

CASE REPORT

A de novo derivative Y chromosome (partial Yq deletion and partial duplication of Yp and Yq) in a female with disorders of sex development

Qing-Song Liu¹  | Xing-Chun Zhu² | Qiang Ma¹ | Cheng He¹ | Jian-Lan Shao³

¹Department of Clinical Laboratory, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China

²Pathogenic Biology and Immunology Experiment Teaching Center, North Sichuan Medical College, Nanchong, Sichuan, China

³Department of Obstetrics and Gynecology, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China

Correspondence

Jian-Lan Shao, Department of Obstetrics and Gynecology Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China.

Email: sssjjlll65@163.com

Funding information

Doctoral startup fund of North Sichuan Medical College, Grant/Award Number: CBY14-QD-08; the Education Department of Sichuan province, China, Grant/Award Number: 16ZA0233

Key Clinical Message

We report an atypical disorders of sex development (DSD) case with no mutation of SYR gene but partial Yq deletion and partial duplication of Yp and Yq. This case emphasizes duplicated region Yp11.2→Yq11.223 with partial deletion of Yq11.223→Yqter most probably perturbed the sex differentiation and led to female phenotype.

KEYWORDS

disorders of sex development, karyotype, SRY

1 | INTRODUCTION

Disorders of sex development (DSD) are defined by congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical.¹ DSD is a rare form of sex reversal in primary amenorrhea female with 46, XY karyotype.² The chromosomal aberrations in lymphocytes of peripheral blood include Y chromosomes translocation with autosome³ and X chromosomes,⁴ and partial deletions/microdeletions of chromosome 9.^{5,6} Typically genetic etiology of DSD is mutations of the sex-determining region on the

Y chromosome (SRY) gene. These mutations lead that SRY gene fails to upregulate the transcription of another key DSD gene and transcription factor SOX9 during early embryonic development. Then, initially immature bipotential gonads fail to differentiate along the male (testicular) pathway.^{7,8} So far, clinical data have shown that 10%-15% of DSD have SRY gene mutations including nearly 40 different mutations described in XY female patients.⁹

It is rare that the rearrangements of Y chromosome resulting in monocentric structure with duplication of large segments of short and long arms of Y chromosome and a

Qing-Song Liu and Xing-Chun Zhu are contributed equally.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. *Clinical Case Reports* published by John Wiley & Sons Ltd.

partial deletion of Yq.¹⁰ Besides, these rare aberrance is present mostly in phenotype male.¹¹ However, the most common cytogenetic aberrations of Y chromosome are isodicentric chromosomes in male and female cases.¹²⁻¹⁴ Phenotypes depend on the location of the breakpoints and on the proportion of aberrant cell lines which vary among male, abnormal female, and individual with ambiguous genitalia.¹³ Due to mitotic instability of idics, patients with idics always develop a mosaic with 45,X cell line, and with a wide spectrum of manifestations regardless of females with Turner symptoms and males with spermatogenic failure.^{15,16} Furthermore, the breakpoints in these patients were found to locate in the 11.2 region of Yq which contains a azoospermic factor (AZF) region. It has been confirmed that most of the patients have AZF microdeletion.¹⁴

Here, we described a primary amenorrhea female who with a derivative Y chromosome [der(Y)] identified by standard cytogenetic analysis. To delineate this der(Y), fluorescence in situ hybridization (FISH) and SNP array characterization was performed. Another, the molecular analysis of the AZF was performed to characterize the aCGH results of Yq11.2 deletion.

2 | METHODS

2.1 | Clinical data

A 17-year-old girl was referred to the cytogenetic laboratory for chromosome analysis for primary amenorrhea. She exhibited a female appearance and voice, with little subcutaneous fat, no beard, or laryngeal prominence. And she exhibited female external genitalia, with hypogenetic labia majora and minora, sparse pubic hair, and a visible vaginal orifice. She was born following a full-term normal delivery to noncon-sanguineous parents, and her mother denied the use of any sex hormone drugs or exposure to radioactive substances during pregnancy. Her younger brother exhibited a normal phenotype.

The B-mode ultrasound showed a promordial uterus, the left Ovarian cannot display, and the right adnexa display 28 × 24 mm cystic separation. Serum sex hormone analysis revealed that the follicle-stimulating hormone level was 58.18 IU/L, the luteinizing hormone level was 18.17 IU/L, the estradiol level was 15 pg/mL, the prolactin level was 18.34 ng/mL, the pregnendione level was 0.3 ng/mL, and the testosterone level was 0.21 ng/mL.

All procedures used in the study confirmed to the tenets of the Declaration of Helsinki. The Ethics Committee of Affiliated Hospital of North Sichuan Medical College approved the protocols used. All participants have known to participate in the study. Written informed consents were obtained from all participants.

2.2 | Karyotype analysis of G-banding in lymphocytes

Chromosomal analysis of peripheral blood lymphocytes was performed according to standard protocols. Peripheral blood (2 mL) was collected in heparin vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Cytogenetic studies were carried out on the patient, her parents, and her younger brother. For cytogenetic analysis, whole blood (0.5 mL) cultures were set up in 5 mL Roswell Park Memorial Institute (RPMI) 1640 media (GIBCO BRL, Grand Island, NY, USA) containing 15% fetal calf serum (GIBCO BRL, Grand Island, NY, USA), antibiotic mixture, and phytohemagglutinin P (GIBCO BRL, Grand Island, NY, USA) for 72 hours. Chromosome preparations were obtained from lymphocyte cultures and analyzed after Giemsa-Trypsin-Giemsa (GTG)-banding. For karyotyping, at least 30 metaphases were analyzed, and for karyotypes of suspected mosaicism, 100 cells were counted. The karyotypes were interpreted using the recommendation of the International System for Human Cytogenetic Nomenclature.

2.3 | Fluorescence in situ hybridization (FISH) in metaphase cells

Metaphase cells were performed on cultured, phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes, according to standard cytogenetic procedures. FISH followed manufacturer's instructions, using centromeric probes for chromosomes CSP 18/X/Y (18p11.1-q11.1, Xp11.1-q11.1, Yp11.1-q11.1; GPMedical, Beijing, China). The slides were analyzed by two independent observers using an Olympus BX50 (Olympus Optical Co., Ltd., Tokyo, Japan) fluorescence microscope equipped with a filter set for FITC, Texas Red, and Aqua. Normal male lymphocyte preparations were used as controls for the FISH assays.

2.4 | Multiplex quantitative fluorescent polymerase chain reaction (MQF-PCR)

Genomic DNA from 200 µL peripheral blood of the propositus, her family members, a healthy fertile man, and a normal woman was extracted using blood DNA extraction Kits (TIANGEN, Beijing, China), using the procedure recommended by the producer. Amplification on 2 STR loci on chromosomes X (DXS981, DXS6809), one common STR loci on chromosomes X and Y, one gender specific loci AMXY, and three STR loci on chromosome 21 specific (D21S1435 D21S1411, D21S11) was conducted using 21-trisome and sex chromosomes polyploid detection kit (Daan Gene, Guangzhou, China), PCR mix created as directed by the producer. The kit contains primer pairs for 4 markers on sex chromosomes, 3 markers for chromosome

21, all in a single multiplex reaction. Fragmental Analysis was conducted on an ABI 3130 Genetic Analyzer, using Run 3130 Data Collection software, using 36 cm capillary array length, and performance optimizing polymer (POP) 7. Run time was set to 1800 seconds. Sizing standard used was ABI LIZ 500. Data analysis and electropherogram creation were carried out using GeneMapper ID v3.2 software.

2.5 | SNP array

Microdeletion/microduplication screening was performed using an SNP array platform (CytoScan HD SNP array; Affymetrix, Santa Clara, CA), following the manufacturer's instructions. The CytoScan HD SNP array has 2.69 million probes, including 1.95 million copy number probes and 0.74 million SNP probes. Array data were analyzed using the Chromosome Analysis Suite (ChAS) software v 2.0 (Affymetrix).

2.6 | Molecular analysis

Genomic DNA from peripheral blood of the proband, her family members, one normal male volunteers, and one normal female volunteers was extracted using blood DNA extraction Kits (TIANGEN, Beijing, China). Sequences of the entire coding regions of SRY was amplified with primers shown in Table S1 and sequenced (Sangon Biotech Co., Ltd, Shanghai, China). The three discrete regions, AZFa, AZFb, and AZFc, located on the long arm of the Y chromosome, were performed by multiplex PCR (Polymerase Chain Reaction) amplification. The set of PCR primers (Table S1)

(Sangon Biotech Co., Ltd, Shanghai, China) for the diagnosis of microdeletion of the AZFa, AZFb, and AZFc region included: sY84, sY86, sY127, sY134, sY254, sY255, SRY, and ZFX/ZFY.

3 | RESULTS

Conventional cytogenetic analysis (G-bands) revealed a der(Y) present in all 30 observed metaphase cells from the patient (Figure 1A). Because her parents and younger brother had normal G-banded karyotypes, it was concluded that the der(Y) was a de novo aberration.

To confirm the der(Y), FISH analysis was performed with Y chromosome centromere probe in metaphase cells from the patient and her father and younger brother. Two adjacent red fluorescence signals (Y chromosome centromere probe) in one chromosome were found in the proband's sample (Figure 1B), but only one red fluorescence signal appeared in her father's and younger brother's sample, respectively. MQF-PCR was also adopted to confirm the duplication of Y chromosome and the results showed the ratio of AMX (106) peak area to AMY (112) peak area was 0.5, and confirmed that the patient's Y chromosome is duplicated (Figure 1C). Based on conventional analysis, the karyotype 46,X,dup(Y)(q11.22;p11.3) was expected.

Furthermore, in order to exclude a cryptic rearrangement of the Y chromosome and to test for potential genomic micro-aberrations a genome-wide study with CytoScan HD array was performed. The array data showed that Yq11.23 region was deleted, while Yq11.223-Yq11.23 and most of Yp arm were duplicated (Figure 2 and Data S1). Thus, the

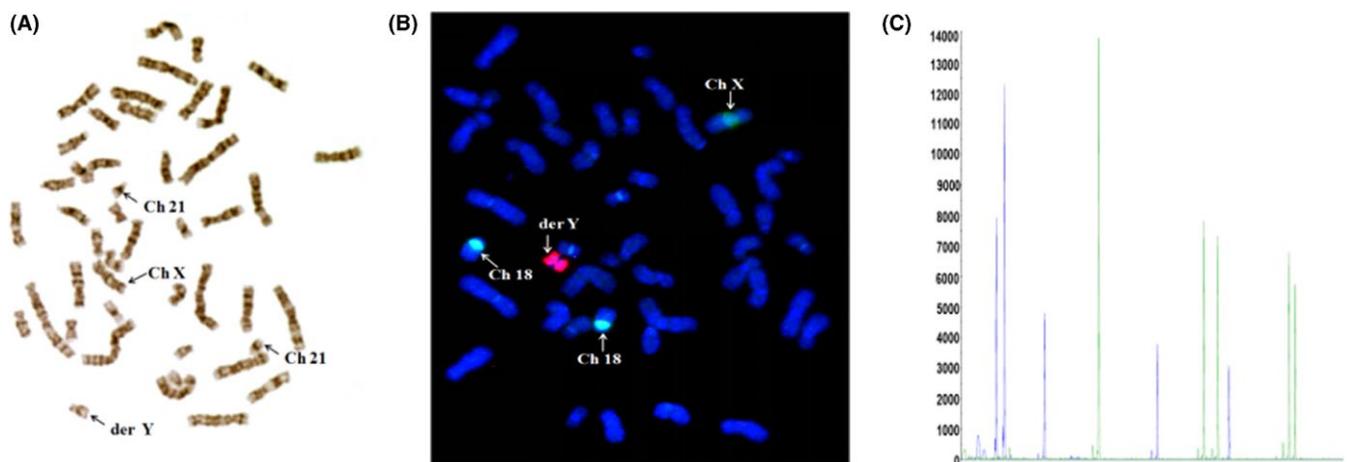


FIGURE 1 Results of karyotype, FISH and QF-PCR. A, In G-banded karyotype, the der (Y) in the patient were seen. B, Fluorescence in situ hybridization on metaphase chromosomes using Y (Yp11.1-q11.1),X(Xp11.1-q11.1), and 18 (18p11.1-q11.1) chromosome centromere-specific probes. Arrows indicate two Y chromosome specific centromeric signals (red) on one chromosome. C, Capillary electrophoresis results for the proband's sample with sex chromosomal aneuploidies (47, XYY) identified in the prospective examination. The results are produced using the GeneMapper software, showing full ranges and all sizes of the detected peaks on the vertical and horizontal axes, respectively, for intuitive intra-sample visual comparison of the peaks. Fragment sizes are shown in bp on the horizontal axis. Arbitrary fluorescence units are shown on the vertical axis

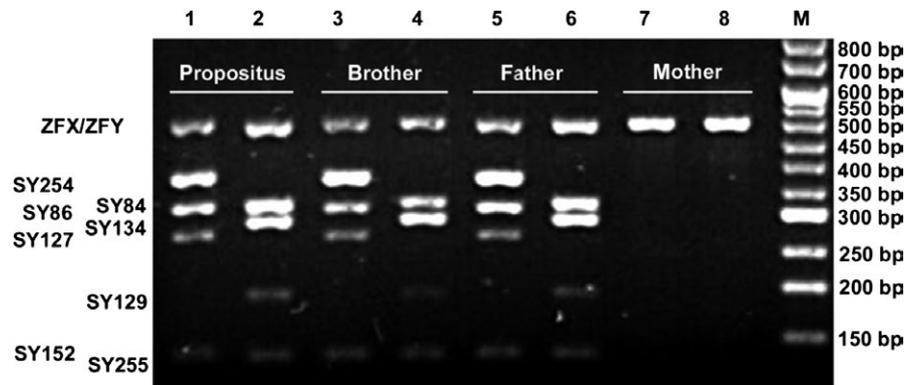


FIGURE 4 Result of SRY gene detected by polymerase chain reaction (PCR). Lines 1 and 2: DNA sample from the proband; lines 3 and 4: DNA sample from the proband's younger brother; lines 5 and 6: DNA sample from the proband's father; lines 7 and 8: DNA sample from the proband's mother; M: DNA Marker I. The bands of the odd lines refer to ZFX/ZFY, SY254, SY86, SY127, and SY152 according to the fragments' sizes from long to short, respectively; the even-numbered sets were ZFX/ZFY, SY84, SY134, SY129, and SY255

gene was present with no mutation (Figure S1). The results of array showed that SRY gene was also present in the duplication region and normal copies region, and the deletion region did not contain the SRY gene. Duplication or haploinsufficiency, such as DAX1, WNT4 or SOX9, SF1, WT1, and DMRT1-DMRT2, has been considered responsible for the development of 46,XY sex reversal,^{22,23} and therefore, the duplication of SRY gene in our patient may play a significant role in the etiology of the disease in this case.

A de novo karyotype 46,X,del(Y)(q11.2) observed in primary amenorrhea female after FISH and aCGH analysis permitted better characterization of derivative Y chromosome with final karyotype description as 46,X,der(Y)del(Y)(q11.2).dup(p11.1→q11.1).ish.arr Yp11.2q11.223(2,650,424-24,636,055)×2, Yq11.223q11.23(24,985,375-28,799,654)×0, Yp11.31q11.23(2,650,424-28,799,654)×1-2. Finally, duplicated region Yp11.2→Yq11.223 with partial deletion of Yq11.223→Yqter most probably perturbed the sex differentiation and led to female phenotype.

ACKNOWLEDGMENTS

We express our sincere gratitude to the patient and his families for their kind co-operation. This work was supported by Doctoral startup fund of North Sichuan Medical College grant: CBY14-QD-08, the Education Department of Sichuan province, China, grant: 16ZA0233.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORSHIP

QL and XZ: wrote the manuscript and collected the clinical data and were involved in scientific input and revision. QM

and CH: involved in scientific input and revision. JS: is the senior author and was involved in scientific input and revision of the final manuscript.

ORCID

Qing-Song Liu  <http://orcid.org/0000-0003-3871-1169>

REFERENCES

- Hughes IA, Houk C, Ahmed SF, Lee PA. Consensus statement on management of intersex disorders. *Arch Dis Child.* 2006;91:554-563.
- Du X, Zhang X, Li Y, Han Y. 46, XY female sex reversal syndrome with bilateral gonadoblastoma and dysgerminoma. *Exp Ther Med.* 2014;8:1102-1104.
- Lee IW, Chou YY, Hsu KF, et al. Complex chromosome rearrangement 46, XY, der(9)t(Y;9)(q12;p23) in a girl with sex reversal and mental retardation. *Urology.* 2011;77:1213-1216.
- Sanlaville D, Vialard F, Thépot F, et al. Functional disomy of Xp including duplication of DAX1 gene with sex reversal due to t(X;Y)(p21.2;p11.3). *Am J Med Genet A.* 2004;128A:325-330.
- Brandt T, Blanchard L, Desai K, et al. 46, XY disorder of sex development and developmental delay associated with a novel 9q33.3 microdeletion encompassing NR5A1. *Eur J Med Genet.* 2013;56:619-623.
- Schlaubitz S, Yatsenko SA, Smith LD, et al. Ovotestes and XY sex reversal in a female with an interstitial 9q33.3-q34.1 deletion encompassing NR5A1 and LMX1B causing features of Genitopatellar syndrome. *Am J Med Genet A.* 2007;143A:1071-1081.
- Chen YS, Racca JD, Phillips NB, Weiss MA. Inherited human sex reversal due to impaired nucleocytoplasmic trafficking of SRY defines a male transcriptional threshold. *Proc Natl Acad Sci U S A.* 2013;110:E3567-E3576.
- Hersmus R, de Leeuw BH, Stoop H, et al. A novel SRY missense mutation affecting nuclear import in a 46, XY female patient with bilateral gonadoblastoma. *Eur J Hum Genet.* 2009;17:1642-1649.

9. Okuhara K, Tajima T, Nakae J, Fujieda K. A novel missense mutation in the HMG box region of the SRY gene in a Japanese patient with an XY sex reversal. *J Hum Genet.* 2000;45:112-114.
10. Wiland E, Yatsenko AN, Kishore A, et al. FISH and array CGH characterization of de novo derivative Y chromosome (Yq duplication and partial Yp deletion) in an azoospermic male. *Reprod Biomed Online.* 2015;31:217-224.
11. Roovere T, Peters M, Horelli-Kuitunen N, et al. Cytogenetic and molecular characterization of the derivative Y chromosome: a case study of an azoospermic patient. *Clin Genet.* 2007;72:460-463.
12. Cui YX, Wang WP, Li TF, et al. Clinical and cytogenomic studies in a case of infertility associated with a nonmosaic dicentric Y chromosome. *Andrologia.* 2015;47:477-481.
13. DesGroseilliers M, Beaulieu BM, Brochu P, Lemyre E, Lemieux N. Phenotypic variability in isodicentric Y patients: study of nine cases. *Clin Genet.* 2006;70:145-150.
14. Kalantari H, Asia S, Totonchi M, et al. Delineating the association between isodicentric chromosome Y and infertility: a retrospective study. *Fertil Steril.* 2014;101:1091-1096.
15. Faure AK, Akinin-Seifer I, Satre V, et al. Fine mapping of rearranged Y chromosome in three infertile patients with non-obstructive azoospermia/cryptozoospermia. *Hum Reprod.* 2007;22:1854-1860.
16. Lehmann KJ, Kovac JR, Xu J, Fischer MA. Isodicentric Yq mosaicism presenting as infertility and maturation arrest without altered SRY and AZF regions. *J Assist Reprod Genet.* 2012;29:939-942.
17. Bergendi E, Plöchl E, Vlasak I, Rittinger O, Muss W. A Turner-like phenotype in a girl with an isodicentric fluorescent Y chromosome mosaicism. *Klin Padiatr.* 1997;209:133-136.
18. Bernstein R, Jenkins T, Dawson B, et al. Female phenotype and multiple abnormalities in sibs with a Y chromosome and partial X chromosome duplication: H-Y antigen and Xg blood group findings. *J Med Genet.* 1980;17:291-300.
19. Fukui S, Watanabe M, Yoshino K. 45, X mosaicism with Y chromosome presenting female phenotype. *J Pediatr Surg.* 2015;50:1220-1223.
20. Wu HH, Lee TH, Chen CD, Yeh KT, Chen M. Delineation of an isodicentric Y chromosome in a mosaic 45, X/46, X, idic(Y)(qter-p11.3;p11.3-qter) fetus by SRY sequencing, G-banding, FISH, SKY and study of distribution in different tissues. *J Formos Med Assoc.* 2007;106:403-410.
21. Wu G-F, Jin R-L, Jia H. Diagnosis of the missense mutation of SRY gene in a patient with 46, XY female sex reverse. *Med Pham J Clin PLA.* 2011;23:4-6.
22. Domenice S, Corrêa RV, Costa EM, et al. Mutations in the SRY, DAX1, SF1 and WNT4 genes in Brazilian sex-reversed patients. *Braz J Med Biol Res.* 2004;37:145-150.
23. Sukumaran A, Desmangles JC, Gartner LA, Buchlis J. Duplication of dosage sensitive sex reversal area in a 46, XY patient with normal sex determining region of Y causing complete sex reversal. *J Pediatr Endocrinol Metab.* 2013;26:775-779.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Liu Q-S, Zhu X-C, Ma Q, He C, Shao J-L. A de novo derivative Y chromosome (partial Yq deletion and partial duplication of Yp and Yq) in a female with disorders of sex development. *Clin Case Rep.* 2018;6:1671–1676. <https://doi.org/10.1002/ccr3.1613>