Journal of Clinical Medicine and Research, Vol. 2(3) pp. 042-048, March 2010
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Full Length Research Paper

Array comparative genomic hybridization (CGH) analysis of sperm DNA to detect copy number variations in infertile men with idiopathic azoospermia

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Accepted 22 January, 2010

Epididymal and testicular spermatozoa of azoospermic patients are frequently used for intracytoplasmic sperm injection (ICSI), so they must be screened for genetic abnormalities. The objective of our study was to investigate whole genome imbalances in immature germ cells found in ejaculates of six males with idiopathic azoospermia and normal karyotype. We used for the first time the most powerful tool for genetic screening - microarray-based technology of comparative genomic hybridization (array CGH) with microarrays, covering all autosomes and sex-chromosomes at a mean density of 1 BAC clone/0.5 Mb. Sub-microscopic copy number variations were found in sperm DNA of all analyzed patients. The most consistent were aberrations in Y-chromosome - they occurred in 5 out of 6 patients (83.3%). These Y micro-aberrations included both micro-deletions and micro-duplications. In addition to Y chromosomal micro-imbalances, we detected several other affected loci. These included 1p36 deletion together with 14q24 gain, 16q24 deletion, 9q34 gain and 3q29 deletion. By array CGH analysis we determined cryptic whole genome imbalances in sperm cells and defined the most precisely the size and the boundaries of aberrations.

Key words: Male infertility, azoospermia, sperm aneuploidy, whole genome analysis, array CGH.

INTRODUCTION

In 30% of the cases of male infertility the causes remain unknown; the men subjected to a semen analysis face the diagnosis of azoospermia (Krausz and Forti, 2000). This generally refers to the inability of the sperm producing part of the testicle (the seminiferous epithelium) to make adequate numbers of mature sperm. It may be an inability of the sperm to complete their development (a "maturation arrest"). The contributions of sperm to normal fertilization and embryogenesis are extensive. The transmission of a haploid chromosome complement is the most fundamental and essential contribution, since embryonic aneuploidy is universally associated with lethality al., 2007). Since epididymal and testicular spermatozoa of azoospermic patients are frequently used for intra- or anomalies in the fetus (Boerke et al., 2007; Carrell et cytoplasmic sperm injection (ICSI), many studies have been carried out to evaluate their karyotype (Burrello et al., 2005).

Sperm chromosome aneuploidy increases risk to offspring and affects infertility therapy outcomes (Gianaroli et al., 2005). Several studies reported the high rate of sperm aneuploidy in some recognizable clinical syndromes, for which sperm chromosome aneuploidy testing may be advisable. It was observed that 1.5 - 7% sperm aneuploidy found in mosaic forms of Klinefelter's Syndrome (Kruse et al., 1998 ; Lim et al., 1999); 2 - 25% in nonmosaic Klinefelter's Syndrome (Rives et al., 2000; Estop et al., 1998); 7 - 36% in the cases with Robertsonian translocation (Ogur et al., 2006; Fryndman et al., 2001); 19 - 77% in the carriers of reciprocal translocation

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(Martin and Spriggs, 1995); 15 - 100% in cases with severe sperm morphology defects - multiflagellar, macrocephalic, tail agenesis (Devillard et al., 2002; Carrell et al., 2004); 15 - 60% in Round Head Only Syn-drome (Carrell et al., 2001; Carrell et al., 1999); 1 - 51% in nonobstructive azoospermia (Burrello et al., 2005); 1 - 34% in unexplained recurrent pregnancy loss (Bernardini et al., 2004 ; Carrell et al., 2003); and 2 - 7% in repeated *in vitro* fertilization (IVF) failure (Petit et al., 2005).

The analysis of sperm chromosome aneuploidy by fluorescent in situ hybridization (FISH) technology has greatly improved our understanding of sperm pathologies (Carrell, 2008). However, the fluorescence microscopy analysis of 5,000 - 10,000 sperm, requiring repeating analyses for limited number of up to 10 chromosomes, makes the sperm aneuploidy analysis by FISH limited, expensive and time-consuming.

Comparative genome hybridization (CGH) has been looked as a potential methodology to improve aneuploidy analysis of gametes due to the fact that it allows simultaneous evaluation of all chromosomes in one experiment, and has the resolution to look at the submicroscopic copy number variations (CNVs). CGH technology is being used now in clinical and research protocols, including single cell studies of oocytes and embryos (Fragouli et al., 2006; Sher et al., 2007).

Modification of the technology to employ microarrays instead of metaphase chromosomes has facilitated the analysis of thousands discreet foci, greatly increasing the ability to identify small, submicroscopic imbalances and improving the accuracy of aneuploidy analysis (Carter, 2007; Pinkel et al., 1998). Literature data have showed that more than half of the variability between human genomes is due to submicroscopic copy number variations of DNA, and that these CNVs are responsible for some complex diseases, even more than single nucleotide polymorphisms (Freeman et al., 2006; McCarroll and Altshuler, 2007). There are currently more than 6,000 known regions of CNV (Redon et al., 2006; Boerke et al., 2007).

Diagnosis of sperm chromosome aneuploidy may reduce risk to the offspring, and in some cases reduce the high financial and emotional expense of repeated IVF (*in vitro* fertilization) failure. The objective of our study was to investigate whole genome imbalances in immature germ cells found in ejaculates of six males with idiopathic azoospermia and normal karyotype.

MATERIALS AND METHODS

Patients

This study included six patients with idiopathic azoospermia. They were selected on the basis of the presence of immature germ cells in ejaculates, investigated microscopically. Hormonal problems and varicocele were excluded as causes of sperm production failure. The study was approved by the local Ethics Committee of the Medical University of Sofia. All participants were asked for and provided their informed consent.

Cytogenetic analysis

G-banded chromosomes were prepared from whole blood samples using standard laboratory protocols. All patients included in the study had a normal male karyotype.

DNA extraction and evaluation

DNA was extracted from sperm ejaculates of the patients by phenol-chloroform after twice washing in phosphate buffered saline (PBS) and centrifugation. Because of the very low DNA concentration in the samples, DNAs were precipitated in sodium acetate and ethanol at -80°C for at least 2 h. DNA concentration was measured by Nanodrop, as well as the purity of DNA was estimated. The ratio 260/280 for the last parameter was in the range of 1.8 - 2.0 for each sample. As an additional quality control, DNA was checked on 1% agarose gel: DNA of high molecular weight (> 50 kbp) indicated it suitable for use.

Genomic arrays

We have used genomic arrays CytoChip (BlueGnome, Cambridge, UK) consisting of Bacterial Artificial Chromosomes (BAC) clones, covering the entire genome at a median density of 1 clone/565 Kbp, a resolution optimised to detect pathogenic imbalances while minimizing polymorphisms. In addition, it investigate sub-telomeres at a median 250 Kb resolution, reliably detect mosiacism and examine 90 known genetic conditions at a median 100Kb resolution. This resulted in an average density of 1 clone/0.5 Mb.

Array-CGH probe labeling, hybridization, image capture and data analysis

Test DNA from patients (400 ng) and reference male DNA from donor with successful reproduction (400 ng) was labeled by random-priming, using BlueGnome Fluorescent Labelling System. The labeled products were purified by AutoSeqTM G50 columns, and incorporation of dyes was evaluated by Nanodrop as the incorporation in range 6 - 15 pmol/ µl and DNA yield in 180 - 325 ng/µl were considered suitable for further analysis. A mix of Cyanine-5 (Cy5) and Cyanine-3 (Cy3) labeled probes and a mix of COT-1 and Herring sperm DNA were ethanol precipitated at -80°C for at least 30 min. Hybridization processing was done dissolving precipitated probes in hybridization buffer. Arrays were washed in standard saline citrate (SSC) solutions with decreasing concentrations and scanned by a GenPix 4100A. The images were analyzed by BlueFuse for Microarrays 3.5 software (BlueGnome, Cambridge, UK). In data processing, base 2 logarithm (log2) ratios of Cy3 and Cy5 intensities are generated for all hybridized clones. Normal copy numbers are considered in ratio between -0.3 and +0.3, values above +0.3 were evaluated as gain/amplification (duplications) and these ones under -0.3 - as losses (deletions). Genomic profiles were represented with logarithmic ratios in Y-axis and along the 23 chromosomes in X-axis. Individual chromosomal profiles are represented with clone positions in Y axis and logarithmic ratios in X axis.

RESULTS

We analyzed genomic imbalances affecting whole genome in sperm samples from 6 males with idiopathic

Table 1. The genomic imbalances, detected in sperms of patients with idiopathic azoospermia.

Patient	Aberration	Cytoband	Start (Mbp)	End (Mbp)
Patient 1	deletion	1p36	11,042,681	28,767,513
	gain	14q24	70,391,490	75,743,300
	gain	Yq11.22	19,853,207	20,143,847
Patient 2	deletion	Yq11.2	10,169,888	26,711,820
Patient 3	deletion	3q29	194,509,639	198,899,968
Patient 4	deletion	16q24	86,493,284	88,559,026
	deletion	Yq11.222	19,212,392	19,716,451
Patient 5	gain	9q34	135,583,152	139,125,153
	gain	Yq11.22	18,563,749	20,143,847
Patient 6	deletion	3q29	196,951,712	198,899,968
	gain	Yp11.2	9,246,143	9,957,613

azoospermia by array-CGH with CytoChip, covering all autosomes and sex-chromosomes at a mean density of 1 BAC clone/0.5 Mega base pair (Mbp). More than 85% of genomic clones were successfully hybridized in each case. Standard deviation in log2 ratios of Cy3 and Cy5 intensities (test -T and normal - N DNA, respectively) ranged between 0.05 and 0,115, depending on the quality of DNA.

We used two approaches to identify BACs that showed significant loss or gain in the analyzed samples: a) observation of loss (log 2 T:N ratio < -0.3) and gain (log 2 T:N ratio > +0.3) after correction with standard deviation; and b) detection of at least one additional adjacent clone with the same aberration in the same probe. Doing this, we eliminated the clones, which were changed due to the procedure errors. The single aberrant clones were excluded from analysis.

We detected copy number variations (microdeletions and microduplications) in spermal cells of all analyzed patients, as the number of alterations ranged between 1 and 3 per sample (Table 1). There were between 2 and 16 affected clones in the aberrations, reflecting on the size of altered regions between 0.5 and 17 Mbp.

The most consistent were aberrations in Y-chromosome. They occurred in 5 out of 6 patients (83.3%). These Y microaberrations included both microdeletions and microduplications (Figure 1 and 2). Y microdeletions were found in 2 patients, and Y microduplications – in 3 patients. The size of Y microdeletion in the first patient expanded region 10,169,888 – 26,711,820 Mbp (16 Mbp deletion in Yq11.2) and involved many of the loci of Y-linked non-obstructive spermatogenic failure, whereas in the second patient the micro-deletion included 3 clones in 19,212,392 - 19,716,451 Mbp interval on Yq11.222 (Figure 3 and 4). The last deletion of 0.5 Mbp completely covered a known locus of Y-linked non-obstructive spermatogenic failure. Microduplications on Y-chromosome in one patient involved 19,853,207 - 20,143,847 Mbp interval (Yq11.22), in the second patient 18,563,749 – 20,143,847 Mbp (Yq11.22), and in the third patient - 9,246,143 - 9,957,613 Mbp (Yp11.2). The last duplication is considered as complex variation (polymorphism), occurring in 16 - 17% of general population.

In addition to Y chromosomal microimbalances, we detected several other affected loci (Table 1). These included:

- 1p36 deletion (17.7 Mbp) together with 14q24 gain (5 Mbp),
- 16q24 deletion (2 Mbp),
- 9q34 gain (3.5 Mbp),
- 3q29 deletion (4 Mbp in one patient and 2 Mbp in another one). The last deletion of 3q29, which has been observed in two patients, comprised several common clones and is considered to be polymorphism with frequeency of 64% in the general population according to the BlueGnome database.

DISCUSSION

Here we report for the first time the results from whole genome array CGH analysis of spermatozoa in infertile men with idiopathic azoospermia.

The most powerful tool for genetic screening is the microarray-based technology of comparative genomic hybridization (array CGH). The capability of array CGH to detect simultaneously DNA copy number changes at multiple loci over the whole genome and to provide high-resolution mapping of variation in copy number has been used in our study. Sub-microscopic copy number variations were found in spermatozoa DNA of all analyzed patients. They included the regions from p and q arm of chromosome Y and sub-telomeric regions of chromosomes 1, 3, 9 and 14.

Other sperm karyotyping studies have demonstrated that human spermatozoa contain higher baseline numerical and structural chromosome aberrations compared to

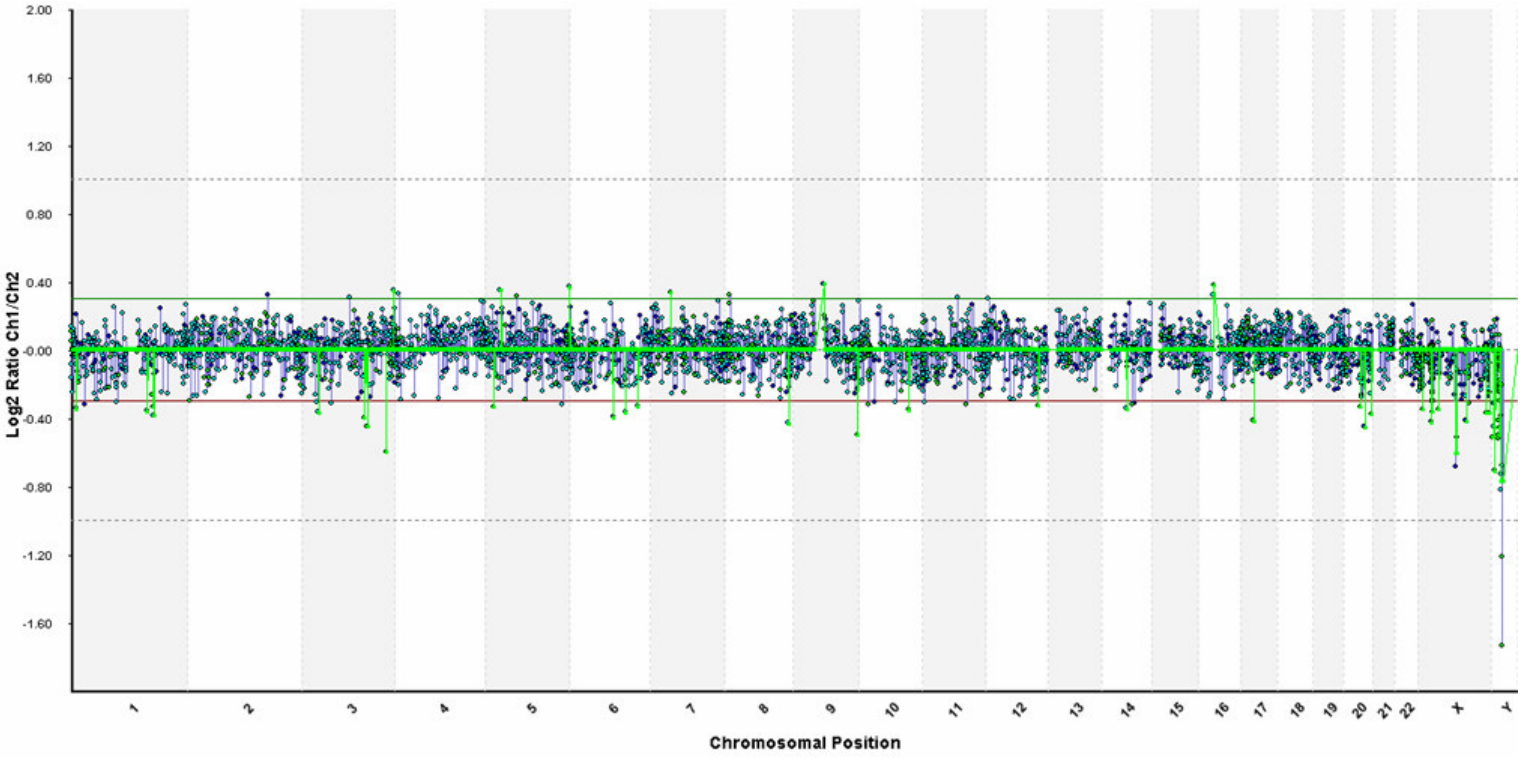

Figure 1. Genomic profile across all chromosomes in patient 2, showing large Yq11.2 deletion. Y-axis - $\log_2$ ratios of test to normal DNA; X-axis – the clones along the autosomes 1-22 and X-chromosome.

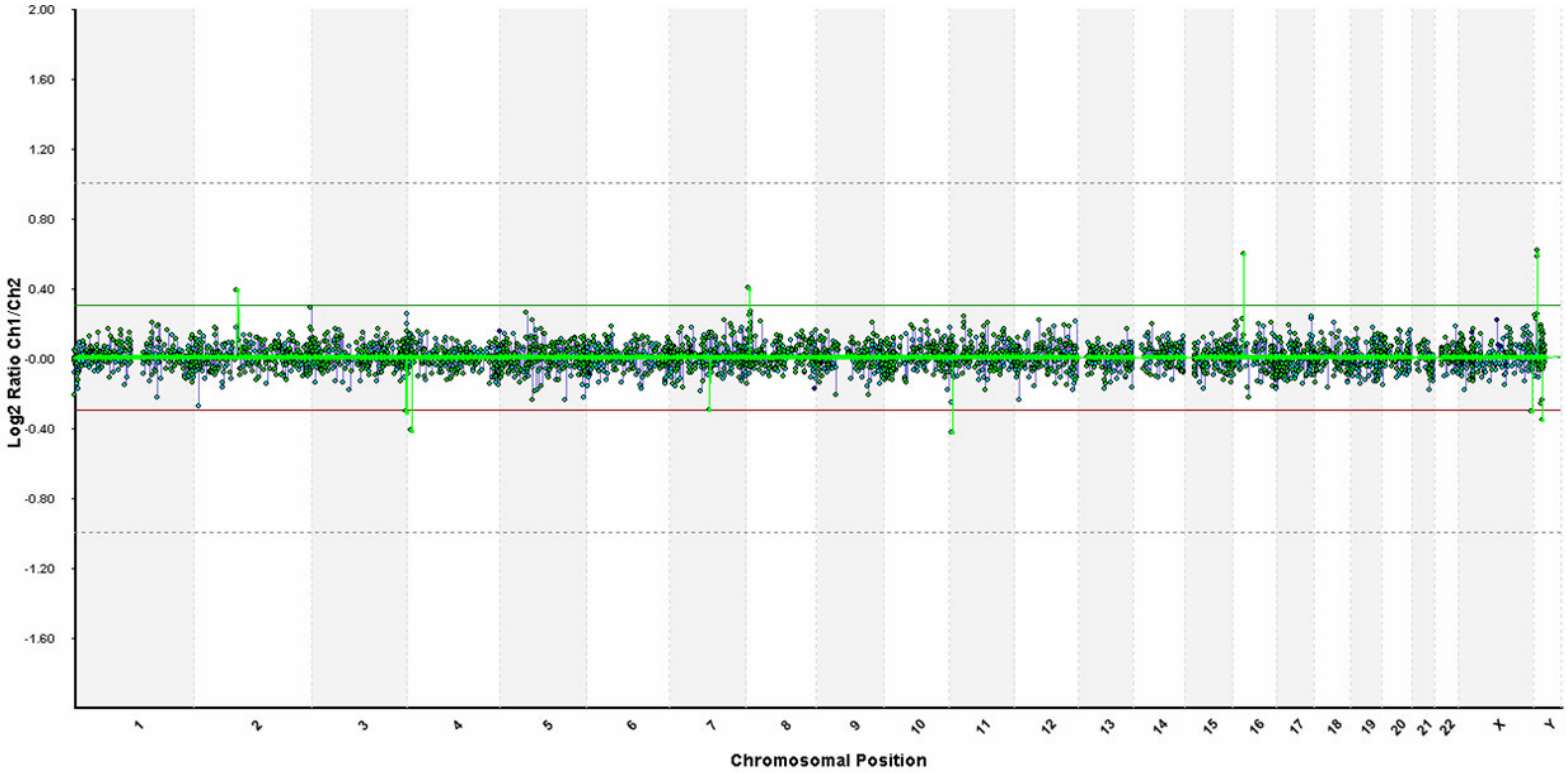

Figure 2. Genomic profile across all chromosomes in patient 6, showing Yp11.2 duplication and 3q29 deletion. Y-axis - $\log_2$ ratios of test to normal DNA; X-axis – the clones along the autosomes 1 - 22 and X-chromosome.

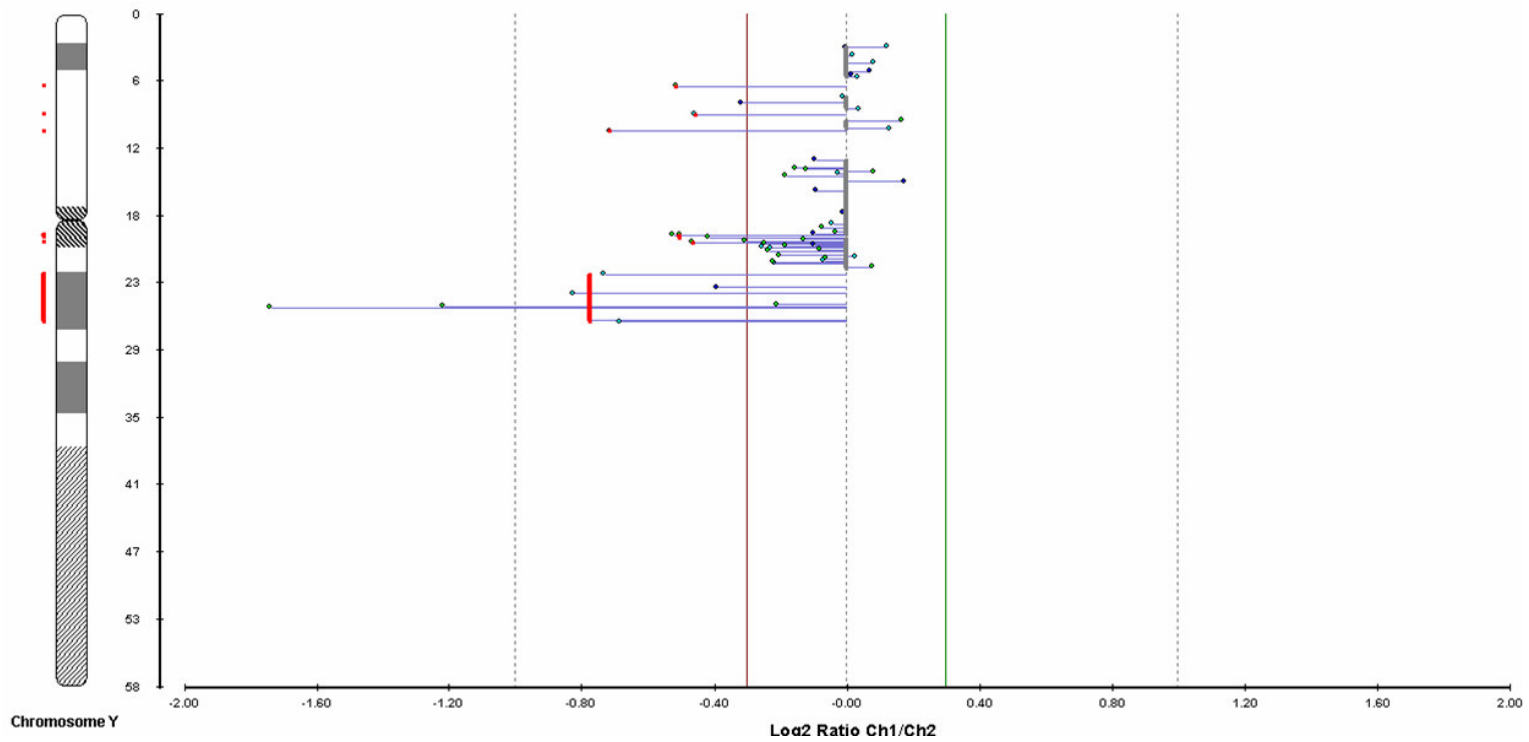

Figure 3. Genomic profile of patient 2 for chromosome Y, showing large Yq11.2 deletion. Y-axis - the clones along the chromosome Y; X-axis – $\log_2$ ratios of test to normal DNA.

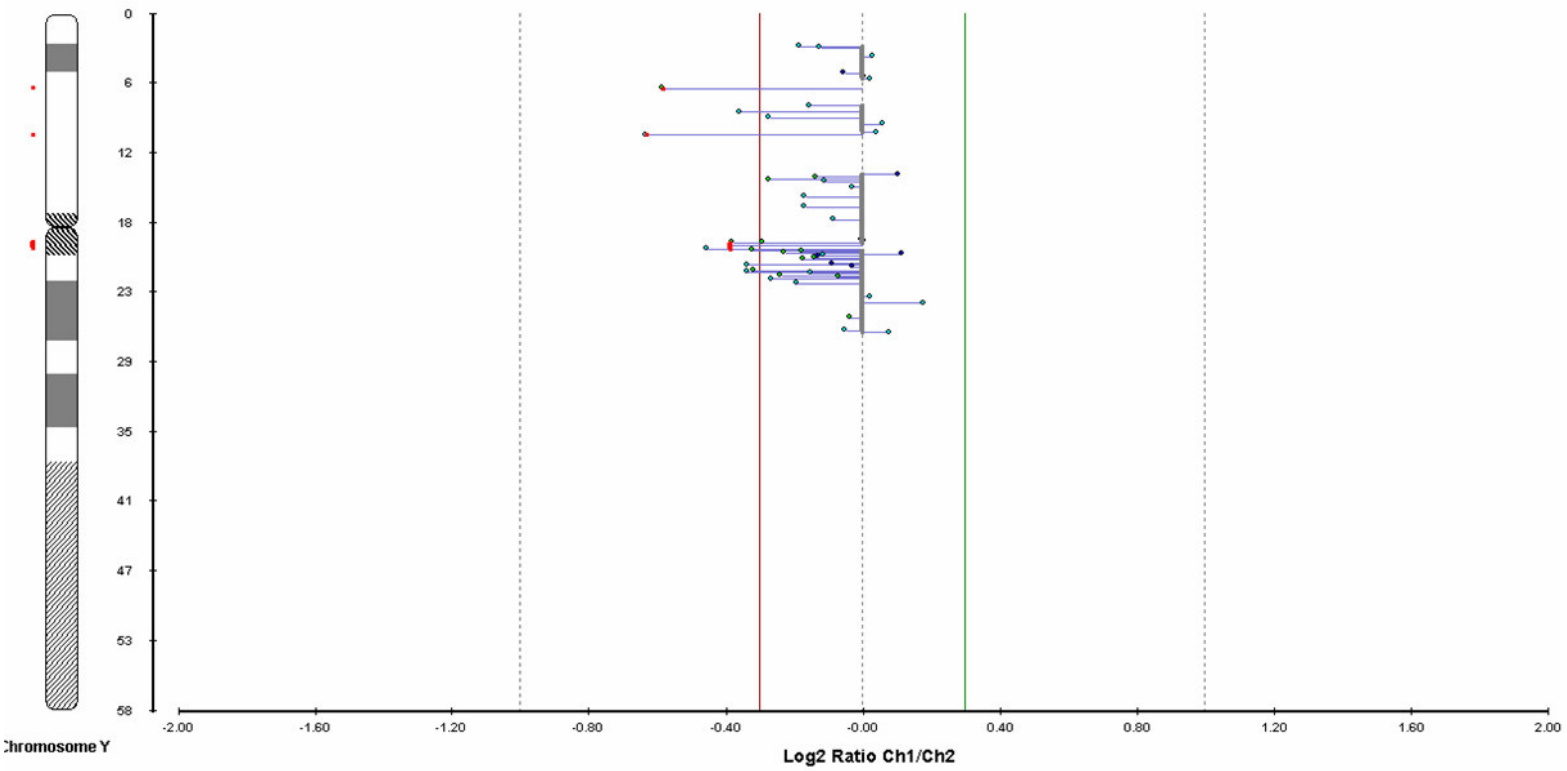

Figure 4. Genomic profile of patient 4 for chromosome Y, showing small Yq11.222 deletion. Y-axis - the clones along the chromosome Y; X-axis – $\log_2$ ratios of test to normal DNA.

somatic cells, as well as a higher incidence of chromosome aberrations after in vitro and in vivo exposure to different mutagens (Genescà et al., 1990; Kamiguchi and Tateno, 2002). They used an inter-specific in vitro fertilization system between human sperm and golden hamster oocytes in order to study sperm-derived chromosomes.

Recently, the method of fluorescent in situ hybridization (FISH) has been used in infertile men for analysis of the rates of sperm aneuploidy using incomplete set of human chromosomes. The data suggest the presence of a correlation between poor semen parameters and aneuploidy of chromosomes 13, 18, 21, X and Y in spermatozoa, as the risk of a chromosomal aneuploidy in spermatozoa seems to be inversely correlated to sperm concentration and total progressive motility (Vegetti et al., 2000; Nagvenkar et al., 2005). Disomy rates for chromo-somes 1, 4, 8, 12, 18, X and Y were ascertained for spermatozoa of infertile patients by means of triple and double FISH experiments (Bernardini et al., 2005). Statistical significance for higher sperm disomy rates was noted for all chromosomes in patients with poor semen quality compared with normal, especially for disomy 1.

Our results from array CGH testing for sperm aneuploidy indicated between 1 and 3 copy number aberrations per sample in azoospermic men. The most frequent aberrations detected in our study were these ones of Y-chromosome. The progress in molecular biology of the Y chromosome in the past years and the intense effort of many laboratories connected to andrology have definitively clarified that Yq microdeletions represent the most frequent genetic cause of severe spermatogenic impairment (Ferlin et al., 2006; Foresta et al., 2005). However, most studies examined Y-microdeletions by polymerase chain reaction, analyzing only azoospermia factor regions (AZF), which are responsible for spermatogenesis. The frequency of different types of Y-microdeletions as constitutional aberrations varied between 10 and 58% of patients with severe oligozoospermia and azoospermia in different studies (Viswambharan et al., 2007; Kent-First et al., 1999). This type of analysis could not determine exactly the size and the boundaries of deletion on Y-chromosome. By array CGH we defined most precisely these parameters. We detected Y-microdeletions in sperm of 2 of our 6 patients. The size was 0.5 Mb in one patient and 16 Mb in another, as the larger deletion corresponded to the poorer quality of semen (very low volume of 0.6ml in the last patient). We detected also Y-microduplications, evolving p and q arm of Y-chromosome, in three of the patients. The size was 0.3 and 1.5 Mb for Yq and 0.5 Mb for Yp. The last aberration is considered as polymorphism according to data base of BlueFuse software (BlueGnome, Cam-bridge, UK). Yq microduplications have not been reported previously and could be associated with spermatogenic failure in the patients.

In addition to Y chromosomal microimbalances, we detected several other affected loci. These included 1p36 deletion together with 14q24 gain, 16q24 deletion, 9q34 gain, and 3q29 deletion. Cryptic aberrations involving the subtelomeric regions of chromosomes are thought to be responsible for idiopathic mental retardation and multiple congenital anomalies, although the exact incidence of these aberrations is still unclear (Caliskan et al., 2005). These aberrations could be polymorphisms, like 3q29 deletion, which was observed in two of our patients. Rearrangements of 1p36 resulting in deletion are observed in 1 in 5,000 live births (Shaffer and Lupski, 2000) and all subjects have mental retardation of varying degrees, delayed language skills, impairment of growth, facial dysmorphism and commonly heart failure. Small distal deletion of chromosome 16 was detected in a child with bilateral coloboma of iris, short stature, moderate developmental delay, and a few minor craniofacial anomalies (Werner et al., 1997). Gain of 14q24 has not been reported in recognizable syndromes so far.

In conclusion, array CGH analysis of spermatozoa of men with azoospermia could be used as a powerful method for high resolution detection of genomic imbalances across whole genome. The data from our analysis indicated that Y microimbalances are the most frequent aberrations, confirming the role of genes located on Y for normal spermatogenesis and sperm maturation. Additionally, sub-telomeric deletions, associated with known syndromes, as well as copy number polymorphisms was detected in our patients. The study should be extended to a larger cohort of patients with clinical follow up.

ACKNOWLEDGMENT

This study was supported by Contract N 5/02.05.2007 of the Committee of Medical Sciences, Medical University – Sofia and Infrastructural project of the Ministry of Education and Science, Bulgaria (contract № 05/01.08.2005).

REFERENCES

Bernardini LM, Calogero AE, Bottazzi C, Lanteri S, Venturini PL, Burrello N, De Palma A, Conte N, Ragni N (2005). Low total normal motile count values are associated with increased sperm disomy and diploidy rates in infertile patients. Int. J. Androl. 28(6): 328-336.

Bernardini LM, Costa M, Bottazzi C, Gianaroli L, Magli MC, Venturini PL, Francioso R, Conte N and Ragni N (2004). Sperm aneuploidy and recurrent pregnancy loss. Reprod Biomed Online. 9: 312-320.

Boerke A, Dieleman SJ, Gadella BM (2007). A possible role for sperm RNA in early embryo development. Theriogenology.

Burrello N, Vicari E, Calogero AE (2005). Chromosome abnormalities in spermatozoa of patients with azoospermia and normal somatic karyotype. Cytogenet. Genome Res. 111: 363-365.

Caliskan MO, Karauzum SB, Mihci E, Tracey S, Luleci G (2005). Subtelomeric chromosomal rearrangements detected in patients with idiopathic mental retardation and dysmorphic features. Genet. Couns. 16(2): 129-138.

Carrell DT, Emery BR, Liu L (1999). Characterization of aneuploidy rates, protamine levels, ultrastructure, and functional ability of round-headed sperm from two siblings and implications for intracytoplasmic sperm injection. Fertil. Steril. 71: 511-516.

Carrell DT, Emery BR, Wilcox AL, Campbell B, Erickson L, Hatasaka HH, Jones KP and Peterson CM (2004). Sperm chromosome

aneuploidy as related to male factor infertility and some ultrastructure defects. Arch. Androl. 50: 181-185.
Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, Campbell B, Branch DW and Hatasaka HH (2003). Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet. Gynecol. 101: 1229-1235.
Carrell DT, Wilcox AL, Udoff LC, Thorp C and Campbell B (2001). Chromosome 15 aneuploidy in the sperm and conceptus of a sibling with variable familial expression of round-headed sperm syndrome. Fertil. Steril. 76: 1258-1260.
Carrell DT (2008). The clinical implementation of sperm chromosome aneuploidy testing: pitfalls and promises. J. Androl. 29(2): 124-133.
Carter NP (2007). Methods and strategies for analyzing copy number variation using DNA microarrrays. Nat. Genet. 39: 16-21.
Devillard F, Metzler-Guillemain C, Pelletier R, DeRobertis C, Bergues U, Hennebicq S, Guichaoua M, Sele B and Rousseaux S (2002). Polyploidy in large-headed sperm: FISH study of three cases. Hum. Reprod. 17: 1292-1298.
Estop A, Munne S, Cieply K, Vandermark K, Lamb A, Fisch H (1998). Meiotic products of a Klinefelter 47,XXY male as determined by sperm fluorescence *in situ* hybridization analysis. Hum. Reprod. 13: 124-127.
Ferlin A, Arredi B, Foresta C (2006). Genetic causes of male infertility. Reprod. Toxicol. 22: 133–141
Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A (2005). Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. J. Clin. Endocrinol. Metab. 90: 152–156
Fragouli E, Wells D, Thornhill A, Serhal P, Faed MJ, Harper JC and Delhanty JD (2006). Comparative genomic hybridization analysis of human oocytes and polar bodies. Hum. Reprod. 21: 2319-2328.
Freeman JL, Perry GH, Feuk L, Redon R, McCarroll SA, Altshuler DM, Aburatani H, Jones KW, Tyler-Smith C, Hurles MEl (2006). Copy number variation: new insights in genome diversity. Genome Res. 16: 949-961.
Fryndman N, Romana S, Le Lorc'h M, Vekemans M, Fryndman R and Tachdjian G (2001). Assisting reproduction of infertile men carrying a Robertsonian translocation. Hum. Reprod. 16: 2274-2277.
Genescà A, Benet J, Caballín MR, Miró R, Germà JR, Egozcue J (1990). Significance of structural chromosome aberrations in human sperm: analysis of induced aberrations. Hum Genet. 85(5): 495-499.
Gianaroli L, Magli MC, Ferraretti AP (2005). Sperm and blastomere aneuploidy detection in reproductive genetics and medicine. J. Histochem. Cytochem. 53: 261-267.
Kamiguchi Y, Tateno H (2002). Radiation- and chemical-induced structural chromosome aberrations in human spermatozoa. Mutat. Res. 504(1-2): 183-191
Kent-First M, Muallem A, Shultz J, Pryor J, Roberts K, Nolten W, Meisner L, Chandley A, Gouchy G, Jorgensen L, Havighurst T, Grosch J (1999). Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. Mol. Reprod. Dev. 53(1): 27-41
Krausz C, Forti G (2000). Clinical aspects of male infertility. Results Probl. Cell Differ. 28: 1-21.
Kruse R, Guttenbach M, Shartmann B, Schubert R, van der Ven H, Schmid M, Propping P (1998). Genetic counselling in a patient with XXY/XXXY/XY mosaic Klinefelter's syndrome: estimate of sex chromosome aberrations in sperm before intracytoplasmic sperm injection. Fertil. Steril. 69: 432-485.
Lim A, Fong Y, Yu S (1999). Estimates of sperm sex chromosome disomy and diploidy rates in a 47,XXY/46,XY mosaic Klinefelter patient. Hum. Genet.. 104: 405-409.
Martin R, Spriggs E (1995). Sperm chromosome complements in a man heterozygous for a reciprocal translocation 46,XY, t(9;13) (q21.1; q21.2) and a review of the literature. Clin. Genet. 47: 42-46.
McCarroll SA, Altshuler DM (2007). Copy-number variation and association studies of human disease. Nat. Genet. 39: 37-42.
Nagvenkar P, Zaveri K, Hinduja I (2005). Comparison of the sperm aneuploidy rate in severe oligozoospermic and oligozoospermic men and its relation to intracytoplasmic sperm injection outcome. Fertil. Steril. 84(4): 925-931
Ogur G, Van Assche E, Vegetti W, Verheyen G, Tournaye H, Bonduelle M, Van Steirteghem A, Liebaers I (2006). Chromosomal segregation in spermatozoa of 14 Robertsonian translocation carriers. Mol. Hum. Reprod. 12: 209-215.
Petit FM, Frydman N, Benkhalifa M, Le Du A, Aboura A, Fanchin R, Frydman R, Tachdjian G (2005). Could sperm aneuploidy rate determination be used as a predictive test before intracytoplasmic sperm injection? J. Androl. 26: 235-241.
Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y (1998). High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat. Genet. 20: 207-211.
Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W et al (2006). Global variation in copy number in the human genome. Nature 444: 444-454.
Rives N, Joly G, Machy A, Simeon N, Leclerc P and Mace B (2000). Assessment of sex chromosome aneuploidy in sperm nuclei from 47,XXY and 46,XY/47,XXY males: comparison with fertile and infertile males with normal karyotype. Mol. Hum. Reprod. 6: 107-112.
Shaffer, Lupski (2000). Molecular mechanisms for constitutional chromosomal rearrangements in humans. Ann. Rev. Genet. 34: 297–329.
Sher G, Keskintepe L, Keskintepe M, Ginsburg M, Maassarani G, Yakut T, Baltaci V, Kotze D and Unsal E (2007). Oocyte karyotyping by comparative genomic hybridization [correction of hybrydization] provides a highly reliable method for selecting "competent" embryos, markedly improving *in vitro* fertilization outcome: a multiphase study. Fertil. Steril. 87: 1033-1040.
Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MM, Bonduelle M, Liebaers I, Van Steirteghem A (2000). Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. Hum. Reprod. 15(2): 351-365.
Viswambharan N, Suganthi R, Simon AM, Manonayaki S (2007). Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome. Singapore Med J. 48(12): 1140-1142
Werner W, Kraft S, Callen DF, Bartsch O, Hinkel GK (1997). A small deletion of 16q23.1-->16q24.2 [del(16)(q23.1q24.2).ish del(16)(q23.1q24.2)(D16S395+, D16S348-, P5432+)] in a boy with iris coloboma and minor anomalies. Am. J. Med. Genet. 70(4): 371-6.