From NSD1 to Sotos syndrome : a genetic and functional analysis

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Chapter 1

General introduction

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Overgrowth syndromes are a group of disorders characterized by the association of increased height or head circumference with a variable combination of dysmorphic features and mental retardation. Over the past two decades, the genetic causes of the majority of these overgrowth syndromes have been identified. This has rapidly advanced our understanding of the spectrum of alterations causing these conditions and delineated phenotype-genotype correlations. However, for each condition there are a considerable number of patients, either with characteristic or non-characteristic manifestations, in whom no molecular alterations are found. Furthermore, in contrast to the accumulated knowledge about genes and genetic defects in overgrowth syndromes, less is known about the function of the proteins they code for, the interacting partners of these proteins and their involvement in longitudinal growth regulation.

One of these overgrowth syndromes is Sotos syndrome. The typical phenotype includes an increased statural height and/or head circumference, characteristic facial dysmorphism and a variable level of learning disability (1). In 2002, haploinsufficiency of the *N*uclear receptor binding *S*ET *D*omain protein *1 (NSD1)* was identified as the cause of Sotos syndrome (2). Most prevalent genetic alterations are intragenic point mutations (~80-85%), whole-gene microdeletions (~10%) and exon-deletions (~5%) (3). In contrast, in Japanese Sotos syndrome patients a recurrent commonly sized microdeletion encompassing *NSD1* and neighbouring genes is detected in ~50% of the patients and intragenic point mutations only account for ~10% (4). The molecular mechanisms causing these commonly sized microdeletions, which may possibly explain the higher prevalence of these microdeletions in the Japanese Sotos syndrome population, are yet unknown.

Overall, *NSD1* abnormalities are found in 60-90% of the patients. No other genes have yet been identified to be responsible for a relatively large series of Sotos syndrome patients or Sotos syndrome-like patients without *NSD1* changes. In comparison, additional genetic causes were recently detected in Marfan and Marfan-like syndromes (5). This further expands genetic screening possibilities in patients with typical and non-typical Marfan syndrome features, although the detection rates have yet to be established.

The protein NSD1 is a co-factor of nuclear hormone receptors and is postulated to either promote or inhibit chromatin transcription (6). It is hypothesized that NSD1 reduces the transcription of growth promoting genes and loss of this activity in Sotos syndrome would consequentially result in overgrowth (2). Nevertheless, the gene regulatory network and the related signaling pathways through which NSD1 modulates its activity remain largely elusive.

Aim of this thesis

The aim of this thesis was:

- 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

Outline of this thesis

Chapter 1 presents a general introduction and the outline of this thesis. **Chapter 2** provides an overview of the classic and new overgrowth syndromes. Discriminating clinical, molecular genetic and functional aspects of these syndromes are presented. **Chapter 3** contains a review about Sotos syndrome in which the clinical features, phenotype-genotype correlations and the functional roles of NSD1 are discussed.

In **chapter 4** the results are described of a breakpoint region analysis of the typical microdeletion detected in Japanese Sotos syndrome patients. Furthermore, the underlying mechanism is addressed. In **chapter 5** the molecular analysis of Sotos syndrome patients, who carry a commonly sized microdeletion but with an uncommon breakpoint region, is described.

Chapter 6 presents a study investigating the promoter region of *NSD1* in Sotos syndrome patients without *NSD1* abnormalities. **Chapter 7** reports on the results of a secondary screening of the *RNF135* gene in patients referred for *NSD1* testing. *RNF135* is a novel gene associated with an overgrowth phenotype, which exhibits overlapping features with Sotos syndrome. An alternative way to search for gene defects that may be related to features of Sotos syndrome is a genome-wide screening for copy number variants. In **chapter 8** the results of a first genome-wide SNP array analysis in patients with features of Sotos syndrome, but without *NSD1* aberrations, are described.

In order to study the role of NSD1 in the pathophysiology of Sotos syndrome, in **chapter 9** the results of a functional study of NSD1 in dermal fibroblasts of Sotos syndrome patients compared with normal individuals are described. With this approach downstream regulators of NSD1 were investigated and NSD1 was mapped into a signaling pathway.

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 causative genes in patients with Marfan syndrome and Marfan-syndrome related phenotypes.

In **chapter 11** the results and outcomes of the reviews and original studies described in the preceding chapters are discussed, including future perspectives. **Chapter 12 and 13** contain the summary of this thesis in English (chapter 12) and in Dutch (chapter 13).

References

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