LETTER TO JMG

SOS1 is the second most common Noonan gene but plays no major role in cardio-facio-cutaneous syndrome


Background: Heterozygous gain-of-function mutations in various genes encoding proteins of the Ras-MAPK signalling cascade have been identified as the genetic basis of Noonan syndrome (NS) and cardio-facio-cutaneous syndrome (CFCS). Mutations of SOS1, the gene encoding a guanine nucleotide exchange factor for Ras, have been the most recent discoveries in patients with NS, but this gene has not been studied in patients with CFCS.

Methods and results: We investigated SOS1 in a large cohort of patients with disorders of the NS–CFCS spectrum, who had previously tested negative for mutations in PTPN11, KRAS, BRAF, MEK1 and MEK2. Missense mutations of SOS1 were discovered in 28% of patients with NS. In contrast, none of the patients classified as having CFCS was found to carry a pathogenic sequence change in this gene.

Conclusion: We have confirmed SOS1 as the second major gene for NS. Patients carrying mutations in this gene have a distinctive phenotype with frequent ectodermal anomalies such as keratosis pilaris and curly hair. However, the clinical picture associated with SOS1 mutations is different from that of CFCS. These findings corroborate that, despite being caused by gain-of-function mutations in molecules belonging to the same pathway, NS and CFCS scarcely overlap genotypically.

To identify novel genes for these disorders, we screened additional functional candidates encoding proteins involved in the Ras-MAPK signalling pathway, including SOS1. While this work was in progress, two other groups reported gain-of-function mutations of SOS1 in 17–20% of PTPN11 mutation-negative patients with NS. SOS1 encodes a guanine exchange factor (GEF) for Ras catalysing the conversion of the inactive GDP-bound form of Ras to its active GTP-bound form. The precise mechanisms of SOS1 activation are incompletely understood. Conformational changes within SOS1 allowing Ras to access its allosteric binding site that, in the inactive state, is blocked by an intramolecular interaction involving the Dbl homology–pleckstrin homology (DH-PH) unit are presumed to play an important role. NS-associated SOS1 mutations have been suggested to result in a release of autoinhibition, followed by an increase in GEF activity subsequently leading to enhanced levels of active, GTP-bound Ras. Indeed, by analysing representative mutant SOS1 proteins, these NS-associated mutations were found to cause a gain-of-function effect, as shown by increased Ras and ERK activation in vitro.

PATIENTS AND METHODS

Study population

Ethics approval for this study was obtained from the Ethics Committee of the University of Erlangen-Nuremberg, and informed consent for the genetic analyses was received from patients or their legal guardians.

Our initial study population (group 1) consisted of 85 clinically well-characterised patients with NS and CFCS, who were assessed by experienced clinical geneticists. In total, 53 patients (group 1A) were classified as having NS according to established diagnostic criteria, and 21 patients (group 1B) were given the diagnosis of CFCS, supported by the published CFC index. In 11 patients (group 1C), a clear-cut assignment to one of these two syndromes was not possible. This subgroup comprised six patients with normal mental development suggestive of NS but ectodermal anomalies similar to those seen in CFCS, and five infants classified as “borderline”, as they had a phenotype that could evolve into either NS or CFCS. All study participants had previously tested negative for mutations in the genes PTPN11, KRAS and HRAS. Patients in groups 1B and 1C had been screened for mutations in BRAF, MEK1 and MEK2 with normal results.

In addition, we included a second cohort (group 2), comprising 80 patients referred for molecular diagnosis of NS.

Abbreviations: CFCS, cardio-facio-cutaneous syndrome; DH-PH, Dbl homology–pleckstrin homology; GEF, guanine exchange factor; NS, Noonan syndrome; OMIM, Online Mendelian Inheritance in Man

Research data accumulated during the past few years have significantly contributed to our current understanding that constitutive activation of the Ras-MAPK signalling cascade is most likely the common pathogenetic mechanism underlying Noonan syndrome (NS; OMIM 163950) and two related disorders, cardio-facio-cutaneous syndrome (CFCS; OMIM 115150) and Costello syndrome (OMIM 218040). These disorders share a common pattern of congenital anomalies, including typical heart defects, craniofacial dysmorphism, short stature, skeletal anomalies and varying degrees of mental retardation. PTEN, encoding the protein tyrosine phosphatase SHP-2, which transduces signals from activated growth factor receptors to Ras and other signalling molecules, was first discovered as the major gene mutated in NS. In contrast, CFCS was found to be associated with mutations in any of the genes BRAF, MEK1 or MEK2, encoding components of the well-known B-Raf-MEK-ERK signalling cascade downstream from Ras. Mutations in the KRAS gene have been found in patients with NS and CFCS, and specific mutations in HRAS have been detected in the vast majority of people with Costello syndrome. Nevertheless, no genetic defect has been found in ~30% of patients with CFCS and in ~50% of those with NS until recently.
and tested negative for a PTPN11 mutation. This group contained a considerable number of cases with a mild or atypical phenotype. A PTPN11 mutation detection rate of 18% in the original cohort, from which group 2 was derived, reflects the clinical and genetic heterogeneity in this group.

**Molecular analysis**

DNA specimens obtained from blood cells were screened for SOS1 mutations by direct sequencing of all coding exons (ABI BigDye Terminator Sequencing Kit V.2.1; Applied Biosystems, Weiterstadt, Germany) using an automated capillary sequencer (ABI 3730, Applied Biosystems). Primer pairs and PCR conditions are available on request. In group 2, we restricted analysis to those exons in which mutations have been found in this and previous studies (exons 3, 6–8, 10–14, 16 and 19).11 12 Where mutations were shown to have arisen de novo, we verified declared relationships by genotyping at 10 microsatellite loci for each patient and both parents. PCR products from one patient showing two sequence alterations in exon 10 were cloned in Escherichia coli (TOPO TA Cloning Kit; Invitrogen, Karlsruhe, Germany and One Shot, Invitrogen) to determine whether these alterations occurred on the same allele.

**Structural analysis of novel SOS1 variations**

The potential effects of novel SOS1 variations detected in this study were analysed in more detail using the known three-dimensional structures of SOS111 13 14 15 and the computer program PyMOL (http://www.pymol.org). Figures were prepared using PyMOL and Adobe Photoshop (Adobe Systems Inc., USA).

**RESULTS**

**SOS1 variations and associated phenotypes**

Overall, we found 18 different heterozygous SOS1 sequence variations predicting amino acid changes of the encoded SOS1 protein in 28 unrelated patients, including 25 sporadic cases and 3 patients with a positive family history for NS (table 1). Three of the observed sequence alterations represent possible polymorphisms: the variation c.1964C→R polymorphism detected in 28 unrelated patients, including 25 sporadic cases and 3 patients with a positive family history for NS (table 1).

**Structural assessment of novel SOS1 variants**

We analysed in more detail the potential effects of the novel predicted SOS1 variants F78C and F623I, as well as those of
substitutions at residues Q477 and P478, using the known three-dimensional structures of SOS1.14 15 19 20 The invariant residue F78 is located in the N-terminal histone folds domain. This residue is predicted to interact indirectly with R552, the most commonly mutated residue, and substitutions of F78 may therefore have similar effects to those of mutations at R552. Q477 and P478 are not located in one of the known mutation clusters. We were unable to predict the precise consequences of substitutions of these residues based on structural modelling. Most likely, they do not directly affect the autoinhibited

Table 1  Missense variants of SOS1

<table>
<thead>
<tr>
<th>Exon*</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Domain</th>
<th>Pathogenic change or polymorphism</th>
<th>Number of affected people</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>c.233T→G</td>
<td>F78C</td>
<td>HF</td>
<td>Possible polymorphism</td>
<td>1 sporadic case†</td>
</tr>
<tr>
<td>6</td>
<td>c.806T→G</td>
<td>M269R</td>
<td>DH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>6</td>
<td>c.806T→C</td>
<td>M269T</td>
<td>DH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>7</td>
<td>c.925G→T</td>
<td>D309Y</td>
<td>DH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1294T→C</td>
<td>W432R</td>
<td>PH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1297G→A</td>
<td>E433K</td>
<td>PH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1300G→A†</td>
<td>M269R</td>
<td>DH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1322G→A</td>
<td>C441Y</td>
<td>PH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1431G→T†; 1433C→T‡</td>
<td>F477H; P478L</td>
<td>PH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1433C→G</td>
<td>P478R</td>
<td>PH</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>10</td>
<td>c.1654A→G</td>
<td>R552G</td>
<td>PH-Rem linker</td>
<td>Pathogenic</td>
<td>2 sporadic cases, 1 familial observation*†</td>
</tr>
<tr>
<td>10</td>
<td>c.1655G→A</td>
<td>R552K</td>
<td>PH-Rem linker</td>
<td>Pathogenic</td>
<td>1 sporadic case, 1 familial observation*‡</td>
</tr>
<tr>
<td>10</td>
<td>c.1656G→T</td>
<td>R552S</td>
<td>PH-Rem linker</td>
<td>Pathogenic</td>
<td>1 sporadic case, 1 familial observation*‡</td>
</tr>
<tr>
<td>11</td>
<td>c.1867T→A</td>
<td>F623I</td>
<td>Rem</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>12</td>
<td>c.1964C→T</td>
<td>P655L</td>
<td>Rem</td>
<td>Polymorphism</td>
<td>4 sporadic cases</td>
</tr>
<tr>
<td>13</td>
<td>c.2104T→C</td>
<td>Y702H</td>
<td>Rem</td>
<td>Pathogenic</td>
<td>1 sporadic case</td>
</tr>
<tr>
<td>16</td>
<td>c.2536G→A</td>
<td>E846K</td>
<td>Cdc25</td>
<td>Pathogenic</td>
<td>4 sporadic cases</td>
</tr>
<tr>
<td>19</td>
<td>c.2999G→A</td>
<td>S1000N</td>
<td>Cdc25</td>
<td>Probable polymorphism</td>
<td>1 sporadic case**</td>
</tr>
</tbody>
</table>

HF, histone-like folds; DH, Dbl homology domain; PH, pleckstrin homology domain; Rem, Ras exchanger motif. Novel variants are printed in bold type.
*Exon 1 refers to the exon containing the ATG starting codon; †unaffected mother carries the same variant; ‡novel nucleotide exchange predicting a known missense mutation on protein level; §both sequence changes occurred de novo on the same allele; *affected mother–child duo; **unaffected father carries the same variant.

Figure 1  (A) Domain organisation of the human SOS1 protein. Histone-like folds, Dbl homology (DH), pleckstrin homology (PH), helical PH-Rem linker (orange), Ras exchanger motif (Rem), Cdc25 and polyproline domains are shown along with previously described Noonan syndrome-associated mutations (black, top of the figure) and the novel missense changes identified in this study (red, bottom). Asterisks mark those variants also detected in one of the healthy parents. (B) Partial amino acid sequence alignments of human SOS1 with its orthologues of different species and human SOS2. Amino acids surrounding the four novel altered amino acids (indicated in red) are shown.
conformation. The highly conserved F623 residue is considered to play an important role in stabilising the Rem domain by forming an aromatic interaction with F958. Interestingly, substitution of F623 by glutamate in a SOS1 mutant protein composed of the Rem-Cdc25 module has been shown to decrease exchange activity.24 Substitution of F623 by isoleucine presumably also abolishes aromatic interaction between F958 and F623 and may therefore lead to a reduced guanine nucleotide exchange activity of SOS1 (see supplementary information for further details; available online at http://jmg.bmj.com/supplemental).

DISCUSSION

By screening genes encoding proteins involved in the Ras-MAPK signalling pathway we have independently discovered SOS1 mutations in patients with classic features of NS (group 1A) and people with NS with more florid ectodermal symptoms and signs (group 1C). Taking these two subgroups of our core study population (group 1) together, our mutation detection rate reaches 28% among clinically well-characterised patients who lacked mutations in the previously reported NS and CFCS genes. This proportion exceeds those in previously published cohorts (Tartaglia et al 17%; Roberts et al 21%).11 12 In aggregate, the two published studies and our study establish SOS1 as the second major gene for NS. A SOS1 mutation detection rate of 5% in group 2 corresponds to the low PTPN11 mutation rate of 18% in this heterogeneous cohort. It is evident that established clinical criteria for NS are valid for patients with SOS1 mutations, as only one patient with an SOS1 mutation did not meet strict diagnostic criteria. Thus, our findings do not support SOS1 as an important gene for people presenting with a mild or even atypical NS phenotype.

Table 2

Clinical manifestations in patients with Noonan syndrome with SOS1 and PTPN11 mutations

<table>
<thead>
<tr>
<th>Patients with:</th>
<th>SOS1 mutations</th>
<th>PTPN11 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present study</td>
<td>Tartaglia et al</td>
</tr>
<tr>
<td>Pulmonic stenosis</td>
<td>20/25</td>
<td>10/16</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>1/25</td>
<td>2/16</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>4/25</td>
<td>4/16</td>
</tr>
<tr>
<td>Thorax deformity</td>
<td>17/24</td>
<td>16/16</td>
</tr>
<tr>
<td>Easy bruising</td>
<td>3/25*</td>
<td>5/16</td>
</tr>
<tr>
<td>Mental retardation/need for special education</td>
<td>5/24*</td>
<td>1/16</td>
</tr>
<tr>
<td>Short stature (&lt;3rd centile)</td>
<td>13/25</td>
<td>2/15</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>5/11*</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, no data.
*p<0.05 (Fisher’s exact test); †combined data from four large studies: Tartaglia et al,11 Musante et al,22 Zenker et al,12 and Jongmans et al; ‡data available only from two of four studies.

Figure 2

Facial appearance of 13 representative patients with Noonan syndrome (NS) carrying SOS1 mutations. The respective missense mutation is indicated below and the clinical assignment above each photograph. The female patient classified as having “mild NS” presented only with short stature and mild facial anomalies. Ichthyosiform skin changes in a patient carrying the mutation E846K are illustrated in the lower right image. Parental/guardian informed consent was obtained for publication of this figure.
SOS1 plays no major role in cardio-facio-cutaneous syndrome

Key points

- We identified mutations of SOS1 in 28% of patients with NS who had previously tested negative for mutations in PTPN11 and KRAS, but not in patients with cardio-facio-cutaneous syndrome.
- Five novel mutations were detected, defining additional mutation hotspots and indicating possible new mechanisms of perturbed SOS1 protein function.
- People with SOS1 mutations commonly show ocular ptosis, curly hair and hyperkeratotic skin, but they seem to have a lower frequency of mental retardation than patients with Noonan syndrome and mutations of PTPN11.

People with SOS1 mutations typically display a distinctive form of NS characterised by frequent ptosis, ectodermal symptoms and generally normal intelligence, an observation that has also been made by Tartaglia et al.12 However, the clinical spectrum is broad (as shown in fig 2) making it difficult to predict the genetic defect of a person with NS based on clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing clinical appearance, thus we will not change our diagnostic strategy of first analysing PTPN11 and KRAS, but not in patients with cardio-facio-cutaneous syndrome.

Differences in patient selection may account for this apparent discrepancy, as several of our patients were recruited from a paediatric endocrinology department where they were seen for growth retardation.

One important finding of this study is the absence of SOS1 mutations in patients with the diagnosis of CFCS, who had tested negative for mutations in the established CFCS genes. Although the ectodermal manifestations in patients with SOS1 mutations are common and may be similar to those typically found in CFCS, the SOS1 phenotype in our study cohort is distinct from CFCS. The most important discriminating feature is the absence of significant mental retardation in patients with SOS1 mutations. Nonetheless, patients with SOS1 mutations may meet published criteria for CFCS.14 This brings into question the usefulness of the “CFCS index” and emphasises the significance of intellectual functioning level in discriminating between NS and CFCS. In young children, however, clinical discrimination between these two conditions, particularly NS caused by SOS1 mutations, is difficult and may even be impossible. In such cases, determination of the molecular defect may have important prognostic implications. SOS1 mutations associated with NS have been shown to result in a gain of function,11 12 consistent with the concept that NS and related disorders are caused by hyperactive Ras and aberrant activation of the B-Raf-MEK-ERK pathway.19 However, to date, few SOS1 mutant proteins have been assessed functionally. This study provides the first hint that NS-associated SOS1 mutations may not uniformly lead to increased GEF activity, but instead may cause a decrease in exchange activity of SOS1, as already suggested by Shannon and Bollag.18 The precise consequences of the F623I mutation remain to be determined by experimental means.

Taken together, our findings reinforce the concept that NS and CFCS scarcely overlap genotypically.27 The pathogenetic basis of this obvious genotype phenotype correlation remains to be elucidated. It has been speculated that the influence of Ras effector pathways other than B-Raf-MEK-ERK or negative feedback mechanisms differentially affecting proximal and distal components may account for the phenotypic differences in patients with NS and CFCS.11 19 These emerging correlations between phenotypes and genotypes in this group of similar disorders is useful in defining new candidate genes for patients with NS or CFCS whose underlying genetic defect has yet to be discovered.

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Electronic database information

- Protein Data Bank, for coordinates of DH-PH-Rem-Cdc25 construct of hSOS1 (molecule B of code 1XD4), ternary Ras-SOS1-Ras-GppNHp complex (1NVW) and Ras-SOS1 (1BKD). http://www.rcsb.org/pdb/home/home.do

Supplementary material is available online at http://jmg.bmj.com/supplemental.

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Parental/guardian informed consent was obtained for publication of figure 2.

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