

### Characterization and Association of Marker Chromosomes with Male Infertility

Dong Suk Kim<sup>1</sup>, Sang Hee Park<sup>2</sup>, Sung Han Shim<sup>2</sup>, Dae Keun Kim<sup>3</sup>, Sang Woo Lyu<sup>4</sup>, Ji Won Kim<sup>4</sup>, Woo Sik Lee<sup>4</sup>, and Seung-Hun Song<sup>1\*</sup>

<sup>1</sup>Department of Urology, Fertility Center of CHA Gangnam Medical Center CHA University, Seoul, Republic of Korea

<sup>2</sup>Genetic Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea

<sup>3</sup>Department of Urology, CHA Fertility Center, Seoul Station, CHA University, Seoul, Republic of Korea

<sup>4</sup>Department of Obstetrics and Gynecology, Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea

**Corresponding Author:** Seung-Hun Song: [shsong02@cha.ac.kr](mailto:shsong02@cha.ac.kr)

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#### ABSTRACT

##### Background and objective

A small supernumerary marker chromosome (sSMC) is a rare structurally abnormal chromosome in which no part can be identified by conventional cytogenetic banding technique. There is little known about the association of marker chromosomes with male infertility. We performed a molecular cytogenetic characterization sSMCs and investigated their association with male infertility.

##### Methods

Among 4230 infertile male patients who underwent cytogenetic analyses from January 2008 to December 2018, the records of 15 patients who were diagnosed with sSMCs were reviewed. After initial infertility evaluation, the patients received additional genetic tests including G-bands by trypsin using Giemsa (GTG-banding), Nucleolar organizer region (NOR) banding, Fluorescence *in situ* hybridization (FISH), and array comparative genomic hybridization (aCGH) analyses to further characterize and identify the origin of their marker chromosome. Testis biopsy was performed for the azoospermic patients to evaluate spermatogenic status.

## Results

Among 15 infertile males with sSMCs, eight had nonobstructive azoospermia, five had severe oligozoospermia, and two had sperm concentrations above the lower normal limit. The marker chromosomes were identified as Y ring chromosomes (n = 8), an isodicentric Y chromosome (n = 1), a neocentromere Y chromosome (n = 1), a derivative chromosome 1 (n = 1), and an acrocentric short arms (n = 4).

## Conclusion

Marker chromosomes appear to be a rare genetic cause of male infertility. Additional cytogenetic tests, including NOR banding, FISH, and aCGH, could help to characterize the origin of the marker chromosome. Appropriate genetic counseling for couples with infertility caused by a marker chromosome should be recommended.

**Key Words:** *Male infertility; Marker chromosome; Azoospermia; Cytogenetic analyses; Genetic counseling*

## INTRODUCTION

Infertility, defined as the inability of a couple to have a baby after 1 year of regular unprotected intercourse, affects 10–15% of couples. Among couples with infertility, it is estimated that 35% of cases are due to female factors alone, 30% are due to male factors alone, 20% are due to a combination of male and female factors, and 15% are due to explained causes.<sup>1</sup> Known causes of male infertility include anatomical abnormalities, hormonal imbalances, and genetic defects. However, various aspects of male infertility are still poorly understood, and many men are diagnosed as having idiopathic infertility. Although researchers believe that genetic background has a substantial effect on male infertility, the ability to diagnose these defects is limited.<sup>2,3</sup>

On the other hand, recent assisted reproductive technologies (ARTs), such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have revolutionized infertility treatment and achieved high pregnancy rates. However, ART can lead to ovarian hyperstimulation syndrome (OHSS), multiple pregnancy, premature birth, and low birth weight. In addition, current ARTs, especially ICSI, can potentially increase the risk of bypassing the natural selection mechanism.<sup>4,5</sup> Therefore, identifying the underlying

genetic basis of male-factor infertility is essential for development of appropriate screening tests that can be used to counsel couples planning for assisted reproduction. Current genetic tests for male infertility, such as karyotyping and Y chromosome microdeletion testing, are widely available and provide a direct benefit to infertile couples with severe male-factor infertility.

A small supernumerary marker chromosome (sSMC) is defined as a rare structurally abnormal chromosome in which no part can be identified by conventional cytogenetic banding technique. The prevalence of sSMCs is estimated to be 0.03–0.05% among infants.<sup>6</sup> The significance of an sSMC marker is variable, depending on its composition, and there is little known about the association of marker chromosomes with male infertility. Herein, we characterized the molecular cytogenetics of sSMCs and investigated their association with male infertility.

## METHODS

This retrospective study analyzed the records of the patients who underwent cytogenetic analyses for male infertility at a single fertility center and was approved by our Institutional Review Board (GCI-20-13). Among the 4230 infertile male patients who underwent cytogenetic

analyses from January 2008 to December 2018, the records of patients diagnosed with marker chromosome abnormality were reviewed. Patients with cryptorchidism, previous scrotal surgery, or exposure to a gonadotoxin such as chemotherapy were excluded.

A total of 15 infertile male patients with marker chromosomes were finally included. Initial fertility evaluation consisted of a thorough personal and family history, physical examination, semen analyses, and profiles of reproductive hormones. Testis volume was measured using an orchidometer by an experienced andrologist. The patients underwent additional genetic tests due to severe male infertility with nonobstructive azoospermia or severe oligozoospermia ( $<5 \times 10^6/\text{mL}$ ) at the initial evaluation.

All semen samples were obtained by masturbation into a wide-mouthed plastic container after at least 2 days of sexual abstinence, and were allowed to liquefy for at least 20 min at  $37^\circ\text{C}$  before further analysis. Then, semen parameters, including sperm concentration, motility, and morphology, were assessed. If sperm were not detected using a conventional microscopic Makler chamber evaluation, the sample was rechecked after centrifugation at  $1500 \times g$  for 10 min to detect any sperm.

Cytogenetic analyses were performed according to the standard operating protocol as previously described.<sup>7</sup> Metaphase spreads from lymphocytes were prepared. G-bands by trypsin using Giemsa (GTG-banding) and Nucleolar organizer region (NOR) banding were used to identify the marker as an acrocentric chromosome or a neocentromere, respectively. Fluorescence *in situ* hybridization (FISH) using specific probes (Vysis, Abbott Park, IL, USA) and aCGH analyses using array probe (Affymetrix, Santa Clara, CA, USA) were used to identify the origin of the marker chromosome. The polymerase chain reaction (PCR) and direct

sequencing were used to clarify the deleted loci of the Y chromosome with Y-specific sequence-tagged sites (STSs). All procedures were followed according to the manufacturer's instructions.

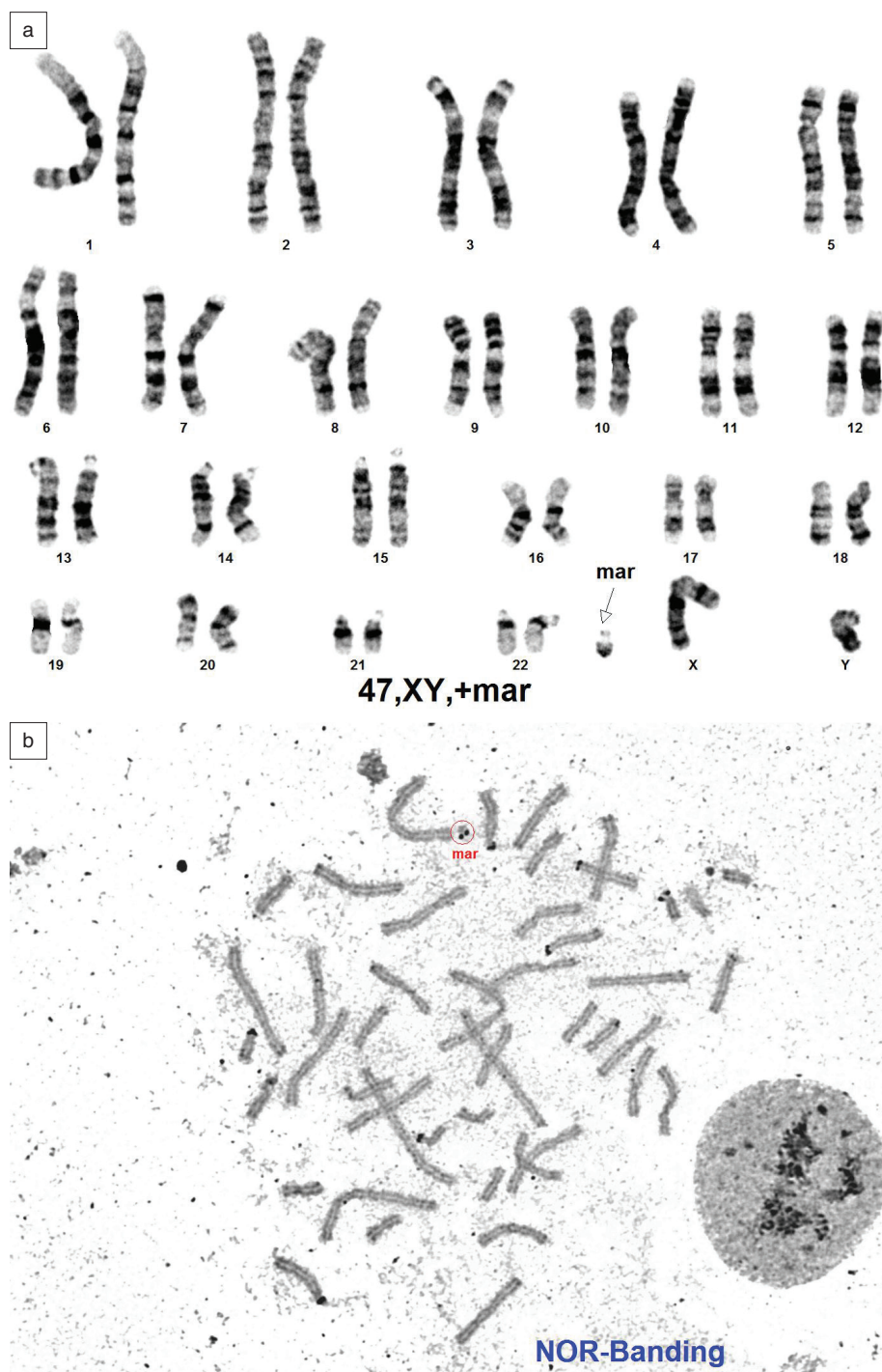
## RESULTS

We retrospectively examined 15 infertile male patients with sSMCs (Table 1). The mean age was 35 years (range: 31–43 years) and the mean duration of infertility was  $12.8 \pm 8.8$  months. Infertility was the main clinical issue in all patients, and none of them had any other abnormality, such as mental retardation, dysmorphic features, or motor disturbance. The mean serum FSH level was  $22.9 \pm 8.4$  mIU/mL (normal: 1.5–12.4), the mean testosterone level was  $4.8 \pm 2.0$  ng/mL (normal: 3–12), and the mean testis volume  $8.4 \pm 5.4$  mL (right) and  $8.0 \pm 5.4$  mL (left). Eight patients had nonobstructive azoospermia, five patients had severe oligozoospermia, and two patients had sperm concentrations above the lower normal limit ( $\geq 15 \times 10^6/\text{mL}$ ).

GTG banding indicated the karyotype was 46, X, +mar or 47, XY, +mar (mostly mosaic forms) in all 15 patients (Figure 1). We performed additional tests to identify the origin of the 15 marker chromosomes (Figures 2 and 3). The results

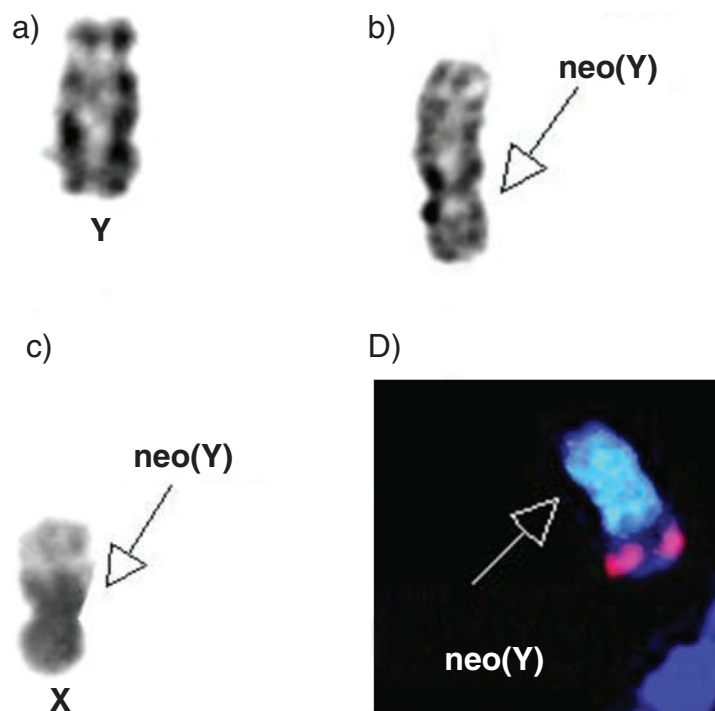
**TABLE 1** Patient Characteristics

Clinical characteristics	
Number of patients	15
Age (years)	35 years (31–43)
Infertility duration (months)	$12.8 \pm 8.8$
Azoospermia (cases)	8
Severe oligozoospermia (cases)	5
Normal ( $\geq 15 \times 10^6/\text{mL}$ ) (cases)	2
Serum FSH (mIU/mL)	$22.9 \pm 8.4$
Serum Testosterone (ng/mL)	$4.8 \pm 2.0$
Rt. testis volume (mL)	$8.4 \pm 5.4$
Lt. testis volume (mL)	$8.0 \pm 5.4$

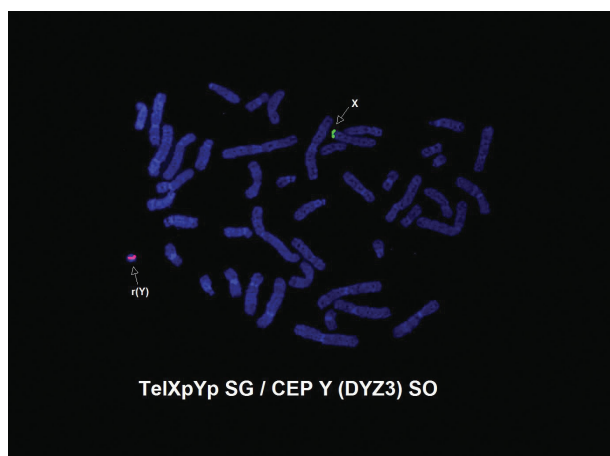


**FIG. 1** Representative images showing GTG-banding of a marker chromosome (a) and NOR-banding with an acrocentric origin of a marker (b).

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**FIG. 2** Representative images showing a normal Y chromosome (a), a neocentromere of a Y chromosome (b), and C-banding and FISH indicating the marker was a neocentromere of the Y chromosome (c) and (d).



**FIG. 3** Representative FISH images with Tel Xp/Yp SpectrumGreen and CEP Y (DYZ3) showing the marker was a ring Y chromosome.

indicated that these marker chromosomes were Y ring chromosomes (n = 8), an isodicentric Y chromosome (n = 1), a neocentromere Y chromosome (n = 1), a derivative chromosome 1 (n = 1), and acrocentric short arms (n = 4) (Table 2).

Eight patients underwent a testis biopsy for pathologic diagnosis. The results showed Sertoli cell only tubules (n = 6), maturation arrest (n = 1), and hypospermatogenesis (n = 1).

## DISCUSSION

We analyzed 15 cases of sSMC-associated male infertility. In all cases, infertility was the main clinical issue and the men were otherwise healthy and with no other concomitant

**TABLE 2** The Characterization of Marker Chromosomes

	<b>Infertility type</b>	<b>Additional tests</b>	<b>Marker type</b>	<b>Final Result</b>
1	Severe oligozoospermia	FISH	inv dup(15)	47,XY,+mar.ish idic(15)(D15Z4+)
2	NOA	FISH	r(Y)	mos 46,X,r(Y)[85]/45,X[15].ish r(Y)(SRY+,DYZ3+,telXp/Yp-)
3	Severe oligozoospermia	NOR	inv dup(acro)	inv dup(acro)
4	NOA	FISH	idic(Y)	46,X,+mar.ish idic(Y)(q11)(TelXp/Yp++,DYZ3++)
5	Normal	NOR	inv dup(acro)	inv dup(acro)
6	Severe oligozoospermia	aCGH, FISH	der(1)	mos 47,XY,+mar[10]/46,XY[10].ish der(1)(D1Z1+)
7	NOA	Y microdeletion	r(Y)	mos 45,X[6]/46,X,r(Y)[44]
8	Normal	NOR	inv dup(acro)	inv dup(acro)
9	NOA	FISH/Y microdeletion	r(Y)	mos 46,X,r(Y)[38]/45,X[12]
10	Severe oligozoospermia	FISH/Y microdeletion	r(Y)	mos 46,X,r(Y)[44]/45,X[6]
11	NOA	Y microdeletion	r(Y)	mos 46,X,r(Y)inv(9)(p12q13)[82]/45,X,inv(9)[18]
12	NOA	Y microdeletion	r(Y)	mos 45,X[68]/46,X,r(Y)[32]
13	NOA	Y microdeletion	r(Y)	mos 45,X[68]/46,X,r(Y)[32]
14	Severe oligozoospermia	C-banding, FISH, Y microdeletion	neo(Y)	46,X,neo(Y)(pter→q12→neo→q12→qter)[110]/45,X[10]
15	NOA	Y microdeletion	r(Y)	mos 45,X[20]/46,X,r(Y)[80]

NOA, *Non-obstructive azoospermia*; FISH, *Fluorescence in situ hybridization*; NOR, *Nucleolar organizer region*; aCGH, *array comparative genomic hybridization*.

abnormalities. Male infertility is an example of a complex phenotype with substantial genetic basis. Previous studies estimated that underlying genetic abnormalities account for 15–50% of male-factor infertility.<sup>3,8</sup> These genetic factors include numerical and structural chromosomal anomalies and several gene mutations. For example, karyotypic abnormalities are present in 5% of patients with fertility problems, and the prevalence increases to 13% when considering men with azoospermia.<sup>2</sup> Most chromosomal abnormalities associated with male infertility involve the sex chromosomes, and Klinefelter syndrome (47, XXY) is the most

commonly detected karyotypic abnormality in infertile men.

Previous studies reported that sSMCs, a specific genetic imbalance, were present in the patients with mental retardation, infertile couples, and prenatal fetuses.<sup>9</sup> We identified 15 of 4230 infertile males as carriers of marker chromosomes, corresponding to a frequency of about 0.35%, similar to previously reported data. sSMCs can occur as ring chromosomes, minute acrocentric chromosomes, or inverted duplications. In our study, eight patients had Y ring chromosomes, one had an isodicentric Y

chromosome, one had a neocentromere Y chromosome, one had a derivative chromosome, and four had acrocentric short arms.

Most publications that examined the association of marker chromosomes with male infertility were case reports.<sup>10,11</sup> One relatively large study analyzed the connection of sSMCs with fertility in 32 patients.<sup>12</sup> However, that study included male and female patients and did not provide detailed clinical characteristics of these patients. Although the correlation between the presence of an sSMC and male infertility is still unclear, these related reports also showed an association of an sSMC with spermatogenesis impairment, especially oligozoospermia. Our study only analyzed infertile males, and clearly showed a strong association between the presence of an sSMC and male infertility due to severely impaired spermatogenesis.

The significance of a marker chromosome can vary, and generally depends on its specific genetic composition. Sometimes, the marker consists of inactive genetic material that has little or no effect. In fact, two patients in our study who had marker chromosomes also had normal sperm concentrations, suggesting that the genetic material in their marker chromosomes did not significantly impact spermatogenesis.

Spermatogenesis is a complex developmental process in adult testes that requires the coordinated expression of many genes. Y chromosome microdeletion is a well-known genetic cause of male infertility due to its impairment of spermatogenesis, and there are concerns for transmission of this trait to male offspring after ART.<sup>5</sup> The long arm of the Y chromosome (Yq) contains the azoospermia factor (AZF) region, which contains genes critical for spermatogenesis; microdeletion of this region is associated with severe oligozoospermia or azoospermia.<sup>13</sup> The ring Y chromosome and the isodicentric Y marker chromosome in our study may have

combined Y chromosome microdeletions, resulting in severe disruption of spermatogenesis.

Accurate transmission of the haploid chromosomal content is fundamental for embryonic vitality and development. Study of the underlying genetic causes of male-factor infertility has become more important because increasing use of ARTs (such as ICSI) may allow fertilization even in men whose infertility has a genetic basis.<sup>14</sup> Correct genetic diagnosis is therefore essential for providing appropriate counseling to infertile couples about the effectiveness and safety of ART treatments, such as IVF/ICSI, and preimplantation genetic diagnosis.

Limitations of this study include the relatively small number of patients and the use of retrospective analysis. Another limitation is that it was mainly cytogenetic analyses, not gene-level analyses. However, cytogenetic studies are essential and important in evaluating the genetic causes of male infertility, and our study provided analysis of rare genetic causes of male infertility due to marker chromosomes. Further research using advanced techniques, such as whole-genome analysis with next-generation sequencing, are needed for more detailed investigations of the genetic association of sSMCs with male infertility.<sup>15,16</sup>

## CONCLUSION

Marker chromosomes appear to be a rare genetic cause of male infertility. Additional cytogenetic tests, including NOR banding, FISH, and aCGH, could help to characterize the origin of the marker chromosome. Appropriate genetic counseling for couples with infertility caused by a marker chromosome should be recommended.

## CONFLICT OF INTEREST

The authors have no potential conflict of interest to declare with respect to the research, authorship, and publication of this article.

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