WNT4 and *RSPO1* Are Not Involved in a Case of Male-to-Female Sex Reversal with Partial Duplication of 1p

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Key Words

Duplication 1p · *RSPO1* · Sex reversal · *WNT4*

Abstract

Some studies suggest that *WNT4* signaling acts to repress the male pathway and a single case of duplication of *WNT4* located in 1p35 has been associated with ambiguous genitalia. Recently, *RSPO1* located in 1p34.3 was shown to be essential in sex determination. We show that neither *WNT4* nor *RSPO1* are duplicated in another case of male-to-female sex reversal with partial duplication of 1p. Therefore, it can be assumed that in male-to-female sex reversal associated with duplication of 1p, other genetic factors may be involved.

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XY gonadal dysgenesis can be caused by a disturbed development of the bi-potential gonad or by failure of differentiation of the bi-potential gonad to testis [for a review see Fleming and Vilain, 2004]. Genes known to be involved in human early gonad development are *WT1* and *SF1*. The key gene of testis differentiation is *SRY*, a transcription factor with hitherto unknown target genes. *SRY* expression in supporting cells leads to their differentiation into Sertoli cells, and it is believed that a critical

threshold number of Sertoli cells are required for testis differentiation. *SOX9* seems to be the next gene downstream of *SRY* that is upregulated in Sertoli cell precursors. *DAX1* is thought to antagonize *SRY* action as duplication of *DAX1* causes male-to-female sex reversal, and Jordan et al. [2001] presented evidence that *WNT4* overexpression leads to upregulation of *DAX1*. In their study, they describe one patient with ambiguous external genitalia and a male karyotype with partial duplication of 1p including *WNT4*. Here, we report on another patient with partial duplication of 1p and complete male-to-female sex reversal, but show that *WNT4* is not duplicated in this case. We also show that *RSPO1*, a gene recently implicated in human sex determination [Parma et al., 2006], is not included in the duplication.

Case Report

The phenotype of the patient has been described by Wieacker et al. [1996]. At 10 years of age growth development was retarded (height and weight <3rd centile) and psychomotor development was severely impaired. Dysmorphic features include epicanthal folds, ptosis, hypertelorism, depressed nasal bridge, anteverted nares, high-arched palate, supernumerary nipples, simian crease on

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Fig. 1. Two-color-FISH with the YAC clone 879f06 situated within the duplicated region on the short arm of chromosome 1 labeled in green and with the P1 clone containing *WNT4* labeled in red. The derivative chromosome shows two green signals but only one red signal, indicating that *WNT4* is not duplicated in this patient.

Fig. 2. Two-color-FISH with the YAC clone 879f06 situated within the duplicated region on the short arm of chromosome 1 labeled in green and with the BAC clone RP11-566C13 containing *RSPO1* labeled in red. As in fig. 1, the derivative chromosome shows two green signals but only one red signal, indicating that *RSPO1* is not duplicated.

the right hand, and nail dysplasia. Computer tomography revealed hypoplasia of the corpus callosum and mild hydrocephalus. External genitalia are female and sonography revealed the presence of a uterus, but gonads could not be visualized. Endocrine parameters demonstrate a hypergonadotropic hypogonadism. Chromosome analysis revealed a male karyotype with partial duplication of 1p: 46,XY,dup(1)(1p22.3p32.3). Parental karyotypes were normal.

Materials and Methods

The YAC clone 879f06 and the *RSPO1*-containing BAC clone RP11-566C13 were obtained from the Molecular Cytogenetic and Positional Cloning Center of the Max-Planck-Institute for Molecular Genetics, Berlin. The P1 clone containing *WNT4* was a gift of E. Vilain (Los Angeles, USA).

DNA probes were labeled directly by nick translation with SpectrumOrange-dUTP or SpectrumGreen-dUTP (Abbott, Illinois, USA). Labeled YAC clones were dissolved in hybridization mixture (50% formamide, 2× SSC, 10% dextran sulfate). After hybridization, metaphases were counterstained with DAPI and viewed with selective filters. Images were captured with the Cytovision digital imaging system (Applied Imaging, Sunderland, UK).

Results

The results of FISH analysis on metaphase spreads from the patient with the P1 clone containing *WNT4* and YAC clone 879f06 which contains the markers *DIS462* and *DIS192* located in 1p31.1 are given in fig. 1. Whereas two signals can be detected with YAC clone 879f06 on the derivative chromosome 1, there is only one *WNT4* signal located distal from the duplication. Similar results were obtained by FISH analysis with BAC clone RP11-566C13 containing *RSPO1*. Only one *RSPO1* signal could be detected on the derivative chromosome 1, whereas two signals for YAC 879f06 were visualized (fig. 2). These results clearly demonstrate that neither *WNT4* nor *RSPO1* are involved in the partial 1p duplication in the patient.

Discussion

Many studies suggest that the supporting cell precursor lineage is bi-potential with the possibility to develop into follicle cells or Sertoli cells. Kim et al. [2006] showed that *Fgf9* (fibroblast growth factor 9) and *Wnt4* are expressed in both XX and XY gonads at 11.25 dpc in mice and proposed that *Fgf9* and *Wnt4* act as antagonistic signals to regulate sex determination. Mice homozygous for

a loss of function mutation in *Fgf9* display male-to-female sex reversal including failure of Sertoli cell differentiation. In contrast, XX gonads with a loss of function mutation in *Wnt4* show some features of testis differentiation [Jeays-Ward et al., 2003]. According to the concept of Kim et al. [2006], *Sox9* upregulates *Fgf9*, and *Fgf9* maintains *Sox9* expression in a feed-forward loop and represses *Wnt4* expression leading to male gonad differentiation. In the absence of a feed-forward loop between *Sox9* and *Fgf9*, as it is the case in XX gonads, *Wnt4* blocks *Fgf9* and the maintenance of *Sox9* expression leading to female gonad differentiation.

On the basis of such an antagonistic system between *Fgf9* and *Wnt4* it can be expected that overexpression of *Wnt4* may lead to male-to-female sex reversal. In fact, Jordan et al. [2001] could show that *Wnt4* upregulates *Dax1*, a gene known to antagonize *Sry*, and *WNT4* was duplicated in a patient with ambiguous genitalia and partial duplication of the short arm of chromosome 1 (1p31p35). However, FISH analysis of *WNT4* in our patient clearly demonstrates that *WNT4* is not duplicated. Therefore, it is unlikely that *WNT4* is involved in the pathogenesis of sex reversal in this patient. A position effect with upregulation of *WNT4* cannot be ruled out, but the distance of more than 28 Mb between the distal duplication breakpoint at 1p32.3 and *WNT4* at 1p35 makes this option highly unlikely.

Recently, Parma et al. [2006] could show that homozygous loss of function mutation of *RSPO1* encoding R-spondin1 causes female-to-male sex reversal associated with palmoplantar hyperkeratosis and predisposition to squamous cell carcinoma of the skin. R-spondin1 is a member of a family of orphan ligands that activate Wnt and β -catenin signaling pathways. Obviously, the absence of any functional *RSPO1* copy leads to testis differentiation of XX gonads, possibly because of inactivation of the ovarian *WNT4* pathway. Because a possible interaction between *WNT4* and *RSPO1* has been suggested [Parma et al., 2006], we performed FISH analysis with a BAC clone containing *RSPO1* that is located in 1p34.3. As expected from the extent of the duplication from 1p22.3 to 1p32.3, *RSPO1* is not involved in the duplication. Thus, sex reversal in our patient is not caused by alterations of *WNT4* or *RSPO1* gene dosage, and we suggest that other loci may be involved in the pathogenesis of this disorder of sex differentiation.

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