A duplication in a patient with 46,XX ovo-testicular Disorder of Sex Development refines the *SOX9* testis–specific regulatory region to 24 kb

Thomas Ohnesorg[1§], Jocelyn A. van den Bergen[1§], Daniele Belluoccio[2], Nandini Shankara-Narayana[3,4], Anne-Maree Kean[3], Arthur Vasilaras[3], Lisa Ewans[5,6], Katie L. Ayers[1,7], Andrew H. Sinclair[1,7] *.

1 Murdoch Childrens Research Institute, Melbourne, VIC, AUS

2 Agilent Technologies Inc., Mulgrave, VIC, Australia

3 Royal Prince Alfred Hospital, Sydney, NSW, AUS

4 ANZAC Research Institute, University of Sydney, Sydney, NSW, AUS

5 Department of Medical Genomics, Royal Prince Alfred Hospital, Sydney, NSW AUS

6 Central Clinical School, Sydney Medical School, University of Sydney, NSW AUS

7 Department of Paediatrics, University of Melbourne, Melbourne, VIC, AUS

[§]These authors contributed equally

* Corresponding author

Murdoch Children's Research Institute
Flemington Rd, Parkville 3052, VIC, Australia
+61 3 8341 6424
andrew.sinclair@mcri.edu.au

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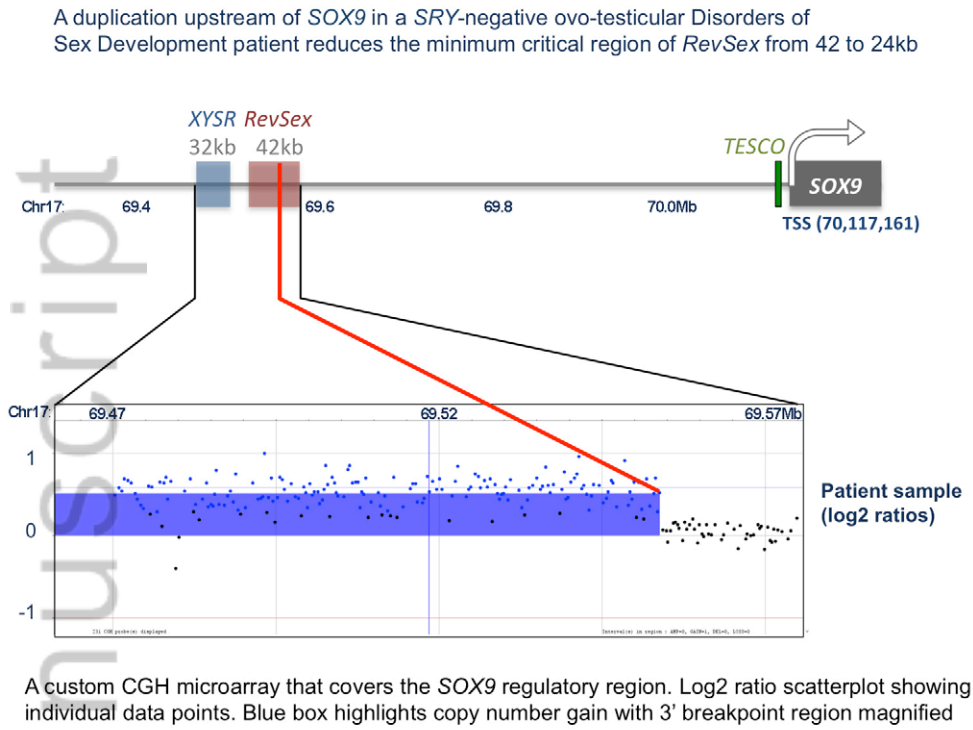
Graphical Abstract



A custom CGH microarray that covers the *SOX9* regulatory region. Log2 ratio scatterplot showing individual data points. Blue box highlights copy number gain with 3' breakpoint region magnified

SRY-negative 46,XX ovo-testicular Disorders of Sex Development (OT-DSD) are rare genetic conditions frequently caused by disruptions to the genes underlying gonad development and differentiation. One of these genes, *SOX9*, is central to male sex differentiation and is essential for testes development. Loss-of-function mutations in *SOX9* cause Campomelic Dysplasia and in 46,XY individuals this can be combined with DSD due to its dual role in the gonads and chondrogenesis [1]. Conversely, duplication of human *SOX9* can lead to 46,XX testicular DSD [2]. Recently, CNVs (copy number variants) of regions upstream of *SOX9*, presumably affecting testis-specific regulatory regions, have been implicated in 46,XX DSD and 46,XY DSD, respectively. Most of these CNVs cover a common 42kb region termed *RevSex* [3], suggesting the presence of *SOX9* gonadal enhancer(s). Further understanding of this region is essential to decipher the complex transcriptional regulation of *SOX9* and to identify its core enhancer(s).

The patient was a 17 year-old Caucasian phenotypic male with normal secondary sexual characteristics and growth presented with pain in his left testis. He was the

only child to a non-consanguineous couple with no relevant family history. Scrotal ultrasound showed a small right testis (3ml) and a larger left testis suggestive of a tumour. No uterus was present. Semen analysis showed azoospermia. The patient had elevated FSH (17.2IU/L) and LH (11.7IU/L) (normal range 1-12IU/L), and low serum total testosterone (5.1nmol/L; normal male 11.5-32nmol/L). An ovo-testis was identified histologically after a partial orchidectomy. Further characterisation of this patient is provided elsewhere (Shankara Narayana *et. al* Submitted). Karyotype was 46,XX and an *SRY* probe test was negative. A diagnosis of *SRY*-negative 46,XX OT-DSD was reached, and the underlying genetic aetiology was investigated. Patient genomic DNA was run on a custom MLPA screen (including 10 genes implicated in DSD and two controls, probe sequences available upon request). This includes four probes upstream of *SOX9:* one within the *TESCO* region [4], and three in hitherto-implicated regulatory regions, *XYSR (XY sex reversal)* [5] and *RevSex* (Figure 1A). MLPA suggested a duplication covering the *XYSR* probe and one *RevSex* probe only. No CNVs were found in the mother.

To determine the extent of the duplication an exon-targeted DSD custom array, including the defined *SOX9* regulatory regions (*RevSex* and *TESCO*), was used. This confirmed the presence of a heterozygous duplication upstream of *SOX9* encompassing a minimal region of 248kb at 17q24.3 (chr17:69,311,111-69,558,832) and a maximum size of 323 kb (Figure 1B). The 3' breakpoint occurs in the *RevSex* region and could be confidently called within a 450bp window, the 5' breakpoint lies outside of the ultra-high resolution region and can only be mapped within a 75kb window. When aligned with the previously described CNVs covering this region, this chromosomal duplication further reduces the *RevSex* region from 42kb to approximately 24kb.

The duplication identified in the individual reported here contains both *XYSR* and *RevSex*. We cannot confirm that the ovo-testicular DSD is due to duplication of *RevSex* alone or whether other elements contained within the 248 to 323kb duplicated region may play a role. However, based on previously published patient data it is likely that the 24kb minimum critical region contains the necessary enhancer elements responsible for the observed phenotype. This finding will facilitate studies aimed at characterising the enhancers that regulate *SOX9* and in particular how they contribute to developmental disorders such as DSD.

Diagnostic tools such as whole exome sequencing, targeted gene sequencing and low-density CNV arrays, often miss CNVs within the *SOX9* regulatory region. Yet, given the numerous reports, it is likely that CNVs in the *SOX9* regulatory region may be a frequent genetic cause of 46,XX DSD. Therefore, we strongly suggest inclusion of CNV analysis of this region in routine diagnostic testing for *SRY*-negative 46,XX DSD patients.

Ethical statement

Informed patient consent was obtained under ethics HREC 22073, approved by the Royal Children's Hospital (Melbourne, Australia) ethics committee.

References

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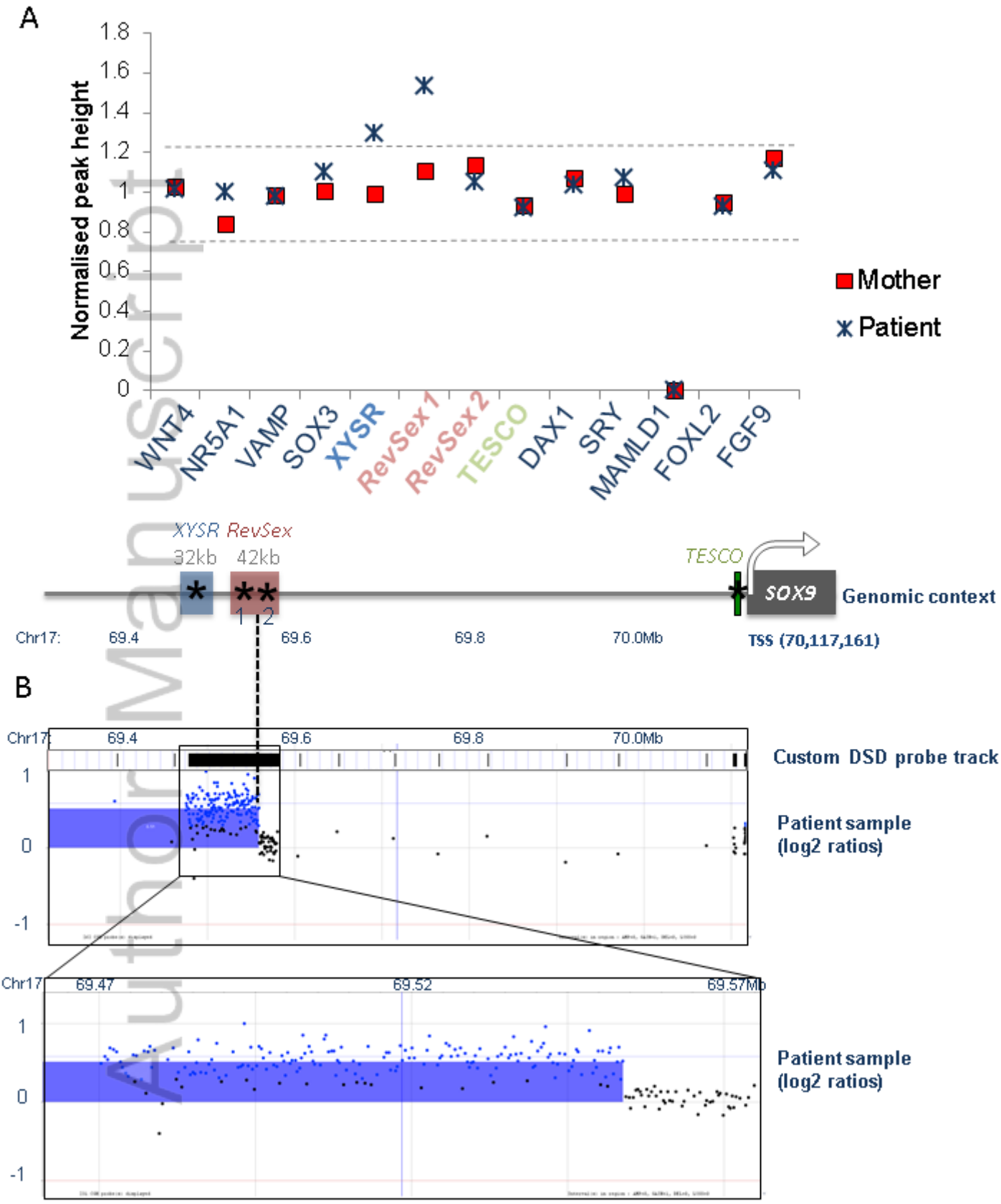
Figure legend

Figure 1. Detection of *RevSex* region duplication in a 46,XX DSD patient.

A. Asterisks denotes MLPA probes covering *SOX9* regulatory regions: *XYSR, RevSex* and *TESCO*. *XYSR* and *RevSex* probe1 showed a normalised peak height greater than 1.25 in the patient suggesting a duplication not detected in the mother.

B. A custom CGH microarray that covers the *SOX9* regulatory region. Log2 ratio scatterplot showing individual data points. Blue box highlights copy number gain with 3' breakpoint region magnified.



Ohnesorg *et. al* Figure 1