Case Report

Cytogenetic and Genome Research

Cytogenet Genome Res
DOI: 10.1159/000366170

Accepted: March 31, 2014
by M. Schmid
Published online: September 10, 2014

De novo Structure Variations of the Y Chromosome in a 47,XXY Female with Ovarian Failure: A Case Report

Bin Lin[a] Fengqin Tan[c] Heng Xu[a] Ping Wang[d] Quan Tang[a] Yufei Zhu[a] Xiangyin Kong[a, b] Landian Hu[a, b]

[a]The Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Jiao Tong University School of Medicine and Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and [b]State Key Laboratory of Medical Genomics, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, and [c]Laboratory of Cytogenetics, Hebei Research Institute for Family Planning, Shijiazhuang, PR China; [d]Department of Pediatrics, Duke University School of Medicine, Durham, N.C., USA

Key Words
De novo structure variation · Ovarian failure · *SRY* · 47,XXY · Y chromosome

Abstract
We report on a patient with a 47,XXY karyotype who presents a normal female phenotype, which is an extremely rare observation worldwide. The patient is infertile. Type B ultrasound scans and other tests suggested that her ovaries had completely failed. Microsatellite DNA marker analysis revealed that the 2 X chromosomes were derived from her mother and that this abnormality was caused by non-disjunction of the maternal X chromosomes during meiosis II. Copy number variation analysis identified 2 large de novo deletions in her Y chromosome. Remarkably, one of the deleted regions includes the *SRY* gene locus, which might explain her female phenotype. However, the genetic mechanism of her ovarian failure remains unclear. This paper is the first report of a 47,XXY female with ovarian failure.

47,XXY is the most common (approximately 80%) karyotype of Klinefelter's syndrome, which is considered as the most frequent genetic reason for male infertility [Lanfranco et al., 2004]. Almost all individuals with 47,XXY karyotypes have male phenotypes because of the Y chromosome, whereas those presenting female phenotypes with this condition are extremely rare. To date, only thirteen 47,XXY cases with female phenotypes have been recorded in the PubMed database [Khandelwal et al., 2010; Frühmesser and Kotzot, 2011]. Among these cases, 8 patients were diagnosed with complete androgen insensitivity syndrome [German and Vesell, 1966; Bartsch-Sandhoff et al., 1976; Gerli et al., 1979; Müller et al., 1990; Uehara et al., 1999; Saavedra-Castillo et al., 2005; Girardin et al., 2009; Frühmesser and Kotzot, 2011]; 2 cases were characterized with complete normal female internal and external genitalia [Thangaraj et al., 1998; Khandelwal et al., 2010]; and only 1 case was reported to be fertile and had 1 son and 2 daughters [Röttger et al., 2000]. Sex-determining region Y (SRY) is a key transcription factor that induces male sex determination [Kashimada and Koopman, 2010]. In the 5 cases in which the *SRY* gene had been tested, it seemed to be normal, with the exception of a mother and daughter pair, whose *SRY* genes were dis-

KARGER

E-Mail karger@karger.com
www.karger.com/cgr

1424–8581/14/0000–0000$39.50/0

Landian Hu
The Key Laboratory of Stem Cell Biology, Institute of Health Sciences
Shanghai Jiao Tong University School of Medicine and
Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences
Shanghai 200025 (PR China)
E-Mail ldhu@sibs.ac.cn

rupted by an aberrant X-Y interchange [Röttger et al., 2000].

The reports mentioned above only focused on the structure of the Y chromosome observed by cytogenetic methods or on the presence of the *SRY* gene; deletions or duplications of the genome were difficult to detect due to the limitations of the applied technology. Here, we present a whole-genome copy number variation (CNV) analysis of a 47,XXY female patient with ovarian failure, revealing several de novo structure variations in the Y chromosome of the patient, including 2 deletions that also contain the locus of the *SRY* gene.

Clinical Report

A 26-year-old female patient was referred to the hospital in 2006 because of infertility. The intelligence level and the height (163 cm) of the patient were normal, and she had a normal female phenotype, including well-developed breasts, a normal vagina and vulva. The patient went through menarche at the age of 14. However, according to the patient's recall, the timing of her menstrual cycle started to delay from September 2004, and before she came to the hospital in May 2006, she had not menstruated for 5 months. A series of type B ultrasound scans of the abdomen from 2005 to 2008 revealed that her uterus had a normal shape and size, but her ovaries were progressively shrinking until they could not be detected by ultrasound in 2006 (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000366170). The values of her sexual hormones measured at that time are shown in online supplementary table 2. These observations suggested that ovarian failure started at the beginning of 2006, and 5 months later, her ovaries had completely failed. Although the patient denied a family history of ovarian failure, the karyotypes of her family members were analyzed using G-banding. Intriguingly, chromosome analysis revealed a 47,XXY karyotype only in the patient (fig. 1a), whereas the karyotypes of her parents and younger brother were normal. The possibility of mosaicism was ruled out. Furthermore, her younger brother is fertile and his daughter's phenotype is normal (fig. 1b).

Materials and Methods

The written informed consent for the genetic analysis was obtained from the patient and her family members. The research was approved by the ethics committee at the Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Genomic DNA was extracted from blood samples taken from the patient and her family using QIAamp DNA Blood Mini Kits (QIAGEN, Cat. No. 51106). CNV analysis was performed using an Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, Calif., USA), and the data were analyzed using Partek software (Partek Inc.) under the HMM algorithm. To filter as many false positive results as possible, we employed a set of rigorous parameters: the CNVs were called according to the presence of different intensity values across at least 10 consecutive probes, and the cutoff of the p value was 0.99. HotStarTaq DNA Polymerase (QIAGEN, Cat. No. 203205) was used for the PCR reactions to amplify the exons and boundaries of several genes *(BMP15*, *FIGLA* and *GDF9)*. The primers were designed according to Parsons et al. [2008]. The PCR products were treated with ExoSAP-IT (Affymetrix, Cat. No. 78201) and then were sequenced using an ABI 3730 Genetic Analyzer. The primers used to amplify segments of several genes on the Y chromosome *(SRY*, *TGIF2LY*, *AMELY*, *TBL1Y*, *TTTY12*, *TTTY19*, and *GAPDH)* are summarized in online supplementary table 3, and HotStarTaq DNA Polymerase was used for the PCR assays. Microsatellite DNA marker analysis was performed using a LI-COR 4200 Genetic Analyzer with the following markers: DXS9900, DXS6810, DXS7132, DXS6789, G10699, D3S2390, and DXS7127.

Results

We initially used microsatellite DNA markers of the X chromosome to determine the parental origin of the abnormal sex chromosomes. The results (table 1) elucidated that both X chromosomes were derived from her mother and that the XXY abnormality was caused by non-disjunction of the maternal X chromosomes during meiosis II. The Affymetrix Genome-Wide Human SNP Array 6.0 was then used to detect the CNVs in the genome of the patient. Two large deletions were identified on the short arm of the Y chromosome (fig. 1c). Deletion 1 was ~6 Mb in size and spanned Yp11.32 to Yp11.2 (positions from the gene chip data: 179,542–6,110,498 bp; hg19) and included the genes *ZBED1*, *SRY*, *ZFY*, *TGIF2LY*, etc; deletion 2 was ~2.6 Mb in size (positions from the gene chip data: 7,127,039–9,731,764 bp; hg19) and included the genes *TTTY12*, *TTTY19*, etc. To validate this result, we selected 4 genes *(SRY*, *TGIF2LY*, *TTTY12*, and *TTTY19)* from the 2 deletion regions and 2 genes *(AMELY* and *TBL1Y)* from outside the deletion region as targets (online suppl. table 3); subsequently, segments of these 6 genes were amplified from the genomes of the patient and her family members using PCR. Consistent with the result of gene chip analysis, the PCR assays revealed that only the PCR products that targeted the genes outside the deleted region were amplified from the genome of the patient, whereas there were no products within the deletion regions (fig. 1c). The results of the other family members were congruent with the expectations.

Discussion

XXY is the most common sex chromosome abnormality among humans, with a prevalence of 2/1000 [Visootsak and Graham, 2006]. Because of the presence of the Y

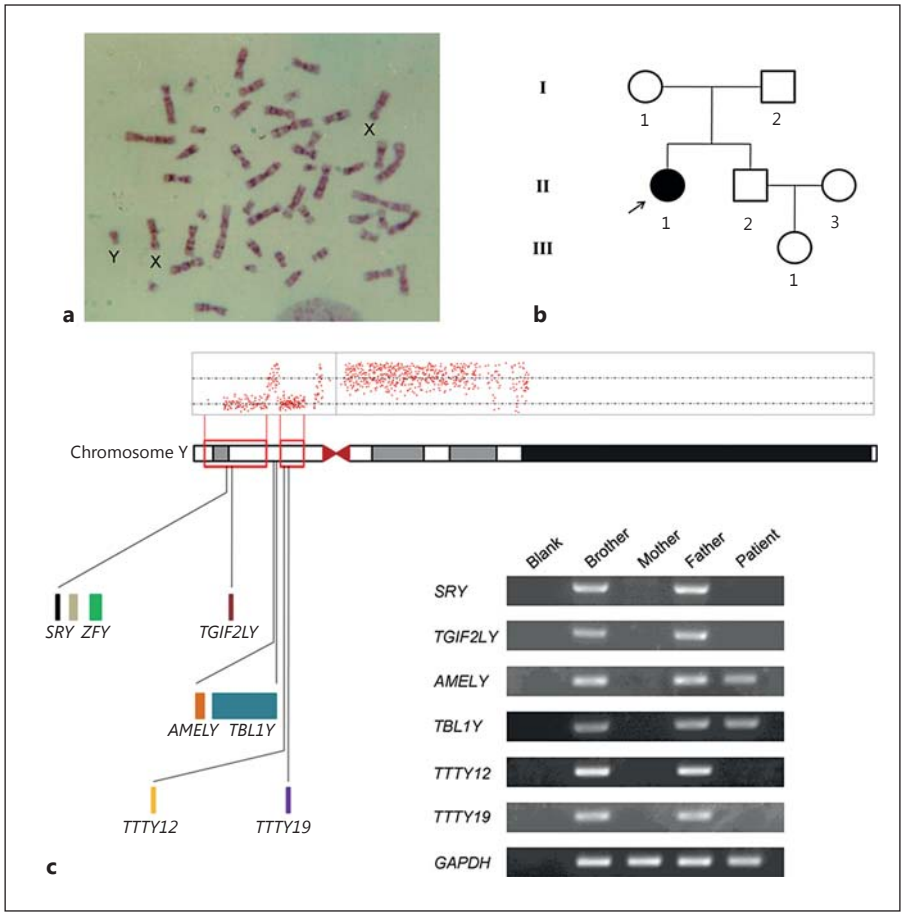

Fig. 1. Two large de novo deletions on the Y chromosome of a 47,XXY female. **a** G-banded karyotype of the patient. The 2 X chromosomes and the Y chromosome are indicated. **b** Pedigree of the family. Only the patient is infertile and has chromosomal abnormalities. **c** CNV analysis using a genome-wide human SNP array detailed 2 large deletions on the Y chromosome. Deletion 1 spans Yp11.32 to Yp11.2; deletion 2 is in Yp11.2. The locations of selected genes for validation assays are denoted by black lines. Different colors indicate different genes. Lower right panel: the PCR assay results are consistent with the CNV analysis. In each lane, the electrophoresis bands represent the segments of the corresponding gene.

chromosome, the vast majority of XXY phenotypes are male. In our case, the patient presents a normal female phenotype, but her ovaries had completely failed. To our knowledge, this is the first case report of a 47,XXY female with ovarian failure. Using a genome-wide human SNP array, we detected 2 large deletions on the Y chromosome of the patient, including the *SRY* locus. The loss of the *SRY* gene can explain why the phenotype of the patient is female despite carrying a Y chromosome. In the previously reported 47,XXY female cases, structure variations on the Y chromosome were only detected in a mother and her daughter, and this mother had already given birth to a normal son and daughter before she was 33 years old [Röttger et al., 2000]. However, in our case, the patient lost her fertility before she was 26 years old, and the genetic mechanism of the patient's ovarian failure remains unknown. Although the possibility that the genetic material from the Y chromosome perturbs the normal function of the ovaries should not be ruled out, it is necessary to explore the potential genetic factors of the patient's ovarian failure. *BMP15*, *FIGLA* and *GDF9* have been reported as disease-causing genes of ovarian failure [Carabatsos et al., 1998; Galloway et al., 2000; Zhao et al., 2008], so we sequenced the coding regions of these genes from the genome, but no mutations were identified. Interest-

Table 1. Analysis of X chromosomal microsatellite DNA markers in the family

Microsatellite marker	Location on X, cM[a]	Patient	Father	Mother	Brother
DXS9900	4.03	1	1	1/2	1
DXS6810	42.75	2	2	1/2	2
DXS7132	52.50	1	2	1/2	1
DXS6789	62.52	1	2	1/3	1
G10699	77.15	2	1	2	2
D3S2390	87.56	2	1	2	2
DXS7127	295.00	2	1	1/2	2

[a] The chromosomal locations of all the markers are from the Marshfield Map, with the exception of DXS7127, which is from the Whitehead-RH Map.

ingly, an ~1-Mb duplication on 10q11.22 was also identified using the SNP array (online suppl. table 4). This region contains the locus of the *GDF10* gene (also known as *BMP3B*). It was reported that *Gdf10* was highly expressed in a subset of ovary cells in rats [Erickson and Shimasaki, 2003] and in the uterus of mice [Zhao et al., 1999], which suggests its potential role in ovarian development. Nevertheless, $Gdf10^{-/-}$ mice are fertile and fail to show any obvious abnormalities in the uterus [Zhao et al., 1999]. It is reasonable to speculate that overexpression of *GDF10* might hamper ovary function in adults, but more evidence is needed to support this hypothesis.

In conclusion, we report on an extremely rare case of a 47,XXY female (the 14th case so far), who presents ovarian failure and carries a Y chromosome with 2 de novo deletions. Further studies are required to uncover the etiology of this case.

Acknowledgements

We are grateful to the patient and her family for their contributions to this study. This work is supported by the National Basic Research Program of China (No. 2007CB512103, 2011CB510100), the National Natural Science Foundation of China (No. 81030015) and the E-Institutes of Shanghai Municipal Education Commission.

References

Bartsch-Sandhoff M, Stephan L, Rohrborn G, Pawlowitzki IH, Scholz W: [A case of testicular feminization with the karyotype 47,XXY (author's transl.)]. Hum Genet 31:59–65 (1976).

Carabatsos MJ, Elvin J, Matzuk MM, Albertini DF: Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. Dev Biol 204:373–384 (1998).

Erickson GF, Shimasaki S: The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. Reprod Biol Endocrinol 1:9 (2003).

Frühmesser A, Kotzot D: Chromosomal variants in Klinefelter syndrome. Sex Dev 5:109–123 (2011).

Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, et al: Mutations in an oocyte-derived growth factor gene *(BMP15)* cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet 25: 279–283 (2000).

Gerli M, Migliorini G, Bocchini V, Venti G, Ferrarese R, et al: A case of complete testicular feminisation and 47,XXY karyotype. J Med Genet 16:480–483 (1979).

German J, Vesell M: Testicular feminization in monozygotic twins with 47 chromosomes (XXY). Ann Genet 9:5–8 (1966).

Girardin CM, Deal C, Lemyre E, Paquette J, Lumbroso R, et al: Molecular studies of a patient with complete androgen insensitivity and a 47,XXY karyotype. J Pediatr 155:439–443 (2009).

Kashimada K, Koopman P: *Sry*: the master switch in mammalian sex determination. Development 137:3921–3930 (2010).

Khandelwal A, Agarwal A, Jiloha RC: A 47,XXY female with gender identity disorder. Arch Sex Behav 39:1021–1023 (2010).

Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E: Klinefelter's syndrome. Lancet 364: 273–283 (2004).

Müller U, Schneider NR, Marks JF, Kupke KG, Wilson GN: Maternal meiosis II nondisjunction in a case of 47,XXY testicular feminization. Hum Genet 84:289–292 (1990).

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, et al: An integrated genomic analysis of human glioblastoma multiforme. Science 321: 1807–1812 (2008).

Röttger S, Schiebel K, Senger G, Ebner S, Schempp W, Scherer G: An SRY-negative 47,XXY mother and daughter. Cytogenet Cell Genet 91:204–207 (2000).

Saavedra-Castillo E, Cortes-Gutierrez EI, Davila-Rodriguez MI, Reyes-Martinez ME, Oliveros-Rodriguez A: 47,XXY female with testicular feminization and positive *SRY*: a case report. J Reprod Med 50:138–140 (2005).

Thangaraj K, Gupta NJ, Chakravarty B, Singh L: A 47,XXY female. Lancet 352:1121 (1998).

Uehara S, Tamura M, Nata M, Kanetake J, Hashiyada M, et al: Complete androgen insensitivity in a 47,XXY patient with uniparental disomy for the X chromosome. Am J Med Genet 86:107–111 (1999).

Visootsak J, Graham JM Jr: Klinefelter syndrome and other sex chromosomal aneuploidies. Orphanet J Rare Dis 1:42 (2006).

Zhao H, Chen ZJ, Qin YY, Shi YH, Wang S, et al: Transcription factor *FIGLA* is mutated in patients with premature ovarian failure. Am J Hum Genet 82:1342–1348 (2008).

Zhao R, Lawler AM, Lee SJ: Characterization of GDF-10 expression patterns and null mice. Dev Biol 212:68–79 (1999).