Identification of an SRY-Negative 46,XX Infertility Male with a Heterozygous Deletion Downstream of SOX3 Gene

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Research Article

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Abstract

Background: A male individual with a non-chimeric karyotype of 46,XX is very rare. We explored the genetic etiology of an infertility male with 46,XX and SRY negative.

Methods: The peripheral blood sample was collected from the patient and subjected to a range of genetic testing, including conventional chromosomal karyotyping, short tandem repeat (STR) analysis for chromosome 13, 18, 21, X, Y contained SRY gene, azoospermia factor (AZF) deletion analysis including SRY gene, fluorescence in situ hybridization (FISH) with specific probes for CSP X/CSP Y/SRY, chromosomal microarray analysis (CMA) for genomic copy number variations (CNVs), and whole-genome analysis (WGA) for SNV&InDel variants, and the X chromosome inactivation (XCI) analysis for AR gene.

Results: The patient was found to have a 46,XX karyotype. Neither AZFa+b+c nor SRY band was detected in the electrophoresis result. FISH results of both interphase cells with CSPX/CSPY probe and metaphase cells with CSPX/CSPY/SRY probe showed two green fluorescence signals at the centromeres of X chromosomes, but no Y chromosome and SRY fluorescence signal. QF-PCR results showed that the patient had only the AMELX fluorescence peak of the X chromosome but no AMELY and SRY fluorescence peak. All results of the Karyotype, FISH, and STR did not suggest limited Y chimerism. CMA showed he had a heterozygous deletion of about 867 kb in Xq27.1 (hg19: chrX: 138,612,879-139,480,163 bp), located at 104 kb downstream of SOX3 gene, including F9, CXorf66, MCF2 and ATP11C; Meanwhile, whole-genome sequencing also found no SNV&InDel mutation associated with abnormal sex development. 75% X chromosome inactivation was detected.

Conclusions: Although the pathogenicity of 46,XX male patients with SRY negative remains unclear, SOX3 expression of the acquired function may be associated with partial testis differentiation. Therefore, copy number variation of SOX3 gene and regulatory region should be performed routinely for these patients.

Background

XX male sex Reversal (46,XX sex Reversal, SRXX) is a disease caused by the abnormalities of ovarian development, characterized by 46,XX karyotype and male phenotype, which is often referred to as 46,XX male[1]. The incidence rate in male newborns is about 1/20,000[2]. It has been reported that over 90% of 46,XX male genomes contain SRY gene that is 46,XX(SRY+) male, which is often caused by the translocation of Y chromosome fragment containing SRY gene to X chromosome[3-7]. Among the remaining 10%, a very few are 46,XX males without SRY gene, namely 46,XX(SRY-) males exclusive of the Y chromosome chimerism[8,9].

In previous reports, the causes of 46,XX(SRY-) male mainly include dose change or mutation of SOX9 gene[9-12], loss of function mutation of ovarian stimulating gene WNT4 or RSP01 gene[13,14], heterozygous mutation of NR5A1 gene[15-17], recently there are also a few reports about the copy number variation(CNV) of SOX3 (SRY-box transcription factor 3) gene[18]. Here, we will proceed with the
molecular genetic identification of a 46,XX(SRY-) male patient admitted to our clinic, and the research results will enrich the theoretical knowledge and guide the clinical treatment of this kind of patient.

**Methods**

**Subject**

A 31-years-old patient, heigh 166cm and weigh 52.5kg, went to our clinic due to primary infertility. Physical examination showed a male appearance, a thin beard, Adam's apple, two broad bean-size of testicles and an average size of the penis. No sperm was found in routine semen examination. Hormone test results were follow: Testosterone: 1.75ng/mL (reference value range(RVR): 2.80-8.00), progesterone: 0.11ng/mL (RVR: 0.20-1.40), prolactin: 208.44uIU/mL (RVR: 86.00-324.00), estradiol: 5.00pg/mL (RVR: 27.10-52.20), luteinizing hormone: 29.32mIU/mL (RVR: 1.70-8.60), follicle-stimulating hormone 37.88mIU/mL (RVR: 1.50-12.40). Deny genetic history. The parents of the patient refused the karyotype analysis.

**Specimen preparation and DNA extraction**

5mL of venous blood was collected from patients with heparin sodium and EDTA-Na$_2$ anticoagulant tubes, respectively, and ready for use. According to the manufacturer's protocols, genomic DNA was extracted from EDTA-Na$_2$ anticoagulated blood using QIAamp DNA Mini Kit (QIAGEN Company). DNA was qualified when the concentration was more than 30ng/uL, the OD260/280 value between 1.8 to 2.0 determined by ultraviolet spectrophotometer Nanodrop 1C (Thermo Fisher Scientific).

**Chromosome karyotype analysis**

Lymphocytes of heparin sodium anticoagulated blood were cultured, harvested, and prepared for microscope slides before Giemsa staining according to conventional cell culture methods. Zeiss karyotype analysis system (Karl Zeiss, Germany) was adopted for chromosome count and karyotype analysis, the same as previous studies[19].

**AZF detection**

Multiplex amplified was performed with AZF detection kit (Yaneng corp.), then 2.0% agarose electrophoresis and imaging, according to manufacturer's instructions.

**QF-PCR detection**

Multiplex PCR amplification was performed with Devyser compact v3 kit (Devyser AB, Sweden). The amplification condition was 95°C for 15 min; 94°C 30 sec, 58°C 1 min 30 sec, 72°C 1 min 30 sec, 27 cycles; 72 °C for 30 min. The amplified products were subjected to capillary electrophoresis with AB 3500Dx gene analyzer, and the electrophoresis data were analyzed by GeneMapper software. The fluorescence peak of AMELX in Xp22.2, AMELY in Yp11.2 and SRY in Yp11.31 were used to evaluate the patient’s gender. The experimental method was referred to in the previous report[20].
FISH analysis

Lymphocytes in EDTA-Na2 anticoagulant blood were isolated by lymphocyte separation solution and hybridized by CSP18/CSP X/CSPY probe (Jin Pujia corp.), as the same method as previously reported[21]. Meanwhile, the metaphase cells harvested from lymphocyte culture were co-hybridized with CSPX/CSPY probe (Jinpuga Company) and SRY probe (Abbott Company). The MIX-1 was prepared by SRY hybridization buffer and SRY probe at a ratio of 9:1, and the MIX-2 was made with CSP hybridization buffer and CSP X/CSP Y centromeric probe at a ratio of 4:1, and then added the MIX-1 and MIX-2 to the metaphase cells loaded on the glass slide. The Glass slide was denaturated at 78°C for 10 minutes and hybridized at 42°C for more than 16 hours. Refer to reagent instruction for the experimental operation. A fluorescence microscope observed the fluorescence signal of hybridization.

CMA analysis

500-1000ng of patient DNA and the equivalent amount of reference DNA was taken for simultaneous experiments. After digestion, the labelled patient sample was mixed with the reference sample and co-hybridized to SurePrint G3 CGH+SNP (180K) chip. Agilent DNA Microarray Scanner was used to scan the fluorescence signals after the slides were washed. Agilent Feature Extraction Software extracted the data from the images(.tif) and converted it to log-ratios data. Agilent CytoGenomics software was used to analyze CNV. Agilent Technologies provide the reagents, chips, instruments and analytical software. Refer to the instructions for specific methods. CMA analysis mainly adopts some online databases such as OMIM (https://omim.org/), DGV (http://dgv.tcag.ca/dgv/), Decipher (https://decipher.sanger.ac.uk/), ClinGen (https://wwW.clinicalgenome.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

WGA analysis

Illumina HiSeq PE150 high-throughput dual-terminal sequencing was performed after random interruption into tiny fragments of DNA, terminal repair, phosphorylation, a-tail addition, connector and library construction. Quality control was carried out on the raw sequencing data to obtain high-quality clean data; Then, fastp software[22] was used for comparative analysis of clean data and human reference genome sequence, and data such as sequencing depth and coverage of the target region were counted and obtained Bam files. Finally, SNP/InDel was detected and annotated based on Bam files to obtain all mutation information. The Haplotyper tool of Sentieo software[23] was used to detect SNP and InDel mutation, and ANNOVAR software[24] was used to annotate the mutation results accompanied by multiple databases (such as dbSNP, 1000G, ESP6500, HGMD, OMIM). Meanwhile, CNVkit software was used to analyze CNV[25].

XCI detection

The sample DNA of undigested and digested by HpaII, which methylation-sensitive restriction enzyme, was amplified by androgen receptor (AR) gene-specific primers and capillary electrophoresis subsequently. MIC2 was used as the reference gene, and the Hpa II enzyme was digested overnight in a 37°C water bath. The samples were amplified by double PCR before and after enzyme digestion. As
reported in the literature[26], FAM fluorescein was added to the 5' end of the forward primer[27]. PCR reaction conditions followed: 95°C for 5min; 28 cycles of 95°C for 45sec, 58°C for 30sec, 72°C for 30sec; 72°C for 7min. PCR products were subjected to capillary electrophoresis. XCI ratio was calculated according to formula (d1/u1)/(d1/u1 + d2/u2), and >70% was determined as XCI bias[28,29].

**Results**

**Cytogenetic analysis**

The patient's karyotype was 46,XX, as shown in Figure.

**AZF**

Electrophoresis results of the amplification products of patients showed no SRY bands nor corresponding bands of AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255) regions.

**QF-PCR**

The gender site of the patient was AMELX but not AMELY, and there was no fluorescence peak at the SRY site, as shown in Figure 2.

**FISH analysis**

FISH results of interphase cells hybridized by the centromere probes of 18, X and Y, showed two 18 and two X fluorescence signals but no Y signal, as shown in Figure 3A. FISH results of metaphase cells hybridized with X, Y, and SRY probes showed two green signals of X chromosome centromere, but neither of SRY nor Y chromosome, as shown in Figure 3b.

**Results of Y chromosome chimerism**

No Y chimerism was detected by karyotype analysis, CSPY analysis in FISH, and AMELY and SRY analysis in QF-PCR.

**CMA**

Taking 46,XX normal females as a reference, the patient’s Xq27.1 (hg19: chrX: 138,612,879-139,480,163 bp) had about 867Kb of heterozygotic deletion, and the deletion region was located at 104Kb downstream of the SOX3 gene, including F9, CXorf66, MCF2, ATP11C four protein-coding genes, as shown in Figure 4a and 4b.

**WGA**

The WGA results showed no pathogenic or likely pathogenic SNV&InDel variant related to sexual development, which can clearly explain the patient phenotype; meanwhile, the results also showed about 892kb heterozygous deletion (hg19: chrX: 138,609,392-139,501,392) in Xq27.1.
The XCI ratio of patients was about 75%, which was non-random inactivation. See Figure 5.

**Comparison of the clinical phenotypes of 46,XX SRY-negative male patients with CNV of SOX3**

Table 1 summarises the clinical features of 46,XX males with SRY-negative individuals involved in the CNV of SOX3. The patients all had the typical male appearance and showed common abnormal phenotypes, including spermatogenous testicular dysplasia because they were absent from the entire Y chromosome. Among the seven patients, five patients (case 1, 3, 4, 5, 6) had microduplications spanning the entire SOX3 gene, another two patients, including the case 2 and our patient, had microdeletions near the SOX3 gene, which were speculated to play a regulatory role for SOX3 expression. Our patient showed a CNV near the SOX3 gene in CMA, and WGA excluded no other SNV&InDel mutations associated with sex development. Meanwhile, he had a skewed X chromosome inactivation, which was not inconsistent with the case 1.

**Discussion**

SRY gene is recognized as the best TDF candidate gene. As long as the SRY gene exists in the individual genome, male gonadal development will occur even without the Y chromosome, the primary mechanism of 46,XX(SRY+) male pathogenesis. Therefore, Y chromosome chimerism in 46,XX(SRY-) males should be excluded first. This study detected no Y chromosome chimerism in the patient's peripheral blood through several experimental analysis methods. Of course, it was impossible to rule out completely the gonad limited chimerism due to the inability to obtain the gonad tissue. Other gene mutations may cause the pathogenesis of 46,XX(SRY-) males in the testicular development pathway, which makes the gonads of individuals without the SRY gene develop towards the testis[4,5].

Some 46,XX(SRY-) male individuals were previously reported to be caused by abnormalities of the SOX3 gene[9] and found the repetition of the SOX3 regulatory region can up-regulate SOX9 gene expression[30]. In our patient, There is a heterozygous deletion near the SOX3 gene in Xq27.1, which may be related to its pathogenesis.

SOX3 gene is one of 20 SOX(SRY-related HMG-box) gene family members. Stevanovic et al. cloned the SOX3 gene and identified its location at Xq27.1 in 1993[31]. SOX3 gene consists of a single exon and contains an HMG box, encoding 446 amino acids of a transcription factor SOX-3 protein[32]. SOX3 gene sequence is highly conserved among different species and has high homology with SRY and other SOX family genes. SOX3 gene is the ancestral gene of the SRY gene[9,33]. SOX3 encodes a transcription factor expressed in the central nervous system of vertebrate embryos[32], which plays a vital role in the pituitary, craniofacial and adrenal development. The variation of this gene is associated with X-linked mental retardation, growth hormone deficiency, X-linked hypothyroidism, 46,XX male sex reversal, and other diseases[9,34-37].
Loss-of-function of SOX3 induced by mutation does not cause sex determination abnormality in mice and humans[38]. However, studies in transgenic mice have shown that in-situ expression of SOX3 in bipotent gonads leads to up-regulation of Sox9 expression, leading to testicular induction and XX male sex reversal. The mechanism of SOX3 rearrangement causes sex reversals, and the frequency of sex development disorders remain unknown. The study of Moalem S et al. provided evidence that the de novo duplication of SOX3 in XX bipotent gonads causes the acquisition of SOX3 function[39]. In XX mice lacking the SRY gene, it was found that the expression of the SOX3 gene with the acquired function was related to partial differentiation of testis. Overexpression of SOX3, synergistically expression with SF1, up-regulated SOX9 stimulated gonad development into testis in XX mice[9,40]. SRY gene was derived from a new mutation in the regulatory region of the SOX3 gene and expressed in the early gonad. The data of transgenic mice indicated that SOX3 and SRY were interchangeable in sex determination function.

The type of variation in SOX3 that has been associated with 46,XX male sex reversal disorders is copy number variation of SOX3 (CNV) in previous reports. To date, 6 cases of SOX3 CNV related 46,XX male patients have been reported. In some cases, CNV duplication, including the SOX3 gene[39,41,42], resulted in the change of gene product dose. Some CNVS do not contain the SOX3 gene but are adjacent to the SOX3 gene region, or the breaking point of CNV falls in the regulatory region of SOX3, so it is speculated that CNV has a positional effect on SOX3 gene expression[9,43]. The elemental clinical manifestations of the patient include sexual reversal and gonadal dysplasia, azoospermia and infertility(adult individual), which may be combined with other abnormal clinical phenotypes if other genes are present. CMA showed that our patient had a heterozygous deletion of about 867kb in Xq27.1 (hg19: chrX: 138,612,879-139,480,163 bp), which was located at 104kb downstream of the SOX3 gene, including F9, CXorf66, MCF2 and ATP11C; Meanwhile, whole-genome sequencing also found an 892kb heterozygosity deletion in Xq27.1 (hg19: chrX: 138,609,392-139,501,392), but no SNV&InDel mutation associated with abnormal sex development. This patient is the first report of whole-genome sequencing in 46,XX male patients, and the use of WGA can entirely exclude pathogenic or likely pathogenic SNV&InDel variants in genes associated with the patient's abnormal sexual phenotype. About this deletion area, no similar report was found in the DGV database of the normal population, and no sexual reversal phenotype was reported in Decipher and ClinVar databases. Similar to previous reports[9,41], we speculated that the deletion region might involve the regulation region of the SOX3 gene, leading to differentiation and development of male testis through weakening inhibition of SOX3 and increasing expression SOX3[9,42].

Since the deletion of the patient in this study occurred on the X chromosome, the inactivation of the X chromosome is also an essential factor affecting the clinical significance. XCI is a dose-compensation mechanism, which usually occurs in early embryonic development when one of the two X chromosomes in a woman is inactivated, resulting in only one paternal or maternal chromosome being expressed in each cell of the female individual. In general, XCI is random, i.e., the ratio of the two X chromosomes inactivated in females is 50%: 50%[44]. However, if the inactivation is not random, it is called skewed inactivation. In 46,XX (SRY+) males, some studies[27,45] showed a high degree of XCI bias (greater than 90%), some were random XCI in other patients[46]. The phenotype of 46,XX males (SRY+) differed with the variable inactivation of the X chromosome carrying the SRY gene[47,48]. However, XCI has not been
reported in 46,XX male(SRY-) patients, so we conducted the XCI analysis in our patient. The XCI test found a moderate XCI bias; about 75% of X chromosomes in the patient’s peripheral blood were inactivated. We speculate that the expression of positive selection of the deficient X chromosomes results in the development of male gonads of the patient, similar to previous reports[49]. Since the sample of the patient’s mother could not be collected, it is unknown whether the patient's X chromosome deletions are de novo or inherited from an unphenotypic mother (the XCI bias inactivates the abnormal X chromosome). The XCI ratio of gonads tissues may differ from peripheral blood[28], but it cannot be accurately known because the sample is inaccessible.

Similar to the two adults patients reported by Sutton E et al.[9], the main features of our patient are azoospermia and infertility[50] because of a deletion of the entire Y chromosome, namely deletion of AZF region, contained USP9Y, DBY, PRY, RBMY, DAZ, BPY2 and other genes related to sperm production, development and maturation. AZF region is recognized as the most common factor of male infertility[51-53], which account for 10%-15% of azoospermia and 5%-10% of severe oligozoospermia[54].

At present, the pathogenesis of SRY positive 46,XX male patients is relatively straightforward. However, the molecular mechanism, signalling pathway and genetic regulation of SRY negative 46,XX male are not understood enough, and the diagnosis and treatment of these patients are still relatively complex. Sutton et al.[9] identified three arrangements including or adjacent to the SOX3 gene, accounting for 19% (3/16) in 16 SRY-negative 46,XX male patients. Subsequently, several case reports indicated that SOX3 is a critical pathogenic factor in SRY negative 46,XX male patients. Therefore, it is crucial to conduct the CNV determination involved in the SOX3 gene in all SRY negative 46,XX male patients. It is noteworthy that the current and reported SOX3 duplications or deletions are below the detection threshold of conventional karyotype and were found only by analyzing CNVs using CMA. Therefore, CMA detection is routinely recommended to detect CNVs, and high-throughput sequencing can simultaneously rule out other SNV/INDEL mutations. In addition, the XCI test in XX diseases also should be considered.

**Declarations**

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**Authors’ contributions**

Shengfang Qin conceived and designed the study, interpreted the data and drafted the manuscript. Xueyang Wang enrolled the patients, performed genetic counseling and provided clinical information. Jin Wang performed the laboratory detection and experimental data acquisition.

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**Availability of data and materials**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The research was approved by the Institutional Committee for the Protection of Human Subjects (Institutional Review Board of Sichuan Provincial Maternity and Child Health Care Hospital, 20201113-98), and the patient signed the informed consent.

**Consent for publication**

The patient had provided his consent for publication.

**Competing interests**

The authors declare that they have no competing interests.

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Tables

Table 1 Comparison of the clinical phenotypes of 46,XX SRY-negative male patients with CNV of SOX3.
| Case | Reference | CNV | Clinical phenotype | Detection method | X chromosome inactivation |
|------|-----------|-----|--------------------|------------------|--------------------------|
| 1    | Sutton E et al. 2011(9) | The patient contained two microduplications of approximately 123 kb and 85 kb, the former of which spanned the entire SOX3 gene. | The patient was an infertility male of 30-year-old. His height was 165 cm, and he weighed 64 kg, with no abnormal symptoms. Infertility was indicated by two spermograms, which confirmed azoospermia. The patient presented with small testicles and normal secondary sexual characteristics. | CMA | X-inactivation studies showed no evidence for skewed inactivation in DNA derived from lymphocytes |
| 2    | Sutton E et al. 2011(9) | The patient contained a single 343 kb microdeletion immediately upstream of SOX3; the coding sequence of SOX3 is not affected. It is suggested that altered regulation (and not increased dosage) of SOX3 is the cause of XY male sex reversal. | The patient was a 35-year-old with gender dysphoria. Height was 167.5 cm, weight 73.5 kg, with no medical problems apart from ongoing gender identity issues. The external genitalia was typical male, apart from small, soft 6 mL testes. There was little body hair. Primary hypogonadism, with FSH and LH elevated, and testosterone low. Histological examination showed atrophic changes in the testes, with loss of normal spermatogenesis, thickening and karyolysis of the tubular basal lamina, and diminished interstitial cells. The patient was SRY-negative in both peripheral blood and testicular tissue. The SOX3 rearrangement was not present in his mother. | CMA | NA |
| 3    | Sutton E et al. 2011(9) | The patient has a large (approximately 1-Mb) duplication that encompasses SOX3 and at least 13 additional distally located genes, overexpression of which probably contributes to the phenotypic complexity. Notably, the proximal breakpoint falls within the SOX3 regulatory region. | The patient was a boy of 19 months who presented with a more complex phenotype that also includes scrotal hypoplasia, microcephaly, small testes on one side, developmental delay(height 75.2 cm [<5th percentile]), and growth retardation. There were no significant problems during pregnancy or the newborn period. No endocrine evaluation or parental DNA was available. | CMA | NA |
| 4    | Moualem S et al. 2012(30) | The patient has a de novo 494 kb copy number gain in region Xq27.1 (NCBI 36/hg18: 139,354,859 - 139,848,664) containing the SOX3 RP1-177G6.2, CDRI and MR32D2 genes. | The patient was a 46,XX male newborn with hypospadias. Ultrasound examination revealed normal testicular size and structure. | CMA | NA |
| 5    | Grinep and RP et al. 2016(31) | The patient has a de novo gain at Xq27.1. The duplicated region was around 0.5 Mb, and encompassed the SOX3 gene (arr[hg19] Xq27.1(139,541,737-140,043,605)x3). Other genes included were RPS7, TP1, CDK1, MR3201D2. | The patient was a two years six months boy He had a trophic phallus 32 mm long and 13 mm wide with ambiguous genitalia and bilateral evotesticular DSD. | CMA | NA |
| 6    | Tasic V et al. 2019(32) | The patient has a 550 kb duplication at Xq27 (ChrX: 139,360,520-139,908,320), involving SOX3, the non-coding RNA LINC00632, AK054921, CDRI1 and the miRNA MR3201D2. | The patient was an 11-year-old boy with right kidney hypoplasia and moderate coronal hypospadias. His testes volume was >4 mL, and the penis length was 5 cm. | CMA | NA |
| 7    | Present study | CMA found the patient contained a heterogeneous deletion of about 867 kb in Xq27.1 (138612879-139480160 bp), located at 104 kb downstream of SOX3 gene, including F9, CXorf66, MCF2 and ATP11C. Whole-genome sequencing also found an 802kb heterozygosity deletion in Xq27.1 (GRCH37: ChrX:13869392-139501392), but no | The patient was a 31-years-old primary infertility patient with two small testicular, his height was 166cm, and he weighed 52.5kg. Physical examination showed a male appearance, a little beard, Adam's apple, the testicles are the size of broad bean, the penis is average size. No sperm was found in routine semen examination. Hormone test results showed testosterone and estradiol were low, FSH and LH were high. The parental sample was unavailable. | CMA, WGA | X chromosome inactivation studies was about 75%, which was non-random inactivation. |
Figures

Figure 1

Chromosome karyotype of the patient. The sex chromosome of 46,XX male sex reversal patient is XX, as the arrow indicated, but no Y chromosome.
Figure 2

QF-PCR electrophoresis diagram of the 46,XX (SRY-) male patient. QF-PCR results of the patient showed a fluorescence peak of AMELX but no AMELXY and SRY. AMELX, AMELXY and SRY represent the loci of Xp22.2, Yp11.2 and Yp11.31, respectively. The solid arrow indicated a specific amplification peak, while the hollow arrow indicated no amplification peak.
Figure 3

(a) Interphase cells FISH of the 46,XX (SRY-) male patient using 18, X, and Y centromeric probes. The blue signal represents the centromeres on chromosome 18, while the green signal represents the centromeres on chromosome X, as indicated by the arrow. 

(b) Metaphase cells FISH of the 46,XX (SRY-) male patient with X, Y and SRY probes. The green signal represents the centromeres of the X chromosome, as indicated by the arrow.
Figure 4

a. CMA results of the 46,XX (SRY-) male patient. There was about 867kb heterozygous loss in Xq27.1 (hg19: chrX: 138,612,879-139,480,163bp), as the line indicated. b. Schematic diagram of gene chip deletion region of the 46,XX (SRY-) male patient. The deletion region (hg19: chrX: 138,612,879-139,480,163 bp) is located at 104kb downstream of the SOX3 gene in Xq27.1. The dotted line is the deletion region with a size of about 867kb.
Figure 5

X chromosome inactivation results of the 46,XX (SRY-) male patient. The figure above and below show the fluorescence peaks of the X chromosome before and after HpaII digestion, respectively. The ordinate and abscissa represent fluorescence intensity and fragment length. The black arrow indicates the amplified products of the reference gene. After complete digestion, there is no amplified products peak (as shown below). The red and green arrows indicate the two alleles of the AR gene in the X chromosomes. X chromosome inactivation was calculated according to the formula $(d_1/u_1)/(d_1/u_1 + d_2/u_2)$. The X chromosome inactivation rate in this patient was about 75%, which was non-random. $d_1$: the height of the higher peak after enzyme digestion, $u_1$: the height of the undigested peak, which corresponding to $d_1$; $d_2$: the height of the shorter peak after digestion; $u_2$: the height of the undigested peak, which corresponding to $d_2$. 