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

JYM Tse, WSB Yeung, EYL Lau, EHY Ng, WWK So, PC Ho

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.

HKMJ 2000;6:143-6

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 Zuffardi1 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.

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 following
internal control primers: sY2, 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 chlor-
ide; 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 oligo-
spermia. 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 candi-
date 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,a
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 populations9-11; how-
ever, 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 al13 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.
Recent studies support the concept of the genetic basis of male infertility. 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. 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. 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.

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.

Acknowledgement

This study was supported by a research grant (CRCG 10201949/31846/20900/301/01) from The University of Hong Kong.

References

19. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. Hum Reprod 1999;14:1722-6.