From NSD1 to Sotos syndrome : a genetic and functional analysis

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Chapter 12

Summary

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Sotos syndrome is an overgrowth disorder which is characterized by an increased statural height and/or head circumference, characteristic craniofacial features and a variable level of learning impairment. The craniofacial features include dolichocephaly and a triangular shaped face, i.e. a broad and high forehead with a prominent chin. Furthermore, (apparent) hypertelorism and downslanting of the palpebral fissures. Sotos syndrome is caused by abnormalities of the *NSD1* gene on chromosome 5q35.2- 5q35.3.

In this thesis a comprehensive "From *NSD1* to Sotos syndrome" analysis was performed. This analysis focused on three main, yet unresolved issues. The first issue is the molecular background of the commonly sized microdeletion encompassing *NSD1* and neighbouring genes, which is much more prevalent in Japanese Sotos syndrome patients in comparison with non-Japanese Sotos syndrome patients. Secondly, since *NSD1* abnormalities are found in 60-90% of the Sotos syndrome patients, there are a considerable number of patients with Sotos syndrome-like features without a molecular diagnosis. In comparison, a similar situation exists for patients with typical and non-typical Marfan syndrome features without changes in the *FBN1* gene. The third issue is the gene regulatory network and the related signaling pathways through which NSD1 modulates its activity.

Chapter 1 presents a general introduction and the outline of this thesis. The following aims of this thesis were formulated:

- to elucidate the molecular basis and mechanisms causing microdeletions in Sotos syndrome
- to identify causative molecular alterations in patients with features of Sotos or Marfan syndrome without identified genetic abnormalities in *NSD1* or *FBN1*
- to unravel the signaling pathways and interacting proteins through which NSD1 exerts its function

In **chapter 2** an extensive overview of the classical and new overgrowth syndromes is presented. Starting point is the identification of genetic causes of overgrowth syndromes which has in the recent years enhanced our understanding and possibilities for diagnosing these syndromes. The syndromes discussed include classical syndromes such as Beckwith-

Wiedemann, Simpson-Golabi-Behmel, Marfan and Sotos syndrome but also recent syndromes such as CATSHL syndrome and patients with overproduction of C-type Natriuretic Peptide. Each syndrome is discussed in detail, including delineation of the characteristic and non-characteristic manifestations, phenotype-genotype correlations and underlying pathophysiological mechanisms.

In **chapter 3** a review about Sotos syndrome is provided. In this review clinical features and the diagnostic criteria of Sotos syndrome are presented. Furthermore, it contains the results of genetic analysis of *NSD1* in different ethnic populations and a discussion of the genotype-phenotype correlations. It also addresses *NSD1* mutations in other patient populations such as Weaver, Beckwith-Wiedemann and Nevo syndrome.

Chapter 4 is the first of two studies (chapter 4 and 5) which focuses on the commonly sized microdeletion which encompasses the whole *NSD1* gene and neighboring genes. This microdeletion is more prevalent in Japanese Sotos syndrome patients and is flanked by regions of high sequence similarity, so called low-copy repeats (LCRs). In this chapter the identification of a recombination hotspot within these LCRs is reported. In this hotspot, deletion breakpoints were mapped in approximately 80% of the patients with Sotos syndrome who carry the common microdeletion. Furthermore, non-allelic homologous recombination (NAHR) between the directly orientated regions (i.e. PLCR-B and DLCR-2B) within the LCRs was found to be the mechanism causing the microdeletions. A heterozygous inversion of the interval between the LCRs was detected in all fathers of the Japanese children carrying a deletion in the paternally derived chromosome. Whether this explains the much higher prevalence of the common microdeletion in the Japanese Sotos syndrome population remains to be elucidated.

In **chapter 5** a molecular analysis was performed of 10 Sotos syndrome patients, who carry a commonly sized microdeletion but do not have a breakpoint in the recombination hotspot (see chapter 4). Two of these breakpoint regions were located in close proximity to the hotspot and two were identified at a further genomic distance. In order to provide a possible explanation for the question why recombination preferably occurs in the hotspot, deletion-junction fragments and hotspots of Sotos syndrome and similar other genomic disorders were analyzed for their DNA stability and the presence of scaffold/matrix attachment regions. All crossover events in the four patients of this study were found to

have occurred within or adjacent to a highly destabilized DNA duplex with a high scaffold/matrix attachment regions probability. In contrast, the Sotos syndrome hotspot and other genomic disorders' recombination hotspots were mapped to stabilized DNA helix regions with a low scaffold/matrix attachment regions probability. This suggests that a specific chromatin structure predisposes for the recombination hotspot in Sotos syndrome.

In **chapter 6, 7** and **8** three studies are presented investigating patients with a Sotos phenotype, but without *NSD1* abnormalities. In **chapter 6**, alterations of the promoter region of *NSD1* were hypothesized to be the underlying cause in these patients. In 18 patients the promoter region of *NSD1* was analyzed for genomic sequence abnormalities or epigenetic changes (hemizygous hypermethylation). However, no sequence aberrations or epimutations could be detected. Although our patient cohort included only 18 patients, it seems unlikely that promoter abnormalities of *NSD1* are a major culprit in patients with phenotypical Sotos syndrome.

In **chapter 7** a new candidate gene, the *RNF135* gene, was screened in 160 patients referred for *NSD1* testing, but without alterations in the *NSD1* gene. *RNF135* is located in the Neurofibromatosis type 1 microdeletion region and recently, *RNF135* aberrations were identified in children who exhibited an overlapping phenotype with Sotos syndrome. However, no alterations were found in 160 patients. In one patient at 4 years of age, a classic Neurofibromatosis type 1 microdeletion was identified. Hence, this phenotypic presentation should be considered in the differential diagnosis of young Sotos syndrome-like patients.

In **chapter 8** a different approach than the candidate gene method is taken. The first genome-wide high-resolution SNP array analysis in patients with features of Sotos syndrome, but without *NSD1* aberrations, is described. In twenty-six Sotos syndrome-like patients four possible pathogenic copy-number variants including 3 deletions and 1 duplication were identified. This results in a detection rate of 15% of novel abnormalities. Therefore, the high-resolution genome-wide SNP array approach is a powerful method to attain a molecular diagnosis in Sotos syndrome-like patients. A possible candidate gene (*PLXDC2)* for overgrowth was found in one of the deletions. Screening of this candidate gene in larger patient populations is necessary in order to further delineate the associated phenotype.

In **chapter 9** a functional study of NSD1 is presented. Expression profiles of dermal fibroblasts of nine Sotos syndrome patients with a confirmed NSD1 abnormality are compared with normal individuals. A significant association was demonstrated with the Mitogen-Activated Protein Kinase (MAPK) pathway. Furthermore, the Ras Interacting Protein 1 (*RASIP1*), a proposed Ras effector, showed upregulated expression in Sotos syndrome. With phosphorylation studies of key kinases, a possible diminished activity state of this MAPK/ERK pathway was found in Sotos syndrome. However, transfection experiments of RASIP1 showed a possible increased state of activity. From short stature syndromes such as hypochondroplasia and Noonan syndrome, it is known that the activation level of the FGF-MAPK/ERK in epiphyseal growth plates is a determining factor for statural growth. Interestingly, in this chapter *NSD1* expression was demonstrated in the terminally differentiated hypertrophic chondrocytes of normal human epiphyseal growth plates at different developmental ages. Therefore, it is proposed that deregulation of the MAPK/ERK pathway in Sotos syndrome results in altered hypertrophic differentiation of *NSD1* expressing chondrocytes and this may be a determining factor in statural overgrowth and accelerated skeletal maturation in Sotos syndrome.

In comparison to Sotos syndrome, in Marfan syndrome, several responsible genes have already been identified. **Chapter 10** contains the results of a comprehensive study of four, i.e. *FBN1, FBN2, TGFBR1,* and *TGFBR2* mutations, in 49 patients with Marfan syndrome and Marfan-syndrome related phenotypes. A total of 27 *FBN1* mutations, one *TGFBR1* mutation, and two *TGFBR2* mutations were identified. No *FBN2* mutation was found. These results demonstrate predominant *FBN1* mutations in patients with a Marfan syndrome phenotype but *TGFBRs* defects may account for approximate 5-10% of the patients. Furthermore, in perspective to the genetic findings and the literature, the clinical manifestations in patients with a gene mutation were critically discussed.

In **chapter 11** the major findings of this thesis are critically reviewed and directions for future research are discussed. First, non-allelic homologous recombination (NAHR) is the underlying mechanism causing commonly sized microdeletions in Sotos syndrome. Second, genome-wide high resolution SNP array is a powerful method to obtain a molecular diagnosis in comparison to a candidate gene approach. Finally, the association of the MAPK/ERK pathway and Sotos syndrome is a significant step in establishing a connection between *NSD1* abnormalities and phenotypic features in Sotos syndrome. A model is proposed in which deregulation of the MAPK/ERK pathway in Sotos syndrome

results in altered hypertrophic differentiation of *NSD1* expressing chondrocytes. This may be an important contributor to statural overgrowth in Sotos syndrome.