Clinical manifestations in patients with *SOS1* mutations range from Noonan syndrome to CFC syndrome

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Abstract Noonan syndrome (NS) and cardio-facio-cutaneous (CFC) syndrome are autosomal dominant disorders characterized by heart defects, facial dysmorphism, ectodermal abnormalities, and mental retardation. There is a significant clinical overlap between NS and CFC syndrome, but ectodermal abnormalities and mental retardation are

more frequent in CFC syndrome. Mutations in *PTPN11* and *KRAS* have been identified in patients with NS and those in *KRAS*, *BRAF* and *MAP2K1/2* have been identified in patients with CFC syndrome, establishing a new role of the RAS/MAPK pathway in human development. Recently, mutations in the son of sevenless gene (*SOS1*) have also

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been identified in patients with NS. To clarify the clinical spectrum of patients with *SOS1* mutations, we analyzed 24 patients with NS, including 3 patients in a three-generation family, and 30 patients with CFC syndrome without *PTPN11*, *KRAS*, *HRAS*, *BRAF*, and *MAP2K1/2* (MEK1/2) mutations. We identified two *SOS1* mutations in four NS patients, including three patients in the above-mentioned three-generation family. In the patients with a CFC phenotype, three mutations, including a novel three amino-acid insertion, were identified in one CFC patient and two patients with both NS and CFC phenotypes. These three patients exhibited ectodermal abnormalities, such as curly hair, sparse eyebrows, and dry skin, and two of them showed mental retardation. Our results suggest that patients with *SOS1* mutations range from NS to CFC syndrome.

Keywords PTPN11 · RAS · Noonan syndrome · Cardio-facio-cutaneous syndrome · RAF

Introduction

Noonan syndrome (NS; MIM#163950) is an autosomal-dominant developmental disorder characterized by facial dysmorphism, including hypertelorism, low-set ears, ptosis, short stature, skeletal abnormalities, and heart defects (Allanson et al. 1985; Mendez and Opitz 1985). Frequently observed features in NS patients are pulmonary stenosis (PS), hypertrophic cardiomyopathy, chest deformities, a webbed and short neck, mental retardation, genitourinary defects, including cryptorchidism in males, and bleeding diathesis due to factor XI deficiency (Mendez and Opitz 1985). The incidence of this syndrome is estimated to be 1 in 1,000–2,500 live births. NS has overlapping features with Costello syndrome and cardio-facio-cutaneous (CFC) syndrome. Patients with Costello syndrome show distinctive coarse facial features, mental retardation, high birth weight, neonatal feeding problems, curly hair, nasal papillomata, loose integuments on the back of the hands, and hypertrophic cardiomyopathy (Hennekam 2003). CFC syndrome is characterized by a distinctive face, mental retardation, heart defects [PS, atrial septal defect (ASD) and hypertrophic cardiomyopathy], ectodermal abnormalities, such as sparse, friable hair, hyperkeratotic skin lesions, and a generalized ichthyosis-like condition (Reynolds et al. 1986; Wiczorek et al. 1997).

The molecular pathogenesis of these syndromes has recently been elucidated. Tartaglia et al. have identified missense mutations in *PTPN11*, a gene encoding protein tyrosine phosphatase (PTP) SHP-2, in 45% of clinically diagnosed NS patients (Tartaglia et al. 2001). In 2005, we discovered *HRAS* germline mutations in patients with Costello syndrome (Aoki et al. 2005). This finding was a

clue for the identification of mutations in *KRAS*, *BRAF*, and *MAP2K1/2* in CFC syndrome (Niihori et al. 2006; Rodriguez-Viciano et al. 2006; Narumi et al. 2007). Mutations in *KRAS* have also been identified in patients with NS (Schubbert et al. 2006). These findings suggest that dysregulation of the RAS/RAF/MEK/ERK pathway causes NS and related disorders, and thus it has been suggested that these syndromes be comprehensively termed the RAS/MAPK syndromes (Aoki et al. 2008). Recently, gain-of-function mutations in the son of sevenless (*SOS1*) gene have been identified in 17–21% of patients with NS (Roberts et al. 2007; Tartaglia et al. 2007). However, mutations in *SOS1* have not been identified in patients with clinically diagnosed CFC syndrome, and the clinical spectrum of patients with *SOS1* mutations has not been clarified.

In this study, we analyzed the *SOS1* gene in 24 patients with NS and 30 patients with CFC syndrome without mutations in *PTPN11*, *HRAS*, *KRAS*, *BRAF*, and *MAP2K1/2*. Clinical manifestations in patients with *SOS1* mutations were examined.

Methods

Patients

Twenty-one sporadic NS patients and three patients from a large family without *PTPN11* mutations were recruited (Niihori et al. 2005) and this study). Also, 30 patients with CFC syndrome were recruited: 18 patients from the previous studies (Niihori et al. 2006; Narumi et al. 2007) and 12 newly referred patients with CFC syndrome. The first diagnoses by clinical geneticists were accepted. In *SOS1*-positive patients, clinical diagnoses were re-evaluated by clinical geneticists (K.N., H.O., and A.V.). Coding exons with flanking intronic sequences in *HRAS*, *KRAS*, *BRAF*, and *MAP2K1/2* were amplified and sequenced as previously described (Niihori et al. 2006; Narumi et al. 2007). No mutations were identified in any of the patients. Control DNA was obtained from 105 healthy Japanese individuals. Control DNA from 105 healthy Caucasian individuals was purchased from Coriell Cell Repositories. This study was approved by the Ethics Committee of Tohoku University School of Medicine. We obtained informed consent from all subjects involved in the study and specific consent for photographs from three patients from an NS family and two sporadic patients.

Mutation analysis in *SOS1*

Genomic DNA was isolated from the patients' peripheral leukocytes. Each exon with flanking intronic sequences in *SOS1* was amplified with primers based on GenBank sequences (Table 1, GenBank accession no. NC_000002.10).

Table 1 Primers used in this study

Gene	Exon	Forward	Reverse	Product length (bp)	Annealing temperature (°C)
<i>SOS1</i>	1	5'-F-ctgttccgcgctgcgagc	5'-R-gcagaccgggaagaaagacg	319	56
	2	5'-F-tgcccgccacaacccac	5'-R-ccctgttctactgacattacaacc	323	58
	3	5'-F-caggatgtgataattcccctag	5'-R-cttctccaccacataaatctctgg	553	58
	4	5'-F-gttgtaagcacaggcctc	5'-R-ctggagatattcccaacac	438	58
	5	5'-F-cagagaacttagagcatttcac	5'-R-gtgcacatgaattaagtcacac	460	58
	6	5'-F-tgcaattgtacacctttgcag	5'-R-agctggaaagaagtaagactc	449	58
	7&8	5'-F-tgtgctcgcatagtcgtgcc	5'-R-cactaatgtgcagggtactcac	512	58
	9	5'-F-gcctgtcacaagataataccc	5'-R-gagggaactgggacccctg	477	58
	10A	5'-F-gtgaataaccttctcagtgcagac	5'-R-ctcttcagctgacttgccag	546	58
	10B	5'-F-ggcagccaagacttctctgtg	5'-R-ggcacaataaacctatgcagg	545	58
	11	5'-F-gtccaaagccttctacttggc	5'-R-gaaaaggatcttagctcaatctc	324	58
	12	5'-F-gactgtgaaaacgtttgtgg	5'-R-ctctctgtttggaaagggtcc	328	58
	13	5'-F-caaacaaggagaggggtgac	5'-R-tactgagcccaatgacatc	452	58
	14	5'-F-tcagggtgtcatccgtgtgac	5'-R-aaccctataaggcagaaatcag	436	56
	15	5'-F-gtttcacagaccttctgttgg	5'-R-gacagagcaaaactccgtctc	395	58
	16	5'-F-ttatactatcgccacccccta	5'-R-ctactgaaaagacaaatgag	422	60
	17	5'-F-gggcgtttctgttagcctag	5'-R-ttctaaagggtctcaggtgc	378	60
	18	5'-F-ggcaactgagatgttacagtg	5'-R-ctcccataaaaataaacctgcc	393	56
	19	5'-F-ggcagggtttatttataggggag	5'-R-ggaagtgggataattcctggac	455	56
	20A	5'-F-tagctgaattttaccaggcac	5'-R-ctgtggtactggaagcaccag	338	58
	20B	5'-F-ccacacctctgcagcaggag	5'-R-gttgtgggagtttagaatttgtc	392	58
	21	5'-F-cttctcaaaagtaagtagtaa	5'-R-gtaccatgctgccagacc	347	59
	22	5'-F-gtctgcatgcttttatggcag	5'-R-gagaactaaactagacagccg	415	56
	23A	5'-F-tagcatcctgccaatagcatg	5'-R-gtccgtgaggagaagggtg	436	56
	23B	5'-F-ccaccacgagaacctgtgag	5'-R-gctggcacattcagtgcatcc	479	56

For: 5'- gtaaacgacggccagt Rev: 5'-aggaaacagctatgacc

The M13 reverse or forward sequence was added to the 5' end of the polymerase chain reaction (PCR) primers for use as a sequencing primer. PCR was performed in 30 µl of a solution containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTP, 10% (v/v) DMSO, 0.4 pmol of each primer, 100 ng genomic DNA, and 2.5 units of Taq DNA polymerase. The reaction condition consisted of 35 cycles of denaturation at 94°C for 15 s, annealing at indicated temperatures for 15 s, and extension at 72°C for 40 s. The products were gel-purified and sequenced on an ABI PRISM 310 automated DNA sequencer (Applied Biosystems).

Results

Clinical manifestations in three-generation family

The pedigree of the NS family (NS13) is shown in Fig. 1. The proband of this pedigree NS13 (III-2) was found to have a systolic murmur at 29 years of age. She was diagnosed as having NS based on her facial appearance, including hypertelorism, ptosis, downslanting palpebral

fissures, and low-set ears. Webbed neck, cubitus valgus, and pectus deformity were not observed. Her height was 1.4 m (−2.5 SD), and she showed normal psychomotor development. Echocardiography of the heart revealed valvar PS and ASD. The patient underwent open-heart surgery for the PS and ASD. The proband's mother [NS 13 (II-6), 55 years old] and daughter [NS13 (IV-1), 5 years old] were found to have similar facial dysmorphisms, short stature, PS, and ASD. Both patients received open-heart surgery for PS and ASD. Chromosome analysis of NS13 (IV-1) showed a normal 46,XX karyotype. A younger sister of NS 13 (III-2) died soon after birth, but the cause of death was unknown. The proband's grandmother [NS 13 (I-1)] had a similar facial appearance with NS13 (II-6), but medical evaluations were not been performed. Other family members were clinically normal. There was no consanguinity in this family.

Mutation analysis in patients

We analyzed *SOS1* in 24 patients with clinically diagnosed NS and identified two mutations in three patients from the

Fig. 1 The *SOS1* D309Y mutation was identified in three patients in a family. **a** Pedigrees of the family. *Square* male, *circle* female, *open symbol* unaffected, *filled symbol* affected, + mutation positive, –

large family and one sporadic NS patient. In patient NS13 (III-2) in the large family, a G to T substitution at nucleotide 925 in *SOS1*, changing D309 to Y, was identified in the heterozygous form. This mutation was also identified in patients NS13 (II-6) and NS13 (IV-1), but not in ten other family members. The D309Y was not detected in 105 normal control individuals. We identified a c.2536G > A (E846 K) mutation in exon 17 in a patient with NS (NS45, Table 2).

From the group of 30 mutation-negative patients with CFC syndrome, we identified three heterozygous mutations in three patients: a c.1656G > T (R552S) mutation in NS 204, a novel in-frame 9-bp insertion in c.1442_1443 of exon 10 in NS84, and E846 K in NS 211 (Fig. 2). R552S was located in the pleckstrin homology (PH) domain and has been identified in four patients with NS (Fig. 3) (Tartaglia et al. 2007; Zenker et al. 2007a). E846 K in the RAS guanine nucleotide exchange factor (GEF) catalytic domain has been identified in eight NS patients (Fig. 3) (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a). The in-frame 9-bp insertion in c.1442_1443 of exon 10 resulted in three amino-acid insertion (P481_G482insRLP). The insertion was not detected in the patient's mother, but the father's DNA was not available. This insertion was not detected in 105 controls. A c.2999G > A (S1000 N) mutation was identified in a patient with CFC syndrome. The substitution was identified in an unaffected mother and

mutation-negative. Facial appearance and sequencing results of NS13 (II-6) (b), NS13 (III-2) (c), and NS13 (IV-1) (d). The positions of nucleotide substitutions are indicated

may represent SNP or a weak allele (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a). A P655L, which has been reported as a SNP or a weak allele (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a), was found in 3 out of 14 CFC patients from France.

Clinical manifestations of *SOS1*-positive patients

Three patients from the large family had similar facial appearances, PS, ASD, short stature, and normal psychomotor development. Patient NS45 was diagnosed as having NS because of her typical facial appearance, PS, pectus deformity, and normal mental development. Patient NS84 was recruited from original CFC patients (Niihori et al. 2006; Narumi et al. 2007). He has the CFC Gestalt, including round face, coarsening of facial traits, curly hair, and sparse eyebrows, as well as aortic valve stenosis and school delay (Table 2; Fig. 2). Patient NS211 is atypical both for NS and for CFC. He has unusually marked hypertelorism, a very short upturned nose with a wide large tip, and small ears (Fig. 2). He also has clinical features that clearly fit the NS to CFC spectrum, including down slanting of the palpebral fissures, ptosis, a deep philtral groove, and a coarse face. Skin and hair anomalies, including deep palmar creases and dry skin, as well as mental retardation resembled those of CFC patients. The

Fig. 2 *SOS1* mutations and clinical features in sporadic patients. **a** Sequencing result of PCR products from control DNA and genomic DNA of NS 211. The positions of nucleotide substitutions are indicated. Photos of NS211 at 6 months (**b, c**) and at 1 year of age (**d, e**). Facial appearance in NS211 with marked hypertelorism, very short upturned nose with a very large tip, and small ears. **f** Sequencing result of a PCR product from genomic DNA of NS84. The PCR product was subcloned and sequenced. The result showed that there is a 9-bp insertion in one allele, resulting in the three amino-acids insertion. The 9-bp insertion is indicated as rectangles. Photos of NS211 at 4 years of age (**g, h**) and at 6 years of age (**i, j**). Clinical features in NS84 show coarsening of facial traits, curly hair, and sparse eyebrows

Fig. 3 Domain organization and identified mutations in the *SOS1* gene. The functional domains are indicated as follows: *HF* histone-like fold, *DH* Dbl homology, *PH* pleckstrin homology, *HL* helical linker, *REM* Ras-exchange motif, *RASGEF* RAS guanine nucleotide exchange factor, *PRD* proline-rich domains. Mutations detected in this study are shown above the domain structure, and mutations identified in the past studies (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a) are shown below the domain structure

phenotype of patient NS204 was between NS and CFC in the early infantile period (1 year and 4 months). He has sparse hair and eyebrows, but this is not that remarkable in a very young child with fair hair. His face is rather large (more similar to CFC than NS).

Discussion

In this study, we identified a novel 3-amino-acid insertion and three missense mutations in *SOS1* in 2 of 22 (9%) families with NS and 3 of 30 (10%) patients from the group

with CFC syndrome. The mutation detection rate was lower than those reported in previous reports: 12/57 (21%) of cases with NS without *PTPN11*, *KRAS* and *BRAF* mutations (Roberts et al. 2007), 22/129 (17%) patients with NS without *PTPN11* and *KRAS* mutations (Tartaglia et al. 2007), and 28% of patients with NS without mutations in *PTPN11*, *KRAS*, *BRAF* and *MAP2K1/2* (Zenker et al. 2007a). The lower detection rate in our study might be due to the smaller size of our cohorts.

SOS1 mutations were identified in three patients with a CFC phenotype. This is in contrast with a previous report in which no mutations in *SOS1* were identified in CFC patients (Zenker et al. 2007a). Clinical manifestations of NS and CFC syndrome have been found to overlap, but several differences have been reported. CFC patients with *KRAS*, *BRAF*, and *MAP2K1/2* mutations have been found to show a high frequency of mental retardation (100%), sparse, curly hair (96%), and skin problems, including follicular keratosis (60%) and hyperkeratosis (56%) (Narumi et al. 2007). In contrast, the rates of mental retardation (24–35%) and skin problems (2–27%) are lower in NS patients (Wieczorek et al. 1997). Our patient NS84 is compatible with CFC syndrome because of his facial appearance, sparse curly hair, and moderate mental retardation. It is noteworthy that he has aortic valve stenosis, which is atypical of NS (Abadir et al. 2007) and has not been reported in patients with *SOS1* mutations (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a). In

Table 2 Clinical manifestations in patients with *SOS1* mutations

Patient ID	NS13 IL-6	NS13 III-2	NS13 IV-1	NS45	NS84	NS204	NS211
Clinical diagnosis	NS	NS	NS	NS	CFC	CFC/NS	CFC/NS
<i>SOS1</i> mutation	D309Y	D309Y	D309Y	E846K	P481_G482 insRLP	R552S	E846K
Origin	Japan	Japan	Japan	Japan	France	France	France
Age at observation	61 years	35 years	11 years	9 years	8 years	1 year 4 month	2 years 8 month
Sex	Female	Female	Female	Female	Male	Male	Male
Birth weight			2,620 g		3,080 g	3,680 g	4,150 g
Cardiac abnormalities	Pulmonic valve stenosis, ASD	Pulmonic valve stenosis, ASD	Pulmonic valve stenosis, ASD	Pulmonic valve stenosis	Aortic valve stenosis	Pulmonic valve stenosis	Pulmonic valve stenosis, pulmonic artery stenosis
Facial features	Short palpebral fissures, left ptosis, low set/posteriorly rotated ears, bulbous nose, hypoplastic alae nasi, long/flat philtrum	Hypertelorism, downslanting of palpebral fissures, bulbous nose, hypoplastic alae nasi, long/flat philtrum	Hypertelorism, downslanting of palpebral fissures, bulbous nose, hypoplastic alae nasi, long/flat philtrum	Hypertelorism, downslanting palpebral fissure, ptosis, low set ear, low posterior hairline	Downslanting palpebral fissure, ptosis, thick ears, large ear lobes, low-set ears, prominent philtrum	Hypertelorism, downslanting palpebral fissures, posteriorly angulated ears, thick helix low-set ears, ogival palate, moderate ptosis, thick lips coarse face, prominent philtrum, lobules of the ears taken off, bulbous nose	Hypertelorism, downslanting palpebral fissures, posteriorly angulated ears, thick helix low-set ears, ogival palate, moderate ptosis, thick lips coarse face, prominent philtrum, lobules of the ears taken off, bulbous nose
Hair and Skin abnormalities	No	No	No	No	Curly hair, sparse eyelashes and eyebrows, hyperpigmentation, dry skin, 2 café-au-lait patches	Curly hair, 1 café-au-lait patch	Sparse eyebrows, deep palmar creases, dry skin, excess skin hands
Developmental delay/mental retardation	No	No	No	No	School delay	No	Moderate
Skeletal features	No	No	No	Pectus deformity, cubitus valgus	No	Delayed bone age	No
Genitourinary	No	No	No	No	No	Cryptorchidism	Cryptorchidism
Short stature (SD)		146 cm (−2.5 SD)	121 cm (−3.5 SD)	No	123 cm (0 SD)	74.5 cm (−1.5 SD)	91 cm (−2 SD)
Macrocephaly	No	No	No	Yes	No	No	No
Short/broad/webbed neck	No	No	No	No	No	Webbed neck	Short and webbed neck
Others				Broad distal phalanges	Hyperextensible fingers	Polyhydramnios, very narrow external auditory canal	

NS Noonan syndrome, CFC cardio-facio-cutaneous, ASD atrial septal defect, SD standard deviation

two other patients with a CFC/NS phenotype, ectodermal abnormalities are evident, including curly hair, sparse eyebrows, deep palmar creases, and dry skin. In a previous report, keratosis pilaris, curly hair, and macrocephaly in patients with *SOS1* mutations were significantly more prevalent compared with typical NS or NS patients with *PTPN11* mutations, suggesting that clinical manifestations of *SOS1* mutations range from those typical of NS to those apparently resembling CFC syndrome (Tartaglia et al. 2007). Another group did not find *SOS1* mutations in 21 CFC patients (Zenker et al. 2007a). Three patients from the NS group exhibited skin manifestations resembling those shown in CFC syndrome, but they were diagnosed as having NS because of normal psychomotor and mental development (Zenker et al. 2007a). Given that the clinical manifestations of NS and CFC syndrome overlap, it is not surprising that *SOS1* mutations were identified in patients diagnosed as having CFC syndrome. Our results suggest that clinical manifestations in patients with *SOS1* mutations range from NS to CFC syndrome.

All of the *SOS1* mutations reported in NS patients have been missense mutations (Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a). We identified a novel 3-amino-acid insertion in the PH domain in patient NS84 with a CFC phenotype. *SOS1* is a ubiquitously expressed GEF, which is responsible for activation of RAS proteins by catalyzing GDP/GTP exchange (Bouguski and McCormick 1993). *SOS1* is a 150-kDa protein that has Dbl homology (DH) and pleckstrin homology (PH) domains in addition to a conserved catalytic region and a putative RAS-binding domain (Fig. 3). Approximately 50% of *SOS1* mutations identified in patients were located in the PH domain (15/29, Fig. 3). This domain has been shown to play an important role in intramolecular regulation. The DH-PH domain has an autoinhibitory effect against catalytic activity of the RAS guanine nucleotide exchange factor (cdc25) (Roberts et al. 2007; Tartaglia et al. 2007). The R552G mutation in the PH domain is the most common mutation in NS patients (Fig. 3) and has been shown to cause a significant increase of RAS activation and phosphorylation of ERK (Roberts et al. 2007; Tartaglia et al. 2007). Another function of the PH domain is to target the proteins to the plasma membrane through interactions with specific phospholipids (Soisson et al. 1998). The novel three amino-acid insertion (P481_G482insRLP) identified in this study is located in $\beta 3$ – $\beta 4$ loop in the PH domain. Two other mutations (477H:P478L and P478R) in the $\beta 3$ – $\beta 4$ loop of the PH domain have been identified in two sporadic patients with NS (Zenker et al. 2007a). The pocket between the $\beta 1$ – $\beta 2$ and $\beta 3$ – $\beta 4$ loops is not likely to affect the autoinhibitory effect (Sondermann et al. 2004), but potentially serves as the binding site of inositol (1,4,5)-triphosphate (Soisson et al. 1998). Recently, the conserved

basic amino acids in the PH domain including P481 and G482 have been shown to have high affinity and specificity to phosphatidic acid (Zhao et al. 2007). The interaction between the PH domain and phosphatidic acid has been found to be essential for EGF-induced SOS membrane recruitment and Ras activation (Zhao et al. 2007). Structural changes caused by the three amino-acid insertion at P481 would alter the membrane translocation of SOS or Ras activation.

We identified the D309Y mutation in three patients of a family with NS. These patients had very similar clinical manifestations: facial dysmorphisms, short stature, ASD, PS, and normal psychomotor development. Although other skeletal abnormalities, including webbed neck, cubitus valgus, and chest deformity, were not evident, the features satisfied the clinical criteria of NS described by van der Burgt (van der Burgt et al. 1994). Two sporadic patients with a D309Y mutation have been reported (Roberts et al. 2007; Zenker et al. 2007a). Clinical variability among the affected members in a family has been reported in families with NS. Van der Burgt et al. reported clinical heterogeneity in a three-generation Dutch family with NS (van der Burgt et al. 1994). Seven of nine patients in the family showed PS, but two had no congenital heart anomalies. Seven had typical faces, and three had pectus carinatus/excavatum. Linkage analysis of this family revealed that a gene for NS was located on 12q24.1, which is the location of *PTPN11* (Jamieson et al. 1994). Schollen et al. reported a four-generation NS family with a *PTPN11* Q79R mutation (Schollen et al. 2003). The patients in this family showed either NS or CFC phenotype. T411 M and G409A mutations in *PTPN11* were identified in two-generation families with clinical variability (Bertola et al. 2004; Zenker et al. 2007b). These results suggested that NS families with *PTPN11* mutations exhibit clinical variability. Clinical manifestations in eight families with *SOS1* mutations have not been described (Roberts et al. 2007; Zenker et al. 2007a). The present report is the first of a large family having a *SOS1* mutation with minimal clinical variability among affected family members. Our findings indicate that clinical manifestations may be similar among family members in a family with a *SOS1* mutation.

In conclusion, we identified four *SOS1* mutations in a three-generation family and four sporadic patients ranging from NS to CFC phenotype. Clinical manifestations in mutation-positive patients revealed that three patients exhibited ectodermal abnormalities and two had mental retardation. These abnormalities have been frequently observed in patients with CFC syndrome. Mutations in *SOS1* were identified in only 10% of our patients with NS and CFC syndrome. Presently unknown genetic causes for mutation-negative NS and related disorders, including recently identified mutations in RAF-1 (Pandit et al. 2007;

Razzaque et al. 2007), remain to be identified in molecules in the RAS/MAPK cascade.

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