

·Original Article·

Y microdeletions in the Istria county, Croatia

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Abstract

Aim: To establish the frequency of Y chromosome microdeletions in an unselected group of infertile Croatian men. **Methods:** An unselected group of 105 patients (male partners of infertile couples), both with idiopathic and non-idiopathic infertility, consecutively referred to the outpatient infertility clinic, gynecology department, General Hospital Pula, Istria County, Croatia, was examined for the presence or absence of Y chromosome microdeletions by polymerase chain reaction analysis. **Results:** One of the 105 men (0.95 %, 95 % CI = 0.17–5.2 %) was found to have a microdeletion. **Conclusion:** A low frequency of Y chromosome microdeletions was found in the group of unselected infertile Croatian men. (*Asian J Androl 2005 Jun; 7: 213–216*)

Keywords: male infertility; Y chromosome; AZF region; microdeletion

1 Introduction

The involvement of the Y chromosome and its genes in both normal and defective spermatogenesis is well established, but several questions still remain. Although Y chromosome microdeletions represent the most frequent molecular genetic cause of infertility in men [1], the data published have often been inconsistent concerning the frequency of microdeletions and genotype-phenotype correlation.

The reported frequency of Y microdeletions in infertile men varies from 1.0 % to 55.5 % [2–7]. Differ-

ences in the study design, especially the inclusion criteria and sample size, as well as ethnic background [1, 8, 9] and selection of genetic markers tested, may explain this variability. Namely, in most studies reporting a high frequency of Y chromosome microdeletions, a selected group of infertile men with severe spermatogenesis failure was included [2, 3, 5, 6, 10, 11]. On the other hand, there are only a few studies analyzing unselected men consecutively attending infertility clinics [4, 8, 11–13]. Therefore, to establish the clinical importance of Y chromosome microdeletions in the evaluation of male infertility, studies analyzing unselected groups of infertile men are needed.

To estimate the frequency of Y chromosome microdeletions in an unselected group of infertile patients, and to evaluate the molecular pathology of Y chromosome mutations in the Croatian population, we examined a group of 105 infertile men, the male partners of infertile couples, unselected and successively referred to the

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Received 2004-04-15 Accepted 2004-11-12

regional infertility clinic for evaluation of infertility.

2 Materials and methods

2.1 Patients

We evaluated all 107 patients, male partners of infertile couples, attending the outpatient infertility clinic at the department of gynecology, General Hospital Pula, the Istria County, Croatia, in the period from 1 January 2001 to 31 December 2002. Cytogenetic analysis was performed in patients with severe impairment of spermatogenesis (sperm count <5 million/mL) and two azoospermic patients with Klinefelter's Syndrome (47, XXY) were diagnosed, thus 105 patients were considered for the final analysis. The patients underwent andrological examination with testicular volume measurements taken with Prader's orchidometer; special attention was paid to the history of hypogonadism, cryptorchidism, varicocele, genitourinary tract surgery, testicular cancer and medication. In all the patients, plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone were measured by immunoradiometric assay (coat-a-count; DPC Diagnostic Products Corporation, Los Angeles, USA).

The control group consisted of 100 men who had fathered at least one child.

Informed consent was obtained from each patient. The study was approved by the Regional Medical Ethics Committee. All the patients were Croatian.

2.2 Spermogram

For each patient, two complete semen analyses were performed according to the WHO guidelines [14] to ascertain volume, sperm count, rapid progressive sperm motility and morphology.

2.3 Molecular genetic methods

Genomic DNA was prepared from peripheral blood samples using standard procedures. Y chromosome microdeletions were detected after polymerase chain reaction (PCR) amplification of the Y chromosome-specific STS and genes.

The patients were tested for the markers suggested by Simoni *et al.* [2]: sY84, sY86, sY127, sY134, sY254 and sY255, the analysis being extended by the following markers: RBMY1, sY147 and sY152, to increase the sensitivity of the diagnostic test [15]. Markers were ampli-

fied in two multiplex PCR reactions, with the ZFX/ZFY as an internal control. Once the deletion in the azoospermia factor a (AZFa) region was detected, the markers sY81, sY82, sY83, sY86, sY85, sY84, DFFRY and sY87 were used to define the extent of the deletion.

PCR was carried out in a total volume of 10 µL. The reaction mixture included 50 ng of each DNA sample, 1 × PCR buffer, 1.5 mmol/L MgCl₂, 200 µmol/L deoxynucleotidetriphosphate (NTP), 1 µmol of each primer pair and 0.5 U Taq DNA polymerase (Promega Corp., Madison, USA and Gibco-BRL, Gaithersburg, USA). The reactions were performed in a thermal cycler (MJ Research Inc., Watertown, USA).

After an initial denaturation step at 94 °C for 5 min, the cycle parameters were: 30 cycles at 94 °C for 60 s, 58 °C for 80 s and 72 °C for 60 s. The programs were followed by the final extension step at 72 °C for 10 min.

The reaction products were then analyzed by electrophoresis at 76 V on 2–4 % agarose gels (Sigma Chemical Co., St Louis, USA) containing ethidium bromide (0.1 mg/mL), and visualized under UV light. We confirmed the deletion of the loci if the product of the expected size was not obtained after three single primer pair PCR experiments. In the third single primer PCR experiment, the annealing temperature was decreased by 3 °C, while the number of cycles was increased by 5. As a control, one sample of female, one sample of fertile male genomic DNA and one sample containing all reaction components, but water instead of DNA, were run with each set of primers.

3 Results

3.1 Clinical analysis

Of the 105 men included in the Y chromosome microdeletion analysis, 33 had azoospermia, 52 oligoasthenoatozoospermia (25 with sperm count 5–20 million spermatozoa/mL, 17 with sperm count 1–5 million spermatozoa/mL and 10 with sperm count <1 million spermatozoa/mL) and 20 were normozoospermic. The clinical characteristics of the patients are summarized in Table 1.

The average age of the patient was 35.2 years (range 20–54 years), the average time of unprotected intercourse was 6.4 years (range 2–20 years). The average testis volume was 17 mL (normal >15 mL). The results of hormonal analysis are summarized in Table 2.

Table 1. Andrological history and examination.

Andrological findings	n
Azoospermic	33
Cryptorchidism and varicocele	1
Hypergonadotropic hypogonadism	4
Cryptorchidism	4
Varicocele	5
Idiopathic	19
Oligozoospermic	52
Cryptorchidism	4
Varicocele	15
Cryptorchidism and varicocele	1
Idiopathic	32
Normozoospermic	20

3.2 Microdeletion analysis

Deletion was detected in one patient (0.95 %, 95 % CI = 0.17 % – 5.2 %) with idiopathic infertility, who was severely oligospermic (sperm concentration 1 million/mL). The molecular analysis in the patient revealed a partial deletion in the AZFa region (sY81, sY82, sY83 present, sY86 and sY85 absent, sY84, DFFRY and sY87 present), the deletion being mapped to a region of 350 kb.

4 Discussion

The results of the present study are consistent with those of previous studies on unselected groups of infertile men in whom the frequency of deletions ranges between 1 % and 3 % [4, 8, 12, 13]. Furthermore, we found a Y microdeletion in a patient with severely impaired spermatogenesis (sperm concentration 1 million/mL), which supports previous observations that microdeletions are mostly found in the population of in-

fertile men with less than 1 million sperm/mL of ejaculate.

The reported microdeletion frequencies range between 10 % and 50 % in azoospermic/idiopathic patients, and from 1 % to 10 % when oligozoospermic patients are also included, while only sporadic patients with Y chromosome microdeletions have been reported in patients with sperm counts of >5 million spermatozoa/mL [3, 10, 11]. Typically, higher frequencies of Y chromosome microdeletions have been found in study groups with less than 100 patients and no reported confidence interval [5, 6, 16].

In addition to the study design, it has been proposed that ethnic differences might be associated with the frequencies of Y chromosome microdeletions [1, 9]. Microdeletions in the Swedish population were reported only in immigrant populations of infertile patients [17]. On the other hand the number of investigated markers has not been significantly associated with the sensitivity of the test [1–3, 15].

We identified a partial deletion of the AZFa region. It has been estimated that non-allelic homologous recombination between endogenous retroviral elements (HERV) flanking the 780 kb region is probably responsible for most of the AZFa microdeletions in men with Sertoli cell-only syndrome (SCOS) [18]. On the contrary, partial microdeletions in the AZFa region were reported less frequently; single cases have so far been reported in French [9] and American [7] studies, and several patients have been reported in the Italian population [19, 20]. With the exception of the deletions described in the Italian population, there is no evidence for common partial AZFa deletion.

Genotype-phenotype correlations are difficult to assess in the case of Y chromosome deletions due to a poor understanding of the molecular pathological basis for the observed association with impaired spermatogenesis. It

Table 2. Hormonal analysis. Data expressed as mean (range).

Patients	n	Follicle-stimulating hormone (FSH) N: 1.1–13.5 IU/L	Luteinizing hormone (LH) N: 1–8 IU/L	Prolactin (PRL) N: <320 mIU/L	Testosterone N: 9.1–55.2 nmol/L
Azoospermic	33	13.8 (1.7–39.2)	5.9 (1.2–16.1)	214.6 (72–746)	14.8 (2.9–32.3)
Oligozoospermic	52	4.6 (1.2–11.7)	3.8 (1.6–9.1)	255.5 (53–1431)	16.8 (7.8–33.7)
Normozoospermic	20	3.5 (1.2–6.6)	3.4 (1.9–9.2)	199.8 (93–440)	15.8 (8.3–20.6)

is even more difficult in the case of relatively small deletions, as identified in this study; chance association of potential genomic polymorphism with infertility can not be excluded. Nevertheless, partial AZFa deletions may be associated with partly preserved spermatogenesis, as was also the case in our study. On the other hand, complete AZFa deletions are as a rule associated with azoospermia/SCOS.

In conclusion, we have found that the frequency of Y chromosome deletions in the unselected group of infertile patients in the Croatian population is 0.95 %. Y chromosome microdeletion screening should be therefore restricted mainly to patients with severely impaired spermatogenesis.

Acknowledgment

This study was supported by the Health Department of the County of Istria, Croatia, and the Ministry of Education, Science and Sport, Grant J3-2370, Slovenia. The authors wish to thank Ms M. Pirc for her English-language assistance.

References

- 1 Krausz C, Forti G, McElreavey K. The Y chromosome and male fertility and infertility. *Int J Androl* 2003; 26: 70–5.
- 2 Simoni M, Kamischke A, Nieschlag E. Current status of the molecular diagnosis of Y-chromosomal microdeletions in the work-up of male infertility. Initiative for international quality control. *Hum Reprod* 1998; 13: 1764–8.
- 3 El Awady MK, El Shater SF, Ragaa E, Atef K, Shaheen IM, Megiud NA. Molecular study on Y chromosome microdeletions in Egyptian males with idiopathic infertility. *Asian J Androl* 2004; 6: 53–7.
- 4 van der Ven K, Montag M, Peschka B, Leygraaf J, Schwanitz G, Haidl G, *et al.* Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. *Mol Hum Reprod* 1997; 3: 699–704.
- 5 Foresta C, Ferlin A, Garolla A, Moro E, Pistorello M, Barboux S, *et al.* High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. *Hum Reprod* 1998; 13: 302–7.
- 6 Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, *et al.* Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. *J Clin Endocrinol Metab* 1996; 81: 1347–52.
- 7 Kent-First M, Muallem A, Shultz J, Pryor J, Roberts K, Nolten W, *et al.* 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* 1999; 53: 27–41.
- 8 Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G, Forti G. Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. *Hum Reprod* 1999; 14: 1717–21.
- 9 Krausz C, Quintana-Murci L, Barboux S, Siffroi JP, Rouba H, Delafontaine D, *et al.* A high frequency of Y chromosome deletions in males with nonidiopathic infertility. *J Clin Endocrinol Metab* 1999; 84: 3606–12.
- 10 Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? *Hum Reprod* 2000; 15: 1431–4.
- 11 Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Shakkebaek NE. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. *J Clin Endocrinol Metab* 2001; 86: 2638–42.
- 12 Van Landuyt L, Lissens W, Stouffs K, Tournaye H, Liebaers I, Van Steirteghem A. Validation of a simple Yq deletion screening programme in an ICSI candidate population. *Mol Hum Reprod* 2000; 6: 291–7.
- 13 Bor P, Hindkjaer J, Kolvraa S, Ingerslev HJ. Y-chromosome microdeletions and cytogenetic findings in unselected ICSI candidates at a Danish fertility clinic. *J Assist Reprod Genet* 2002; 19: 224–31.
- 14 World Health Organization. Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge: Cambridge University Press; 1999.
- 15 Peterlin B, Kunej T, Hristovski D. Diagnostic test for Y chromosome microdeletion screening in male infertility. *Genet Test* 2004; 8: 45–9.
- 16 Foresta C, Moro E, Garolla A, Onisto M, Ferlin A. Y chromosome microdeletions in cryptorchidism and idiopathic infertility. *J Clin Endocrinol Metab* 1999; 84: 3660–5.
- 17 Osterlund C, Segersteen E, Arver S, Pousette A. Low number of Y-chromosome deletions in infertile azoospermic men at a Swedish andrology centre. *Int J Androl* 2000; 23: 225–9.
- 18 Kamp C, Hirschmann P, Voss H, Huellen K, Vogt PH. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet* 2000; 9: 2563–72.
- 19 Ferlin A, Moro E, Garolla A, Foresta C. Human male infertility and Y chromosome deletions: role of the AZF-candidate genes DAZ, RBM and DFFRY. *Hum Reprod* 1999; 14: 1710–6.
- 20 Foresta C, Ferlin A, Moro E. Deletion and expression analysis of AZFa genes on the human Y chromosome reveal a major role for DBY in male infertility. *Hum Mol Genet* 2000; 9: 1161–9.