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Title: Refining the regulatory region upstream of *SOX9* associated with 46,XX testicular Disorders of Sex Development (DSD)

Running title: Regulatory region upstream of *SOX9* and 46,XX DSD

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Abstract

Disorders of Sex Development (DSD) are a heterogeneous group of disorders affecting gonad and/or genito-urinary tract development and usually the endocrine-reproductive system. A genetic diagnosis is made in only around 20% of these cases. The genetic causes of 46,XX-*SRY* negative testicular DSD as well as ovotesticular DSD are poorly defined. Duplications involving a region located ~600 kb upstream of *SOX9*, a key gene in testis development, were reported in several cases of 46,XX DSD. Recent studies have narrowed this region down to a 78 kb interval that is duplicated or deleted respectively in 46,XX or 46,XY DSD. We identified three phenotypically normal patients presenting with azoospermia and 46,XX testicular DSD. Two brothers carried a 83,8 kb duplication located ~600 kb upstream of *SOX9* that overlapped with the previously reported rearrangements. This duplication refines the minimal region associated with 46,XX-*SRY* negative DSD to a 40.7-41.9 kb element located ~600 kb upstream of *SOX9*. Predicted enhancer elements and evolutionary-conserved binding sites for proteins known to be involved in testis determination are located within this region.

Key words

46,XX DSD, duplication, regulatory element, *SOX9* gene, CNV

INTRODUCTION

Disorders of sex development (DSD) are congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical [Hughes et al., 2006]. DSD covers a wide spectrum of phenotypes. 46,XY DSD includes 46,XY complete or partial gonadal dysgenesis as well as undermasculinization of an XY male due to a defect in androgen synthesis or action. At the opposite, most 46,XX DSD correspond to the virilization of an XX female due to androgen excess. Most often, 46,XX testicular DSD, a rare pathology affecting 1 in 20,000-25,000 newborn males, [Kousta et al., 2010] are *SRY* positive and have Y-to-X translocations while most cases of 46,XX ovotesticular DSD are mosaics or chimeras [López et al., 1995]. However, some 46,XX males do not carry the *SRY* gene and the genetic cause of these DSD cases remains poorly defined.

Human sex determination is a tightly controlled and highly complex process where the bipotential gonad anlage develops to form either a testis or an ovary. In 46,XY males the expression of the testis determining gene *SRY* leads to the upregulation of the *SOX9* gene and the development of Sertoli cells that produce AMH and initiate the series of events that lead to testis formation. Prior to the expression of *Sry* in mice, *Sox9* expression is at basal levels in both male and female gonadal primordial cells and at 11.5 days dpc, following the expression of *Sry*, it is up-regulated in males and down-regulated in females. Upregulation in males is due, at least in part, to a synergistic action of *Sry* with NR5A1/SF1, through binding to a testis-specific *Sox9* enhancer named TESCO (Testis-specific Enhancer of *SOX9* Core), located approximately 13 kb upstream of *Sox9* [Sekido and Lovell-Badge, 2008]. In XX-*SRY* negative males duplication of the entire *SOX9* gene was described in a boy with severe penile hypospadias and XX karyotype [Huang et al., 1999]. Rearrangements involving other closely related members of the *SOX* gene family have also been associated with testis development in 46,XX individuals including the *SOX3* and *SOX10* genes [Polanco et al., 2010; Sutton et al., 2011; Moalem et al., 2012]. Mutations or deletions in *RSPO1* and *WNT4*, genes involved in the *WNT4*- β catenin pathway leading to *SOX9* downregulation, have been described in syndromic forms of 46,XX *SRY*-negative testicular DSD [Parma et al., 2006; Mandel et al., 2008].

As well as being essential for testis formation, *SOX9* plays a key role in chondrogenesis [Bell et al., 1997; Ng et al., 1997]. Consequently, mutations in the coding sequences of *SOX9* are associated with campomelic dysplasia and, in about 70% of affected 46,XY individuals, with a range of anomalies of testis development [Foster et al., 1994; Wagner et al., 1994]. Translocations and copy number variation both 5' and 3' to *SOX9* gene, are associated with a milder phenotype as compared with the intragenic mutations. Large

duplications (>1Mb) 5' to *SOX9* are associated with brachydactyly-anonychia (Cooks syndrome) and deletions located >1.3 Mb 5' and 3' to *SOX9* are associated with micrognathia, cleft palate and glossoptosis (Pierre-Robin sequence) [Benko et al., 2009; Kurth et al., 2009]. In both examples there is an apparently normal testis development in 46,XY men and the precise phenotype associated with *SOX9* mutations depends on the position and size of the rearrangement. Indeed, four recent studies have reported 46,XX-*SRY* negative individuals who presented with testis development. Each carried duplications involving a chromosomal region located approximately 600 kb upstream of *SOX9* [Benko et al., 2011; Cox et al., 2011; Vetro et al., 2011; Xiao et al., 2013]. In contrast, 46,XY females with gonadal dysgenesis have been reported to carry overlapping deletions of this region [Benko et al. 2011; Bhagavath et al. 2014]. These individuals presented with no other associated somatic anomalies, suggesting that a regulatory element involved specifically in human sex-determination (termed *RevSex*) is located in this region [Benko et al., 2011].

Here, we describe three novel 46,XX-*SRY* negative patients, two brothers and an unrelated man with testicular DSD and azoospermia, who carry a microduplication upstream of *SOX9*. The detailed analyses of these patients and the incorporation of the published datasets have enabled the minimal critical region located ~600 kb upstream of *SOX9* to be resolved to an approximately 40kb element that contains putative enhancers elements and DNA-binding sites for known factors to be involved in early testis formation.

MATERIEL AND METHODS

Clinical reports

Patients 1 and 2 were two brothers referred for infertility at the age of 30 and 31 respectively. Both had normal male genitalia without any signs of undervirilization. Clinical examination in patient 1 revealed a reduced testicular volume (10 ml; normal range 18-30 ml). Semen analysis revealed an azoospermia. Ultrasound examination of the scrotum in patient 1 was normal and pelvic ultrasound examination showed a normal male genital tract without Mullerian remnants. Testicular biopsy was performed to rule out a possible 46,XX/46,XY chimera. It revealed atrophic seminiferous tubules containing only eosinophilic Sertoli cells suggestive of testicular dysgenesis. There was no evidence of spermatogenesis and the interstitium showed hyperplasia of Leydig cells.

Patient 3 was a 45 years old male who was referred for clinical investigation after five years of infertility. He presented with a normal male phenotype and had a normal libido and no erectile dysfunction. Ultrasound investigation showed bilaterally hypotrophic testes (right 15mm x 7mm and left 18mm x 12mm) with calcifications, seminal vesicle hypoplasia and normal prostate. Semen analysis showed azoospermia.

Genetic analysis

Genetic analyses were performed after obtaining patient's informed consents. Cytogenetic analysis was performed using GTG-banding techniques on metaphase chromosomes obtained by standard procedures from peripheral blood lymphocytes. FISH analysis was carried out using chromosome X specific probe DXZ1 and probes for *SRY* and *SOX9* locus.

Genomic DNA was extracted for each patient from peripheral blood samples using FlexiGene DNA Kit (Qiagen, Valencia, CA) according to manufacturer's instructions.

For patients 1, 2 and 3, SNP array analysis was performed using the HumanCytoSNP-12 BeadChip from Illumina (San Diego, CA). Data analysis was performed using Illumina's Genome Studio Genotyping Module software allowing the identification of both copy number variations (CNVs) with the Log R ratio (LRR) and regions of copy-neutral loss of heterozygosity (LOH) with the B allele frequency. Previous array CGH for patient 3 was performed using the Human Genome CGH Microarray Kit 105A from Agilent (Santa Clara, CA). DNA sequence information was made according to the UCSC Genome Browser (<http://genome.ucsc.edu/>; February 2009, Assembly, hg19).

Boundaries of the duplication for patient 2 were confirmed by long-range PCR using primers in the 5' and 3' parts of the putative duplicated region (Supplemental data). The long-range PCR amplicon was generated using Roche's Expand Long Template PCR System (Roche Diagnostics, Meylan, France) according to the following conditions: 5 µl of 10x Expand Long Template Buffer 3 (including 27.5 mM MgCl₂ and detergents), 8.75 µl of a 2 µmol/L of dNTPs, 0.75 µl of Expand Long Template Enzyme Mix, 3 µl of a 5 µmol/L of each primer, 150 ng of genomic DNA, 26.5 µl of water in a total reaction volume to 50 µl. Reaction was cycled under following conditions: 94°C for 2 minutes; 94°C for 10 seconds; annealing temperature of 60°C for 30 seconds; 68°C for 10 minutes; cycle 10× from step 2; 94°C for 15 seconds; annealing temperature of 60°C for 30 seconds; 68°C for 10 minutes plus 20 seconds per cycle; cycle 30× from step 5; 68°C for 7 minutes; and hold at 4°C. Internal primers were used to sequence the amplicon. Data were analyzed using SeqScape® Software v2.7 (Applied Biosystems) and Sequencing Analysis Software (Applied Biosystems).

Quantitative PCR

For the qPCR analysis, genomic DNA from the patients 1 and 2 and two other individuals with two copies of the region (as indicated by the array analysis) were evaluated for quantity and quality via an ND-8000 Spectrophotometer (NanoDrop®) and agarose gel electrophoresis. Only intact genomic DNA was used for qPCR

analysis. Six sets of Taqman® CNV assays were custom designed, each at approximately 9 kb interval, to span the entire duplication (Supplemental data, Table 1).

Each DNA sample was analyzed in triplicate and reactions were conducted in a MicroAmp fast 96-well optical reaction plate (P/N 4346906; Applied Biosystems) sealed with an optical adhesive cover (P/N 4311971; Applied Biosystems) on 7900HT System (96-Well Block) real-time PCR System. Each individual reaction contained CNV assay (containing two primers and a FAM™ dye labeled MGB probe), RNaseP assay (containing two primers and aVIC® dye-labeled TAMRA™ probe), TaqMan® Genotyping Master Mix (containing AmpliTaq Gold® DNAPolymerase, and dNTPs), 15ng genomic DNA and water to a final reaction volume to 20 µl. Reactions were held at 95 °C for 10 min and then cycled 40 times through 95 °C for 15 s and 60 °C for 1 min. The data was collected using Sequence Detection Software v2.3 (Applied Biosystems). qPCR data was analysed using a delta delta Ct algorithm with RnaseP as an endogenous control. Calibration with a control sample with two copies of the region was used to calculate delta delta Ct values and assign copy numbers by CopyCaller® software.

RESULTS

For all the patients initial cytogenetic standard G-banding analysis (550 bands) showed an homogenous 46,XX karyotype. FISH studies with both the *DXZI* and *SRY* probes confirmed the existence of two X chromosomes and the absence of the *SRY* gene in all cells analysed (Supplemental data, Figure 1A). Hybridization with the *SOX9* probe was normal for patients 1 and 2 (not performed for patient 3) (Supplemental data, Figure 1B).

In patient 1 and 2, SNP array analysis identified a 77,078 to 85,175 bp duplication in chromosome 17 long arm, upstream from *SOX9* gene, extending between 69,490,856 (normal)-69,493,863 (duplicated) to 69,570,941 (duplicated)-69,576,031 (normal) (Figure 1). For patient 3, array CGH identified an overlapping duplication of 194 kb in the same region which was reduced to a 140,572-152,536 bp between 69,435,809 (normal)-69,441,277 (duplicated) and 69,581,849 (duplicated)-69,588,345 (normal) using the SNP array (Figure 1). These duplications were confirmed by independent qPCR analysis for patients 1 and 2 (data not shown) and sequencing the boundaries of the duplication for patient 2 which allowed us to identify the exact size of the duplication extending between 69,491,366 and 69,575,195 (83,829 bp duplication) (Figure 2).

Xiao et al, described an XX male with a 74 kb duplication overlapping with this region which proximal breakpoint was located between 69,533,305 bp (normal) and 69,534,526 bp (duplicated) [Xiao et al., 2013]. Taking this data and our breakpoint into account we can redefine the minimal region duplicated to a minimum

size of 40,669 bp and a maximum size of 41,890 bp (Figure 3). Detailed analysis of this region indicates that it contains two non-overlapping putative enhancer elements. The Encyclopedia of DNA Elements (ENCODE) project datasets indicate a weak or poised enhancer at chr17:69,568,380-69,569,850. Chromatin at this position is enriched for experimentally determined CTCF-binding from human mammary epithelial cells (HMEC). The primary role of CTCF is thought to be in regulating the 3D structure of chromatin. CTCF creates boundaries between topologically-associating domains in chromosomes and anchors DNA to cellular structures like the nuclear lamina and thereby facilitates interactions between transcription regulatory sequences [Murrell, 2011]. This region is also associated with acetylation of histone H3 at lysine 27 (H3K27ac) and enriched in monomethylation of histone H3 at lysine 4 (H3K4me1) from a variety of cell lines (Figure 3). Both H3K4 methylation and H3K27 acetylation are epigenetic marks that are generally associated with gene activation [Eissenberg and Shilatifard, 2010; Smith and Shilatifard, 2014].

A strong putative enhancer element is located at chr17:69,544,206-69,546,005 and it is associated with H3K27Ac and H3K4me1 enrichment, as well as the repressive mark H3K9me3 which was identified experimentally by ChIP-seq. The histone acetyltransferase EP300, which is a known co-activator of SOX9-dependent gene expression, binds to this element (chr17:69,544,741-69,545,056) [Furumatsu et al., 2009]. This was determined experimentally using neuroblastoma cell line treated with retinoic acid (SK-N-SH_RA).

Multiz alignment results obtained from the UCSC genome browser show 12 short evolutionary-conserved regions that are enriched for transcription factors binding sites that are present in the human, mouse and rat (Figure 3). These include factors that are strongly expressed in the male supporting cell lineages of the mouse testis at the time of sex-determination (E11.5 to E13.5) and include NFIL3 (also known as E4BP4; chr17:69,544,862-69,544,873), PBX1 (chr17:69,563,419-69,563,427) and GATA1 (chr17:69,560,189-69,560,198 and chr17:69,560,189-69,560,198). In addition, to these factors TRANSFAC and TFSEARCH analysis of the human sequence predicted multiple DNA-binding sites for transcription factors known to be involved in the early stages of sex-determination such as SRY, WT1, SOX9, NR5A1 and LHX9 [Bashamboo and McElreavey, 2013]. The region also contains 4 predicted binding sites for the evolutionary conserved transcription factor DMRT1 at chr17:69,543,454-69,543,468, chr17:69,554,542-69,554,559, chr17:69,559,047-69,559,062 and chr17:69,569,634-69,569,648.

DISCUSSION

Multiple tissue specific enhancers located upstream and downstream from *SOX9* have been described [Velagaleti et al., 2005; Bagheri-Fam et al., 2006; Gordon et al., 2009]. In the human, a large duplication

(46,XX,dup(17)(q23.1q24.3)/46,XX) involving *SOX9* was associated with female to male sex reversal in a 46,XX individual [Huang et al., 1999], however mutations or translocations involving the *TESCO* region have not yet been reported [Georg et al., 2010]. This suggests that in human gonad additional regulatory elements may be involved in the regulation of *SOX9* expression. This hypothesis is supported by recent reports that identified rearrangements involving elements situated ~600 kb upstream of *SOX9* in association with 46,XX testicular DSD and termed the RevSex element [Benko et al., 2011; Cox et al., 2011; Vetro et al., 2011; Xiao et al., 2013]. The phenotypes associated with these rearrangements are reported in Table 1. In this study, through the analyses of three XX males and comparison with the published data, we redefined the minimal critical region located ~600 kb upstream of *SOX9* associated with XX sex-reversal. The two microduplications that we describe overlap those previously reported in 46,XX testicular DSD as well as deletions in 46,XY undermasculinized DSD patients. Therefore, we can define the critical duplicated region to 69,533,305 bp (normal) and 69,534,526 bp (duplicated) to 69,575,195 bp. This corresponds to a minimum size of 40,669 bp and a maximum size of 41,890 bp. Although this considerably reduces the extent of the RevSex regulatory element, it is unclear precisely how the rearrangements of this region result in changes in the expression of *SOX9* in the early developing gonad.

The duplicated region itself contains two predicted enhancer motifs. Although interpretation of ENCODE datasets should be taken with caution since the data was generated using cell lines that are not necessarily relevant for DSD, there are potentially interesting observations. The proximal strong enhancer motif (chr17:69,544,206-69,546,005) is enriched for H3K4 methylation and H3K27 acetylation that are epigenetic marks characteristic of gene activation [Eissenberg and Shilatifard 2010; Smith and Shilatifard, 2014]. This enhancer element binds the histone acetyltransferase EP300 that regulates transcription via chromatin remodelling and it is important in the processes of cell proliferation and differentiation [Ogryzko et al., 1996]. EP300 is strongly expressed in the somatic and germ cell lineages of the XX and XY gonad during sex-determination and it can act as a co-activator of both NR5A1 and *SOX9* [Ito et al., 1998; Furumatsu et al., 2009; Munger et al., 2013]. The CH3 domain of EP300 directly associates with the C-terminal PQ-rich transactivation domain of Sox9, and activates Sox9-dependent transcription in chondrogenesis by induction of histone acetylation [Furumatsu et al., 2009]. NR5A1 (also known as SF-1) was originally identified as a master-regulator of steroidogenic enzymes in the early 1990s and controls many key aspects of adrenal and reproductive functions [El-Khairi and Achermann, 2012]. Mutations involving *NR5A1* are one of the most common causes of 46,XY DSD and they are associated with a range of phenotypes including 46,XY gonadal dysgenesis and adrenal

failure, 46,XY DSD with apparently normal adrenal function, 46,XY infertile male and 46,XX female with ovarian insufficiency [El-Khairi and Achermann, 2012]. It is possible that *SOX9* expression may be regulated through this element involving both NR5A1 and EP300 proteins. Furthermore, this enhancer motif is located between two predicted binding sites for DMRT1 (chr17:69,543,454-69,543,468 and chr17:69,554,542-69,554,559). DMRT1 controls many aspects of testicular development in the mouse and human, including the differentiation, proliferation, migration, and pluripotency of germ cells, and also proliferation and differentiation of Sertoli cells [Matson and Zarkower, 2012]. Haploinsufficiency of *DMRT1* in the human is associated with a failure of testis-determination, whilst in the mouse DMRT1 is essential for maintaining mammalian sex-determination by antagonising the ovarian gene regulatory pathway [Ottolenghi and McElreavey, 2000; Matson et al., 2011]. In avians, where *DMRT1* is the best candidate testis-determining gene, it is necessary for the expression of *SOX9* [Smith et al., 2009; Lambeth et al., 2014]. ChIPseq experiments using mice using cells from P28 testis have demonstrated that *Dmrt1* binds to both upstream and downstream of *Sox9* and bind near to the genes *Wnt4*, *Foxl2* and *Rspo1* suggesting that DMRT1 may initiate or maintain male cell fate by directly activating *Sox9*, whilst repressing female-promoting genes [Murphy et al., 2010; Matson et al., 2011]. The localisation of DMRT1-binding sites within a putative enhancer element within the RevSex element opens the possibility that the testis formations associated with these duplications could be initiated through DMRT1 activation.

Evolutionary conserved predicted DNA-binding sites for other proteins involved in sex-determination are located in the minimal region including PBX1. PBX1, encodes a TALE (three amino acid loop extension) class homeodomain protein that participates in multimeric transcriptional complexes to modulate gene expression [Longobardi et al., 2014]. A role for PBX1 in gonad development is highlighted by *Pbx1*-deficient mice which exhibit embryonic lethality at E15 and have severe adrenal hypoplasia together with pancreatic dysfunction, skeletal abnormalities and impaired gonadal development [Schnabel et al., 2003].

Narrowing the region encompassing gonad specific regulatory elements of *SOX9* gene to an approximately 40 kb interval containing evolutionarily conserved elements is of potential interest for further studies to delineate precisely the sequences and mechanism(s) by which this region may regulate the expression of *SOX9* during human testicular development. As hypothesized by Lybaek and colleagues, the RevSex element may interact with other known regulatory elements of *SOX9* such as the TESCO motif or the minimal promoter region of *SOX9*. In a study of a familial case of XX sex-reversal carrying a 148 kb RevSex duplication, the chromatin landscape at the TESCO enhancer element was found to be modified in cells from XX masculinized

individuals [Lybæk et al., 2014]. The authors speculate that the RevSex duplication was acting to induce long-range epigenetic changes including a more open chromatin landscape at the TESCO element resulting in the upregulation of SOX9 expression. The analyses of further DSD patients with duplications or deletions of this region should delimit further the minimal region involved and provide insights into the mechanism of sex-reversal. Moreover, the description of two novel duplications of this region, in two different families, suggests that it is likely to be a recurrent genetic cause of 46,XX-*SRY* negative males in humans although the exact mechanism of duplications/deletions remains to be elucidated.

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Table 1. Phenotypic features and characteristics of the rearrangement of individuals with rearrangement upstream of *SOX9*.

	Cox <i>et al</i> , 2011	Vetro <i>et al</i> , 2011	Benko <i>et al</i> , 2011				Xiao <i>et al</i> , 2013	Bhagavath <i>et al</i> , 2014	Our patients		
			DSD1	DSD2	DSD3	DSD4 Case 1	DSD4 Case 2	Case III3	Patients 1&2	Patient 3	
Karyotype	46,XX	46,XX	46,XX	46,XX	46,XX	46,XY	46,XY	46,XX	46,XY	46,XX	46,XX
SRY	-	-	-	-	-	NA	NA	-	+	-	-
Phenotype	- Male	- Male - Hypotrophic testes	- Hypospadias - Incurved short penis - Bifid scrotum	- Perineal hypospadias - Asymmetric scrotum	- Perineal hypospadias - Incurved penis - asymmetric scrotum	- Urogenital sinus - Phallus	- Female	- Male - Hypotrophic testes - Hypospadias	- Female - Left adnexal mass - Primary amenorrhea	- Male - Hypotrophic testes	- Male - Hypotrophic testis
Internal genitalia	- NA	- NA	- Epididymal structures - Bilateral fallopian tubes - Ovotestes	- Right : testis - Left : ovarian remnant with fallopian tube structures	- Vagina, uterus - Right : streak gonad partially differentiated toward ovary - Left : ovotestis, epididymal structure and fallopian tube	- Right : normal testis - Left : streak gonad, fallopian tube, hemi-uterus	- Right : streak gonad with gonadoblastoma - Left : ovary	- NA	- Small uterus - Left adnexal mass - Right streak gonad	- No mullerian remnants	- NA
Sex of rearing	Male	Male	Male	Male	Female	Female	Female	Male	Female	Male	Male
Gonadal histology	- Sertoli and Leydig cells - Atrophied seminiferous tubules - No spermatogenesis	- Germinal cell aplasia	- NA	- NA	- Right : dispersed primordial ovocytes and rare follicles - Left : - Testicular portion : numerous seminiferous tubules and Sertoli cells; - Ovarian portion : abundant primordial follicles	- Right : small testis and normal architecture with spermatogonia in seminiferous tubules - Left : streak gonad	- NA	- NA	- Streak gonad	- Eosinophilic Sertoli cells - Atrophy of seminiferous tubules - Leydig cells hyperplasia - No spermatogenesis	- NA
Type of rearrangement	Duplication	Duplication	Duplication	Duplication	Duplication	Deletion	Deletion	Duplication	Deletion	Duplication	Duplication
Size of the rearrangement	178 kb	96 kb	693 kb	148 kb	780 kb	240 kb	240 kb	74 kb	349 kb	83,8 kb	140 kb

NA : Not available

Legends to figures

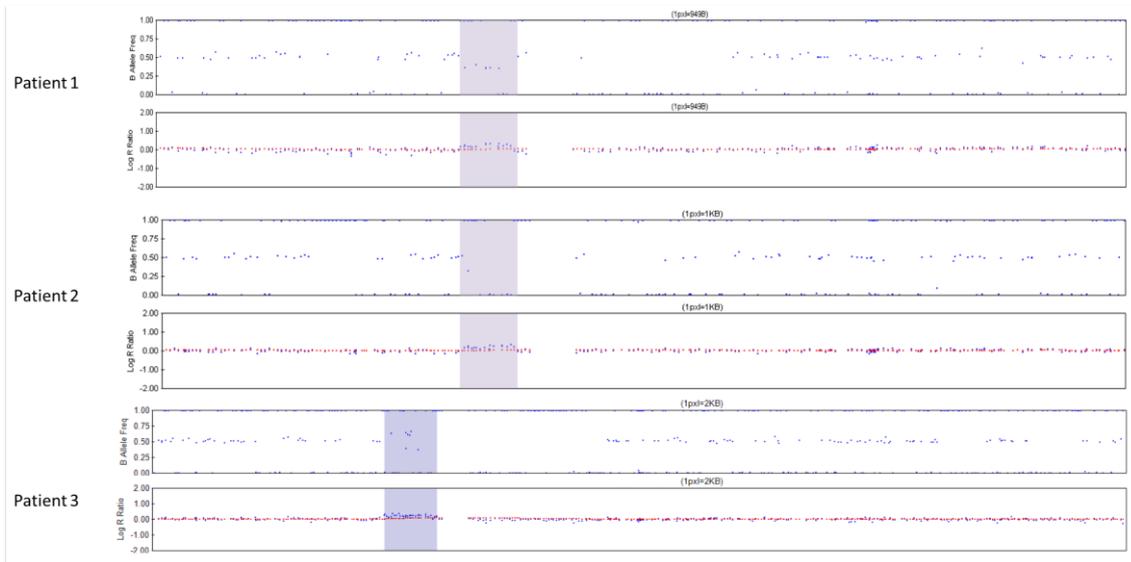


Figure 1. SNP array results for patients 1, 2 and 3 of the duplicated region. Note the increase in the log R Ratio within the duplicated region (grey box) (Scales: for patient 1 and 2: 1pixel=1kb, patient 3: 1pixel=2kb)

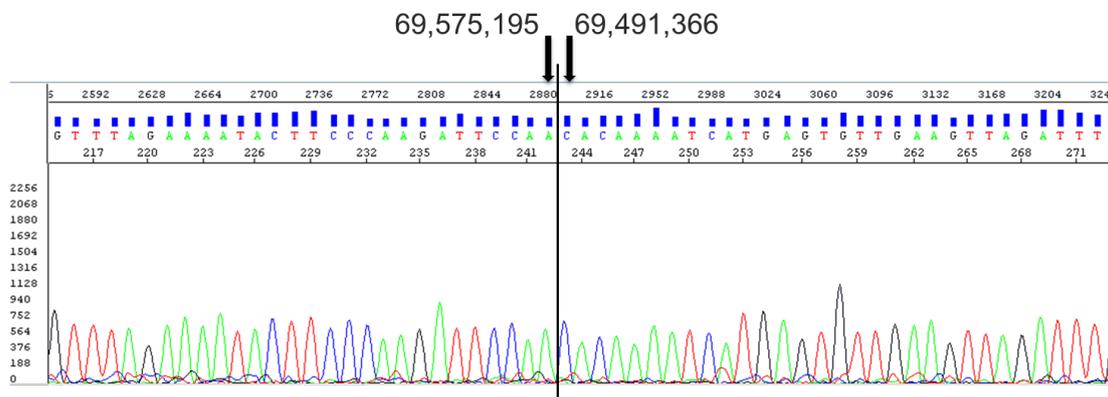


Figure 2. Exact boundaries of the duplication for patient 2 (according to the UCSC Genome Browser (<http://genome.ucsc.edu/>; February 2009, Assembly, hg19)).

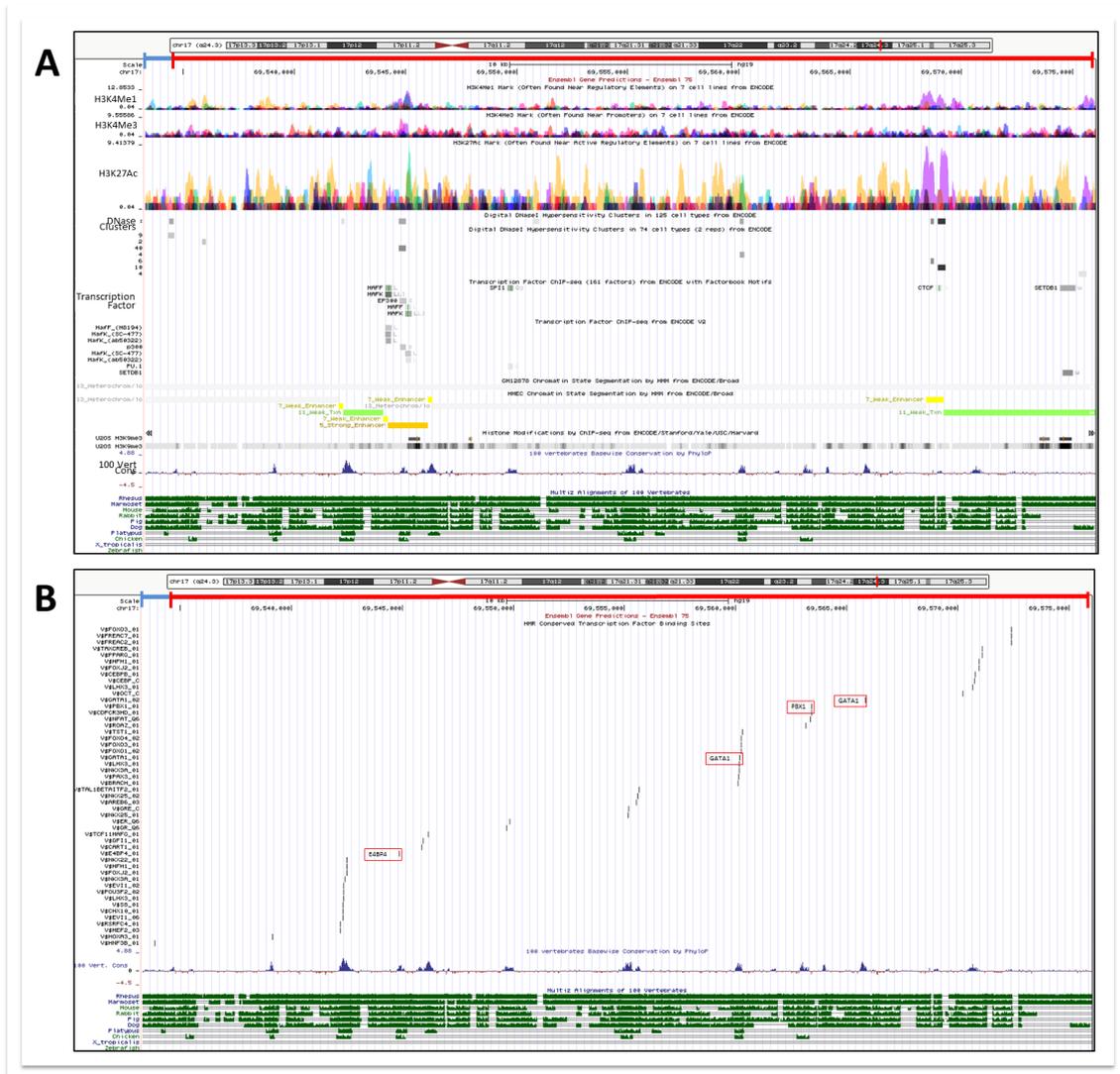


Figure 3 (A). UCSC genome browser panel displaying the minimal (red bar) and maximal (blue bar) extend of the revised RevSex duplication upstream of *SOX9*. Panels show H3K4me1, H3K4me3 and H3K27Ac sites within this region from a variety of cell lines. DNase I hypersensitive sites and transcription factor ChIP-seq binding sites are indicated. Proximal and distal enhancer elements are shown as well as Multiz conservation alignments. **(B)** UCSC genome browser panel displaying evolutionary conserved DNA transcription factor binding sites (see text for details). Histone modifications correspond to the following cells and cell lines –

GM12878	H1-hESC	HSMM	HUVEC	K562	NHEK	NHLF

GM12878 - B-lymphocyte, lymphoblastoid, International HapMap Project - CEPH/Utah - European

Caucasion, Epstein-Barr Virus

H1-hESC – human embryonic stem cells

HSMM – human skeletal muscle myoblasts

HUVEC – human umbilical vein endothelial cells

K562 – human leukaemia cell line

NHEK – normal human epidermal keratinocytes

NHLF – normal lung fibroblasts

Color figure can be viewed in the online issue, which is available at

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