A case of an infertile male with a small supernumerary marker chromosome negative for M-FISH and containing only heterochromatin

Wei Wang · Yali Hu · Haiyan Zhu · Jie Li ·
Ruifang Zhu · Ya-ping Wang

Received: 31 July 2008 / Accepted: 23 April 2009 / Published online: 14 May 2009
© Springer Science + Business Media, LLC 2009

Abstract

Aim To describe the case of a 32-year-old infertile male with small supernumerary marker chromosome (sSMCs) in 80% of peripheral lymphocytes.

Methods G-banding, C-banding, STRP analysis, M-FISH and molecule diagnosis of Y-chromosomal microdeletions were performed to determine the origin of sSMCs.

Results The karyotype of this patient was established as 47, XY, +mar/46, XY. C-banding showed that the marker chromosome was composed of heterochromatin without visible euchromatic material. No positive result was obtained in STRP, M-FISH and the microdeletion analysis of Y-chromosome.

Conclusions The small supernumerary marker chromosome could play a causative role in male infertility although the mechanism remains to be elucidated.

Keywords Infertility · Small supernumerary chromosome · M-FISH · Heterochromatin

Introduction

Small supernumerary marker chromosomes (sSMCs) are reported in 0.043% of newborn infants [1]. sSMCs are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are (in general) equal in size or smaller than a chromosome 20 of the same metaphase spread [1]. It is difficult to correlate a particular marker chromosome with a particular phenotype because of the wide variety of marker chromosomes. We know little about small supernumerary marker chromosomes in humans so far.

We recently found an infertile male who showed a karyotype seeming to be a trisomy 21 because of the marker chromosome in the same size of G group. However, no positive result was obtained when we used various techniques to identify the origin of this marker chromosome.

Materials and methods

Clinical data

A 32-year-old male was referred because of infertility. He was born at term and was well at birth. He always felt faint and experienced shortness of breath when he exercised, which limited his athletic activities. However, no conclusive result was obtained in cardiopulmonary examinations. Several lipomas (about 40×50 mm²) were found in his waist and scapulars. Azoospermia was diagnosed with

Capsule We report a case of an infertile male with heterochromatic sSMCs, and analyze the relationship between infertility and heterochromatic sSMCs.

W. Wang · Y.-p. Wang (✉)
Department of Medical Genetics, School of Medicine,
Nanjing University,
22 Hankou Road,
Nanjing 210093, China
e-mail: wangyap@nju.edu.cn

Y. Hu · H. Zhu · J. Li · R. Zhu
Affiliated Drum Tower Hospital, School of Medicine,
Nanjing University,
Nanjing 210008, China

W. Wang · Y. Hu · H. Zhu · J. Li · R. Zhu · Y.-p. Wang
Jiangsu Key Laboratory of Molecular Medicine,
Nanjing University,
Nanjing 210093, China

sperm examination. Neurological examination did not demonstrate any neurological abnormal signs. The chromosome karyotypes of his parents were unavailable because his father had deceased and his mother refused to be taken blood for test. No family history could be traced.

Conventional cytogenetic analysis

For the preparation of the metaphase cells, we used standard cytogenetic procedures [2]. Then conventional protocols were used for G-banding and C-banding.

Short tandem repeats polymorphism (STRP) analysis

Genomic DNA was extracted from peripheral blood using QIAamp DNA blood mini kit (Qiagen, Germany) according to the manufacturer's instructions [3]. The five STRP markers on chromosome 21 were selected for the copy number analysis with multiplex PCR (the primer sequences are shown in Table 1) [4]. PCR amplification was performed in 25 μ L volumes for 30 cycles (PTC-200 peltier thermal cycler, Bio-RAD). The PCR products were analyzed by vertical denaturing polyacrylamide gel electrophoresis with 1 $\times$ TBE and visualized by silver staining.

Fluorescence in situ hybridization (FISH)

Multiplex fluorescence in situ hybridization (M-FISH) was performed with whole chromosome painting probes directly labeled with five different fluorophores including SpectrumGoldTM, SpectrumFRedVIIITM, Spectrum AquaTM, SpectrumRedTM and Spectrum GreenTM i(Vysis, Downers Grove, USA). They were in a combinatorial label format to provide 24 distinct colors when analyzed with the SpectraVysion Imaging System (Cytovision3.9, AI Company).

The special chromosome painting probes for chromosomes 15 and 19 were used for retesting the result of M-

FISH. The painting probes for the two chromosomes were gifts from Dr Hervé Avet-Loiseau, Laboratoire de Cytogénétique Hematologique, Centre Hospitalier Universitaire de Nantes, France. The probes were labelled by SpectrumGreen-dUTP, SpectrumRed-dUTP (Roche, Germany) with nick translation tagging technique (Vysis USA).

Molecular diagnosis of Y-chromosomal microdeletions

The molecular diagnosis of Y-chromosomal microdeletions was performed with following STS primers: sY84, sY86 (AZFa); sY113, sY116, sY127, sY134 (AZFb); and sY254, sY255 (AZFc), which were routinely used for testing in male infertility with azoospermia or severe oligozoospermia [5].

Results

Chromosome G-banding unveiled that the index case had the karyotype 47, XY, +mar/46, XY. The marker chromosome was approximately the size of a G-group chromosome and appeared in 80% of peripheral lymphocytes observed (Fig. 1a, b). The test on five high polymorphism markers of STRs for chromosome 21 showed no evidence that it was a trisomy 21. C-banding result showed that the marker was composed of C-band positive heterochromatin without visible euchromatic material (Fig. 1c). The results of M-FISH suggested that the sSMCs lacked hybridization with all fluorochrome painting-probes in original image. It was negative for all chromosome-specific sequences (Fig. 1d, e). Nevertheless, the analysis with imaging software showed that sSMCs in two metaphase spreads seemed to have parts of homologous sequences with normal chromosomes, one mainly derived from chromosome 15 and the other from chromosome 19 (Fig. 1f, g). However, FISH analysis with the special painting probes for chromosomes 15 and 19 presented a negative result in the marker chromosome. (Fig. 1h). No Y-chromosomal microdeletion could be found with the deletion analysis of STS known to define the regions of AZFa, AZFb and AZF.

Discussion

We report a 32-year-old infertile male with mitotic stable small supernumerary marker chromosomes (sSMCs) containing only heterochromatin in 80% of peripheral lymphocytes. We analyzed carefully twelve metaphase spreads with M-FISH. The hybridized signal of normal chromosomes was clear and visible.

M-FISH kit contains whole chromosome painting probes and Cot-1 DNA, which blocks chromosome-nonspecific

Table 1 The STRP marker and PCR primers for checking chromosome 21

Marker	Primer sequence, 5' to 3'	Annealing
D21S1442	Forward: ctctccccactgcagac Reverse: tctccagaatcacatgagcc	55°C, 40 s
D21S20 ^a	Forward: tgaggtaggttccttagccc Reverse: aaggcctccctatgttatg	59°C, 40 s
D21S21 ^a	Forward: ttggcgaatcatgacactaa Reverse: gggagagcctcaaggaataac	56°C, 40 s
D21S34 ^a	Forward: cacaaggcagaataaaggga Reverse: agcatgtgtcctgaatcttg	56°C, 40 s
D21S2052 ^a	Forward: gcaccctttatacttggtg Reverse: tagtactctaccatccatctatccc	58°C, 40 s

^a Reference from Yan J [4]

Fig. 1 A supernumerary marker chromosome with heterochromatin detected in an infertile man. **a** Metaphase spread of G-banded chromosomes from the proband's peripheral lymphocytes. The arrow shows the SMC. **b** Karyotype analysis of the metaphase spread. **c** Metaphase spread of C-banded chromosomes from the proband's peripheral lymphocytes. The arrow shows the sSMC. **d** A metaphase spread before performing M-FISH. **e** The sSMC without hybridiza-

tional signal in the metaphase spread after performing M-FISH. The arrow shows the sSMC. **f** Analysis by imaging analysis software showed that sSMC was hybridized with multichromosome probes. **g** The sSMC seems to have been derived from chromosome 15 mainly in a metaphase spreads. The arrow shows the sSMC. **h** FISH: the red and green fluorochrome signal indicates the origin of chromosome 15 and 19 respectively

sequences during hybridization. The lack of hybridization with the painting probes suggests that no homologous sequences or few unique sequences exist in sSMCs. Twenty four kinds of centromeric probes are used to determine the origin of the marker chromosome. However they cannot

give any information about the chromosome-specific expression sequences.

Several laboratories also reported that the chromosome markers can not be hybridized by chromosomal painting probes. Mackie Ogilvie *C et al* showed that a sSMC was

negative for whole chromosome libraries, centromeric probes, chromosome specific telomere regions, and the C-banding [6]. It supposed that the sSMC might represent a multifold amplification of a very small region of the genome, analogously to that have been seen in homologously staining regions (HSRs) and double minutes (DMs) described in some types of tumour cells [7–10]. Liehr *T et al* have reported a patient from whom the similar results were obtained [11]. They suggested that a minority of sSMC show similarities to B-chromosomes.

In our report, the patient who carried a small supernumerary marker chromosome had infertility as a primary symptom. The marker chromosome was made of heterochromatin only. Whether the sSMC is a cause of infertility or a coincidental finding is still a question. Klinefelter syndrome and Y chromosomal microdeletion has been excluded for this case. We suspect the marker chromosome could have a role in infertility.

The sSMCs with the regions of chromosome 15 or 20 have been reported with male infertility [12, 13], which indicates that male infertility may have no direct relation with genetic abnormality of specific genes. However, the frequency of sSMCs detected in infertile patients is higher than that in general population (0.125% versus 0.043%) [14], and it is also different between male (0.165%) and female infertility (0.022%). sSMCs are preferentially maternally transmitted [15], suggesting either a reduced fertility in male carriers or that the marker is excluded in spermatogenesis.

Increasing evidences show the role of this supernumerary chromosome in infertility. Eggermann and his colleagues reported that six infertile carriers of heterochromatic sSMCs, five of whom suffered from azoospermia [12]. The other one presented with a severe OAT syndrome and had a history of a seminoma. They also have studied the sperms of an infertile male with supernumerary marker derived from chromosome 15, and found that more than 82.7% of sperm are unisomic, 17% disomic and 0.3% trisomic with respect to chromosome 15. A study of meiotic segregation in the sperms of two men with sSMCs also showed a significantly increased frequency of aneuploidy in the sperms compared to that in control donors [16]. The marker chromosome could disrupt normal disjunction by pairing with a similarly sized chromosome. Pairing failure at meiosis has been postulated as the cause of gametogenic arrest in gametes with balanced chromosome rearrangements and unbalanced chromosome constitutions [17], with male gametogenesis being more vulnerable. Additionally, autosomes with numerical and constitutional abnormalities are common causes of male infertility [18–20], such as Down syndrome [21]. It suggests that pairing failure may be a common mechanism in male infertility.

On the other hand, some evidences showed no increased risk for trisomic offspring or spontaneous abortion when one of the parents carries sSMC [22]. Liehr *et al* suggested that the individuals with infertility might not be regarded as clinically abnormal as this condition could be multifactorial and would not be due to the presence of sSMC alone [23].

On the study of this case, we suggest that sSMC without euchromatin could have a role in male infertility and might affect the gametogenetic process by interacting with other factors. However, the correlation between the phenotypes, lipomas and shortness of breath when exercising, and sSMC remains to be clarified.

Acknowledgments We are grateful to Dr. Yimei Fan and Dr. Huimei Chen for their valuable advice on preparing the manuscript. This work is supported by grants from Jiangsu Health Department (Grant number: H2006), China.

References

1. Liehr T, Claussen U, Starke H. Small supernumerary marker chromosomes (sSMC) in humans. *Cytogenet Genome Res*. 2004;107:55–67. doi:10.1159/000079572.
2. Kirsch IR, Morton CC, Nakahara K, Leder P. Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. *Science*. 1982;216:301–3. doi:10.1126/science.6801764.
3. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*. 1991;10:506–13.
4. Yan J, Wu J, Li Y, Wang H, Huang Z, Zhou X, et al. A novel diagnostic strategy for trisomy 21 using short tandem repeats. *Electrophoresis*. 2006;27:416–22. doi:10.1002/elps.200500349.
5. Dada R, Gupta NP, Kucheria K. Yq microdeletions—azoospermia factor candidate genes and spermatogenic arrest. *J Biomol Tech*. 2004;15:176–83.
6. Mackie Ogilvie C, Harrison RH, Horsley SW, Hodgson SV, Kearney L. A mitotically stable marker chromosome negative for whole chromosome libraries, centromere probes and chromosome specific telomere regions: a novel class of supernumerary marker chromosome? *Cytogenet Cell Genet*. 2001;92:69–73. doi:10.1159/000056871.
7. Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, van Kessel AG. Comparative genomic hybridization analysis of human sarcomas: I. Occurrence of genomic imbalances and identification of a novel major amplicon at 1q21–q22 in soft tissue sarcomas. *Genes Chromosomes Cancer*. 1995;14:8–14. doi:10.1002/gcc.2870140103.
8. Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, van Kessel AG. Comparative genomic hybridization analysis of human sarcomas: II Identification of novel amplicons at 6p and 17p in osteosarcomas. *Genes Chromosomes Cancer*. 1995;14:15–21. doi:10.1002/gcc.2870140104.
9. Muleris M, Almeida A, Gerbault-Seureau M, Malfroy B, Dutrillaux B. Identification of amplified DNA sequences in breast cancer and their organization within homogeneously staining regions. *Genes Chromosomes Cancer*. 1995;14:155–63. doi:10.1002/gcc.2870140302.

10. Pandita A, Godbout R, Zielenska M, Thormer P, Bayani J, Squire JA. Relational mapping of MYCN and DDX1 in band 2p24 and analysis of amplicon arrays in double minute chromosomes and homogeneously staining regions by use of free chromatin FISH. *Genes Chromosomes Cancer*. 1997;20:243–52. doi:[10.1002/\(SICI\)1098-2264\(199711\)20:3<243::AID-GCC4>3.0.CO;2-2](https://doi.org/10.1002/(SICI)1098-2264(199711)20:3<243::AID-GCC4>3.0.CO;2-2).
11. Liehr T, Mrasek K, Kosyakova N, Ogilvie CM, Vermeesch J, Trifonov V, et al. Small supernumerary marker chromosomes (sSMC) in humans; are there B chromosomes hidden among them. *Mol Cytogenet*. 2008;1:1. doi:[10.1186/1755-8166-1-12](https://doi.org/10.1186/1755-8166-1-12).
12. Eggermann K, Mau UA, Bujdosó G, Koltai E, Engels H, Schubert R, et al. Supernumerary marker chromosomes derived from chromosome 15: analysis of 32 new cases. *Clin Genet*. 2002;62:89–93. doi:[10.1034/j.1399-0004.2002.620113.x](https://doi.org/10.1034/j.1399-0004.2002.620113.x).
13. McNerlan SE, Morrison PJ, McClure N, Nevin NC. A supernumerary chromosome 20, identified by FISH, in a male with azoospermia—cause or coincidence? *Am J Med Genet A*. 2003;117:188–90. doi:[10.1002/ajmg.a.10899](https://doi.org/10.1002/ajmg.a.10899).
14. Liehr T, Weise A. Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med*. 2007;19:719–31.
15. Buckton KE, Spowart G, Newton MS, Evans HJ. Forty four probands with an additional “marker” chromosome. *Hum Genet*. 1985;69:353–70. doi:[10.1007/BF00291656](https://doi.org/10.1007/BF00291656).
16. Martin RH, Hildebrand KA, Yamamoto J, Peterson D, Rademaker AW, Taylor P, et al. The meiotic segregation of human sperm chromosomes in two men with accessory marker chromosomes. *Am J Med Genet*. 1986;25:381–8. doi:[10.1002/ajmg.1320250225](https://doi.org/10.1002/ajmg.1320250225).
17. Miklos GL. Sex-chromosome pairing and male fertility. *Cytogenet Cell Genet*. 1974;13:558–77. doi:[10.1159/000130307](https://doi.org/10.1159/000130307).
18. Zuffardi O, Tiepolo L. Frequencies and types of chromosome abnormalities associated with human male infertility. In: Crosignani PG, Rubin BL, editors. *Genetic Control of Gamete Production and Function*. New York: Academic; 1982. p. 261–73.
19. Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. *Hum Reprod*. 2002;17:13–6. doi:[10.1093/humrep/17.1.13](https://doi.org/10.1093/humrep/17.1.13).
20. De Braekeleer M, Dao TN. Cytogenetic studies in male infertility: a review. *Hum Reprod*. 1991;6:245–50.
21. Sheridan R, Llerena J Jr, Matkins S, Debenham P, Cawood A, Bobrow M. Fertility in a male with trisomy 21. *J Med Genet*. 1989;26:294–8. doi:[10.1136/jmg.26.5.294](https://doi.org/10.1136/jmg.26.5.294).
22. Steinbach P, Djalali M. Ineffectivity of accessory bisatellited marker chromosomes in inducing meiotic nondisjunction. *Hum Genet*. 1983;64:402–3. doi:[10.1007/BF00292376](https://doi.org/10.1007/BF00292376).
23. Liehr T, Mrasek K, Weise A, Dufke A, Rodríguez L, Martínez Guardia N, et al. Small supernumerary marker chromosomes—progress towards a genotype-phenotype correlation. *Cytogenet Genome Res*. 2006;112:23–34. doi:[10.1159/000087510](https://doi.org/10.1159/000087510).