonan syndrome from a clinical geneticist. Participants and their primary caregivers signed written informed consents prior to enrollment. Families were enrolled in the study at the 2007 family meetings of the CFC International support group (n = 20) and The Noonan Syndrome Support Group (TNSSG) (n = 12), Children's Hospitals and Clinics of Minnesota (n = 24), Children's Hospital Boston (n = 20), the Waisman Center at the University of Wisconsin (n = 3), and via direct contact from families who learned about our study online via our laboratory website (n = 10). The study was approved by the Internal Review Board (IRB) at each of the participating institutions.

Genetic testing results were obtained from medical records requested from the child's primary physician or geneticist using Health Insurance Portability and Accountability Act (HIPAA) authorizations signed by the families. For the purposes of this study, only individuals for whom a disease-causing mutation was found are reported. Our final cohort included 89 individuals heterozygous for a missense mutation in *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *BRAF*, *MEK1* or *MEK2*. The cohort included 22 individuals who were clinically

diagnosed with CFC (10 males, 12 females). Nineteen individuals with CFC had confirmed mutations in *BRAF*, one individual had a mutation in *MEK1*, and two individuals had mutations in *MEK2*. The remaining 67 individuals were clinically diagnosed with NS (33 males, 34 females). This group included fifty individuals with *PTPN11* mutations, eleven individuals with *SOS1* mutations, three individuals with *RAF1* mutations, two individuals with *KRAS* mutations, and one participant with a *BRAF* mutation. Supplementary Tables I and II display the mutations identified among participants in the study.

Individuals with a clinical diagnosis of CFC syndrome in this study (n = 22) ranged in age from 1 to 21 years (M = 9.33, SD = 4.75 years). Cardiac disease was present among sixteen CFC participants (72%). Eleven participants (50%) were born pre-term (prior to 37 weeks gestation). Parents of all but one individual with CFC syndrome (95%) reported that their child had experienced delays in motor milestones relative to their peer group. Age at first word ranged from 9 months to 42 months (M = 22.05, SD = 10.07 months). Two participants (ages 13 and 21 years) did not produce any spoken language. All participants with CFC had received or were currently receiving special education services such as speech/language therapy, physical therapy, or special assistance in the classroom.

Participants with a clinical diagnosis of Noonan syndrome (n = 67) ranged in age from 1 to 24 years (M = 8.0, SD = 5.33 years). Cardiac disease was present among 60 participants (90%) and pre-term births had occurred among 26 (39%). Motor milestones were reported to be delayed in 51 participants (76%). Age at first word ranged from 6 months to 60 months (M = 15.92, SD = 9.65 months). All participants with NS over 18 months of age at the time of enrollment in the study produced spoken language. The majority of participants with NS (72%) had received or were currently receiving some form of special education services. Cognitive abilities of a portion of this NS cohort (n = 42; 63%) have been described previously [Pierpont et al., 2009].

Procedures

Parents of individuals diagnosed with NS or CFC completed the Vineland Adaptive Behavior Scales, Second edition (Vineland-II): Parent/Caregiver Rating Form. The Vineland-II [Sparrow et al., 2005] provides a reliable, well-standardized assessment of adaptive behavior in three main areas: communication, daily living skills, and social skills. For younger individuals (< 7 years), a fourth subscale assesses motor skills. Each subscale has a mean of 100 and a standard deviation of 15. Domain scores on these subscales are combined to derive an overall Adaptive Behavior Composite.

In addition to this adaptive behavior instrument, data were collected pertaining to several possible risk factors for difficulties in adaptive behavior. These variables include the participants' clinical diagnosis, chronological age, severity of cardiac disease, gestational age at birth, and parental education levels. Parents completed a demographic form to obtain information about the participant's educational and family background. Medical information was requested from the child's physician or geneticist using HIPAA authorizations signed by the families. Based on a review of each participant's medical record by a pediatric cardiologist (MEP), individuals were assigned a rating of medical severity of cardiac disease. The score was based on the Cardiologist's Perception of Medical Severity (CSEV) scale [DeMaso et al., 1991]. This scale indexes cardiac disease severity on a scale of 1 to 5.

Statistical analyses for this study were conducted using the PASW Statistics package version 17.0 (formerly SPSS Statistics).

RESULTS

Profile of adaptive skills in CFC and NS

In order to investigate the pattern of adaptive behavior in individuals with CFC and NS, the syndromes were first analyzed separately based on clinical diagnosis. Overall adaptive functioning in the CFC group (M = 58.50, SD = 12.91, range: 27–76) was significantly lower than expected based on normative data (M = 100, SD = 15), $t(21) = -15.08$, $p < .001$. Scores of male and female participants with CFC did not differ significantly, $t(20) = .03$, $p = .97$. Figure 1a displays the scores for the younger CFC participants (ages 1–6) in the communication, daily living skills, social and motor domains. Because scores in the motor domain were only available for this younger group, a comparison of gross motor skills (e.g., crawling, jumping, throwing a ball) with fine motor skills (e.g., drawing, stacking blocks, turning doorknobs) was conducted for these individuals only. Gross motor skills were significantly more delayed among young children with CFC than fine motor skills, $t(5) = -2.77$, $p < .05$. Figure 1b displays the scores for older children and adolescents (ages 7–21) on the remaining three subtests.

Due to the small number of participants in the CFC group as a whole, scores for the communication, daily living skills and social domains were examined across the full age range. A within-subjects ANOVA was conducted to compare scores across these three domains. The multivariate analysis was used to evaluate the significance of the differences between the subtests because the assumption of sphericity was violated (Bartlett's test of sphericity: $\chi^2 = 72.3$, $p < .001$). With a large violation of sphericity the statistical power of multivariate techniques tends to be greater than univariate techniques [Keppel, 1991; Mendoza et al., 1974]. The overall difference among the subtests was significant, Wilks' $\lambda = .501$, $F(2,20) = 9.97$, $p < .001$. Pairwise comparisons with a Bonferroni correction (α-level = .016) were conducted to determine how performance differed across the three domains. Results indicate that individuals with CFC syndrome have significantly stronger abilities in the communication domain than the daily living skills domain, $t(21) = 3.20$, $p = .004$. No other pairwise comparisons reached levels of significance. The communication subscale of the Vineland-II was probed further to determine whether performance differed for production vs. comprehension of language in participants with CFC. Individuals with CFC syndrome showed a marked difficulty with expressive language relative to receptive language, $t(21) = 4.58$, $p < .001$. This difference is consistent with the finding that some individuals with CFC are unable to produce spoken language, but have some receptive comprehension.

Adaptive scores of individuals with Noonan syndrome (M = 88.01, SD = 14.87, range: 62–124) were also significantly lower than the normative population, $t(66) = -6.60$, $p < .001$. Scores of participants with NS did not differ significantly as a function of gender, $t(65) = 1.69$, $p = .10$. Scores of younger children with NS (ages 1–6) were analyzed first. A within-subjects ANOVA comparing performance on the four subdomains indicated a significant difference across the subtests, $F(3,96) = 10.43$, $p < .001$. Pairwise comparisons with a Bonferroni correction (α-level = .008) indicated that motor skills were significantly weaker than communication skills, $t(32) = 3.76$, $p = .001$ and social skills, $t(32) = 4.48$, $p < .001$. Social skills were also significantly better than daily living skills, $t(32) = 3.21$, $p = .003$. Taken together, these analyses indicate that early on in development, individuals with NS tend to have more difficulties with motor and daily living skills than with social and communication skills (Figure 2a). In this young group, social and communication skills did not differ significantly from the normative population ($p > .20$), although daily living skills ($t(32) = -2.89$, $p < .01$) and motor skills ($t(32) = -4.79$, $p < .001$) were significantly delayed. Within the motor domain, gross motor skills were significantly more delayed among young children with NS than fine motor skills, $t(32) = -2.68$, $p < .05$. In the

communication domain, receptive language skills were significantly stronger than expressive language skills, $t(32) = 3.27$, $p < .01$.

Among school-aged individuals and young adults with NS (ages 7–24), a within-subjects ANOVA revealed no significant differences across the three adaptive domains, $F(2,66) = 1.18$, $p = .315$. Thus, the older children and young adults with NS in our cohort did not show an uneven pattern of abilities across communication, daily living skills and social skills (Figure 2b). Adaptive functioning in this group was significantly below the normative population average, $t(33) = -7.17$, $p < .001$. When examining communication skills in this older group, no reliable difference was seen between expressive and receptive language, $t(32) = -.80$, $p = .43$. Thus, older individuals with NS do not show the marked delays in expressive language relative to receptive skills seen in younger children with NS and individuals with CFC. In order to confirm this finding that expressive language becomes more commensurate with receptive language over time in NS, a test of the relationship between chronological age and the difference between receptive and expressive skills (RL-EL) was conducted. Difference scores between receptive and expressive language showed a significant negative correlation with age, $r(66) = -.34$, $p < .01$, such that expressive skills became stronger relative to receptive skills as the age of children with NS increased.

In order to determine whether there was an overall difference in adaptive behavior between the groups diagnosed with CFC and NS, standard scores of the these two groups were compared for the full Vineland-II scale. Individuals diagnosed with NS scored significantly higher in adaptive functioning than individuals diagnoses with CFC, $t(87) = -8.33$, $p < .001$. Note that figures 1 and 2 are depicted on different scales.

Predictors of adaptive functioning in CFC and NS

A multiple regression approach was employed to examine possible risk factors for difficulties in adaptive behavior. Predictor variables included the participant's clinical diagnosis (CFC vs. NS), chronological age (in months), severity of cardiac disease (CSEV rating), gestational age at birth (in months), and socioeconomic status (average of parent's years of education). Genotype was not included as a predictor in this analysis due to low numbers in some genotype groups. Intercorrelations among the predictor variables are listed in Table III. The significant correlations between clinical diagnosis and other predictor variables indicate that: (1) individuals with NS had more severe cardiac disease ratings than individuals with CFC, and (2) individuals in the NS group had a lower rate of premature births (i.e., higher gestational age at birth) than individuals in the CFC group. No other correlations among the predictors were significant.

Results of the regression analysis predicting adaptive skills in NS and CFC are shown in Table IV. Four variables accounted for 62% of the variance in adaptive behavior scores: clinical diagnosis, chronological age, gestational age at birth and parental education. Results corroborated our earlier finding that individuals with a clinical diagnosis of NS had higher adaptive scores than individuals with a CFC diagnosis. On average, participants who were older demonstrated more delays relative to their peers than participants who were younger. Individuals who were born pre-term were at greater risk for adaptive difficulties than individuals who were born on time, and individuals whose parents had more education had higher adaptive scores. The severity of cardiac disease was not a significant predictor of adaptive skills in participants with CFC or NS.

Age-related differences in adaptive behavior

We examined how adaptive skills vary at different ages in our cohort. The relationship between chronological age and raw scores on the Vineland-II were examined first, to

determine whether improvement in adaptive skills could be observed across the age range studied. Across the subtests, raw scores increased significantly with chronological age in both the CFC group, $r(22) = .42$, $p = .05$ and the NS group, $r(67) = .85$, $p < .001$. This indicates that for both syndromes, individuals are likely to develop more sophisticated adaptive skills as they get older. However, the relationship between standardized scores of adaptive behavior and chronological age showed a negative correlation for both the CFC group, $r(22) = -.48$, $p < .05$ and the NS group, $r(67) = -.31$, $p < .05$. These correlations suggest that as children with CFC and NS get older, the gaps between their abilities and the abilities of their typically developing peers may become more pronounced.

Genotype-phenotype analysis

As a first step in understanding gene-behavior correlations within our cohort, adaptive behavior of individuals with mutations in different genes was compared (Figure 3). The sample size and age distribution of participants having mutations in each gene are presented in Table V. Statistical comparisons were limited to the three most common genes (*BRAF*, *PTPN11* and *SOS1*). An ANOVA was conducted to examine whether these three gene groups differed, with the Vineland-II Adaptive Behavior Composite as the dependent variable. The overall group effect was significant, $F(2,78) = 30.75$, $p < .001$. Pairwise comparisons with a Bonferroni correction (α-level = .016) were conducted to examine the group differences. The *BRAF* group had significantly lower adaptive scores than the *PTPN11* group ($t(69) = 7.73$, $p < .001$) and the *SOS1* group ($t(30) = 5.08$, $p < .001$). The *PTPN11* and *SOS1* groups did not differ significantly in adaptive behavior, $t(60) = .71$, $p = .48$.

Several analyses were conducted to compare more specific mutations within each gene. Results for individuals with gene mutations that are typically associated with CFC syndrome were examined first. Standard scores for adaptive behavior among the 20 individuals with *BRAF* mutations ranged from 27 to 86 ($M = 58.35$, $SD = 14.13$). The distribution of adaptive scores subdivided according to each mutation within the *BRAF* gene is shown in Figure 4a. Six of the seven highest scores were obtained by individuals with the p.Q257R mutation. Scores of individuals with the p.Q257R mutation ($M = 65.00$, $SD = 7.84$) were higher than individuals with other mutations ($M = 52.91$, $SD = 16.05$), but the comparison did not reach the cut-off for significance, $t(20) = 2.06$, $p = .54$. When the single *BRAF*-positive participant with a clinical diagnosis of NS (who was an outlier, scoring 2 standard deviations higher than the mean of *BRAF*-positive participants) was removed from this analysis, the group with p.Q257R mutations scored significantly higher than the group with other *BRAF* mutations, $t(19) = 3.20$, $p < .01$. In order to ensure that this effect was not due to chronological age differences among the groups, both age and mutation type (p.Q257R vs. other *BRAF* mutations) were entered into a regression analysis predicting adaptive skills. Mutation type remained a significant predictor of adaptive skills ($\beta = .45$, $p < .05$), with higher scores in the p.Q257R group even after controlling for age. This indicates that among *BRAF*-positive individuals with CFC syndrome, the p.Q257R mutation is likely to have a less severe developmental impact than other mutations.

In order to determine whether performance of individuals with rarer CFC mutations differed from individuals with *BRAF* mutations, a single-case methodology developed by Crawford and Howell [1998] was employed. This approach utilizes modified t-tests to compare an individual's test score to norms derived from a small group of controls. Individuals with a clinical diagnosis of CFC and a mutation in *BRAF* comprised the control group. Scores of the three individuals with *MEK1* and *MEK2* mutations were compared individually to this CFC control group. None of these three patients scored significantly differently from the control group in levels of overall adaptive functioning ($p > .05$). Due to the clinical ambiguity of some *KRAS* cases, scores of individuals with mutations in this gene were also

examined. One of the *KRAS*-positive (p.Y71D) individuals scored significantly higher than the CFC control group, $t(19) = 1.82$, $p < .05$; however, the second *KRAS*-positive (p.T58I) individual did not have a significantly different score ($p > .05$). A final comparison examined whether the participant with NS who had a *BRAF* mutation differed significantly from the CFC control group. This *BRAF*-positive NS participant scored significantly higher than the CFC group, $t(19) = 2.20$, $p < .05$.

The genotypes typically associated with Noonan syndrome were examined next. Wide variation in adaptive behavior scores was seen among individuals with *PTPN11* mutations (Figure 4b). Standard scores ranged from 62 to 124 (M=89.10, SD = 15.37). Previous studies have reported that individuals with the p.N308D mutation in *PTPN11* commonly have average range intellectual functioning and attend regular education classrooms [Pierpont et al., 2009; Tartaglia et al., 2002]. In our cohort, individuals with this mutation ($n = 6$) all had scores indicating average range adaptive skills (M = 96.5, SD = 9.95, range: 87–111). Scores of individuals with the p.Y63C genotype ($n = 4$) were also in the average to high-average range (M = 111.8, SD = 12.25, range: 97–124). Individuals with other more common genotypes ($n \geq 4$) such as the p.N58D mutation (M = 85.7, SD = 7.0, range: 79–95) and the p.M504V mutation (M = 87.5, SD = 16.4, range: 71–104) scored similarly to the *PTPN11* group as a whole. The eleven individuals with *SOS1* mutations had scores ranging from 67 to 111 (M=85.54, SD = 14.49) (Figure 4c). Due to small numbers of individuals with the same mutation in *SOS1*, statistical power was too low to detect significant differences among the different *SOS1* genotypes.

Using the Crawford and Howell [1998] method, single cases of individuals with *RAF1* ($n = 3$) and *KRAS* ($n = 2$) mutations were compared with a control group of individuals with a clinical diagnosis of NS and mutations in *PTPN11* or *SOS1*. None of the scores of the *RAF1* or *KRAS* participants differed significantly from the mean of the NS control group ($p > .05$). The individual with a *BRAF* mutation who had a clinical diagnosis of NS also did not differ significantly in adaptive behavior from the NS control group.

DISCUSSION

This study aimed to better characterize the adaptive behavior of individuals with mutations of the Ras/MAPK pathway. Adaptive behavior is a highly useful construct in investigating developmental disabilities because it provides a measure of the impact of a specific disorder on real world functioning [Mervis et al., 2001]. Indeed, adaptive functioning has been shown to be an even better predictor of placement in special programs and services in individuals with intellectual disabilities than mental age [Futterman and Arndt, 1983]. Our results indicate that individuals with CFC and NS exhibit wide variation in language comprehension and production, social relationships, fine and gross motor abilities, and other skills necessary for daily life. However, several consistent patterns emerged from the data. First, on average, both the CFC and NS groups had significant delays in adaptive behaviors relative to their peers. However, only one-third of participants with a clinical diagnosis of NS scored below the average range, while all of the participants with CFC had significant delays in adaptive skills. Second, in both groups, raw scores for adaptive behavior showed a positive correlation with age, indicating that older individuals had developed more sophisticated adaptive skills than younger individuals. Although longitudinal data would be necessary to examine growth trajectories for individual participants, this initial cross-sectional finding suggests that significant progress over time is likely for most individuals with CFC and NS. However, as they get older, participants with these syndromes may fall farther behind their peers in some adaptive skills, as indicated by the negative correlation between chronological age and standardized scores on the Vineland-II. This finding

highlights the need for ongoing intervention to target difficulties with communication, social and daily living skills that may persist past childhood.

Our research also identified several strengths and weaknesses in adaptive behavior in individuals with CFC and NS. Across the cohort, individuals with a clinical diagnosis of CFC tended to have greater difficulty with daily living skills than with the other domains. This result may reflect the fact that many individuals with CFC have severe physical limitations, and may require assistance to complete activities such as feeding themselves, bathing, and dressing. Although communication was a relative strength for individuals with CFC, production of language was found to be significantly more difficult than comprehension of language. This finding suggests that in order to improve communication abilities, many individuals with CFC syndrome may benefit from augmentative communication strategies such as use of simplified sign language or a picture-pointing paradigm. For those individuals with CFC who can produce spoken language, ongoing speech and language training may prove beneficial for developing expressive skills.

Among participants with a NS diagnosis, the pattern of abilities differed in younger and older individuals. Younger children with NS (ages 1–6) had more significant delays in the areas of motor skills and daily living skills than in the communication and social domains. This suggests that young children with NS may benefit greatly from physical and occupational therapies to overcome everyday challenges in these areas. Children at these ages also had more difficulty with expressive language relative to their receptive language abilities. However, our results indicated that receptive and expressive skills did not differ among older individuals with NS (ages 7–24). This finding is supported by a recent study reporting no difference between expressive and receptive language skills on standardized behavioral assessments of language in 4–18 year-old individuals with NS [Pierpont et al., in press]. A correlational analysis confirmed the finding that expressive skills increased relative to receptive skills as the age of individuals with NS in this study increased. Interestingly, this pattern of better skills in expressive language relative to receptive language over time is the opposite pattern that is seen in individuals with some other developmental disorders like Down syndrome, where weaknesses in expressive language tend to become increasingly pronounced as children get older, rather than becoming more commensurate with other abilities [Miller, 1992].

In addition to investigating the profile of adaptive abilities in CFC and NS, we also examined several potential predictors of adaptive functioning. These analyses indicate that, not surprisingly, both medical and environmental variables contribute to development of adaptive skills (Table IV). A regression analysis indicated that individuals with a diagnosis of CFC are at greater risk for adaptive difficulties than those with NS, and the delays in adaptive behavior relative to age mates may increase as affected individuals get older. In addition, gestational age at birth was a significant predictor of adaptive functioning, such that children born pre-term were at a higher risk for adaptive delays than those born full-term. Finally, individuals whose parents had higher education levels were at slightly less risk for adaptive difficulties, indicating that home environment can contribute to development of important functional skills.

The genotype-phenotype analyses conducted in this study lend further support to previous reports [Cesarini et al., 2009; Pierpont et al., 2009] that aspects of learning and behavior are correlated with the specific genetic mutations of the Ras/MAPK pathway. On average, participants in this study with mutations in *BRAF*, *MEK1* and *MEK2* had more impairment in adaptive behavior than participants with mutations in more upstream components of the pathway. However, specific findings of this study reveal a more nuanced story. First, it is important to highlight the wide variation in abilities observed among individuals with

mutations in the same gene. Scores of individuals with mutations in *PTPN11*, which constituted the largest group, ranged across greater than 4 standard deviations of the normative spectrum (62 standardized points). Hence, much of the variation in adaptive behavior must be explained by factors other than the gene where a mutation is located.

Secondly, classification of individuals as CFC or NS based on the gene mutation may result in some inconsistencies with regard to expected adaptive development. Our results confirm the notion that individuals with *RAF1* mutations do not tend to differ significantly in adaptive behavior from individuals with *SOS1* and *PTPN11* mutations, and therefore most likely fit best with others with a NS diagnosis in terms of development in the areas we measured. Likewise, scores of individuals with *MEK1* and *MEK2* mutations did not differ significantly from the mean of *BRAF*-positive CFC participants. Nevertheless, there are a few cases which did not fit neatly with the range of the expected diagnosis. The most striking case of this was the participant with a p.L597V mutation in *BRAF*. This participant (who had been diagnosed with NS) had adaptive functioning in the average range, with abilities that were statistically similar to the NS group but not the CFC group. This finding is consistent with some recent evidence that the spectrum of *BRAF* mutations may include individuals with unimpaired cognitive development. Koudova, Seemanova and Zenker [2009] recently reported on an individual with the p.L245F mutation in *BRAF* who had a clinical diagnosis of LEOPARD syndrome and high-average intellectual abilities. Other studies have reported *BRAF* involvement in NS and LEOPARD syndrome [Nystrom et al., 2008; Razzaque et al., 2007; Sarkozy et al., 2009]. Sarkozy and colleagues [2009] reported *BRAF* mutations among 5 individuals with clinical diagnoses of NS and 1 with LEOPARD syndrome; in this study, one NS patient had the same p.L597V mutation as the participant in our study.

An additional source of nosological ambiguity arises from cases of individuals with *KRAS* mutations. In our study, the two individuals with *KRAS* mutations differed somewhat in their ability, such that one *KRAS*-positive participant scored within the range of the NS group but not the CFC group, and the other *KRAS*-positive participant scored within the range expected for both syndromes. Both participants scored somewhere between the mean of the CFC and NS groups. This observation suggests that description of individuals with *KRAS* mutations as having an intermediate clinical phenotype [Nystrom et al., 2008; Schubbert et al., 2006] may be extended to aspects of learning and behavior. Combined with our finding that adaptive skills can change over time in these syndromes, it is plausible that an individual with a *KRAS* mutation could at different ages demonstrate adaptive skills more commensurate with a NS or a CFC profile, depending on the chronological age at which they are assessed. So few cases have been evaluated that it remains to be seen if there is a wider breadth of ability among larger groups of individuals with *KRAS* mutations.

One limitation of the current study is that (as is the case when utilizing any parent report measure) there is the potential for parents to overstate or understate their child's true ability. This may be especially relevant when parents are administered the Vineland-II using the Parent Rating Form rather than the interview format, as a psychologist or clinician may probe or reframe certain questions during an interview in order to reduce potential bias. However, attempts were made to reduce bias in this study by providing a detailed explanation of the instructions to the families and by following up on items that were unclear. Further, the instrument was administered using the same form for all of the participants in the cohort.

Although individuals with other Ras/MAPK pathway syndromes were not assessed in this study, previous work has been published on adaptive behavior in Costello syndrome and neurofibromatosis type 1 (NF1). In one study of 16 *HRAS*-positive patients with Costello

syndrome (ages 4–19), standardized adaptive behavior scores obtained using the Vineland-II assessment ranged from 51 to 86 (M = 68, SD = 10), with the highest scores in the socialization domain and the lowest scores in the daily living skills and motor domains [Axelrad et al., 2007]. This result is quite similar to the range and profile of scores observed in CFC participants in our study. A study of 30 patients with NF1 (ages 4–15) using a previous version of the Vineland assessment reported a mean score of 81.9 (SD = 21), with no significant differences among the communication, socialization and daily living skill domains [Fisch et al., 2007]. Although there are slight differences between the earlier Vineland assessment and the more current one used in our study, this finding is remarkably similar to the profile of abilities we observed in individuals with NS. Thus, we can tentatively speculate that additional cross-syndrome comparisons will provide further support for the claim that mutations in more upstream components of the Ras/MAPK pathway (including *NF1*) are less deleterious to cognitive and adaptive skill development than more downstream components (including *HRAS*).

In summary, our study is the first to investigate adaptive behavior in individuals diagnosed with CFC and NS. Our results show robust correlations between development of functional skills such as communication and social relationships and the disease-causing genes. However, wide variation in adaptive skills was still observed among individuals with mutations in the same gene, suggesting that non-genetic factors as well as other genetic factors (e.g., genetic background coded by the rest of an individual's genome) contribute greatly to outcomes. Indeed, the significant association of medical and environmental variables with adaptive behavior reveals that much can be done to foster development in these syndromes, regardless of an individual's genotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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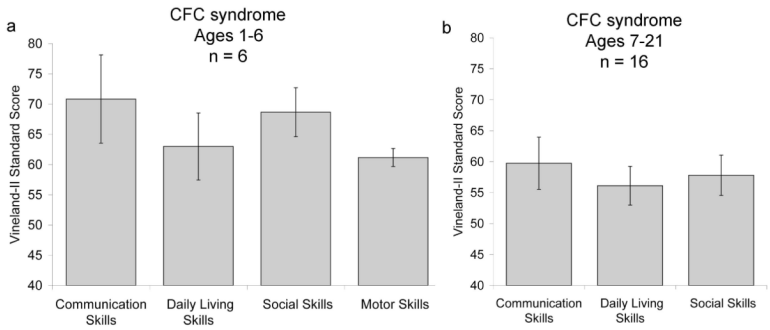

Figure 1.

Pattern of adaptive skills in individuals with cardiofaciocutanous syndrome (a) ages 1–6 and (b) ages 7–21, showing strengths in communication and social skills relative to daily living skills.

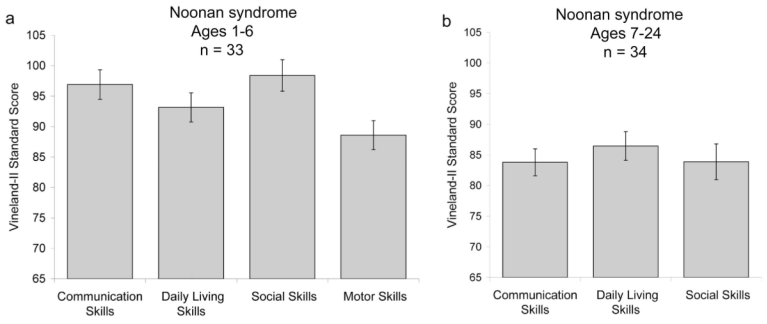

Figure 2.

Pattern of adaptive skills in individuals with Noonan syndrome, showing (a) strengths in communication and social skills relative to motor and daily living skills in individuals ages 1–6, and (b) a flat profile across all domains in individuals ages 7–24.

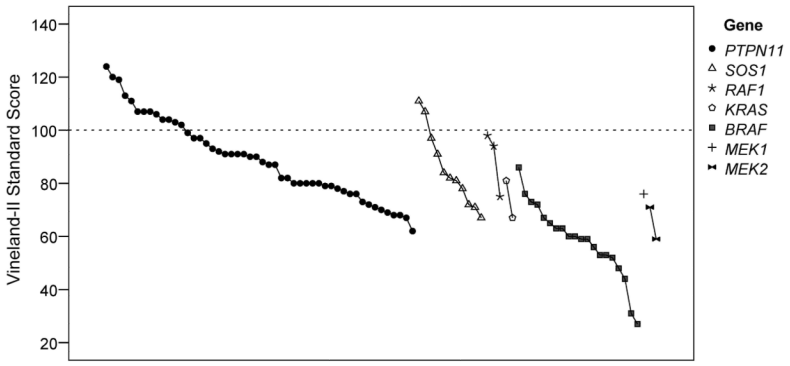

Figure 3.
Distribution of adaptive behavior scores in individuals with CFC and NS, grouped by the Ras/MAPK pathway gene in which a mutation was identified.

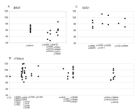

Figure 4.
Plots of adaptive behavior scores in individuals with CFC and NS, grouped according to specific locations where a mutation was identified, in the (a) *BRAF*, (b) *PTPN11* and (c) *SOS1* genes.

Table I

Ras/MAPK pathway mutations in 67 individuals with NS

n	Gene	Exon	Nucleotide Substitution	Amino Acid Change
1	PTPN11	1	C>T	T2I
4	PTPN11	3	A>G	N58D
2	PTPN11	3	A>G	D61G
2	PTPN11	3	G>A	D61N
1	PTPN11	3	T>G	Y62D
4	PTPN11	3	A>G	Y63C
1	PTPN11	3	G>C	E69Q
1	PTPN11	3	A>T	E69V
2	PTPN11	3	G>T	A72S
1	PTPN11	3	C>T	T73I
1	PTPN11	3	G>T	E76D
2	PTPN11	3	A>G	Q79R
2	PTPN11	3	A>C	D106A
2	PTPN11	4	G>C	E139D
1	PTPN11	7	C>T	L261F
3	PTPN11	7	A>G	I282V
1	PTPN11	8	T>C	F285S
6	PTPN11	8	A>G	N308D
2	PTPN11	8	A>G	N308S
1	PTPN11	8	A>C	N308T
1	PTPN11	13	C>T	P491L
2	PTPN11	13	C>T	P491S
1	PTPN11	13	C>T(2)*	P491F
1	PTPN11	13	G>C	G503R
4	PTPN11	13	A>G	M504V
1	PTPN11	13	C>G	Q510E
1	SOS1	6	C>A	T266K
3	SOS1	6	T>G	M269R
1	SOS1	10	G>C	G434R
1	SOS1	10	G>A	C441Y
2	SOS1	10	A>C	S548R
1	SOS1	15	A>T	I733F
2	SOS1	16	G>A	E846K
1	RAF1	7	C>T	S257L
1	RAF1	7	T>C	S257P
1	RAF1	7	C>T	P261S
1	KRAS	3	C>T	T58I
1	KRAS	3	T>G	Y71D
1	BRAF	15	C>G	L597V

* In this individual, two heterozygous missense mutations (C>T) were discovered adjacent to each other (confirmed to be in cis)

Table II

Ras/MAPK pathway mutations in 22 individuals with CFC syndrome

n	Gene	Exon	Nucleotide Substitution	Amino Acid Change
9	BRAF	6	A>G	Q257R
2	BRAF	11	G>A	G469E
1	BRAF	12	A>G	K499E
1	BRAF	12	A>G	E501G
1	BRAF	12	G>A	E501K
1	BRAF	14	A>G	N581D
1	BRAF	15	T>G	F595L
1	BRAF	15	G>T	G596V
1	BRAF	15	C>G	L597V
1	BRAF	15	C>G	T599R
1	MEK2	2	T>G	F57C
1	MEK2	3	A>G	Y134C
1	MEK1	3	A>G	Y130C

Table III

Intercorrelations among predictor variables

Predictor variable	1	2	3	4	5
1. Clinical diagnosis (CFC/NS)	-	−0.12	.28**	.27*	−0.04
2. Chronological age (months)		-	0.06	0.03	0.02
3. Cardiac disease severity (CSEV rating)			-	−0.01	−0.06
4. Gestational age (months)				-	0.03
5. Parental education (years)					-

* $p < .05$

** $p < .01$

Table IV

Standardized regression coefficients for medical and environmental predictors of Adaptive Behavior (Vineland-II) skills among individuals with Ras/MAPK pathway gene mutations

	Beta	*t*	*p*
Clinical diagnosis	.56	6.55	<.001
Chronological age	−.33	−4.30	<.001
Cardiac disease severity	.06	.69	n.s.
Gestational age	.21	2.52	<.05
Parental education	.19	2.52	<.05

n.s. = not significant

Table V

Clinical diagnosis, sample size and age distribution of participants with gene mutations affecting the Ras/MAPK signaling pathway

Clinical diagnosis	Gene	*n*	Age range	Mean age
Noonan syndrome				
	PTPN11	50	1.0–24.5	7.6
	SOS1	11	3.2–16.9	10.3
	RAF1	3	1.7–9.8	4.8
	KRAS	2	1.4–3.8	2.6
	BRAF	1	14.3	14.3
CFC syndrome				
	BRAF	19	1.1–21.6	9.1
	MEK1	1	9.8	9.8
	MEK2	2	10.4–14.3	12.3