Deletions within the azoospermia factor subregions of the Y chromosome in Hong Kong Chinese men with severe male-factor infertility: controlled clinical study

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Objective. To determine the patterns and the prevalence of microdeletions in the azoospermia factor subregions of the Y chromosome in Hong Kong Chinese men with severe male-factor infertility.
Design. Controlled clinical study.
Setting. Reproductive centre of a university teaching hospital, Hong Kong.
Participants. Fifty-eight men with severe male-factor infertility who participated in the in vitro fertilisation programme from May 1998 through March 1999, and 46 male volunteers with proven fertility.
Main outcome measures. Polymerase chain reaction analysis of DNA from peripheral blood lymphocytes using six loci spanning the *AZFa*, *AZFb*, and *AZFc* subregions of the Y chromosome.
Results. No microdeletions were detected in the fertile controls or in patients with obstructive azoospermia. Deletions within the *AZFc* subregion were found in 9% (4/44) of men with non-obstructive azoospermia or severe oligospermia. Neither *AZFa* nor *AZFb* deletions were detected in any participants.
Conclusion. Deletions within the azoospermia factor subregions of the Y chromosome are associated with severe male-factor infertility in Hong Kong Chinese men.

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Key words: Chromosome deletion; Infertility, male; Polymerase chain reaction; Y chromosome/genetics

Introduction

Infertility affects approximately 15% of all married couples in the general population, and half of the cases are male-related.[1,2] Little is known, however, about the genetic basis of male infertility. Tiepolo and Zuffardi[3] reported that 0.5% of a group of 1160 infertile men had macroscopic deletions of the distal long arm of the Y chromosome (Yq). They proposed the presence of an azoospermic factor (AZF) in this region. The putative genes were subsequently mapped to three close subregions within the Yq11.22-23 region, named *AZFa*, *AZFb*, and *AZFc*.[4,5] The *AZFa* subregion was found in the proximal portion of deletion interval 5; *AZFb* was found in the proximal end of deletion interval 6, extending into the distal part of deletion interval 5; and *AZFc* was found in the distal portion of deletion interval 6. By using the polymerase chain reaction (PCR) and constructing Y-chromosome sequence-tagged site (STS) maps, Y-chromosome microdeletions have been detected in the *AZF* region of DNA from infertile men.[6] This study used the same methodology to detect Y-chromosome microdeletions in Hong Kong Chinese men with severe male-factor infertility.

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Methods

Participants

Infertile men were recruited from the in vitro fertilisation programme of the Department of Obstetrics and Gynaecology at The University of Hong Kong from May 1998 through March 1999. The study was approved by the university ethics committee, and informed consent was obtained from each participant. Fifty-eight

males with severe oligospermia (<1 x 10^6 spermatozoa per millilitre of ejaculate [n=9]), non-obstructive azoospermia (n=35), or obstructive azoospermia due to congenital or acquired causes (n=14) were recruited. Semen analysis was performed according to the 1992 World Health Organization guidelines.[7] Forty-six healthy men of proven fertility were recruited as positive controls.

Screening for Y-chromosome microdeletions

All tests were performed on genomic DNA that was extracted from peripheral blood lymphocytes by using a commercially available DNA-isolation kit for mammalian blood (Boehringer Mannheim Corp., Indiana, United States). For each participant, Y chromosome–specific STSs that spanned the *AZFa* (sY84 and sY86), *AZFb* (sY127 and sY132), and *AZFc* (sY254 and sY255) subregions were used to amplify six specific regions of the Y chromosome using PCR. These markers were concentrated in the subintervals 5 and 6 of Yq11, in which microdeletions have been reported. To prevent false-negative results, each PCR sample was co-amplified with one of the two following internal control primers: sY72, a control marker for the presence of Y chromosome–specific DNA; or globin, a marker indicating the quality of the DNA preparation and successful PCR amplification. For each PCR sample, water and female genomic DNA were used as controls to verify that no cross-contamination had occurred.

The PCR amplification comprised a total volume of 25 μL, which contained 100 to 200 ng of human genomic DNA; 1.5 to 2.0 mmol/L magnesium chloride; deoxyribonucleoside 5'-triphosphates (200-400 μmol/L each of dTTP, dCTP, dGTP, and dATP); 0.2 to 1.0 μmol/L primer; PCR buffer; and 1 U *Taq* polymerase (Boehringer Mannheim Corp., Indiana, United States). Thermocycling consisted of initial denaturation of 4 minutes at 94°C and 35 cycles of incubation at 94°C for 30 seconds, at 49 to 50°C for 30 seconds, and at 72°C for 1 minute. The PCR reaction products were stored at 4°C, separated on 2% to 3% agarose gels, and visualised by staining the gel with ethidium bromide. A sample was considered positive for an STS marker when the PCR product of the expected size was present; it was considered negative if a product of the expected size was not amplified after three PCR attempts.

Results

Among the 58 infertile men examined, neither *AZFa* nor *AZFb* microdeletions were detected by PCR analysis in any DNA samples. Furthermore, no *AZF* deletions were detected in any of the 14 patients with obstructive azoospermia (congenital or acquired). However, *AZFc* microdeletions corresponding to the STSs sY254 and sY255 were detected in the DNA samples of four infertile men: three with non-obstructive azoospermia and one with severe oligospermia. They constituted 6.9% (4/58) of all the infertile men investigated or 9.1% (4/44) of those with non-obstructive azoospermia or severe oligospermia. All four patients had microdeletions in subinterval 6 of Yq11, which included the region of the *AZF* candidate gene, *DAZ* (deleted in azoospermia). The PCR results of some patients with deletions are shown in the Figure. None of the 46 men with proven fertility showed deletions at any of the STSs tested.

Discussion

The prevalence of Y-chromosome microdeletions in this study population was 6.9%. In contrast to studies of Caucasian populations, in which Y-chromosome microdeletions were observed in a small proportion (1%) of infertile men with obstructive azoospermia,[8] no such microdeletions were detected in this study among the men whose infertility had an obstructive cause. On the other hand, of the participants with non-obstructive azoospermia or severe oligospermia, 9.1% (4/44) had microdeletions (Fig, lanes 1 and 2). This figure is slightly lower than the frequencies (10%-15%) quoted in studies of Caucasian populations[9-11]; however, these studies examined more loci. The frequency of microdeletions found in this study is similar to that found in a report from Taiwan (9%).[12] Although both studies used similar loci to detect the presence of Y-chromosome microdeletions, the study from Taiwan detected six *AZF* deletions in six different patients: three with deletions within the *AZFa* region and the other three with deletions within the *AZFc* region. The microdeletions of the four patients in this study were all within the *AZFc* region.

Zheng et al[13] recently reported the prevalence of Y-chromosome microdeletions in *AZF* subregions of 13.3% (12/90) in azoospermic or oligospermic men.[13] The figure is higher than that found in this study, but their results were not confirmed by including an internal control primer in their PCR assay; hence, the absence of amplification might have been because of PCR failure or poor DNA quality. Furthermore, they did not include an additional primer within the *AZFc* region in their PCR assay. In this study, *AZFc* deletion was confirmed by performing triplicate PCR amplifications with two sets of primers within the *AZFc* region.

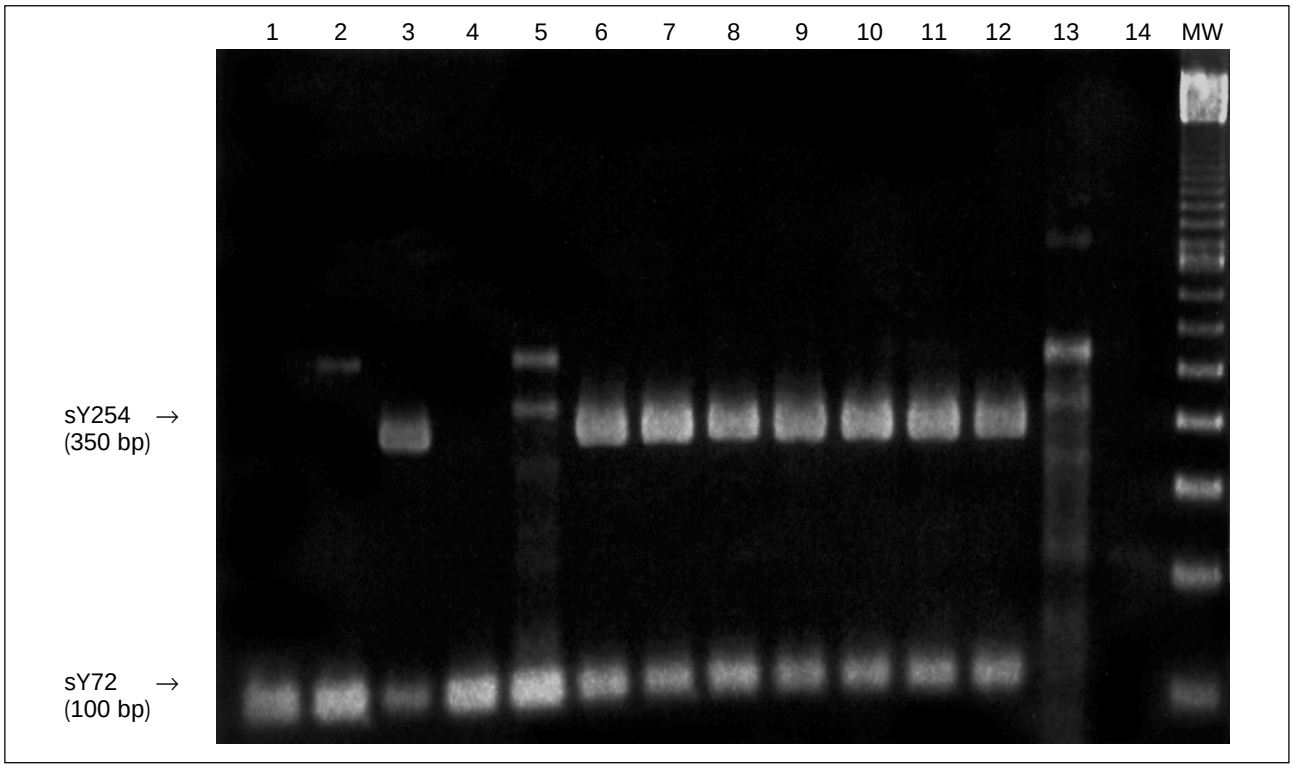

Fig. Results from multiplex polymerase chain reaction analysis
Lanes 1, 2, 4, and 5 show a deletion of sY254; lane 1: man with severe oligospermia; lane 2: intracytoplasmic sperm injection–derived baby from the patient in lane 1; lanes 3-5: men with non-obstructive azoospermia; lanes 6-12: fertile controls; lanes 13 and 14: negative control samples of female DNA and water, respectively; MW = 100-bp molecular weight markers

Recent studies support the concept of the genetic basis of male infertility.[14-16] The high prevalence rates of *AZFc* deletions among the local population and in Caucasian populations suggest that this region of the Y chromosome consists of one or more important genes that are responsible for normal spermatogenesis. The *DAZ* gene, which clusters at the *AZFc* region, is one of the most frequently deleted genes in patients with severe male-factor infertility.[16,17] This gene is expressed specifically in the testis and encodes an RNA-binding protein. Although its precise biological function is not yet known, the DAZ protein is believed to play a role in male germ-cell development.

The development of intracytoplasmic sperm injection (ICSI) has allowed many males with severe male-factor infertility to father a child. This technique, however, cannot cure the underlying spermatogenic problem, and it is possible that the genetic defect is transmitted from the father to his offspring by virtue of the ICSI. We have recently found evidence for this process in one patient (Fig, lanes 1 and 2): the same *AZFc* microdeletion was detected in the oligospermic father and his ICSI-derived baby.[18] Hence, a spermatozoon with a Yq deletion in the *AZFc* subregion can fertilise an oocyte and result in pregnancy by means of assisted reproduction techniques. [18,19]

This is the first study to investigate the pattern and prevalence of Y-chromosome deletions in *AZF* subregions in Hong Kong Chinese men with severe male-factor infertility. The prevalence of microdeletions in the local population needs to be confirmed by screening more infertile men and using more STS markers. Although Y-chromosome microdeletions occur in a subgroup of infertile men, routinely screening microdeletions in all male patients before ICSI treatment is an important prerequisite to their appropriate counselling.[20]

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