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ARTICLE

Variants of *SOS2* are a rare cause of Noonan syndrome with particular predisposition for lymphatic complications

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Abstract

RASopathies are caused by variants in genes encoding components or modulators of the RAS/MAPK signaling pathway. Noonan syndrome is the most common entity among this group of disorders and is characterized by heart defects, short stature, variable developmental delay, and typical facial features. Heterozygous variants in *SOS2*, encoding a guanine nucleotide exchange factor for RAS, have recently been identified in patients with Noonan syndrome. The number of published cases with *SOS2*-related Noonan syndrome is still limited and little is known about genotype–phenotype correlations. We collected previously unpublished clinical and genotype data from 17 individuals carrying a disease-causing *SOS2* variant. Most individuals had one of the previously reported dominant pathogenic variants; only four had novel changes at the established hotspots for variants that affect protein function. The overall phenotype of the 17 patients fits well into the spectrum of Noonan syndrome and is most similar to the phenotype observed in patients with *SOS1*-related Noonan syndrome, with ectodermal anomalies as common features and short stature and learning disabilities as relatively infrequent findings compared to the average Noonan syndrome phenotype. The spectrum of heart defects in *SOS2*-related Noonan syndrome was consistent with the known spectrum of cardiac anomalies in RASopathies, but no specific heart defect was particularly predominating. Notably, lymphatic anomalies were extraordinarily frequent, affecting more than half of the patients. We therefore conclude that *SOS2*-related Noonan syndrome is associated with a particularly high risk of lymphatic complications that may have a significant impact on morbidity and quality of life.

Introduction

The RASopathies constitute a group of developmental disorders characterized by short stature, congenital heart defects, variable developmental delay and intellectual disability, and characteristic facial features. Noonan syndrome (NS; MIM #163950) is the most common entity within this group and caused by changes in various genes encoding members or modulators of the RAS/MAPK signaling pathway [1]. *PTPN11*, *SOS1*, and *RAF1* are the three major genes, in which disease-causing variants account for about 65–80% of cases [2, 3]. Heterozygous variants in the gene *SOS2* (MIM 601247) have recently been identified in a small number of patients with NS [4, 5].

SOS1 and SOS2 share 70% amino acid homology [6]. Both function as RAS-specific guanine nucleotide exchange factors (GEFs). GEFs activate RAS by facilitating the release of GDP bound to the GTPase thereby enabling the binding of GTP, which is more abundant in the cytoplasm [7]. SOS1 and SOS2 share a structure characterized by two tandemly arranged histone folds, a Dbl-homology (DH)

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domain, and pleckstrin-homology domain at the *N*-terminus, followed by a RAS-exchanger motif, a CDC25 domain, and a tail containing docking sites for adapter proteins [6].

NS-associated missense changes affecting three different conserved amino acid positions of SOS2, p.Thr264, p.Met267, and p.Thr376, have been previously described in a total of 13 individuals from nien families [4, 5, 8]. All amino acid substitutions are located in the DH domain, and substitutions at p.Thr264 and p.Met267 are predicted to disrupt the inhibitory interdomain bonding network, resulting in an unstable inactive conformation of SOS2 [4]. Similar mechanisms of activation had previously been shown for SOS1 [9]. In functional experiments, the cells expressing SOS2 c.791C>A (p.(Thr264Lys)), c.800T>G (p.(Met267Arg)), and c.1127C>G (p.Thr376Ser)) showed enhanced GEF activity, leading to increased levels of GTP-bound RAS and hyperactive RAS/MAPK pathway signaling [4].

Whereas variants in *SOS1* account for approximately 10% of NS-affected cases, *SOS2* variants are assumed to be less frequent [4, 5]. The phenotype associated with *SOS2* variants has been described as fitting the general NS spectrum and resembling the phenotype of *SOS1*-related NS. Affected individuals more commonly have normal height and cognitive functioning. They frequently present with ectodermal anomalies including keratosis pilaris, sparse scalp hair, and ulerythema ophryogenes [4].

Here, we report on the spectrum of genetic variants and phenotypic features in 17 subjects with *SOS2*-related NS from 15 families. We identified three novel NS-associated *SOS2* variants and documented a high prevalence of lymphatic anomalies in our cohort.

Material and methods

Subjects and phenotyping

Patients with the clinical diagnosis of a RASopathy who were found to carry a pathogenic/likely pathogenic variant in the *SOS2* gene by diagnostic genetic testing and who have not been reported with complete clinical details previously were eligible for this study. We included a total of 17 affected individuals, 13 sporadic cases and two families with two affected individuals each. One of the sporadic patients was previously included in a large multicentric study on dermatological manifestations in NS but no further clinical details were provided there [10]. All other individuals are previously unpublished. Patients were clinically assessed by experienced clinical geneticists. Standardized patient data were collected via the NSEuroNet database (www.nseuronet.com). This database uses a comprehensive online questionnaire to facilitate the collection and improve the standardization of clinical information regarding RASopathies. It has previously been used in other genotype–phenotype correlation studies [11, 12].

Clinical data and samples for genetic testing were obtained from all individuals with informed consent of the patients' parents/legal guardians or the patients themselves, and all studies were performed in accordance with the Declaration of Helsinki and the national legal regulations. Specific written permission was obtained for the use of clinical photographs for publication in this report.

Molecular analysis

SOS2 (NM_006939.3) variants were identified by routine diagnostic testing of the known RASopathy genes using Sanger sequencing, targeted gene panel sequencing, or whole-exome sequencing of DNA extracted from venous blood samples. Where available, DNA samples from parents and additional affected family members were investigated for the variant discovered in the index case in order to demonstrate de novo occurrence or segregation of the variant with the RASopathy phenotype (Table 1). Further details on methodology are available on request.

Results

SOS2 variants

The 17 individuals from 15 unrelated families were found to carry one of seven different pathogenic or likely pathogenic *SOS2* variants in the heterozygous state. In two families, the variant was transmitted from a clinically affected mother to an affected child, while all the others were sporadic cases. All variants were classified according to the ACMG criteria adapted for RASopathies [13] (Table 1). The missense changes c.791C>A p.(Thr264Lys), c.800T>G p.(Met267Arg), c.800T>A p.(Met267Lys), and c.1127C>G p.(Thr376Ser) have previously been reported [4, 5, 8] and were identified in 13 affected individuals from 12 families. The missense change c.791C>G; p.(Thr264Arg) was found in two unrelated patients and the missense change c.800T>C; p.(Met267Thr) was found in one sporadic case. Both are novel changes at the same position of a previously described NS-associated variant [4]. The *SOS2* variant c.798_800delinsCAA; p.(Glu266_Met267delinsAspLys) that was detected in one sporadic case alters two neighboring highly conserved amino acid residues, including p.Met267 a major hotspot for NS-associated variants in

Table 1 *SOS2* variants identified in the study cohort.

DNA	Protein	Number of observations	Reference	ClinVar	gnomAD	REVEL	Variants at corresponding position in SOS1	ACMG criteria[a]	Classification
c.791C>G	p.(Thr264Arg)	2	Previously unreported	Absent	Absent	0.675	c.797C>A; p.(Thr266Lys) (pathogenic)	PS2, PM2, PM5_strong	Pathogenic
c.791C>A	p.(Thr264Lys)	1	[4]	VCV000684626.1: likely pathogenic	Absent	0.713	c.797C>A; p.(Thr266Lys) (pathogenic)	PS1, PM2, PM6, PP3, PP5	Pathogenic
c.798_800delinsCAA	p.(Glu266_Met267delinsAspLys)	1	Previously unreported	Absent	Absent	NA	NA	PM2, PM5_strong	Likely pathogenic
c.800T>G	p.(Met267Arg)	6 (5 families)	[4]	VCV000577079.2: likely pathogenic	Absent	0.919	c.806T>G; p.(Met269Arg) (pathogenic)	PS1, PS3, PM2, PM5, PP3, PP5	Pathogenic
c.800T>A	p.(Met267Lys)	4	[5, 8]	VCV000209092.2: pathogenic	Absent	0.802	c.806T>G; p.(Met269Arg) and c.806T>C; p.(Met269Thr) (pathogenic)	PM2, PM5_strong, PM6, PP3, PP5	Pathogenic
c.800T>C	p.(Met267Thr)	1	Previously unreported	VCV000373114.2: pathogenic	Absent	0.868	c.806T>C; p.(Met269Thr) (pathogenic)	PS1, PM2, PM6, PP3, PP5	Pathogenic
c.1127C>G	p.(Thr376Ser)	2 (1 family)	[4, 5]	VCV000209091.3: pathogenic	Absent	0.368	c.1132A>G; p.(Thr378Ala) (pathogenic)	PS3, PM2, PM5, PM6, PP5	Pathogenic

All *SOS1* and *SOS2* variants mentioned in this table are considered to cause NS in a dominant manner.

NA not available.

[a]All variants were classified according to the ACMG criteria adapted for RASopathies (Gelb et al. [13]).

SOS2 [4]. All variants were submitted to ClinVar (www.ncbi.nlm.nih.gov/clinvar/).

Phenotype analysis

Clinical findings in 17 individuals with a *SOS2* variant (10 females and 7 males) are summarized in Table 2. Overall, the clinical phenotype was characteristic of NS. In all subjects, the craniofacial phenotype has been evaluated by experienced RASopathy specialists (MZ, MT, MCD, and HC) based on personal examination or photographs. In all of them, we classified the craniofacial dysmorphism to be typical of NS. Broad forehead, hypertelorism, downslanted palpebral fissures, ptosis, broad nasal bridge, low-set ears, and short neck were common features in patients with *SOS2* variants (Fig. 1 and data not shown). In 8 of 14 patients, for which appropriate information was available (57%), prenatal abnormalities were recorded. The most common prenatal finding was polyhydramnios ($n = 7$) followed by nuchal edema ($n = 4$) and hydrops fetalis ($n = 2$). In one case, cardiovascular anomalies were detected prenatally. Twelve of fifteen subjects (80%) experienced feeding difficulties in infancy, and one patient required tube feeding for more than one year. Congenital heart defects were present in 12 out of 17 patients (71%), with atrial or ventricular septal defects (ASD/VSD) being the most common (6/17; 35%), followed by hypertrophic cardiomyopathy (HCM) (5/17; 29%) and pulmonary valve stenosis (PST) (3/17; 18%). Short stature (height SD below −2.00; 3rd centile) was found in 4/14 individuals (29%), and three additional individuals had a height below −1.25 SD (10th centile). One patient had received growth hormone treatment. Motor developmental delay was recorded in 5/12 subjects (42%) and was mild in the majority of them. However, two individuals were reported to have substantial motor delay (unaided walking later than 36 months of age). Mild learning or intellectual disabilities were reported in 1/13 (8%) of subjects aged 5 years or older. Patient 5 required special schooling for children with visual disabilities, but had a normal IQ. She had a complex visual impairment (estimated visual acuity of 0.3–0.4 on both eyes) with nystagmus, hypermetropia, asymmetric ptosis, and amblyopia.

Four out of eight male patients (50%) had cryptorchidism. Skin and hair abnormalities were present in 14/17 individuals (82%), with curly hair and keratosis pilaris as the most common findings. Hemangioma was found in four patients and multiple nevi in three. A short, broad, or webbed neck was present in 9/15 (60%) individuals and pectus deformities in 7/15 (47%). In two out of eleven individuals (18%) easy bruising was reported, and one of them had a confirmed platelet dysfunction. Ocular

Table 2 Clinical features of individuals with *SOS2* mutations.

	1a	1b	2	3	4	5	6	7
Gender	Female	Female	Male	Male	Female	Female	Female	Male
Age at last evaluation	2 y 0 m	30 y 0 m	0 y 3 m	44 y 0 m	7 y 1 m	14 y 6 m	27 y 10 m	21 y 10 m
SOS2 variant	c.1127C>G p.(Thr376Ser)	c.1127C>G p.(Thr376Ser)	c.798_800delinsCAA p.(Glu266_Met267delinsAspLys)	c.800T>G p.(Met267Arg)	c.800T>A p.(Met267Lys)	c.800T>A p.(Met267Lys)	c.800T>C p.(Met267Thr)	c.800T>A p.(Met267Lys)
Segregation	Inherited from affected mother	Unknown	Unknown	Unknown	De novo	Unknown	De novo	Unknown
Prenatal anomalies	PH	ND	NE, HF, PH	ND	PH	ND	PH	No
Feeding difficulties	PF	ND	ND	no	PF	GERD, TF > 12 months	PF	PF
Heart defects/ anomalies	VSD, PFO, HCM	HCM	PFO, HOCM	MVA	PST	PST, ASD	No	HCM
Lymphatic anomalies	No	No	CHT	aLE, CHT[a], LC, IL	aLE	aLE	aLE	aLE
Height SD	−0.46	−0.48	ND	−1.26	−4.5 before GH treatment	−0.59	1.75	−1.71
Development	MD	MD, no ID	ND	MD ND, no ID	MD, SD, mild ID	MD, SD, ID	MD ND, SD, no ID (IQ 97)	MD, SD, mild ID
Genito-urinary anomalies	No	No	CRY	CRY, PD	No	No	No	CRY, nephrolithiasis
Skin and hair	CH, HE	CH, KP	HK	CH, KP, MN	No	No	ND	SH, KP, HK, MN, ML, HE
Thoracic anomalies	WSN	Hypermobile joints	SN	TH, SN	TH, SN	TH	TH, SC	TH, SN, achilles tendon contractures
Hemato-oncology and Immunology	ND	Cystic dermoid (leg)	ND	No	No	Frequent respiratory infections, low immunoglobulin levels	EB, autoimmune thyroiditis	Platelet dysfunction, splenomegaly, neurofibroma
Ocular	PT	PT	ND	PT, RE, ST	PT, RE	PT, RE, NY, amblyopia, visual impairment	ND	PT, RE, AS
Other malformations/ anomalies	No	No	Died at 3 m	Depression, hearing deficit (not congenital)	Mild conductive hearing deficit (not congenital)	No	No	Conductive hearing deficit (not congenital, due to recurrent otitis media)

	8	9	10[b]	11a	11b	12	13	14	15
Gender	Male	Female	Male	Female	Male	Female	Female	Female	Male
Age at last evaluation	8 y 0 m	0 y 4 m	13 y 6 m	21 y 0 m	5 y 10 m	55 y 8 m	7 y 8 m	1 y 7 m	26 y 9 m
SOS2 variant	c.800T>G p.(Met267Arg)	c.800T>A p.(Met267Lys)	c.800T>G p.(Met267Arg)	c.800T>G p.(Met267Arg)	c.800T>G p.(Met267Arg)	c.800T>G p.(Met267Arg)	c.791C>G p.(Thr264Arg)	c.791C>A p.(Thr264Lys)	c.791C>G p.(Thr264Arg)
Segregation	De novo	Unknown	Unknown	Unknown	Inherited from affected mother	Unknown	Unknown	De novo	De novo
Prenatal anomalies	No	NE, HCM, PCE, ADV	PH, HF, NE	PH	No	No	No	NE, PH	No
Feeding difficulties	No	PF	PF	PF, FTT	No	PF, FTT	PF, GERD	PF, GERD	PF

Table 2 (continued)

	8	9	10[b]	11a	11b	12	13	14	15
Heart defects/ anomalies	No	PST, HCM, ASD	No	ASD	ASD, VSD	AY	MVA, TVA	VSD	No
Lymphatic anomalies	No	CHT	No	aLE	aLE	No	No	No	aLE
Height SD	0.89	ND	−2.68	−2.20	−1.27	−3.60	−1.04	ND	−1.11
Development	ND	ND	No MD, no SD, no ID	No MD, SD, no ID	No MD, mild SD, no ID	No MD, no SD, no ID	No MD, SD, no ID	No MD	No MD, no SD, no ID
Genito-urinary anomalies	No	Kidney failure	No	No	CRY	PD	No	ND	No
Skin and hair	KP, erythema, dry skin	HE	CH, brittle hair, CAL, dry skin	CH, MN, dry skin	CH, SH	CH, HP	KP	No	CH, KP, hemangioma
Skeletal	SN	ND	SC, hyperlaxity of hands	SC, hyperlaxity with recurrent dislocation of kneecaps	SN	TH, SN, hyperlaxity of hands	No	SN	SN, TH, hyperlaxity of hands
Hemato-oncology and Immunology	No	No	Frequent infections (otitis)	ND	ND	No	No	No	No
Ocular	No	No	AS	No	PT, RE	PT, RE	PT	No	No
Other malformations/ anomalies	Sleep disorder	Died at 4 m	Hepatosplenomegaly, biliary lithiasis	No	Hearing deficit	Hashimoto hypothyroidis, sleep hypopnea, hypertrophic neuropathy	No	Neonatal bowel obstruction	Venous insufficiency (varicose veins left leg)

[a]Chylothorax 6 m after thorax surgery at 28 years of age, treated with 3 weeks of drainage.

[b]Patient was already published in Bessis et al. [10] .

ADV absence of ductus venosus, *aLE* acquired lymphedema, *AS* astigmatism, *ASD* atrial septal defect, *AY* arrhythmias, *CAL* café-au-lait spot, *CH* curly hair, *CHT* chylothorax, *CRY* cryptorchidism, *EB* easy bruising, *FTT* failure to thrive, *GA* gestational age, *GERD* gastro-esophageal reflux disease, *HCM* hypertrophic cardiomyopathy, *HE* hemangioma, *HF* hydrops fetalis, *HK* hyperkeratosis, *HOCM* hypertrophic obstructive cardiomyopathy, *HP* hyperpigmentation, *KP* keratosis pilaris, *LAM* lymphangiomatosis, *LC* lymphcysts, *LD* learning difficulties, *m* months, *IL* intestinal lymphangiectasia, *MD* motor delay, *ML* multiple lentigines, *MN* multiple nevi, *MVA* mitral valve anomaly, *N* none/normal, *NA* not applicable/not available, *ND* no data, *NE* nuchal edema, *NY* nystagmus, *OFC* occipitofrontal head circumference, *PCE* pericardial effusion, *PD* pubertal delay, *PF* poor feeding reported, *PFO* persistent foramen ovale, *PH* polyhydramnios, *PST* pulmonary valve stenosis, *PT* ptosis, *RE* refractive error, *SC* scoliosis, *SD* speech delay, *SH* sparse hair, *SN* short neck, webbed neck, *ST* strabismus, *TF* tube feeding (>4 weeks), *TH* thorax anomalies, *TVA* tricuspid valve anomaly, *VSD* ventricular septal defect, *WSN* wide-spaced nipples, *y* years.

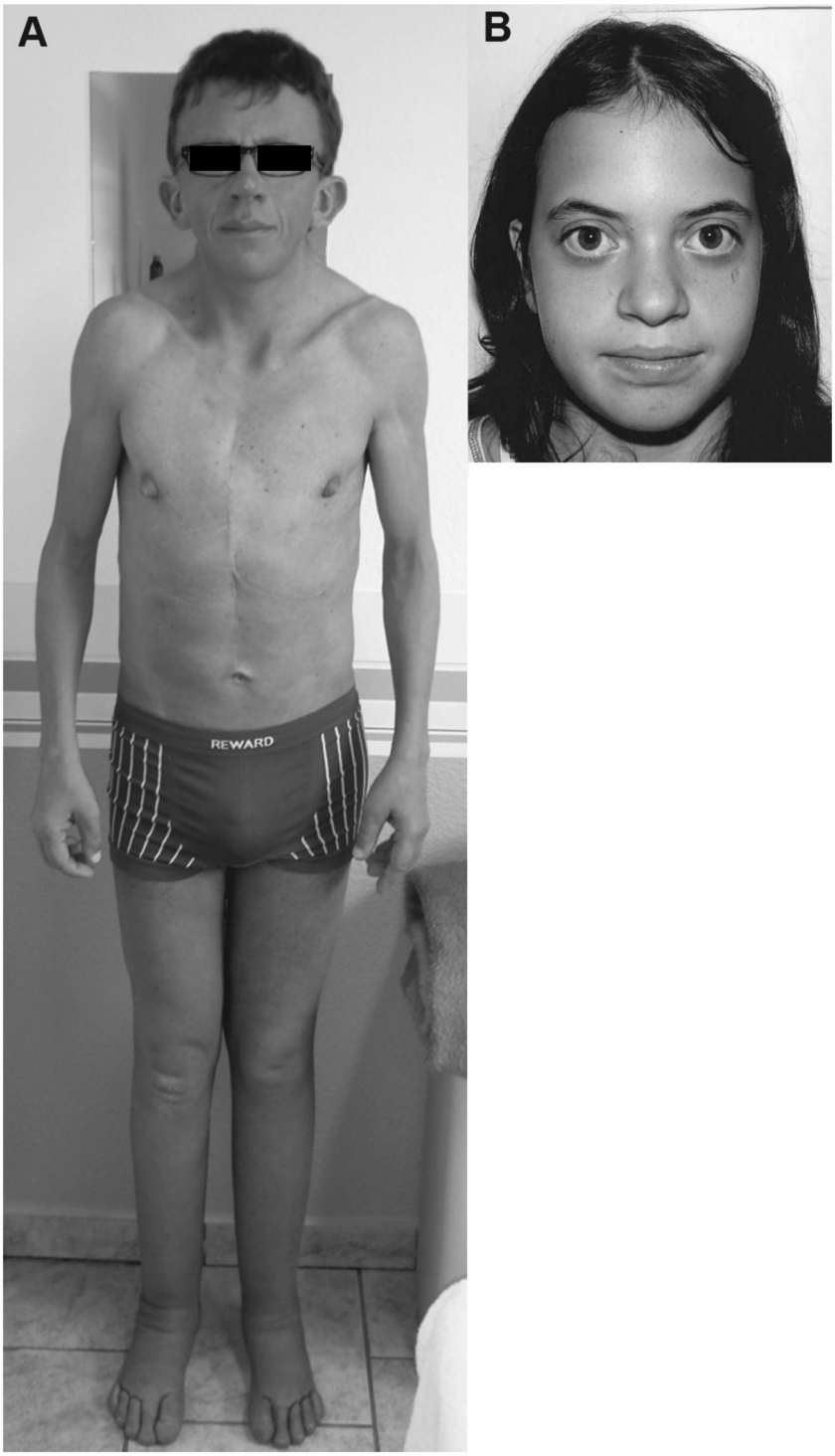

Fig. 1 Clinical photographs documenting the craniofacial and clinical phenotype of patients with SOS2-related NS. A Typical facial features of adult NS, thorax deformity and chronic lymphedema of lower limbs in patient 3 at age 44 years. **B** Craniofacial appearance patient 15 at age 7.7 years.

abnormalities were seen in more than two thirds of the patients (11/14 individuals; 79%), with ptosis being the most common feature observed in nine out of 15 subjects (60%). In two patients benign neoplasias were reported, including a cystic dermoid in the leg (patient 1b) and a neurofibroma (patients 7). Patient 7 additionally had keratosis pilaris, hyperkeratosis, multiple nevi, multiple lentigines, and a hemangioma, but no café-au-lait macules. The neurofibroma was located at right forearm along the course of the posterior branch of the antebrachial cutaneous nerve. No case of malignancy was recorded. Four subjects were reported to have hearing deficits, which were acquired beyond childhood in three of them and of unknown onset in the fourth. The hearing

deficit was severe enough to require hearing aids in two individuals.

Patient 12, a 55-year-old woman, had fusiform hypertrophy of multiple spinal nerve roots in the cervical and lumbar region and hypertrophy of the roots of the cauda equina. At cervical level, nerve hypertrophy extended to the upper limbs. The patient complained of paresthesia of the lower limbs. Neurological examination revealed a distal motor deficit (interosseous muscles, short abductor of the thumb, and foot extensors). Electrophysiological testing revealed a mixed axonal and demyelinating damage on lower limbs.

Postnatal lymphatic anomalies were common and affected 11/17 (65%) of the patients with *SOS2* variants, including two cases with congenital chylothorax and one with persistent chylothorax following thoracotomy. Notably, lymphedema occurring after newborn period, such as lymphedema of lower limbs and genitalia, was found in 8 out of 17 individuals (47%). Localization, age of onset, and treatment of lymphedema, however, were highly variable. Two patients with the most severe expression of lymphatic anomalies are presented in more details below; among them is one individual who passed away in infancy. The lymphatic anomalies contributed to the unfavorable outcome.

Patient 2 (c.798_800delinsCAA; p.(Glu266_Met267delinsAspLys)) was born after a pregnancy complicated by increased nuchal translucency and polyhydramnios. Amniocentesis revealed a normal male karyotype. After birth, facial features of NS were noticed, including downslanted palpebral fissures, low-set ears, and a pterygium colli. He also had cryptorchidism and muscular hypotonia. Cardiac evaluation showed a persistent foramen ovale and a dysplastic pulmonary valve. He later developed hypertrophic obstructive cardiomyopathy, chylothorax, and pleural effusions and died at 3 months of age due to worsening lymphatic problems. Parental DNA samples were not available for testing.

Patient 3 (c.800T>G; (p.Met267Arg); Fig. 1a) developed chronic lymphedema at age 8 years, first presenting in the left leg along with erysipelas. Around the age of 15 or 16 years, the lymphedema slowly spread to the right leg. He was treated with manual lymph drainage and compression garments. Genital lymphedema first developed at the age of 20 years. Six months after surgical correction of the pectus excavatum at 28 years of age, the patient developed chylothorax and was treated with drainage for 3 weeks followed by repeated punctures and ultimately pleurodesis. In addition, the patient followed a medium chain triglyceride diet for 4 years. He developed chronic restrictive pulmonary dysfunction and chronic lymphopenia. The patient also reported intestinal malabsorption and pain suggesting the possibility of intestinal lymphangiectasia, but this has not yet been confirmed. Due to the severity of his lymphatic issues, the patient was unable to work.

Discussion

Herein we present the largest cohort of patients with NS caused by heterozygous pathogenic *SOS2* variants published to date. We identified *SOS2* changes in 17 patients from 15 families. We confirm that variants in *SOS2* are a very rare cause of NS. Based on the number of individuals who had a primary screening for *SOS2* variants with a multigene panel at the collaborating institutions, we calculated that the number of cases with NS caused by mutated *SOS2* is more than 30 times lower than for *PTPN11* and approximately eight times lower than for *SOS1* (Supplementary Table S3). Considering that 40–50% of patients with a definite clinical diagnosis of NS have *PTPN11* variants, this would mean that *SOS2* accounts for less than 2% of cases with definite NS.

In 13 patients from 12 families presented here we identified one of the previously published variants (Table 1) [4, 5]. Four patients were discovered to have a novel change at one of the two known hotspots: c.791C>G (p.Thr264Arg) in two unrelated individuals, c.800T>C (p.Met267Thr) in one, and c.798_800delinsCAA; p.(Glu266_Met267delinsAspLys) in one. All of the previously published and the novel variants described here cluster at codons 264, 267, and 376 in SOS2, predicting amino acid changes in the DH domain [4]. Amino acid substitutions at p.Thr264, p.Met267, and p.Thr376 have been shown to lead to gain of GEF function of SOS2 and increase RAS/MAPK pathway activation, similar to variants in other genes found in NS-affected individuals [4]. The corresponding positions in SOS1 (p.Thr266, p.Met269, and p.Thr378) are also known hotspots for NS-causing missense changes. However, NS-associated missense variants in SOS1 are more widely distributed throughout the protein and not limited to distinct amino acid residues in the DH domain [4].

Phenotype comparison in the present cohort of 17 individuals and in the 13 previously reported individuals with *SOS2*-related NS showed consistent frequencies for most items analyzed (Supplementary Table S1, Supplementary Fig. S1) [4, 5, 8, 14]. Short stature (height below −2.0 SD or 3rd centile) was relatively uncommon (31% in total) compared to its general prevalence in NS. Even when including those patients with a height in the low normal range (height below −1.25 SD or 10th centile) only 50% were affected. A relatively low percentage of subjects with *SOS2*-related NS was recorded to have intellectual/learning disabilities (15% in total).

Remarkably, ectodermal abnormalities such as curly hair and hyperkeratosis were quite frequent in *SOS2*-positive subjects (Supplementary Table S1 and Fig. S1). Cardiovascular abnormalities were seen in about two thirds of individuals with a *SOS2* alteration (Supplementary Table S1 and Fig. S1). No particular cardiac anomaly predominated. Considering a considerable frequency of HCM, however, careful cardiological follow-up has to be recommended also for individuals with SOS2-related NS. Benign neoplasias have been recorded in two individuals from the present cohort but not in published individuals. This discrepancy could be due to small sample size and ascertainment bias. The observation of nerve hypertrophy in one adult individual (patient 12) is compatible with hypertrophic neuropathy that has been reported in a few cases with NS and NS with multiple lentigines before [15, 16].

Acquired lymphatic abnormalities have already been reported in the literature as relatively frequent findings in patients with *SOS2* alterations (50%; Supplementary Table S1, Supplementary Fig. S1) [4, 8, 14]. Of particular note are two recent case reports [8, 14]. Ding et al. reported a 9-year-old girl with chronic bilateral lower limb lymphedema and lymphedema of the vulva [8]. Lymphatic imaging showed primary lymphatic dysplasia. The patient described in detail by Bobot et al. was previously included in the series reported by Cordeddu et al. [4]. He was a 44-year-old man with Charcot–Marie–Tooth disease and symptoms of drug reaction with eosinophilia and systemic symptoms and diffuse lymphadenopathy [14]. Whole body MRI showed mediastinal and retroperitoneal lymphangiomatosis. Both of these reports are in line with the observations in the present cohort indicating that lymphatic anomalies are frequent in patients with *SOS2*-related NS and have significant impact on morbidity and mortality. In 2016, Joyce et al. studied the lymphatic phenotype in RASopathies in eleven individuals with NS [17]. Their cohort included individuals with *PTPN11*, *KRAS*, *BRAF*, and *RIT1* variants, and all patients had bilateral lower limb edema, some with genital involvement. Some of these patients also had lymphangiectasia, pleural effusions, and/or chylous reflux. This spectrum is consistent with the patient cohort presented here and thus seems to represent the lymphatic phenotype of NS. Treatment options have been limited to conventional therapies, like compression garments and a low-fat, high protein high-calorie diet [17, 18]. However, recently trametinib, a MEK inhibitor, was successfully used to treat a 12-year-old patient with severe lymphatic disease caused by a recurrent germline missense variant in *ARAF*, a member of the RAS/MAPK pathway, in which variants have been associated with central conducting lymphatic anomaly (CCLA) without signs of NS [19]. *ARAF*-associated lymphatic disease might be a model for RASopathy-related lymphedema and lessons from this extremely rare condition could also lead to novel treatment options for patients affected by RASopathies. Indeed, CCLA with retrograde flux of lymphatic fluid or abnormal drainage of lymphatic fluid is also a typical finding in patients with NS and lymphatic disease [20]. The nature of the lymphatic anomalies in NS, however, and how the RAS/MAPK pathway affects development or function of the lymphatic system remain to be investigated further.

We also compared the frequency of phenotypic features in our *SOS2*-positive cohort with the respective frequencies in patients harboring a causative variant in other *PTPN11*, *SOS1*, *RAF1*, or *RIT1*, using the data from the NSEuroNet database previously reported by Kouz et al. (Supplementary Table S2, Supplementary Fig. S2) [12]. Statistical analysis confirmed that the prevalence of acquired lymphatic anomalies in *SOS2*-related NS was significantly higher compared to *PTPN11*, *SOS1*, and *RAF1* cohorts. The frequency of keratosis pilaris, one of the typical ectodermal signs of NS, was in the same range as for patients with *SOS1* variants and significantly higher than in individuals with *PTPN11* and *RIT1* variants (Supplementary Table S2, Supplementary Fig. S2). An ectodermal phenotype resembling *SOS1*-associated NS was previously noted in the literature [4, 5]. Similarly, the relatively low frequencies of short stature and intellectual disability in the patients with *SOS2* variant was most comparable to those with *SOS1* variants, although differences to other genes did not reach statistical significance in this analysis. In contrast, the spectrum of heart defects in individuals with a *SOS2* variant differed from those with a *SOS1* variant where PST was more frequently observed. The spectrum of heart defects in *SOS2*-related NS seems to be less distinct as for cohorts with NS of other genetic etiologies (Supplementary Table S2 and Fig. S2).

There is currently no obvious evidence of an increased malignancy risk in individuals with *SOS2*-related NS, but cancer risks in a dimension similar to NS of other genetic etiologies [21] are impossible to identify in the limited number of reported patients. All of the neoplasias observed in individuals with *SOS2* variants of the present cohort were benign. Notably, two individuals were reported to have neurofibromas, a typical tumor entity of neurofibromatosis type 1 (MIM 162200) [22] but rarely observed in other RASopathies. *NF1* was among the genes analyzed in our patient cohort but no variants were identified, thus suggesting that abnormal SOS2 function itself was a main driver for neurofibroma development in these cases. Multiple giant cell lesions of the bone, a quite frequent tumor-like lesion in

SOS1-related NS have not been recorded so far in individuals with *SOS2* mutations [23]. Further studies are required to determine the tumor risk in this particular subgroup of NS patients.

We conclude that *SOS2* variants are a rare cause for NS. The *SOS2*-associated phenotype fits well into the NS spectrum and is most similar to the phenotype associated with *SOS1* variants, especially regarding the prevalence of hair and skin anomalies and a relative low frequency/mild expression of growth and developmental deficits. Heart defects are frequent, but not as specific as observed for the other NS-causing genes. Lymphatic abnormalities, both antenatal and acquired later in life, are frequent and are a significant cause of morbidity and mortality in patients with *SOS2*-related NS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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