Molecular cytogenetic characterization of an isodicentric Yq and a neocentric isochromosome Yp in an azoospermic male

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Abstract. Isodicentric Y chromosomes are considered one of the most common structural abnormalities of the Y chromosome. Neocentric marker chromosomes, with neocentromeres, have drawn increasing attention in recent years. The present study reported an azoospermic male with a neocentric isochromosome Yp, neo(Yp), and an isodicentric Yq, idic(Yq). The karyotype was analyzed using G-banding, chromosome microarray analysis (CMA), and fluorescence *in situ* hybridization (FISH) with various detection probes, including sex-determining region on the Y chromosome (SRY) and Y centromeric, applied at the same time. G-banding initially revealed the karyotype 47,X,i(Y)(q10),+mar. CMA indicated the presence of an extra Y chromosome, seemingly equivalent to 47,XYY males. FISH delineated the existence of two centromeres on the idic(Yq). For the marker chromosome, two SRY signals were detected instead of the Y-specific centromere signal, and a visual centromere was observed. This indicated the possible existence of a neocentromere in the marker chromosome, located in the connected region in Yp11.2 band. Finally, the patient's karyotype was established as 47,X,idic(Y)(p11.2), neo(Y)(pter→Yp11.2::Yp11.2→pter). The findings suggested that both idic(Yq) and neo(Yp) could be the main causes of the patient's azoospermia, despite the fact that the partial disomy of Ypter to Yp11.2 did not lead to any major malformations. The present study not only improves the understanding of karyotype/phenotype relationships between neocentric marker Y chromosomes and male infertility, but also supports the hypothesis that the combined application of molecular cytogenetic analysis could aid in reliably confirming breakpoints, origins, and the constitution of the marker chromosomes.

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Introduction

Isodicentric Y chromosomes, containing two centromeres and two symmetrically identical arms, are recognized as the most common structural abnormalities of the human Y chromosome (1). Due to the instability of isodicentric chromosomes during cell division, different proportions of chromosomal mosaics will usually occur, ordinarily in association with a 45,X cell line (2,3). These mosaic karyotypes can exhibit various clinical manifestations, ranging from females with Turner symptoms to males with spermatogenic failure (4-7).

Small supernumerary marker chromosomes (sSMCs), smaller than, or equal in size to, chromosome 20, are structurally abnormal chromosomes which cannot be usually identified through the conventional banding technique (8). The incidence rate in the general population is ~0.03-0.05%; however, in patients with fertility problems, the rate of sSMCs is increased to 0.125%, and the frequency of sSMC carriers is increased in males compared with females (0.165% vs. 0.022%) (8,9). sSMCs are usually derived from chromosome 15, i(12p), der(22), inv dup (22), and i(18p) (10). Although the karyotype/phenotype relationship between sSMCs and male infertility remains to be elucidated, a previous study demonstrated that spermatogenesis failure, for example oligoasthenozoospermia, may be associated with the presence of sSMCs (11).

For marker chromosomes without any harbored repetitive α-satellite (or alphoid) DNA, these uncommon marker chromosomes are described as neocentric marker chromosomes, in which the newly derived centromeres (termed neocentromeres) can form functional kinetochores and maintain mitotic stability of the chromosome (12,13). It has been reported that neocentric marker chromosomes are formed when acentric chromosomal fragments are rescued through the formation of neocentromeres (12). Currently, more than 100 cases of sSMC with neocentromeres have been reported in literature; however, cases of neocentromeres deriving from the Y chromosome are rare, which has led to the failure in establishing karyotype/phenotype relationships (14-16).

In general, the concrete constitution and origins of the sSMC or neocentric marker chromosome were associated with clinic phenotypic diversity, ranging from normal to severely abnormal (8). The combined application of molecular techniques could offer increasingly reliable references for genetic counseling and therapeutic strategy in clinics (9,10). The present study described a case presenting a neocentric isochromosome Yp, neo(Yp), and an isodicentric Yq, idic(Yq), in an azoospermic male using a combination of G-banding, chromosome microarray analysis (CMA), and fluorescence *in situ* hybridization (FISH) analysis.

Materials and methods

Patient. A 27-year-old Chinese male accepted infertility consultation in The Center for Reproductive Medicine and Center for Prenatal Diagnosis at The First Hospital of Jilin University after 1 year of regular unprotected coitus and no pregnancy in June, 2015. The patient's height and weight were 176 cm and 80 kg, respectively. His penis development and growth were normal, and testicular volume was ~4 and ~6 ml for the left and right testes, respectively. No other physical abnormalities were observed. A series of routine examinations were conducted. Semen analysis and levels of sex hormones are presented in Table I. The male was eventually diagnosed with azoospermia according to routine semen examination (17). The Ethics Committee of the First Hospital of Jilin University approved the study protocol (permit no. 2016-416), and the patient provided written informed consent and agreed to participate in this study.

Karyotype analysis. For the patient, a karyotype analysis of peripheral blood lymphocytes was conducted: Peripheral blood (0.5 ml) was cultured in lymphocyte culture medium containing 30 U/ml heparin for 72 h at 37˚C (Yishengjun; Baidi Biotechnology) and subsequently treated with 50 μg/ml colchicine (Yishengjun; Baidi Biotechnology) 1 h before culture termination to arrest mitoses. The lymphocytes were hypotonically treated in 0.075 M KCl for 20 min at 37˚C and fixed in methanol:acetic acid (3:1) for 10 min at room temperature, G-banding of metaphase chromosomes and chromosomal karyotype analysis were performed according to a previous study (18). The karyotype was described according to the International System for Human Cytogenetic Nomenclature 2016 (19).

CMA analysis. Following written consent from the patient, 5 ml peripheral blood was collected from the patient using a standard vacuum extraction blood-collecting system containing EDTA and heparin. Genomic DNA was isolated from whole blood using a QIAamp DNA Mini kit (Qiagen GmbH) according to the manufacturer's protocol. CMA was performed using a CytoScan 750K array (Affymetrix; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The procedure included genomic DNA extraction, digestion and ligation, PCR amplification, PCR product purification, quantification and fragmentation, labeling, array hybridization, washing and scanning. In the PCR amplification process, 250 ng DNA samples was digested with Nsp1, amplified with TITANIUM Taq DNA polymerase (cat. no. 190118EA; Clontech Laboratories, Inc.), fragmented with Affymetrix fragmentation reagent, and labeled with biotin end-labeled nucleotides. PCR was performed using a Veriti Thermal Cycler 96-well, Alpha-SE (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions consisted of an initial denaturation step of 3 min at 94˚C, followed by 30 cycles of 30 sec at 94˚C, 45 sec at 60˚C, and 15 sec at 68˚C, with a final extension step at 68˚C for 7 min. The platform used included 550,000 nonpolymorphic probes and 200,436 single nucleotide polymorphic probes. Thresholds for genome-wide screening were set at ≥200 kb for gains and ≥100 kb for losses. The detected copy number variations were comprehensively estimated by comparing them with published literature and public databases, including Database of Genomic Variants (DGV; http://dgv.tcag.ca/dgv/app/home), DECIPHR v9.28 (http://decipher.sanger.ac.uk/), ISCA (https://www.iscaconsortium.org/), ECARUCA (http://www.ecaruca.net), and OMIM (http://www.ncbi.nlm.nih.gov/omim).

Table I. Semen analysis and levels of sex hormones.

Hormone	Results	Reference range
FSH, mIU/ml	10.00	1.5-12.4
LH, mIU/ml	12.40	1.7-8.5
E2, pg/ml	24.59	28-248
PRL, uIU/ml	287.00	86-258
T, nmol/l	6.60	9.9-27.8
Semen volume, ml	2.0	1.5-5.5
Sperm count, million/ml	0	>20

The reference values were obtained from electrochemiluminescence immunoassays using Roche Elecsys1010 (Roche Diagnostics) according to the manufacturer's instructions. FSH, folliclestimulating hormone; LH, luteinizing hormone; E2, estradiol; PRL, prolactin; T, testosterone.

FISH. Based upon the karyotype analysis and CMA results, FISH analysis was carried out to further confirm the characterization of chromosomal Y anomalies. Two sets of probes were used according to the manufacturers' instructions in the present study: Centromere probes specific for chromosomes X, Y (cat. no. F01001, CSPX, spectrum green; CSPY, spectrum red; Beijing GP Medical Technologies, Ltd.) and SRY probe (cat. no. RU-LPU026; Cytocell). The SRY probes, labelled in red, consists of two non-overlapping probes, 30 and 50 kb. The probes cover the entire SRY gene and flanking DNA, including the RPS4Y1 gene. The probe mix also contains control probes for the X centromere (DXZ1), labelled in blue, and for chromosome Y (DYZ1, the heterochromaticblock at Yq12), labelled in green. Lymphocytes cell suspensions fixed with methanol:acetic acid (3:1) for the karyotype analysis were further used for FISH analysis. The cell suspensions were centrifuged (931 x g for 5 min) at 4˚C, then were dropped onto dry glass slides (Shitai; Citotest Labware Manufacturing Co., Ltd.) using a dropper. The procedure of slide preparation (including probe application, co-denaturation, hybridization and post-hybridization washes) was performed according to

Table II. Primer sequences used in polymerase chain reaction analysis of sequence-tagged sites for the azoospermia factor region of the human Y chromosome.

STS	Primer sequences	Product size (bp)
ZFX/Y	Forward: 5'-ACCR(A,G)CTGTACTGACTGTGATTACAC-3'	
	Reverse: 5'-GCACY(C,T)TCTTTGGTATCY(C,T)GAGAAAGT-3'	495
SY14(SRY)	Forward: 5'-GAATATTCCCGCTCTCCGGA-3'	
	Reverse: 5'-GCTGGTGCTCCATTCTTGAG-3'	472
SY84	Forward: 5'-AGAAGGGTCTGAAAGCAGGT-3'	
	Reverse: 5'-GCCTACTACCTGGAGGCTTC-3'	326
SY86	Forward: 5'-GTGACACACAGACTATGCTTC-3'	
	Reverse: 5'-ACACACAGAGGGACAACCCT-3'	320
SY127	Forward: 5'-GGCTCACAAACGAAAAGAAA-3'	
	Reverse: 5'-CTGCAGGCAGTAATAAGGGA-3'	274
SY134	Forward: 5'-GTCTGCCTCACCATAAAACG-3'	
	Reverse: 5'-ACCACTGCCAAAACTTTCAA-3'	301
SY143	Forward: 5'-GCAGGATGAGAAGCAGGTAG-3'	
	Reverse: 5'-CCGTGTGCTGGAGACTAATC-3'	311
SY152	Forward: 5'-AAGACAGTCTGCCATGTTTCA-3'	
	Reverse: 5'-ACAGGAGGGTACTTAGCAGT-3'	125
SY157	Forward: 5'-CTTAGGAAAAAGTGAAGCCG-3'	
	Reverse: 5'-CCTGCTGTCAGCAAGATACA-3'	285
SY254	Forward: 5'-GGGTGTTACCAGAAGGCAAA-3'	
	Reverse: 5'-GAACCGTATCTACCAAAGCAGC-3'	380
SY255	Forward: 5'-GTTACAGGATTCGGCGTGAT-3'	
	Reverse: 5'-CTCGTCATGTGCAGCCAC-3'	123

SRY, sex-detemining region Y; STS, sequence-tagged sites.

the manufacturer's instructions. The probes were denatured for 2 min at 75˚C. The hybridization mixture (1 μl each probe and 7 μl hybridization solution) was applied to each slide and covered with a coverslip 20x20 mm. Then, the slides were sealed with rubber cement before hybridization was carried out overnight in a moist chamber at 37˚C. After hybridization, the slides were washed for 2 min in a solution of 0.4X SSC at 72˚C and a second time for 30 sec in a solution of 2X SSC, 0.05% Tween-20 at room temperature. Following the final wash, slides were air dried and counterstained with a solution of 4',6'-DAPI in the dark at 4˚C overnight. Appropriate viewing, analysis and imaging of FISH results was accomplished using a fluorescent microscope (Leica DM4000B; Leica Microsystems GmbH; magnification, x1,000).

Azoospermia factor (AZF) microdeletion analysis. Microdeletions in the AZF region were detected using PCR, as previously described in accordance with the recommendations of The European Academy of Andrology and the European Molecular Genetics Quality Network. Specific sequence-tagged sites were mapped in the AZF region, including SY84 and SY86 for AZFa; SY27, SY134, and SY143 for AZFb; SY157, SY254, and SY255 for AZFc; and SY152 for AZFd. Human zinc-finger protein-encoding genes (ZFX/ZFY) located on the X and Y chromosomes were selected as internal control primers (20). Genomic DNA was isolated with the Tiangen Blood DNA Extraction Mini kit (Tiangen Biotech Co., Ltd.) using peripheral blood lymphocytes according to the manufacturer's protocol. The PCR process was performed according to a previous study, and primer sequences are presented in Table II (21). Each 20 ml reaction mix contained 50 ng genomic DNA, 5-10 pmol of each primer (Shanghai Sangon Pharmaceutical Co., Ltd.), 100 mM potassium chloride, 200 mM Tris-HCl (pH 8.8), 15 mM magnesium chloride, 1% Triton X-100, 500 mM of each dNTP and 2 U Taq polymerase (Beijing Dingguo Changsheng Biotechnology Co., Ltd.). Multiplex PCR was carried out using a Veriti 96-well PCR thermal cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.). The cycling program involved preliminary denaturation at 94˚C for 6 min, followed by 35 cycles of denaturation at 95˚C for 40 sec, annealing at 58˚C for 45 sec, and elongation at 72˚C for 60 sec, followed by a final elongation step at 72˚C for 10 min. Classical AZF reaction products were analyzed by electrophoresis in 1.5% agarose gels at 80 V for 30 min (Biowest). All gels contained 0.5 mg/ml ethidium bromide and PCR products were visualized under ultraviolet light.

Selection of neocentric chromosome Y cases. The present study focused on cases presenting neocentric chromosome Y

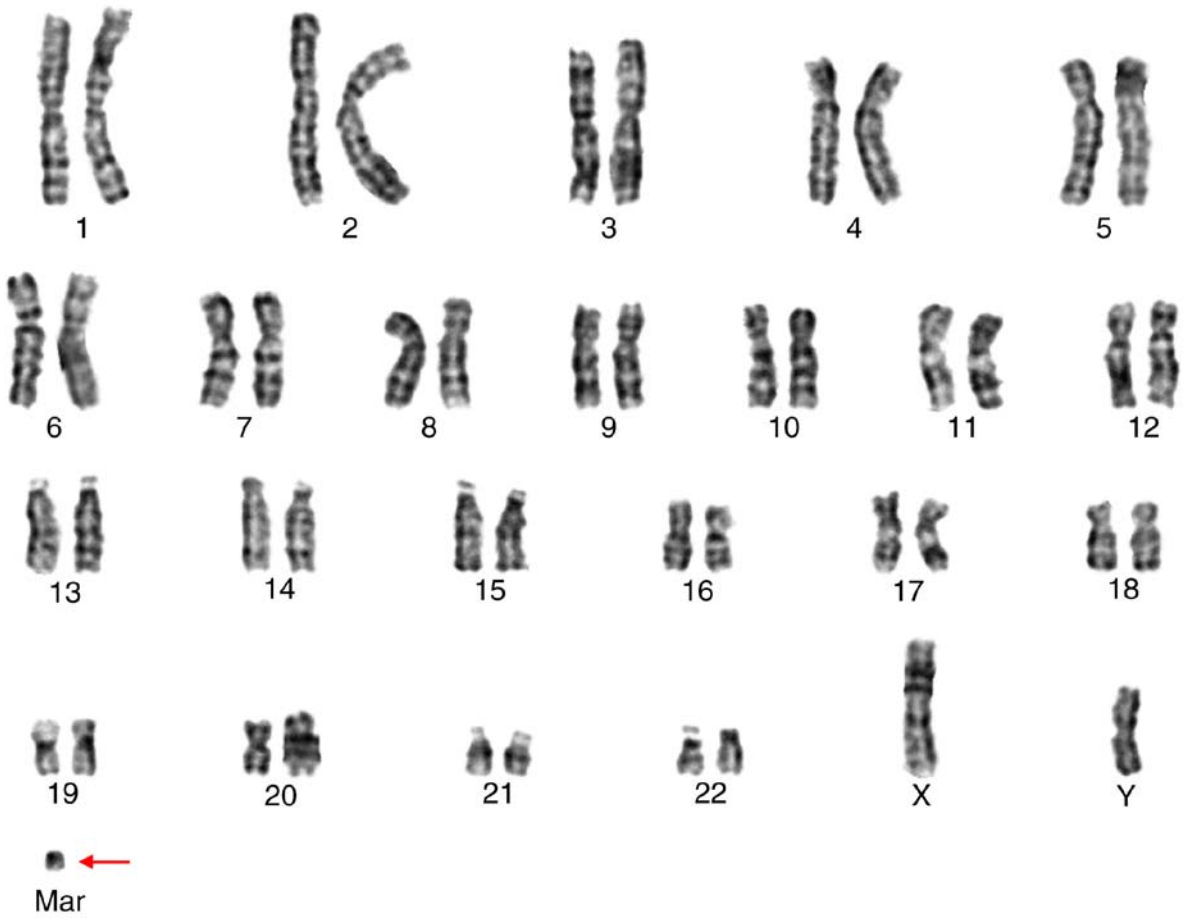

Figure 1. Karyotype of the proband identified by the G-banding technique: 47,X,i(Y)(q10),+mar. Mar, marker chromosome.

associated with different clinical manifestations. Based upon this selection criterion, a systematic literature search was conducted by means of a PubMed literature search (https://www.ncbi.nlm.nih.gov/pubmed/) using relevant terms and their combinations [including neo(Y), neocentric chromosome Y, chromosomal Y neocentromere, neocentric marker chromosome Y and marker chromosome Y neocentromere], and by searching the sSMC database (http://ssmc-tl.com/sSMC.html) (22).

Results

Karyotype analysis. Cytogenetic G-banding analysis initially described the karyotype as 47,X,i(Y)(q10),+mar (Fig. 1), consisting of an isochromosome Yq [i(Yq)] and an sSMC.

Molecular cytogenetic analysis. In order to delineate the origin of the marker chromosome, CMA analysis was performed, describing the sSMC as arr[hg19]Yp11.31q11.23 (2,650,424-28,799,654)x2 (Fig. 2), indicating that the patient had an extra Y chromosome but that the karyotype was not 47,XYY. Based on this, the karyotype was characterized as 47,X,i(Y)(p10),i(Y)(q10). The existence of two forms of the Y chromosome was further investigated with SRY and alphoid probes for the Y centromere. FISH with centromere probes showed two close but clearly distinct chromsomal Y centromere signals (red) in the middle region of i(Yq) (Fig. 3A), but no Y centromere signal was detected on the sSMC. Furthermore, two DYZ1 signals (green) and no SRY signal indicated that i(Yq) was actually an idic(Yq) (Fig. 3B). These results imply that two identical Y chromosomes, with partially missing short arms, had integrated together symmetrically (Fig. 4). The sSMC(Y) with two SRY signals (red) revealed the presence of neo(Y)(pter→Yp11.2:: Yp11.2→pter) derived from Ypter to Yp11.2 without centromeric material (Fig. 3B), which might indicate the existing breakpoints in Yp11.2. The existence of the neocentromere requires further verification. In addition, no microdeletions in the AZF region were detected.

Summary of neo(Y) cases. A literature review on the cases with neocentric chromosome Y was conducted, which are presented in Table III (13,14,22-28). Of all cases, 4/9 were female, 3/9 were male, and the sex in 2/9 cases was not available. Among their chromosomal karyotypes, 4/9 were mosaic, 4/9 were non-mosaic and 1/9 was not reported. The clinic manifestations of these patients were varied, ranging from normal fetal ultrasound findings to abnormal phenotypes in teenagers and adults.

Discussion

The present study reported an azoospermic male and established the karyotype as a non-mosaic 47, X, idic(Y)(p11.2), neo(Y)(pter→Yp11.2::Yp11.2→pter), consisting of an idic(Yq)

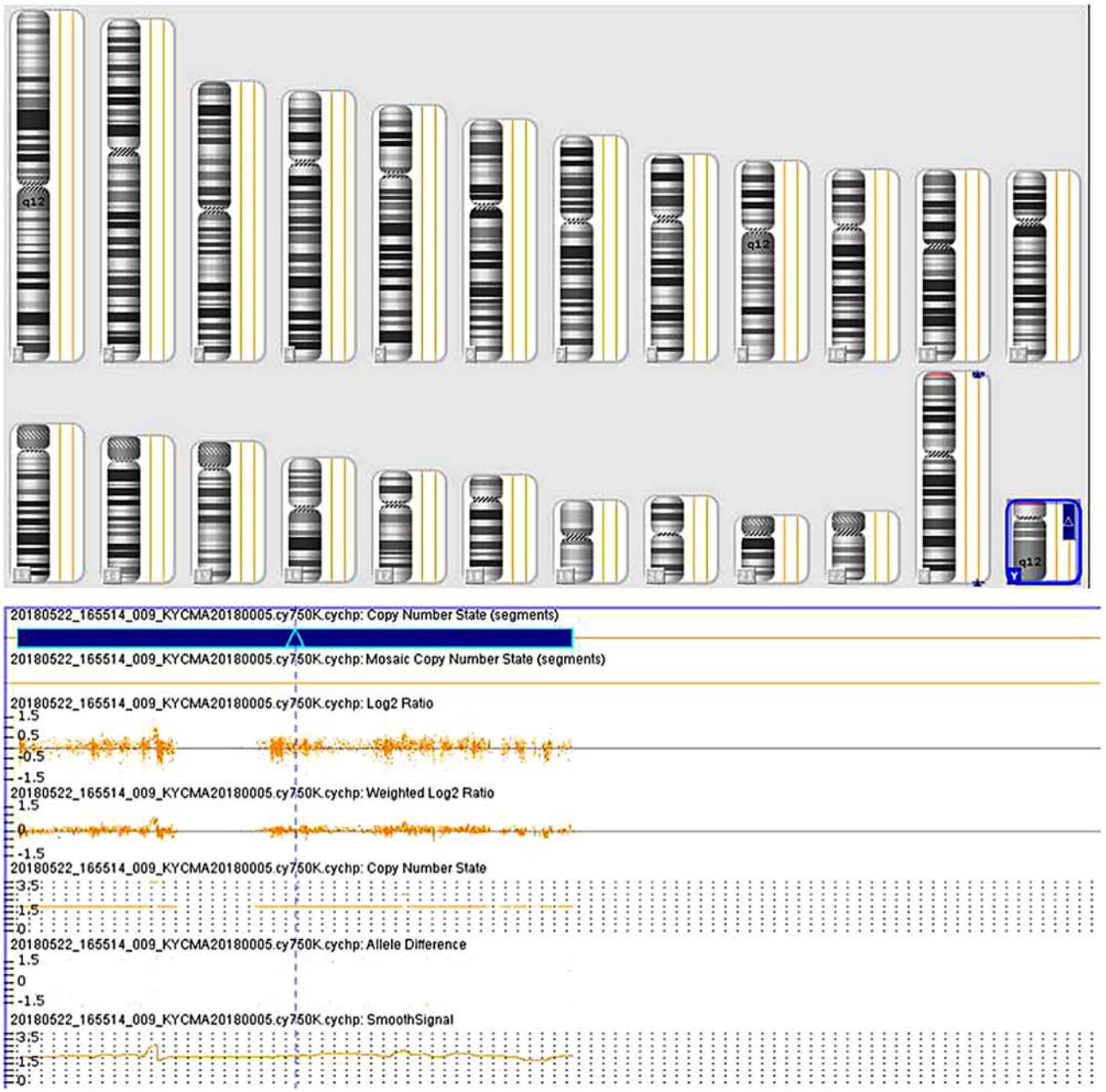

Figure 2. Chromosome microarray analysis results depicting a 26,149 kb duplication of chromosome Y location.

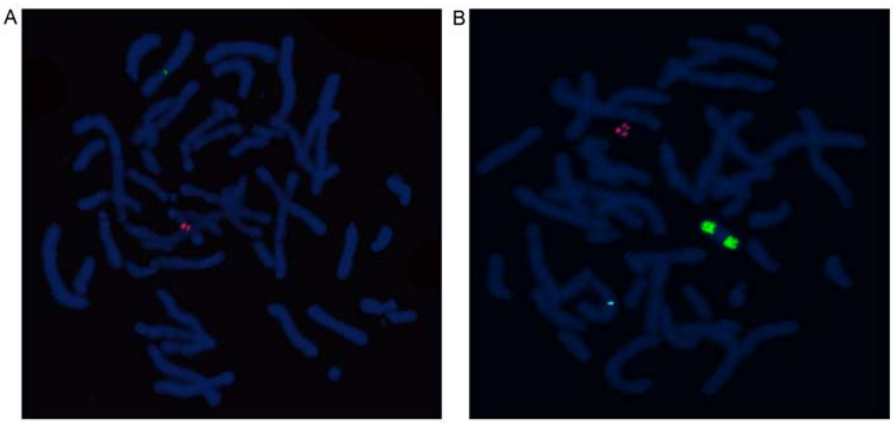

Figure 3. Metaphase-FISH analysis using centromere probes specific for chromosomes X, Y and an SRY probe. (A) Metaphase FISH results showed the idic(Y)(p11.2) with two centromere signals (red). (B) SRY probe showing the two SRY signals in the marker chromosome (red) and two signals of heterochromatic region (green; DYZ1) of Yq12 (magnification, x1,000). FISH, fluorescence *in situ* hybridization; SRY, sex-determining region Y.

Table III. Clinical review and manifestations of chromosome Y with neocentromere.

Author, year	Case	Sex/age	Karyotype	Involved neocentric Y	Other description	Clinical feathers/ Reason to study	(Refs.)
Rivera *et al*, 1993	1	N.R./12 y	45,X(37.5%)/46, X, + mar(30.5%)/46, X, psu dic(Y) (q11) (7.5%)/47,X, psu dic(Y) (q11), psu dic(Y) (q11) (4.5%)	psu dic(Y) (q11)	N.R.	Turner syndrome phenotype	(27)
Bukvic *et al*, 1996	2	F/29 y	45,X/46,XY/47, XY,+mar	A rearranged Y chromosome carrying an inactivated normal centromere.	N.R.	Short stature (138 cm); removed hypoplastic uterus and streak gonads (2 y)	(23)
Warburton *et al*, 1997	3	N.R.	N.R.	inv dup Y(q) (qter-q11.2::q11.2-qter)	Consisting of Yq heterochromatin and distal Yq euchromatin but lacking detectable a-satellite DNA	N.R.	(25)
Floridia *et al*, 2000	4	F/fetus	45,X[8]/46,X, rea(Y)[18]	rea(Y) (qter-q11.2::q11.2-qter)	Consisting of an inverted duplication of the long arm heterochromatin and a small amount of euchromatin, but lacking a normal centromere	Cystic hygroma; intrauterine fetal death	(26)
Assumpção *et al*, 2002	5	F/18 y	46,X,+mar	rea(Y)(qter→q11::q11→qter)	Lacking Yp and Y-centromere sequences	Primary amenorrhea; hypogonadism	(24)
Sheth *et al*, 2009	6	M/fetus	47,XX,+mar	inv dup(Y) (pter→Yp11.2::Yp11.2→pter)	N.R.	Normal in ultrasound; pregnancy terminated; no abnormalities observed	(14)
Liehr, 2019	7	F/28 y	47,XX,+mar	inv dup(Y) (qter→q11.23::q11.23→qter)	N.R.	Normal female; infertile	(22)
Chen *et al*, 2011	8	M/newborn	46,X,+mar[8]/ 46,XY[16]	r(Y)(::p11.31/q11.1::) (Ypter-, SRY+, DYZ3+,DYZ1-, Yqter-,mBand Y+,SKY+)[8]	Lacking Yp and Yq telomeric signals and DYZ1 signal	Advanced maternal age	(28)

Table III. Continued.

Author, year	Case	Sex/age	Karyotype	Involved neocentric Y	Other description	Clinical feathers/ Reason to study	(Refs.)
Pasantes *et al*, 2012	9	M/11 y	47,X,i(Y)(p10), i(Y)(q10)	inv dup(Y) (qter→q11.221::q11.221→ q12→neo→q12→qter)	ish idic(Y)(wcpY+,TEL++, SHOX++,SRY++,TSPY++, RBMY++,DYZ3++,UTY+, XKRY-,DAZ-,CDY-,DYZ1-, SYBL-), inv dup(Y)(wcpY+, TEL++,SYBL++,DYZ1++, DAZ+, CDY+,RBMY+, XKRY+, SHOX-,TSPY-, DYZ3-,UTY-).	an elevated blood serum AFP in the mother; an urachal cyst (2 m)	(13)
The present study	10	M/27 y	47,X,i(Y)(q10), +mar	neo(Y) (pter→Yp11.2::Yp11.2→pter)	47,X,idic(Y)(p11.2), neo(Y) (pter→Yp11.2::Yp11.2→pter)	azoospermia	N/A

M, male; F, female; N.R., not reported; m, months; y, years.

Figure 4. Diagrams of chromosomal Y anomalies in the patient. Normal chromosome Y (left), the idic(Y) (center) and neo(Y) (right) derived from Y chromosome. The position indicated by the arrowhead suggests a neocentromere. Y chromosome band nomenclature follows the International System for Human Cytogenetic Nomenclature (2016).

and a neo(Yp). In the karyotype, idic(Y) was shown to contain a copy of the entire long arm of the Y chromosome, Y centromere, and partial euchromatic part of Yp11.2. The neo(Yp) could be described as neo(Y)(pter→Yp11.2::Yp11.2→pter), including two copies of SRY and Yp11.2 to Ypter, lacking the original Y centromere. The primary constriction appeared in the connecting regions, making it possible to associate it with a functional neocentromere. However, further tests will be required to confirm this. To the best of the authors' knowledge, this is the first report of an infertile man with a neo(Yp) in addition to an idic(Yq).

Considering the amount of Y chromosomal duplication from CMA results, this azoospermic proband could be seen as being equivalent to 47,XYY males. However, this did not reasonably explain the male's spermatogenesis impairment, as patients with XYY syndrome can have various spermatozoa counts, ranging from normal to azoospermia (29-31). It is worth mentioning that CMA in the present study serves a critical role in identifying the fact that the marker chromosome is originated from chromosome Y, which offers guidance for the following verification of the neo(Y) using FISH.

Idic(Y) is one of the most common structural abnormalities of the Y chromosome, most of which usually exist in a mosaic form with 45,X (5,32). Patients with idic(Y) exhibit various abnormal/ambiguous sexual developments, due predominantly to differences in karyotypic mosaic proportions, breakpoints and Y chromosome fusions (1,32,33). Lange *et al* (6) proposed that the formation of idic(Y) results from homologous recombination between opposing arms of male-specific region of the Y(MSY) palindromes, leading to outcomes ranging from dyszoospermia to sex reversal

or Turner syndrome. Idic(Y) can be generally divided into idic(Yp) and idic(Yq); idic(Yp) with breakpoints in the long arm of the Y chromosome can lead to spermatogenic failure due to the deletion or rearrangement of the AZF region (5,34). DesGroseilliers *et al* (5) described two males with no observed genital abnormalities that presented 46,X,idic(Y)(q11.21) separately, who lacked most of Yq and were positive for the Y centromere DYZ3 and SRY, but negative for the Yq heterochromatin DYZ1. The inactivation or synergic action of the centromeres may be responsible for the stable formation of idic(Y) in the process of early paternal gametogenesis (5,35). Kuan *et al* (36) identified a non-mosaic 46,X,idic(Y)(q12) in a fetus and concluded that an i(Y) chromosome can result from breakage and fusion at the Yq pseudo-autosomal region. Kumar *et al* (37) reported a 32-year-old non-obstructive azoospermia man with non-mosaic 46,XY, whose dicentric Y fused at Yq12 [proximal to pseudoautosomal region (PAR)2]. However, the association between non-mosaic idic(Yq) and infertility remains unclear. Cui *et al* (7) described a non-mosaic 46,X,idic(Y)(p11.32) male presenting short stature and severe oligozoospermia. They concluded that PAR1 serves a critical role in regulating the meiotic pairing and sperm production, the aberrations of which could result in spermatogenic failure. Lehmann *et al* (32) reported an azoospermic male with idic(Y)(p11.3), speculating that extra copies of the AZF region might be a contributing factor for spermatogenic failure. In the present study, the proband presented no microdeletions in AZF region, located in Yq11. The gain of Yp11.31q11.23 detected by CMA, which was further testified using two sets of FISH probes, additionally demonstrated the existence of idic(Yq) with two integrated Yq arms, as well as two AZF regions. The copy of AZF regions in the present study appeared to support the hypothesis to some extent. However, the correlation between extra copies of AZF regions and spermatogenesis still needs to be investigated.

Chromosome Y is often involved in the formation of marker chromosomes (23). The majority of published neocentric sSMCs can be classified as small inverted duplicated chromosomes, with the neocentromere forming on an acentric fragment (14,16). If neocentromerization occurs in the same cell cycle, a stable non-mosaic state, resulting from the formation of an acentric fragment at meiosis, will develop (38). In the present study, the formation of a neocentromere on the stable neo(Y)(pter→Yp11.2::Yp11.2→pter) was accompanied by the idic(Y), which suggests that sequences in Yp might exist that facilitate the combination of the centromeric proteins; this requires further investigation. For the proband in the present study, it is probable that the inactivation of one centromere in idic(Y) and the activation of the neocentromere in neo(Y) may have happened simultaneously after the formation of both idic(Y) and neo(Y) (13). Currently, the clinical phenotypes of sSMCs with neocentromeres include facial dysmorphisms, renal defects, short stature and delayed development (15). The genotype-phenotype correlation on neocentric chromosome Y was summarized. Among them, newborns or fetuses showed normal or no evident abnormalities (cases 4, 6 and 8). Five cases presented gonadal dysgenesis or spermatogenesis dysfunction (cases 1, 2, 5, 7 and 9). In addition, the majority of the neocentric sites were located between Yq11.2 and Yq12 (cases 1,3-5,7 and 9), which demonstrated that neocentromeres arising on the Y chromosome were inclined to emerge on the Yq instead of Yp. In case 6, an unborn boy with karyoype 47,XX,+mar was described, with marker chromosome identified as neo(Y)(pter→Yp11.2::Yp11.2→pter). Partial disomy of Ypter to Yp11.2 might not be associated with any major malformations (14), but fertility in adulthood could not be established. In general, there might be phenotypic diversity associated with neo(Y) carriers and the potential risk of infertility for such carriers should be taken seriously.

An azoospermic male with a neocentric sSMC(Y) accompanied by idic(Yq) was characterized according to karyotype analysis, CMA and FISH. The sSMC was determined to be neo(Y)(pter→Yp11.2::Yp11.2→pter). The present study contributed to the understanding of clinical phenotypes associated with neocentric Y chromosomes and provided information for the genetic counseling of such aberrations.

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Availability of data and materials

The data and materials of this study are available from the corresponding author on reasonable request.

Authors' contributions

YJ obtained the clinical information, collected data from the literature and wrote the manuscript. FY and RW critically analyzed the data and revised the manuscript. HZ, LeL and LiL performed the cytogenetic study, CMA and FISH experiments and analyzed the results. SL and RL conceived and designed the study. RL performed the final review and editing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Medical Ethics Committee of the First Hospital of Jilin University (permit no. 2016-416). The patient provided written informed consent for participating in this study.

Patient consent for publication

The current case report was published with the informed consent of the patient, whose anonymity was preserved.

Competing interests

The authors declare that they have no competing interests.

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