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High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates

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BACKGROUND: The Y chromosome gr/gr microdeletion eliminates two copies of the DAZ gene and several additional transcriptional units and has been associated as a risk factor for infertility. Our objective was to study the presence of the gr/gr deletion in ICSI candidates in our population and to determine whether the laboratory, clinical and ICSI outcome were different in the gr/gr deleted patients. METHODS: Two hundred and eighty-three ICSI candidates were studied. Semen analysis, serum FSH, LH, testosterone, inhibin B, karyotype and detection of sequence tagged sites in the Y chromosome were performed. RESULTS: gr/gr deletions were detected in 11 (5.07%) of 217 oligospermic and in one (1.52%) of 66 azoospermic consecutive ICSI candidates, but in none of 232 controls ($P = 0.002$). The fertility rate was not different in the four patients of the gr/gr deleted group treated by ICSI (64.38%; 47/73) as compared to average results at our center (65.49%; 2393/3654). CONCLUSIONS: gr/gr deletions are a risk factor for spermatogenic failure at our population, but the prognosis of the four patients of the gr/gr deleted group treated by ICSI is not different from that of other ICSI patients.

Key words: azoospermia/gr/gr deletion/ICSI treatment/oligospermia/Y chromosome

Introduction

Infertility in men affects 5–10% of the whole population. Spermatogenic failure is the most common form, of which ~10% are Yq microdeletions (Vollrath *et al.*, 1992; Reijo *et al.*, 1995; Vogt *et al.*, 1996; Oliva *et al.*, 1998; Moro *et al.*, 2000; Foresta *et al.*, 2001; Yen, 2001; Tyler-Smith and McVean, 2003). The most common Yq microdeletion is that involving the AZFc region in distal Yq11, a region that contains genes expressed in testis and long direct and indirect repeats called amplicons (Yen, 2001; Skaletsky *et al.*, 2003). The amplicons are organized into palindromic structures and often have >99.9% identity. Homologous recombination between amplicons often causes deletions and duplications that explain 12% of all azoospermias and 6% of severe oligospermias (Oliva *et al.*, 1998; Kuroda *et al.*, 2001; Fernandes *et al.*, 2004). However, the recently described 1.6 Mb deletion polymorphism turned out to be the most frequent large Y chromosome deletion (Repping *et al.*, 2003). This deletion, named gr/gr, affects the AZFc region of the Y chromosome and removes two copies of the DAZ gene and several additional transcriptional units. Deletion of the DAZ gene copies, DAZ1 and DAZ2, has been previously reported as causing a decrease in male's sperm number (de Vries *et al.*, 2002; Fernandes *et al.*, 2002; Repping *et al.*, 2003). Thus, rather than causing severe spermatogenic failure, this deletion turned out to be only a genetic risk factor for spermatogenic failure. We initiated the present study in order to determine whether the presence of the gr/gr deletion was also a risk factor for spermatogenic failure at our population and to determine whether the clinical, laboratory and ICSI outcomes of the gr/gr deleted patients were different or comparable to those of the average ICSI patients at our center.

Methods

Studied subjects

The presence of the gr/gr chromosome Y deletion was studied in different groups: 232 controls of which 34 were semen donors, 75 were fertile men with demonstrated paternity and 123 were patients with conditions unrelated to infertility, and 283 consecutive patients with spermatogenic failure, who were ICSI candidates at our infertility clinic. Sixty-six of the patients with spermatogenic failure were azoospermic and 217 patients were severe oligozoospermic (<5million sperm/ml). All the DNA samples were isolated from venous blood at the Unitat de Genètica, Hospital Clínic de Barcelona, Barcelona, using standard extraction procedures. All

azoospermic and severe oligozoospermic patients were ICSI candidates at the Assisted Reproduction Unit at the Hospital Clinic de Barcelona. A G-banded karyotype analysis and a Y chromosome microdeletion analysis were performed routinely in most cases. Y chromosome microdeletions were determined through the analysis of the following markers in multiplex reactions: sY254, sY134, sY143 and sY84. We excluded from the present study all patients with an AZFc complete microdeletion removing all copies of the DAZ gene.

Clinical analysis

Semen analysis was performed according to the World Health Organization guidelines and under internal and external quality control. Serum FSH was measured by an immunoenzymatic assay (Immuno1; Technicion, Bayer, Tarrytown, NY). Dimeric inhibin B was measured by a solid phase sandwich enzyme-linked immunosorbent assay (Serotec, Oxford, UK). Ovarian stimulative and ICSI treatments were performed according to protocols previously reported (Balasch *et al.*, 1996; Ballesca *et al.*, 2000). Oocyte aspiration was performed with vaginal ultrasonography 35–36 h after HCG administration. ICSI procedure was performed according to the method previously described (Palermo *et al.*, 1992).

Molecular analysis of the gr/gr deletion

The chromosome Y gr/gr deletions were identified as described (Repping *et al.*, 2003) by the following results: sY1291 negative, sY1161, sY1191, sY1206 and sY1201 all positive. Initially a multiplex reaction including sY1291 and sY1191 was performed on all samples. The conditions for this PCR reaction were the following: 50 ng of DNA, 150 μM dNTPs (each one), 1 μM each primer, 1× buffer and 0.1 units/ml Taq pol (Roche) in a total volume of 10 μl. The primers for sY1191, yielding a PCR product 385 bp long, were forward 5′-CCAGACGTTCTACCCTTTCG-3′ and reverse 5′-GAGCCGAGATCCAGTTACCA-3′, and the primers for sY1291, resulting in a 527 bp long PCR product, were forward 5′-TAAAAGGCAGAACTGCCAGG-3′ and reverse 5′-GGGAGAAAAGTTCTGCAACG-3′ (Repping *et al.*, 2003; Gene Bank accession numbers G72340 and G73809, respectively). Female negative controls were included in all cases. After the PCR reaction, the samples were electrophoresed in a 2% agarose gel including a 1 kb DNA ladder as a marker (Invitrogen; Invitrogen Corporation Life technologies, Inc.; Figure 1). All samples in which the sY1191 STS was present but the sY1291 STS was absent, were subsequently studied for the presence of the STSs sY142, sY1201, sY1258, sY1161, sY1197 and sY1206 to confirm that they were gr/gr deletions and not b1/b3 deletions or longer deletions of the AZFc region. Statistical analyses were performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL).

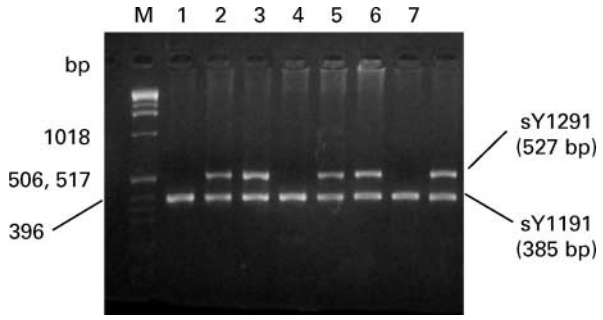

Figure 1. Detection of the gr/gr deletion. Ethidium bromide-stained products from a multiplex PCR reaction of sequence tagged sites Y1191 and sY1291 separated by a 2% agarose gel. The products correspond to azoospermic patients (lanes 1 and 2) and oligozoospermic patients (lanes 3–8). Cases 1, 4 and 7 have the sY1291 marker deleted. M = 1 kb DNA ladder.

Results

A total of 12 gr/gr Y chromosome deletions were found in the spermatogenic failure population of 283 individuals. This represented the 4.24% of all patients with spermatogenic failure (<5 million sperm/ml; Table I). The difference as compared to the controls (0 gr/gr deleted from 232 controls) was statistically significant [χ^2 (Pearson) = 0.002, Fisher = 0.001]. If the spermatogenic failure group was stratified into azoospermic and oligospermic patients it turned out that a total of 11 out of 217 individuals (5.07%) of the oligospermic group were positive for the gr/gr deletion while only one of the 66 individuals (1.52%) of the azoospermic population was positive for the gr/gr deletion. The sperm count of the gr/gr deleted patients (average 1.38 million/ml) was significantly lower ($P < 0.001$) to the sperm count of the semen donor controls (average 77.19 million/ml).

The outcome of ICSI treatment was also compared to average outcome of ICSI treatments available for 2003 at our assisted reproduction unit. The rate at which ICSI-treated oocytes proceeded to zygotes was not different between the gr/gr deleted group and the average treatments performed at our center (64.38% or 47/73 in the gr/gr deleted group compared to 65.49% or 2393/3654 in the complete series; Table II).

Discussion

In this paper we report the gr/gr Y chromosome deletion screening of 283 consecutive ICSI candidates and the laboratory data and clinical outcomes after ICSI treatment. A total of 12 gr/gr deletions were detected in the 283 ICSI candidates (4.24%) while no gr/gr deletions were detected in the control groups ($n = 232$; Table I). The differences between the frequency of deletions between the spermatogenic failure and control groups were highly significant ($P = 0.001$; Fisher) consistently with the previous reports (Repping *et al.*,

Table I. Detection of the gr/gr chromosome Y deletion in spermatogenic failure and control groups

Group	Genotype (gr/gr deletion)		% of gr/gr deletion	*P*
	Absent	Present		
All controls	232	0	0	
Semen donors	34	0	0	
Paternity cases	75	0	0	
Conditions unrelated to infertility	123	0	0	
Spermatogenic failure	271	12	4.24	0.002
Oligospermia	206	11	5.07	0.003
Azoospermia	65	1	1.52	

Table II. Clinical data and ICSI results corresponding to the gr/gr deleted patients

Case	Physical exam	Semen	FSH	B inhibin	Karyotype	Oocytes Metaphase II	Zygotes	% zygotes/punctioned oocytes	Transfers	Pregnancies
1	Severe bilateral testicular hypoplasia	Criptozoospermia C = 0.001	30.02	<15	46, XY					
2	Bilateral testicular hypoplasia. Hypospadia	Oligoastenozoospermia C = 2.8	30.09	27.2	46, XY					
3	Bilateral testicular hypoplasia	Oligoastenozoospermia C = 2.8	8.04	75	46, XY	18	12	66.6	2	2
4	Bilateral testicular hypoplasia	Astenozoospermia C = 1.9	22.36	27		12	8	44.44%	3	0
						14	13	92.87	3	2
5	Bilateral testicular hypoplasia	Oligozoospermia C = 0.01	10.18	31						
6	Normal	C = 0.4	6.04	127	46, XY					
7	One testis normal, other removed by testicular torsion	Oligoastenozoospermia C = 4.6	10.73	42	46, XY der(13–14) (q10;q10)					
8	Normal	C = 2.2	4.97	98	46, XY	4	3	75.00		0
						5	4	80.00		0
9	Bilateral testicular hypoplasia	Cryptozoospermia C = 0.001	31.04	<15						
10	Only one hypoplasic testis	Oligoastenozoospermia C = 1.8	14.15	48	46, XY					
11	Bilateral testicular hypoplasia	Azoospermia C = 0	52.66	<15						
12	Normal	C < 0.1	6.28	82	46, XY	6	3	50.00	3	0
						14	4	28.57	3	1
Total gr/gr						73	47	64.38	14	5 (35.71%)
2003 activity at the center						3654	2393	65.49	438	177 (40.4%)

Testicular hypoplasia is defined as a testicular volume <10 ml. Counts (C) are in million/ml sperm. ICSI data was available for patients 3, 4, 8 and 12. Data for independent treatments (patients 4, 8 and 12) have been indicated on independent lines.

2003). Of the 12 gr/gr deletions, only one corresponded to an azoospermic patient (1.52%; 1/66) and 11 out of 217 (5.07%) corresponded to the oligospermic group. This frequency of gr/gr deletion at our population is higher than that reported by Repping *et al.* (2003) (3.18%; 15/471 in the initial screening, and 3.66%; 9/246 in the association study). However these differences are not significant and, in addition, could be due to slight differences in the type of patients included in the respective studies and/or to differences in the respective populations. The high frequency of gr/gr deletions detected in the oligospermic group (5.07%) contrasts with the low frequency detected in the azoospermic group (1.52%; 1/66). This situation is opposite to that present in the detection of full Y chromosome microdeletions previously performed at our center (16% microdeletions; 8/50 in azoospermia patients as compared to 1.5%; 2/136 in oligospermic patients; Oliva *et al.*, 1998) as well as by other groups (Vollrath *et al.*, 1992; Reijo *et al.*, 1995; Vogt *et al.*, 1996; Foresta *et al.*, 2001; Repping *et al.*, 2003; Fernandes *et al.*, 2004). These results are consistent with the idea that Y chromosome microdeletions cause severe spermatogenic failure while the gr/gr deletion is only a risk factor (rather than a cause) for spermatogenic failure. The fact that the AZFc Y chromosome microdeletion removes all copies of the DAZ gene and 21 transcriptional units of the Y chromosome, while the gr/gr deletion removes only two copies (half) of the DAZ gene and nine transcriptional units is also consistent with this idea (Repping *et al.*, 2003).

We have also determined the ICSI outcomes in the four patients (numbers 3, 4, 8 and 12) of the gr/gr deleted group treated by ICSI as compared to the average ICSI outcomes obtained at our assisted reproduction unit (Table II). The rate at which ICSI-treated oocytes proceeded to zygotes was not different to that derived from the 2003 activity at our center (65.49%; 2393/3654 vs 64.38%; 47/73). No significant differences were detected either at the rate at which pregnancies were achieved in both groups (Table II). These results are also consistent with those obtained using ICSI sperm from AZFc microdeleted patients (Oates *et al.*, 2002). Thus, prognosis of the three patients with a gr/gr deletion and treated by ICSI is not different to that of the average ICSI patients at our center. This result fits well with the known expression of the transcriptional units deleted in the gr/gr deleted patients and its lack of function in the initial stages of development.

In the vast majority of cases of spermatogenic failure in man a clear cause cannot be identified. The gr/gr deletions reported present in 3–5% of oligospermics (Repping *et al.* 2003; and present paper) cannot be considered as a cause, but rather a risk factor for spermatogenic failure. In addition to this gr/gr deletion there are now an increasing number of risk factors for spermatogenic failure being considered (Rovio *et al.*, 2001; Mengual *et al.*, 2003a). It must also be taken into account that spermatogenesis is a complex differentiation process resulting in profound biochemical, morphology and functional changes sensitive to environmental factors (Hecht, 1990; Oliva and Dixon, 1991; de Yebra *et al.*, 1993; Mengual *et al.*, 2003b). Thus, the opportunity is now open to look for the potential interaction between the presence of gr/gr deletions as a genetic risk factor and other genetic or environmental factors.

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