Chapter 5.2

Genome-wide SNP array analysis in patients with features of Sotos syndrome

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Abstract

Background Sotos syndrome is characterized by overgrowth, facial dysmorphism and learning impairment. Haploinsufficiency of NSD1 accounts for approximately 60%-90% of the patients. Consequently, a considerable number of patients with features of Sotos syndrome remain without a molecular diagnosis. To date, target-gene approaches in these patients have not been successful.

Methods Twenty-six Sotos syndrome-like patients were analyzed with a high resolution whole genome SNP array and segregation was studied in the parents.

Results Four possible pathogenic copy-number variants including deletions of 10p12.32-p12.31, 14q13.1, Xq21.1-q21.31 and a duplication of 15q11.2-q13.1 were detected. They varied in size from 155 kb to 13.36 Mb. The 10p12.32-p12.31 deletion revealed a candidate gene (PLXDC2) for overgrowth. The 14q13.1 deletion affected only the NPAS3 gene and the patient carrying this deletion displayed mental retardation as the main feature. The Xq21.1-q21.31 deletion and the 15q11.2-q13.1 duplication encompassed multiple genes of which several could be associated with phenotypic expression.

Conclusion The high resolution genome-wide SNP array approach resulted in a detection percentage of 15% of novel abnormalities and is therefore a powerful method to attain a molecular diagnosis in Sotos syndrome-like patients. Identified candidate genes provide directions for future screening of larger patient cohorts.

Introduction

Sotos syndrome (SoS; MIM #117550]) is an autosomal dominant overgrowth disorder characterized by three cardinal features: height and/or head circumference > +2.0 SDS, facial dysmorphism and learning disability [1]. The typical craniofacial features include macrodolichocephaly, a broad forehead with a receding hairline, a prominent chin and downslanting of the palpebral fissures [2]. SoS is caused by haploinsufficiency of the NSD1 gene at 5q35.2-35.3. The detection percentage varies but overall NSD1 abnormalities are detected in ~60% - 90% of the cases [3]. Therefore, there are a considerable number of patients suspected of SoS but without a molecular explanation. The phenotypic spectrum of these patients is usually broad, varying from a classical SoS phenotype to patients exhibiting only a few Sotos features. The latter group is sometimes referred to as “Sotos-like”[4;5].

In order to identify genetic alterations in SoS patients without NSD1 abnormalities, several gene-targeted approaches have been performed [6-8]. In 78 overgrowth syndrome patients in whom NSD1 abnormalities were excluded, the NSD-gene-family members NSD2 and NSD3 were screened but no aberrations were detected [6]. Furthermore, screening of the NSD1 interacting protein 1 gene (NIZP1) did not reveal any sequence abnormalities in 97 patients referred for NSD1 analysis (Visser et al, unpublished data) and no NIZP1 deletions were detected in 12 typical non-NSD1 SoS patients [7]. In 38 patients with SoS features, a duplication of IGF1R was found in a single patient [8]. Recently, RNF135 (MIM #611358) abnormalities were identified as the cause of a new overgrowth syndrome in combination with learning disability [9]. However, analysis of RNF135 in 160 patients referred for NSD1 screening, did not reveal any alterations [10]. These results render the gene-targeted approach rather unsuccessful in detecting a molecular diagnosis in these patients. Therefore, in this study we have chosen a high resolution genome-wide approach using a Single Nucleotide Polymorphism array (SNP array) with the objective to detect pathogenic copy-number variants (CNVs) in 26 patients with certain features of SoS.
Subjects and methods

Patients
The present study includes 26 patients described previously by de Boer et al. [11] in whom \textit{NSD1} mutations or microdeletions were excluded. From family B of the original study only the proband (nr. 41) was included. From 3 patients (nr. 21, 29 and 52), DNA was no longer available. In patient 17 a duplication of \textit{IGF1R} had been previously found and this patient was therefore excluded from the present analysis [8]. According to the clinical scoring system previously used [11], this study included 3 “typical” SoS patients, 15 from the “dubious” SoS group, and 8 from the “atypical” SoS group. Phenotypic details and results of the clinical scoring system are shown in Table 5.2.1. Approval was obtained from the Medical Ethical Committee of the Leiden University Medical Center and consent was given by the patients and/or their parents or legal guardians. Standard deviation scores (SDS) for growth are expressed in reference to the Dutch population [12] using the Growth Analyser version 3.5 software (http://www.growthanalyser.org/). Weight SDS refers to the SDS for weight corrected for height. Target height was calculated with a correction for the secular trend as described previously [13]. Growth parameters at birth were corrected for gestational age using Swedish references [14].

SNP arrays
Genome-wide high density SNP array screening was performed with HumanCNV370-Duo Genotyping BeadChips (Illumina Inc., Eindhoven, The Netherlands). These chips contain ~318,000 SNP probes and ~52,000 non-polymorphic probes per chip, which results in a median spacing of 1 probe per 5 kb. Procedures were performed according to manufacturer’s protocol (http://www.illumina.com). Fluorescence intensities were read with the BeadArray Reader (Illumina) and data-files were analyzed with the Beadstudio Data Analysis Software Version 3.2.

CNV validation
Copy-number polymorphisms were excluded with the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/). Detected CNVs were mapped to the human genome assembly (NCBI build 36.1) using the UCSC-genome browser (http://genome.ucsc.edu/). They were confirmed using a SNP-array from a different manufacturer, i.e. the GeneChip Human Mapping 500k array Set (Affymetrix Inc., Santa Clara, CA). These chips contain another set of SNP probes, either using the restriction enzyme \textit{NspI} (~262,000 SNPs) or \textit{StyI} (~238,000 SNPs), and are prepared and analyzed following different practical procedures and analysis methods. If available, parental DNA was analyzed as well using the Affymetrix-platform. Practical procedures were conducted following manufacturer’s guidelines (http://www.affymetrix.com). SNP copy-number was assessed using CNAG v2 software [15].

X-inactivation study
X-inactivation was investigated at the Androgen Receptor locus as described previously [16].

Results
Genome-wide SNP array was performed in 26 patients with SoS features. In total we identified five deletions (patients 6, 39, 45, 54, 58) and one duplication (patient 49) which were not found in the DGV. The CNV plots are shown in Figure 5.2.1 and are
Table 5.2.1 Clinical scores for 26 patients diagnosed as having Sotos syndrome and the results of the molecular studies. Adapted from De Boer et al. [11] with permission from Karger AG, Basel.

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Group 1 = Typical Sotos syndrome; Group 2 = Dubious Sotos syndrome; Group 3 = Atypical Sotos syndrome; Fac = Facial characteristics (1 = down-sloping palpebral fissures, 2 = high arched palate, 3 = prominent jaw, 4 = dolichocephaly, 5 = frontal bossing, 6 = high hairline); Gr = Growth; Ba = Bone age; Hc = Head circumference; Dev = Development; Mut = Mutation; Del = Deletion; Dup = Duplication; *Numbering of patients is according to the original article of De Boer et al. [11]; b For the details about the marks used in the clinical scoring system we refer to De Boer et al. [11].
### Table 5.2.2 Identified CNVs by genome-wide SNP array screening

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<th>SNP-end</th>
<th>Start-position</th>
<th>End-position</th>
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<th>Type</th>
<th>Genes</th>
<th>Inheritance</th>
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<td>90759007</td>
<td>13.36Mb</td>
<td>Deletion</td>
<td>25</td>
<td>de novo</td>
</tr>
<tr>
<td>45</td>
<td>10p12.32-p12.31</td>
<td>rs10827906</td>
<td>rs1012086</td>
<td>20262098</td>
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**Likely non-pathogenic CNVs**

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*a* N.D. Not Determined because parental DNA was not available; *b* Only maternal DNA available

### Table 5.2.3 List of Refseq genes involved in the deleted or duplicated regions

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<td>CYSLTR1, ZCCHC5, LPAR4, P2RY10, GPR174, ITM2A, TBX22, FAM46D, BRWD3, NSBP1, SH3BGRL, POU3F4, CYLC1, RPS6KA6, HDX, APOOL, SATL1, ZNF711, POF1B, CHM, DACH2, KLHL4, CPXCR1, TGIF2LX, PABPC5</td>
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<td>TUBGCP5, CYFIP1, NIPA2, NIPA1, GOLGA8E, MKRN3, MAGEL2, NDN, C15orf2, SNRPN, SNURF, UBE3A, ATP10A, GABRB3, GABRA5, GABRG3, OCA2, HERC2, GOLGA8G, FLJ32679</td>
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**Likely non-pathogenic CNVs**

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N.B. Genes are ordered according to their genomic position.
summarized in Table 5.2.2. Table 5.2.3 lists the genes involved per CNV. Based on the size, the *de novo* character and/or the genes involved, the chromosomal alterations identified in patients 39, 45, 49 and 58 were categorized as possible pathogenic.

The deletion in patient 6 was inherited from a phenotypically normal mother suggesting absence of a causal correlation between the deletion and the phenotype in the child. Furthermore, in patient 54 the father (possibly affected) and two of his siblings (normal phenotype) were carrier of the deletion detected. Therefore, the chromosomal imbalances detected in patients 6 and 54 were considered to be likely non-pathogenic and these two patients will not be described here.

In patient 39 a 13.36 Mb deletion was found encompassing Xq21.1-q21.31. The deletion involved 25 Refseq genes and occurred in the paternally derived chromosome. X-inactivation study showed bi-allelic activity, with the paternal chromosome being more active (71% versus 29%). Although chromosome analysis at the age of three had been described as a normal 46,XX karyotype, with the current techniques the deletion was visible in the repeated karyogram (data not shown).

In patient 45 a 550 kb deletion was detected at 10p12.32-p12.31 deleting the last 12 exons, out of a total of 14 exons, of the Plexin Domain Containing 2 (*PLXDC2*) gene. *PLXDC2* encodes a transmembrane protein and is expressed in mouse brain, limbs, spinal cord, dorsal root ganglia, lung buds and heart [17]. Unfortunately parental DNA was not available to test inheritance. In the DGV, variants were reported in four individuals, including loss of exon 1 (variant 9140), loss of exon 2 (variant 2855), loss of exon 2 and 3 (variant 9141) and gain of exon 2 (variant 2855) [18;19].

A 6.53 Mb-sized duplication was found in patient 49 encompassing 15q11.2-q13.1 and harboring 20 Refseq genes. The proximal and distal breakpoints were mapped within two known low copy repeats (LCRs) [20]. Non-allelic homologous recombination between LCRs at 15q11-q13 cause interstitial deletions which account for approximately 70% of the patients with Prader-Willi syndrome or Angelman syndrome [21]. Reciprocal duplications of 15q11-q13 occur less frequent and breakpoints are clustered in the same LCRs as Prader-Willi and Angelman syndromes: BP1 or BP2 for proximal and BP3 for distal breakpoints [22]. The same breakpoints, BP1 and BP3 respectively, were found in our patient.

In patient 58 a 155 kb deletion of 14q13.1 was identified comprising the first exon of the Neural Pas Domain Protein 3 (*NPAS3*) gene, which was also detected in his mother. Two of his brothers were tested and both were confirmed to be non-carriers. To exclude compound heterozygosity, sequence analysis of *NPAS3* was performed in our patient but no pathogenic changes were detected (data not shown). *NPAS3* has two transcript isoforms (NM_022123 and NM_173159) which both include exon 1 and encode for a neuronal transcription factor [23].

Discussion

Studies examining *NSD1* abnormalities in SoS patients have refined the clinical diagnostic criteria which has resulted in high detection rates for *NSD1* abnormalities in classical SoS patients. The diagnostic challenge nowadays lays therefore in the heterogeneous group of patients with a few phenotypic features of SoS, but without *NSD1* abnormalities. With a genome-wide approach four possible pathogenic CNVs were detected in patients previously diagnosed as “dubious” SoS (patients 39, 45, 49) and “atypical” SoS (patient 58) [11]. Phenotypic details and possible phenotypic expression of the genes involved are discussed per patient below.
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Patient 6

Patient 39

Patient 45

Patient 49

Chromosomes

Chr.11

Chr.X

Chr.10

Chr.15
Patient 39

This female patient was born as the first child of healthy, non-consanguineous white Dutch parents at 35 + 2 weeks of gestation after premature rupture of membranes and gestational diabetes. At birth, weight was 3240 grams (+1.9 SDS) and length 50 cm (+1.5 SDS). Parental heights were 192.2 cm (+1.2 SDS) and 168.3 cm (-0.4 SDS) for the father and mother, respectively, which results in a target height of 178.2 cm (+1.2 SDS). Her motor milestones were within the normal range. Audiologic examination at 2.2 years of age was normal. At the age of 2.9 years, her height was 107 cm (+3.2 SDS) and occipital frontal circumference (OFC) was 54.4 cm (+3.4 SDS). She showed dysmorphic features including dolichocephaly, frontal bossing, parietal balding, a high, narrow palate (Figure 5.2.2A-C) and there was an advanced osseous maturation of 6 months. Two IQ-tests at around three years of age showed an IQ of 57 and 74. Concerning her family history, her father’s growth pattern and head circumference were reportedly similar, although his OFC measured 60 cm (+1.4 SDS). Two younger siblings of the proband showed normal development. Our patient was last seen at the age of 9.4 years, with a height of 152.4 cm (+1.0 SDS), weight 49.5 kg (+1.2 SDS) and OFC 57.5 cm (+3.0 SDS). She receives special education. During a recent routine check-up for amblyopia, abnormalities of the retina were detected and retinitis pigmentosa was suspected.

Figure 5.2.1 Copy-number analysis plots of the CNVs detected. The log R ratio's (Y-axis) of the SNP probes for the detected CNVs and their normal flanking regions are shown. A log R ratio of >0.3 or < -0.3 indicates a duplication or deletion respectively. Corresponding chromosomes, chromosomal bands and positions (in base pairs) are depicted below each CNV and are according the NCBI Build 36.1 genome assembly in the UCSC genome browser.
An Xq21.1-q21.31 deletion with a size of 13.36 Mb was detected encompassing 25 genes. In literature several genes in this region have been associated with phenotypic expression. Firstly, deletions of Xq21 in male patients were reported to cause a contiguous gene syndrome including choroideremia (*CHM* gene), mental retardation (possibly *RPS6KA6*) and X-linked deafness type 3 (*POU3F4* gene) [24]. In our patient audiologic testing at the age of two was normal and presently she had no apparent hearing loss. However, recently retinal abnormalities were found which might be the first signs of choroideremia. The variable phenotypic expression of deleted genes in our patient could be explained by the incomplete skewed X-inactivation.

Secondly, *BRWD3* is another gene possibly related to our patient’s phenotype, because loss of function mutations were identified in four male patients with X-linked mental retardation, macrocephaly and dysmorphic features [25]. Interestingly, one of them showed also tall stature (> +1.9 SDS) and his affected uncle was tall with a final height of +1.3 SDS. Thirdly, a gene which might also be associated with tall stature, is *ITM2A*. This gene encodes a transmembrane protein which has been shown to be involved in chondrogenesis and a significant association with height was recently found in a genome-wide association study [26;27].

Thus, although the implication of multiple genes complicates linking individual genes to the phenotypic features in our patient, the deleted region contains likely candidate genes explaining the retinal alterations, overgrowth and mental retardation.
**Patient 45**
This male patient was born as the first child of healthy, non-consanguineous white Dutch parents at a gestational age of 35\(^{+3}\) weeks with a birth weight of 3500 grams (+2.2 SDS). Mother was hospitalized for preeclampsia, but the delivery was uneventful. Parental heights were 185.0 cm (+0.2 SDS) and 179.7 cm (+1.4 SDS) for the father and mother, respectively which results in a target height of 193.4 cm (+1.3 SDS). Although his motor development milestones were normal, his gross and fine motor skills were assessed as delayed during childhood. He spoke his first words at the age of 30 months and talked complete sentences at the age of 60 months. For this language and speech development delay he attended special education, after which he proceeded to a mainstream elementary school. During childhood he had an increased statural growth near the +2.5 SDS curve. Bone age at the age of 2.5 was conform calendar age. At the age of 6.2 years his height was 133.1 cm (+2.4 SDS), weight 29.9 kg (+0.6 SDS), OFC 55 cm (+1.9 SDS) and arm span 131.5 cm (+2.0 SDS). His facial features included frontal bossing, a receding hairline and a high arched palate. A simian crease was found in his right hand. His IQ was tested to be 84. Chromosome analysis showed a normal male 46,XY karyotype.

A small deletion was found, affecting the last 12 exons out of 14 exons of *PLXDC2*. A patient (patient KK) with a 4.3 Mb-interstitial deletion of 10p12.1-p12.31 was described, including amongst others *PLXDC2* in the deleted region [28]. This patient showed also overgrowth with height, weight and OFC > +1.6 SDS, mild dysmorphic features and a delay in language development [28]. Patients with larger interstitial deletions or 10pter deletions have been reported but they showed postnatal growth retardation [29]. In addition to the much larger size of the deletion, involvement of *PLXDC2* in these deletions could not always be confirmed (Figure 5.2.3).

**Figure 5.2.3** Schematic representation of reported patients with deletions of the short arm of chromosome 10. On top, the chromosomal bands of 10p are shown in scale with the physical distance in Mb according the NCBI Build 36.1 genome assembly in the UCSC genome browser (below). The deletions of patient 45, KK, GM3470 and MAR comprise *PLXDC2* (horizontal black line), while the deletions of patients MEG, TAT, KAN and ROB might include *PLXDC2*. For each patient, horizontal black bars depict non-deleted chromosomal regions. White bars represent the undetermined breakpoint regions and the blank spaces in between the deleted region. Mapping data for patient KK was obtained from [26] and for patients GM3470, MAR, MEG, TAT, KAN and ROB from [27] and references therein.
Due to the missing parental DNA for inheritance studies and the detection of variants within \textit{PLXDC2} in four control individuals [18;19], the contribution of haploinsufficiency of \textit{PLXDC2} to a clinical phenotype remains equivocal. However, all the more since growth and height data of the four control individuals is lacking, phenotypic expression should be considered as well. The findings in our patient suggest then that \textit{PLXDC2} is a candidate gene to be involved in growth regulation and/or intellectual development.

\textbf{Patient 49}
This male patient of Indonesian descent was first seen at the age of 45.2 years. His height was 1.78 cm (-0.8 SDS), weight 78.5 kg (+0.3 SDS) and OFC 60 cm (+1.4 SDS). The patient presented with a long face, a prominent jaw, a high arched palate and strabismus divergens (Figure 5.2.2D). He had a thoracic kyphosis and scoliosis. He was institutionalized for his moderate to severe mental retardation. In his forties he developed periods of depression followed by severe behavioural problems such as screaming, restlessness and aggression. His medical history showed hypertension and Parkinsonism and his family history was normal. Chromosome analysis revealed a normal 46,XY karyotype. The patient died at the age of 52, due to complications after surgery for a femoral fracture.

A duplication of 15q11-q13 was identified, which is a region containing imprinted and non-imprinted genes. The clinical phenotype of the 15q11-q13 duplication is variable even within families and is associated with varying levels of mental retardation and problems in motor coordination [30]. On physical examination, hypotonia, decreased deep tendon reflexes and joint laxity are frequently found. Distinct dysmorphic features are not present, although an antimongoloid-slant of the eyes and thick or pouting lips have been described [30]. An association with autism spectrum disorders has been postulated, but this is not a common feature [30;31]. Paternal inherited duplications have been reported to associate with a normal or less severe phenotype [30;32], but affected patients have been reported as well [22;33;34]. Considering the phenotypic features associated with 15q11-q13 duplications, it is likely that the chromosomal imbalance in our patient is the cause of his mental retardation and behavioural problems.

\textbf{Patient 58}
This male patient was born as the 7th child of healthy non-consanguineous white Dutch parents at 42 weeks of gestational age after an uneventful pregnancy. At birth, weight was 3550 grams (-0.5 SDS) and length 54 cm (+1.2 SDS). His target height was 186 cm (+0.3 SDS). Although he was hypotonic for which he received physiotherapy from 7 till 14 months of age, his motor development milestones were within normal range. He followed two years of mainstream elementary school, but continued with special education. His IQ was tested to be 76 at the age of 7.3 years. He was last seen at the age of 15.8 with a height of 180.3 cm (+0.3 SDS), weight 84 kg (+1.9 SDS) and OFC 57.4 cm (+0.6 SDS). He did not show any major dysmorphic features, although he had short fingers and a simian crease in his left hand. His father died at the age of 40 years, due to a ruptured abdominal aneurysm. Both his father and mother attended normal elementary schools and followed lower secondary education. He had nine siblings who were healthy, but learning problems were prevalent. There was no history of psychiatric problems.
A small deletion of the first exon of *NPAS3* was identified and his mother was confirmed to be a carrier. Previously, a mother and daughter have been described who carried a balanced reciprocal translocation t(9;14)(q34;q13) disrupting *NPAS3* on chromosome 14 without disrupting genes on chromosome 9 [35]. The mother showed mild learning disability and was diagnosed with schizophrenia [36]. The daughter had severe learning impairment and suffered from schizophreniform psychosis [35]. Additionally, a recent study revealed four *NPAS3* related haplotypes which were associated with bipolar disorder and schizophrenia [37]. The association of *NPAS3* with these psychiatric disorders remains subject for discussion since our patient and mother did not show signs of psychiatric illness. Yet, there is still a possibility that our patient will develop schizophrenia since the onset of schizophrenia in males is usually in their late teens and early twenties [38]. Another possibility is that due to the translocation, the disruption of *NPAS3* resulted in a dominant negative effect and therefore caused a more severely affected phenotype [36]. More pronounced behavioural abnormalities were also seen in homozygous knock-out mice, while only a non-significant trend was observed in the heterozygous mutants [39]. Compound heterozygosity in our patient was excluded.

The findings in our patient support the postulated association of *NPAS3* with intellectual development, although admittedly the intelligence of the mother of our patient was borderline normal. In contrast, they are not in support of a causative relation of *NPAS3* with psychiatric disorders and more patients with *NPAS3* abnormalities are necessary to elucidate this association.

**Conclusion**

A genome-wide SNP array analysis detected four possible pathogenic CNVs in 26 (15%) patients with features of SoS. This approach is therefore a powerful method to provide a molecular explanation in patients with features of SoS but without *NSD1* alterations, although still molecular validation is required to establish a definite causal relationship. A candidate gene for overgrowth (*PLXDC2*) was identified and a deletion of *NPAS3* in two carriers without psychiatric illness warrants caution of linking this gene to schizophrenia. Screening of these genes in larger patient populations is necessary in order to further delineate the associated phenotype.

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