Malan syndrome (Sotos syndrome 2) in two patients with 19p13.2 deletion encompassing NFIX gene and novel NFIX sequence variant

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Background and Aim. Sotos syndrome 2 (MIM #614753), known also as Malan syndrome, is caused by heterozygous mutations/deletions of the NFIX gene located on chromosome 19p13.2. It manifests in developmental delay, intellectual impairment, macrocephaly, central nervous system anomalies, postnatal overgrowth, and craniofacial dysmorphism. Unusual behavior with/without autistic traits, ophthalmologic, gastrointestinal, musculo-skeletal, and hand/foot abnormalities are also frequent. Due to the limited number of such cases, no definitive conclusions about genotype-phenotype correlations have been possible. In the following paper, we discuss physical features consistent with Sotos syndrome 2 based on literature review and two new cases (a patient with de novo 19p13.2 deletion encompassing a part of the NFIX gene and a patient with de novo (not described so far) heterozygous missense mutation c.367C>T (p.Arg123Trp) in the NFIX gene).

Results. Apart from overgrowth and psychomotor developmental delay, the most consistent physical features of our two patients are dysmorphism including high forehead, downslanting palpebral fissures, pointed chin, and abnormalities of the pinna. Both show abnormal behavior and present with long, tapered fingers and toenail defect. No severe congenital malformations were noted.

Conclusions. We hope these data will serve as a material for further studies and provide an opportunity to make more reliable genotype-phenotype correlations.

Key words: Malan syndrome, Sotos syndrome 2, NFIX gene, 19p13.2 deletion, NFIX mutation

INTRODUCTION

Aberrations in 19p13.2 (or 19p13.13 according to the hg18) result in diverse clinical phenotypes depending on the size of the affected fragment and involved genes (in large deletions/duplications) as well as type of point mutations. Descriptions of patients presented so far in the literature comprise several cases of chromosomal aberrations in 19p13.2/19p13.13 band reported by Lysy et al., Dolan et al., Malan et al., Bassuk et al., Wangensteen et al., Natiq et al., Schwemmle et al., Klaassens et al., Shimojima et al., and point mutations in NFIX gene (localized within 19p13.2) reported by Malan et al., Klaassens et al., Priolo et al., Yoneda et al., in patients with Sotos-like and Marshall-Smith (MRSHSS) syndromes. Unfortunately, due to the limited number of these cases, no definitive conclusions about the genotype-phenotype correlations can be made.

As patients with 19p13.2 and NFIX gene aberrations have been described in previous papers, we will not present another detailed comparison. Instead our goal is to discuss physical features consistent with Sotos syndrome 2 (Malan syndrome) based on literature review and two new cases. We hope these data will serve as a material for further studies, involving larger group of patients, and hence will provide an opportunity to make more reliable genotype-phenotype correlations.

MATERIAL AND METHODS

After obtaining informed consent, the genomic DNA was extracted from probands’ and their parents’ peripheral blood samples using the automatic method (MagnaPure, Roche).

High-resolution chromosomal analyses were performed using the whole-genome oligonucleotide microarrays, i.e., PerkinElmer CGX™ HD v1.1 v1.1 4-plex (180 K array; hg19) and NimbleGen CGX-3 v1.0 3-plex (720 K array; hg18). Digestion, ligation, PCR, labeling, and hybridization of test and reference DNA were performed according to the manufacturer’s recommendations. The slides were scanned into image files using the MS 200 Microarray Scanner (NimbleGen). Feature extraction and primary data analysis were performed using Agilent CytoGenomics Edition 2.7.22.0 software or NimbleGen...
DEVA software (depending on the type of microarray). Signature Genomics’ Genoglyphix genome browser software was used for detailed analysis and data visualization.

NGS (next-generation sequencing) analysis was performed using TruSightOne Sequencing Panel (Illumina) according to the manufacturer’s instructions. The samples were run on 1/12 of lane each on HiSeq 1500 using 2x75 bp paired-end reads. Bioinformatics analysis was performed as previously described\textsuperscript{12}. Briefly, after initial processing by the CASAVA, the generated reads were aligned to the hg19 reference genome with Burrows-Wheeler Alignment Tool\textsuperscript{13} and further processed by Genome Analysis Toolkit\textsuperscript{14}. Base quality score recalibration, indel realignment, duplicate removal and the SNP/INDEL calling were done as described\textsuperscript{15}. The detected variants were annotated using Annovar\textsuperscript{16} and converted to MS Access format for final manual analyses. In the analyzed probe 76.6% of target was covered minimum 20 times. Alignments were viewed with Integrative Genomics Viewer v.2.3.40 (ref.\textsuperscript{17}).

**Patient NM (Deletion of 19p13.2)**

The girl was referred for genetic counseling because of psychomotor delay. Her family history is unremarkable. She is a first child born at 40 weeks gestation after a pregnancy complicated by preterm contractions, by caesarean section (because of breech position) with 10 points in the Apgar scale, a weight of 3020 g (15th-50th centile), length of 56 cm (>97th centile) and occipitofrontal circumference (OFC) of 36 cm (85th-97th centile) (Table 1).

During further development, delay in language and motor skills as well as poor coordination were noted. She presents poor wound healing, bruising susceptibility, accelerated skeletal maturation (+2.5 years), dental malocclusion, strabismus, and constipation. The craniofacial examination revealed: triangular face with high forehead, hypertelorism, short and downslanting palpebral fissures, smooth philtrum, abnormality of the pinna, narrow mouth with open-mouth appearance, and pointed chin (Fig. 1). Moreover, long and tapered fingers, aplasia/hypoplasia of the palmar creases, clinodactyly of the 4th finger as well as overlapping toe and abnormality of the toenails were observed (all features are listed in Table 2).

She also presents behavioral anomalies (anxiety, tantrums, autoagression), but in general, she is rather a cheerful child. Even though she can speak only a few words (mummy, daddy), she understands much more and executes commands. She also has a preference for water-related items.

<table>
<thead>
<tr>
<th>Table 1. Growth parameters of patient NM (with 19p13.2 deletion, including NFIX gene).</th>
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<tr>
<td><strong>Prenatal growth: 40 Hbd</strong></td>
</tr>
<tr>
<td>Weight</td>
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<tr>
<td>Length / height</td>
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<tr>
<td>OFC</td>
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The previous genetic diagnostics testing: GTG-banding karyotyping, MLPA (Multiplex Ligation-dependent Probe Amplification) for subtelomeric regions (SALSA MLPA P036 and P070 kits; MRC-Holland), MLPA for MECP2, CDKL5, ARX, NTNG1 genes (SALSA MLPA P015 kit) and MS-MLPA toward Angelman syndrome (SALSA MS-MLPA ME028 kit) gave normal results.

PerkinElmer 180 K array revealed deletion within 19p13.2 (with genomic coordinates chr19:13020206-13178390; hg 19).

PerkinElmer 180 K array revealed deletion within 19p13.2 (with genomic coordinates chr19:13020206-13178390; hg 19). of about 158.2 kb, including 7 genes: SYCE2, FARS2, CALR, RAD23A, GADD45GIP1, DAND5, and NFIX (Fig. 2). NimbleGen 720 K array showed deletion of 19p13.13 region (with genomic coordinates chr19:13020206-13178390; hg 19).
Table 2. Clinical features present in Sotos syndrome 2 described in the literature (symptoms observed in both our patients are marked in bold).

<table>
<thead>
<tr>
<th>Development</th>
<th>NM</th>
<th>AB</th>
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<tr>
<td>Deletion of 19p13.2</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NFIX mutation</td>
<td>+</td>
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**Motor retardation**

**Hypotonia**

**Speech delay**

**Mental deficiency**

**Behavioral anomalies**

**Autistic traits**

**Craniofacial features**

**Long / narrow face**

**Downslanting palpebral fissures**

**Hypertelorism**

**Proptosis**

**Epicanthal folds**

**Small mouth**

**Thin upper lip**

**Everted lower lip**

**Prognathia**

**Small nose**

**Short nose**

**Anteverted nares**

**Low nasal bridge**

**High forehead**

**Frontal bossing**

**Complex craniosynostosis**

**Flat occiput**

**Eyes**

**Hypermetropia**

**Strabismus**

**Nystagmus**

**Astigmatism**

**Optic nerve hypoplasia**

**Musculo-skeletal abnormalities**

**Abdominal wall hypotonia**

**Pectus excavatum**

**Coxa valga**

**Scoliosis**

**Advanced bone age**

**Hand / foot abnormalities**

**Long fingers**

**Clinodactyly of the 5th finger**

**Overlapping toes**

**Brain MRI**

**Ventricular dilatation**

**Hypoplasia of the corpus callosum**

**Mild atrophy**

**Chiari I malformation**

**Seizures / EEG anomalies**

**Abnormal EEG**

**Seizures**

**Gastrointestinal abnormalities**

**Chronic diarrhea**

**Abdominal pain**

**Constipation**

**Vomiting**

**Poor feeding**

**Celiac disease**

**FTT (G-tube)**

**Other abnormalities**

**Malformed nails**

**Premature eruption of teeth**

**Generalized livedo**

**Hearing loss**
chr19:12881047-13048119; hg18) of about 167.1 kb, encompassing the same genes (Fig. 3). No other potentially pathogenic losses or gains in other chromosome regions were identified except for known copy number variations. Parental chromosome investigations for the 19p13.2 deletion (array CGH by PerkinElmer) gave normal results, proving the de novo occurrence of this aberration in patient NM.

PATIENT AB (NFIX MUTATION)

The girl was diagnosed because of psychomotor delay. Her family history is unremarkable. She was born after infertility treatment (with intrauterine insemination) at 40 weeks gestation, after an uneventful pregnancy with 10 points in the Apgar scale, a weight of 3350 g (50th-75th centile), length of 56 cm (>97th centile) and OFC of 33 cm (15th-50th centile) (Table 3).

During further development, delay in language and motor skills, muscular hypotonia, broad-based gait with poor coordination, and generalized joint laxity were noted. The craniofacial examination revealed: long face with high and broad forehead, downsloping palpebral fissures, short columella and short philtrum, thin vermilion border, abnormality of the pinna, and pointed chin (Fig. 4). Moreover, long, tapered fingers and abnormality of the toenails were observed. She presents behavioral abnormal-
at least two of the following features: 1. advanced bone age, 2. dysmorphic craniofacial features, 3. congenital malformations led to identification of first two cases with 19p13.1 monosomy. Both patients had advanced bone age, long/narrow faces with high forehead, long fingers, slender habitus, and presented behavioral anomalies with autistic traits. Since the deleted region involved a single common gene, \text{NFI}X (Nuclear Factor I, X-type), it was considered to be a strong candidate in the cause of overgrowth in the investigated patients.

Subsequently, these authors screened for \text{NFI}X mutations 76 patients with unexplained syndromic overgrowth, confirming a heterozygous de novo nonsense mutation in a patient previously diagnosed with Sotos-like syndrome. Moreover, based on the observation of Nfix-deficient mouse model presenting a phenotype similar to Marshall-Smith syndrome, the group screened 9 patients with MRSHSS for \text{NFI}X mutations that allowed for the identification of 7 independent frameshift mutations and 2 different mutations within the donor splice site of exon 6.

Links between \text{NFI}X gene disturbances and phenotype resembling Sotos syndrome were also verified shortly after by Yoneda et al. who identified different heterozygous missense mutations in 2 patients negative for \text{NSD1} mutation. In 2012, Priolo et al. found in frame deletion of \text{NFI}X gene in a patient with overgrowth and suspected a mild form of Marshall-Smith syndrome (i.e. blue sclerae, mild proptosis, slightly bullet-shaped phalanges, and sleep apnea that spontaneously resolved with age) (ref.10). As is now recognized, mutations in the DNA binding/dimerization domain of \text{NFI}X (leading to \text{NFI}X haploinsufficiency) are likely to cause Sotos-like syndrome, while Marshall-Smith syndrome is rather due to mutations with dominant negative effect [mutated RNAs escape nonsense-mediated decay (NMD)] (ref.3,10,11). However, the NMD mechanism pertains exclusively to mutations leading to premature

**Discussion**

In 2010, 244 K oligonucleotide arrays were performed by Malan et al. in 18 patients from non-consanguineous parents with developmental delay, height >95th centile and(or) occipitofrontal circumference >95th centile, and
stop codons, and hence is not expected in our patient AB. As noted by Klaassens et al., variants found in the Marshall-Smith syndrome are frameshift and splice site within 6-8 exons\(^6\). In patient AB, the novel heterozygous missense mutation c.367C>T (p.Arg123Trp) in the DNA-binding/dimerization domain of \(NFIX\) was identified. Arginine 123 is a very highly conserved residue across species (Fig. 6). This substitution is predicted to be disease-causing. Thus it might be assumed that the missense mutation affects DNA-binding/dimerization domain of \(NFIX\) gene leading to its haploinsufficiency.

As was just recently proposed by Klaassens et al., who reviewed cases with Sotos-like overgrowth, a phenotype caused by whole gene deletions, nonsense variants and missense variants affecting the DNA-binding domain may be referred to as Malan syndrome\(^5\). Referring to these authors’ results, we can support the data on the high frequency of strabismus among cases with Sotos syndrome 2, while none of described herein patients present with nystagmus or optic disc pallor/hypoplasia [noted in 25% of cases by review by Klaassens et al.\(^1\)]. In none of our patients pectus excavatum or scoliosis were observed, noted by cited authors as other recurrent features (respectively in 40% and 25% of cases).

As shown in Tables 1 and 3, birth weight was within normal limits in both our patients, and no overweight in postnatal period is observed. On the other hand, in both girls, birth length was above 97th centile, and constantly tends to be within 75th-90th centile. Patient NM (diagnosed with 19p13.2 deletion) was born with larger OFC than patient AB (with point mutation in \(NFIX\) gene) – 36 cm (85th-97th centile) vs. 33 cm (15th-50th centile), what may result from the fact that the deletion also includes other than \(NFIX\) genes. This measurement increased later in patient AB to 50th-75th at the age of 6. The postnatal onset in weight and OFC gain are noteworthy features of Malan syndrome, in contrast to Sotos syndrome (caused by mutation in the nuclear receptor binding SET domain protein 1, \(NSD1\) gene), where the overgrowth is usually in prenatal period and is more significant.

Recognition of Malan syndrome on a clinical basis is however difficult. The differential diagnosis should take into account other overgrowth conditions that may be confused with the Sotos syndrome, such as: Weaver syndrome, Beckwith-Wiedemann syndrome, in males – Simpson-Golabi-Behmel syndrome as well as fragile X syndrome or benign familial macrocephaly in neurologically normal individuals.

Looking at Table 2, where clinical features described in patients with Sotos syndrome 2 are specified, it can be noted that, apart from overgrowth and psychomotor developmental delay, the most consistent physical features of our patients are dysmorphism including high forehead, downsloping palpebral fissures, pointed chin, and abnormalities of the pinna. Moreover, both present long, tapered fingers and abnormality of the toenails. No severe congenital malformations were noted. In general, they share a limited number of dysmorphic features.

On the contrary, they have a similar spectrum of motor abnormalities, such as poor coordination, which is known to be characteristic for patients with Sotos syndrome and \(NSD1\) mutation. Likewise abnormal EEG was noted in both, however only patient with deletion 19p13.2 suffers from seizures. Also both our probands show abnormal behavior, such as aggression, self-injurious behavior and – noted in patient NM – tantrums. In contrast to the observation of Malan et al.\(^3\), no autistic traits were observed in our patient with \(NFIX\) mutation. This is consistent with the observation of Yoneda et al. that missense mutations in the DNA-binding/dimerization domain do not lead to autistic traits\(^11\). Our patients show only some central nervous system anomalies – persistent cavum septum pellicudum and pineal cyst in patient NM and unilateral ventriculomegaly in patient AB.

When we compare the craniofacial appearance of our two probands, their gestalt is consistent with Sotos syndrome 2. However, it is quite clear that patient NM (diagnosed with 19p13.2 deletion) has more pronounced dysmorphism, which, moreover, more closely corresponds to the facial characteristics found in Sotos syndrome 2 (regarding the ocular region, philtrum, and chin).

The clinical status of patient NM is probably influenced by the absence of other than \(NFIX\) genes located within the 19p13.2 region – SYCE2, FARSA, CALR, RAD23A, GADD45GIPI, and DAND5. The \(DAND5\) gene (*609068) encodes a member of the bone morphogenetic protein antagonist family and may play a role in regulating organogenesis, body patterning, and tissue differentiation\(^11\). The \(CALR\) gene (*109091) encodes a chaperone protein mediating the attenuating effect of glucocorticoids in Wnt signalling in osteoblastic cells and thus plays an important role in controlling osteoblastogenesis\(^49\). It is also located in neurons in the human small intestine and therefore might play a role in the gastrointestinal issues\(^2\). The \(NFIX\) gene (*164005), which is known to be essential for normal brain development and is probably required for neural stem cell homeostasis, is also associated with the features observed in our presented patients\(^30\). As for the rest of mentioned genes, based on current data on their function we found no correlation to the phenotype of patient NM.

The \textit{de novo} heterozygous missense mutation c.367C>T (p.Arg123Trp) in \(NFIX\) gene in patient AB affects highly
CONCLUSIONS

This paper provides insight into the phenotype of Sotos syndrome 2, providing new clinical data important for distinguishing patients with NFIX mutations and deletions within 19p13.2 region. We hope that it will help in uncovering the molecular pathway leading to the Sotos-like phenotype and more precise diagnostics based on patients’ clinical features.

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Author contributions: AJS: study design, data interpretation, critically revised the manuscript; MKuc, DJ: data interpretation and analysis; DW, MKug, AC: clinical evaluation of the patients and their parents; EC, RP: data interpretation, critically revised the manuscript; MKW: final approval.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

Study approval: This study was approved by the Bioethics Committee of the Children’s Memorial Health Institute in Warsaw.

REFERENCES