The Application of Chromosome Abnormality Chip Detection in Male Infertility

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ABSTRACT

Objective*: To discuss the application of microarray technology in the diagnosis of male infertility.*
Methods*: Sixteen loci, including a sex-determining region on the Y chromosome, were investigated by polymerase chain reaction (PCR) in infertile male patients. Chromosome abnormality chip with 180 000 probes was used to detect small deletion, small amplification and loss of heterozygosity.*
Results*: By PCR, nine of 103 infertile patients were found to have sequence-tagged site microdeletions. Microdeletions were not observed in control samples. The deletions detected by PCR were present in six azoospermic men (6/44, 13.6%) and in three oligoasthenoteratozoospermic (OATS) men (3/59, 5%). The overall frequency of microdeletions in infertile men was 8.7% (9/103). Chromosome abnormality chip detection 500+ detected more amplification or deletion in 51 infertile patients and the overall frequency of microdeletions in infertile men was 49.5% (51/103).*
Conclusion: *Chromosome abnormality chip detection system provides a sensitive, economic and high-throughput method for detecting the deletion or amplification of genomic DNA sequences of infertile patients. Not only can it identify Yq deletions, but it can also find other chromosome abnormalities and facilitate the understanding of male infertility.*

Keywords: Chip detection, chromosome abnormality, infertility, polymerase chain reaction

Aplicación de la Detección de la Anormalidad del Cromosoma Mediante Biochips Genéticos en la Infertilidad Masculina

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RESUMEN

Objetivo: *Analizar la aplicación de la tecnología de los microarreglos en el diagnóstico de la infertilidad masculina.*
Métodos: *Dieciséis loci, incluyendo una región determinante del sexo en el cromosoma Y, fueron investigados mediante reacción en cadena de la polimerasa (RCP) en pacientes hombres con problemas de infertilidad. Un biochip de la anormalidad cromosómica, con 180000 sondas, fue utilizado a fin de detectar pequeñas delecciones, pequeñas amplificaciones y pérdidas de heterocigosidad.*
Resultados: *Por medio de la RCP, se halló que nueve de 103 pacientes con infertilidad presentaban microdelecciones de sitios de secuencia marcada. Las microdelecciones no fueron observadas en las muestras de control. Las delecciones detectadas mediante RCP, estuvieron presentes en seis hombres azoospérmicos (6/44, 13.6%) y en tres hombres con oligoastenoteratozoospermia (OAT) (3/59, 5%). La frecuencia general de las microdelecciones en los hombres infértiles fue 8.7% (9/103). La detección con biochip de la anormalidad cromosómica de 500+ detectó más amplificación y delección en 51 pacientes, y la frecuencia general de microdelecciones en los hombres infértiles fue 49.5% (51/103).*
Conclusión: *El sistema de detección de la anormalidad del cromosoma mediante biochips genéticos representa un método sensible, económico, y de alto rendimiento, para detectar la delección o amplificación de las secuencias genómicas de ADN de pacientes infértiles. Este método puede no sólo*

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identificar las delecciones Yq, sino también hallar otras anormalidades cromosómicas, facilitando así la comprensión de la infertilidad en los hombres.

Palabras claves: Detección mediante biochips, anormalidad cromosómica, infertilidad, reacción en cadena de la polimerasa

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INTRODUCTION

In infertile couples, male factors account for almost half of the cases of infertility (1). These male factors can be ascribed to infection, immunological factors, anatomical malformations, chemical or genetic factors. Y-chromosomal deletions represent the most frequent genetic alterations in infertile men (2−3). Analysis of these deletions in the azoospermia factor (AZF) region demonstrates four non-overlapping loci, AZFa, AZFb, AZFc and AZFd (4–12).

The deletion of these loci results in spermatogenic arrest and is associated with azoospermia and oligozoospermia (4–12). Schlegel *et al* first demonstrated that the deletion of different AZF regions appears to have different effects on spermatogenesis (13). The correlation between the deletion of sperm in testicular tissue and specific AZF deletion has been made in a few studies with small numbers of subjects. Y chromosome microdeletion most frequently involves the AZFc region (60%), less frequently the AZFb region (16%), and only rarely the AZFa and AZFd interval (5%). Larger Y chromosome microdeletions involving two or three AZF regions are diagnosed in 14% of cases. At present, multiplex polymerase chain reaction (PCR) is the most commonly used method to detect Y chromosome microdeletions.

However, the Y deletion studies did not fully explain the various phenotypes. In addition to Y chromosome deletion, male sterility is also related to other chromosome deletion or mutation. Y chromosome-linked copy number variants (CNVs) and Y-linked genes have also been demonstrated as important contributors to impaired sperm production in infertile humans. So, a high resolution array comparative genomic hybridization (array-CGH) technology was developed and used to identify the variation of spermatogenesis related to autosomes and sex chromosomes (14). It is gradually replacing the traditional karyotyping, chromosome banding technique and PCR because of its high resolution, short detection time, high automatic advantages and it has become the main tool for the analysis of cytogenetic study of chromosome abnormalities (15). In this study, we used the chromosome abnormality chip detection 500+ (CGH+SNP chip) developed by Boao Company to screen for Yq microdeletions and other deletions or amplifications on autosomes of infertile male patients.

SUBJECTS AND METHODS

The patients were selected from Yantai Yuhuangding Hospital from March 2010 to June 2011. One hundred and three infertile patients with non-obstructive azoospermia (NOA) or severe oligoasthenoteratozoospermia (OATS) were enrolled in this study. All of the men underwent a thorough history and comprehensive physical evaluation. Forty-six healthy fertile men were used as controls. Only normal subjects with at least one child and no history of assisted reproductive technology were included in the control group. Each PCR product identified by gel electrophoresis was used as a positive control. All samples were collected with the patient's consent.

Sequence-tagged site analysis by polymerase chain reaction

Genomic DNA was extracted from peripheral blood using the Genomic DNA Purification Kit (Promega, USA). Fifteen sequence-tagged sites (STS) within the long arm of the Y chromosome were selected in the AZFa, b and c regions. The testing of the short arm on the Y chromosome (Yp) was performed with the sex-determining region Y (SRY). Previously published primer sequences were used for each STS (16, 17). Multiplex PCR was performed for analysis of microdeletions. The internal control used was SRY. Samples from normal fertile men without Y chromosome microdeletions were used as positive controls. Water and DNA from a female served as negative controls. Polymerase chain reaction products were run by electrophoresis on a 4% agarose gel. This analysis was performed at least three times on microdeleted samples.

Patients who were diagnosed as having chromosomal abnormalities or Y chromosome microdeletions were given genetic counselling. Statistical analysis was carried out by the Statistical Package for Social Sciences for Windows, version 11.0 (SPSS; Chicago, IL, USA). The unpaired *t*-test, Mann Whitney U test and Chi-squared test were used appropriately. $P < 0.05$ was considered significantly different.

Chromosome abnormality chip detection

Chromosome abnormality detection chip 500+ was developed by the scientists of CapitalBio Corporation. It covers the latest GRCP (Platform database of Chinese nation's health and genetic related diseases resources) information for chromosome abnormal detection. The chip contains 180 000 probes combined by CGH probe and single nucleotide polymorphisms (SNP) probe and covers each segment of chromosome and can effectively detect small deletions, small amplifications and loss of heterozygosity. The standard scheme of non-amplified labelling and hybrid and CytoGenomics software were used in the whole experimental process according to manufacturer's instructions (Agilent Technologies, Santa Clara, CA, USA).

RESULTS

Diagram of Y chromosome deletion

By UCSC genome browser on human February 2009 assembly, we obtained the Y chromosome gene mapping and annotation of the corresponding gene. The gene deletion of Y chromosome occurred mainly in the area of Yq11.221. The detection focussed on three non-overlapping regions of AZFa, AZFb and AZFc in Yq. The size of AZFa, AZFb and AZFc deletion fragments was 800 kb, 3.2 Mb and 3.5 Mb, respectively. The corresponding genes of the three regions is AZFa for USP9Y, DBY and UTY gene; AZFb for SMCY, EIF1AY and RBMY1A1 gene; and AZFc for BPY2, DAZ, CDY1, PRY, CSPG4LY, GOLGA2LY, TTY3, TTY4, TTY5 and TTY6 gene. In the three regions, the sequence-tagged sites for PCR amplification were sY82, sY84 and sY86 in AZFa region, sY124, sY127, sY128, sY133, sY134, sY143 in AZFb region and sY152, sY239, sY254, sY255, sY242 in AZFc region.

Y chromosome microdeletion analysis by polymerase chain reaction

One hundred and three infertile patients and forty-six normal subjects were selected as previously described. Genomic

Table: Frequency of chromosome microdeletion in 103 infertile patients

Phenotype	n	Deletions (%)	
		PCR	Array
Azoospermia	44	6 (13.6)	34 (77.3)*
Oligoasthenoteratozoospermia	59	3 (5)	17 (28.8)*
Total	**103**	**9 (8.7)**	**51 (49.5)***

Multiplex polymerase chain reaction (PCR) and array technology (chromosome abnormality chip detection 500+) were performed for analysis of microdeletions. The internal control used was sex-determining region Y (SRY). Samples from normal fertile men were used as positive controls. Water and DNA from a female served as negative control. *$p < 0.05$

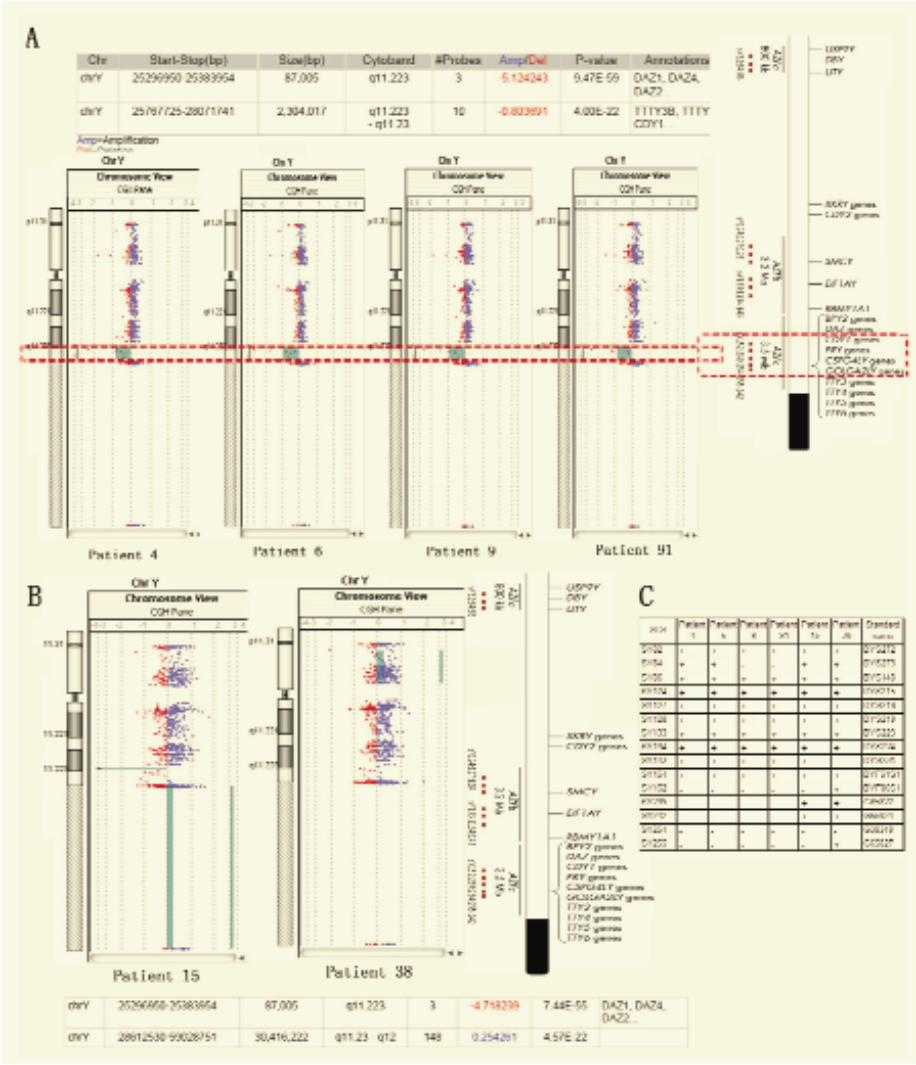

Fig. 1: Chromosome abnormality chip detection for azoospermia. A: Deletion of the Y chromosome in samples of patients 4, 6, 9 and 91 who are azoospermic men. B: Deletion of the Y chromosome in samples of patients 15 and 38 who are azoospermic men. C: The sequence-tagged site (STS) deletions type in the samples of patients 4, 6, 9, 91, 15 and 38.

DNA from healthy controls and from infertile patients was first screened individually by PCR analysis of which nine subjects were found to have microdeletions. Microdeletions were not observed in control samples. The deletions were present in six azoospermic men (6/44, 13.6%) and in three men with oligoasthenoteratozoospermia (OATS) [3/59, 5%] (Table). The overall frequency of microdeletions in infertile men was 8.7% [9/103] (Table).

Chromosome abnormality chip detection

We tested the 103 infertile patients with the chromosome abnormality chip detection 500+ technology. Fifty-one subjects were found to have microdeletions and the overall frequency of microdeletions in infertile men was 49.5% [51/103] (Table). Samples of patients 4, 6, 9 and 91 with five STS deletions on Y chromosome were also found to have two adjacent segment deletion with the size of 87 kb and 2.3 Mb (Fig. 1A, 1C). Samples for patient 15 and patient 38 were also detected to have small amplification or deletion on the Y chromosome (Fig. 1B). All six patients were azoospermic men.

Patients 5, 69 and 85 had OATS. They were found to have larger fragment deletions on the Y chromosome. The result coincided with that of the PCR examination but yielded more information (Fig. 2A, 2B). Chip detection allows one to see the size of the deletion or amplification fragment, the cytoband and the related genes in the deletion region (Fig. 2C). The nine samples all had amplification or

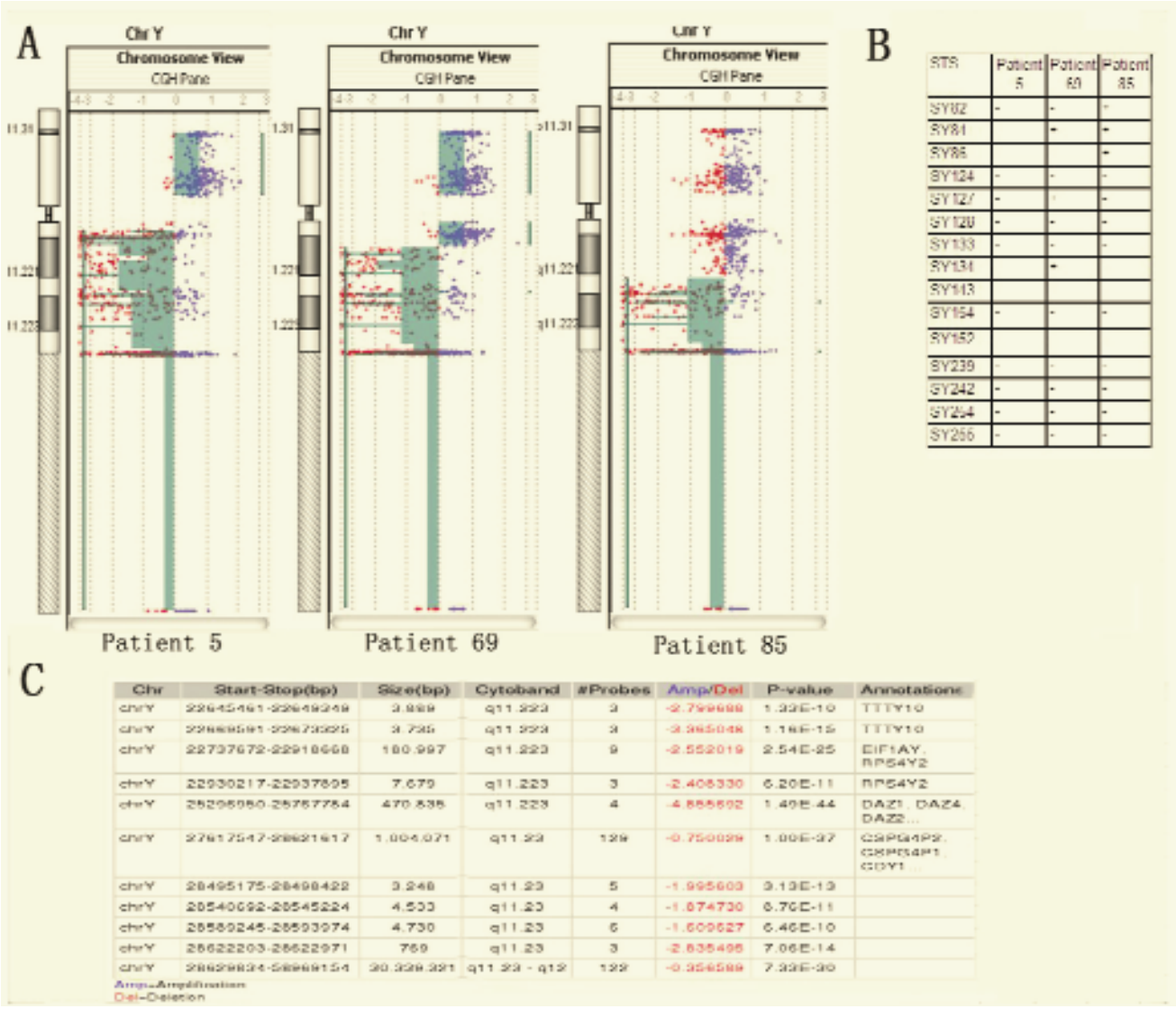

Fig. 2: Chromosome abnormality chip detection for oligoasthenoteratozoospermia. A: Deletion of the Y chromosome in samples of patients 5, 69 and 85 who are all oligoasthenoteratozoospermic. B: The sequence-tagged site (STS) deletions type of the samples of patients 5, 69 and 85. C: The fragment size, cytoband and the related genes in the deletion regions.

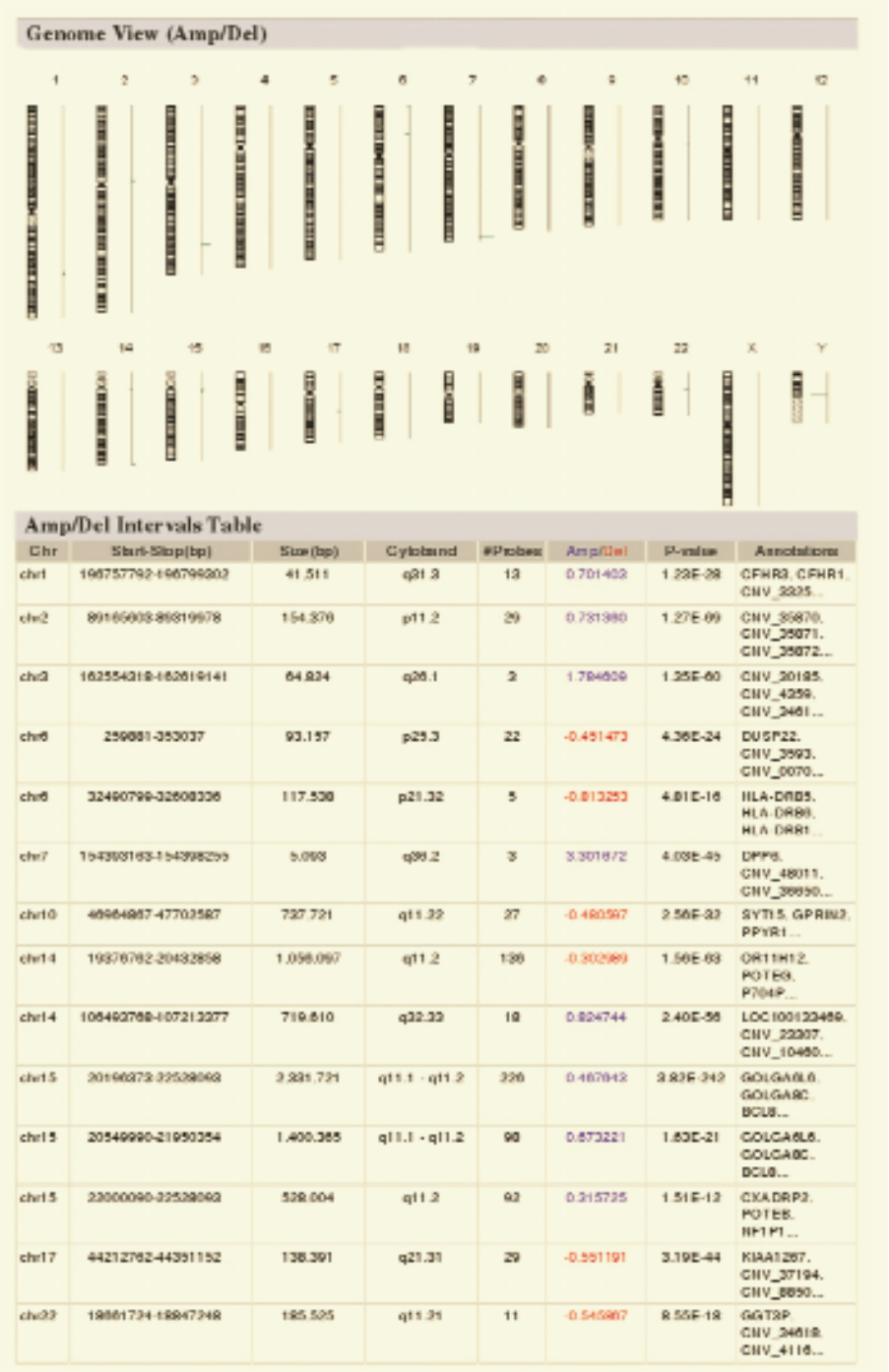

Fig. 3: The abnormality of autosome by chromosome abnormality chip detection. Autosome abnormality in patient 69 is shown. The fragment size, cytoband and the related genes in the deletion regions are also shown.

deletion on other chromosomes such as 1, 2, 3, 6, 7, 10, 14, 15, 17, 22 besides the Y chromosome, but their clinical significance has not been analysed (Fig. 3).

DISCUSSION

Among numerous aetiologic factors, genetic factor plays a key role in male infertility with abnormal semen parameters (18). Spermatogenesis is regulated by a number of genes on the Y chromosome and autosomes that act at different stages of germ cell development. Y chromosome deletions are emerging as a prevalent cause of male factor infertility. The frequency of Y chromosome deletions increases with the severity of spermatogenic defect (1, 19). The reported incidence of Y chromosome deletion varies among the studies:

approximately 15% of azoospermic and 5%–10% of oligozoospermic men. Balkan *et al* reported that the frequency of chromosomal aberrations (11.2%) exceeded the incidence of microdeletions of the Y chromosome [1.3%] (20). Therefore, new technology is needed for chromosome abnormality detection.

In this study, Yq microdeletion was detected in six (13.6%) of 44 azoospermic cases and three (5%) of 59 OATS cases but not in other fertility cases. The frequency of AZF deletions in severe oligozoospermia was found to be lower than those in azoospermia and the results are similar to the published data. The most common microdeletion found in our patient population was AZFc. The association between AZFc deletion and impaired spermatogenesis has been demonstrated previously (21). The DAZ gene family is located in the AZFc region and is reported to be the most frequently deleted AZF candidate gene in infertile males. Azoospermia factor (AZFc) deletions appear to remove the DAZ gene cluster and have been associated with a variety of spermatogenic alterations, ranging from azoospermia due to Sertoli cell-only to oligozoospermia with different testicular phenotypes (22).

Analysis of conventional multiple PCR products on a gel is quite complicated and troublesome. In the present study, we obtained more amplification or deletion fragments on the Y chromosome microdeletion patients by chromosome abnormality chip detection (CGH+SNP). The results might give more accurate results than gel electrophoresis analysis because of their sequence specific hybridization and high-throughput screening of human genome, and can provide a useful tool for the molecular diagnosis of male infertility in clinical laboratories. In this study, we detected multiple deletions on the Y chromosome in the sample of patient 5 and amplification in the sample of patient 85. Deletion was also detected on other autosomal chromosomes. It can also be used in prenatal screening and diagnosis of congenital diseases and developmental disorders.

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