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Reproductive Genetics

No partial *DAZ* deletions but frequent gene conversion events on the Y chromosome of fertile men

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***Purpose*:** Recently, partial *DAZ* deletions on the Y chromosome were identified in infertile men. To determine the clinical importance of partial *DAZ* deletion, we studied the number of *DAZ* copies in a well-defined population of 47 fertile men.
***Methods*:** The number of *DAZ* gene copies was determined by PCR assays, qualitative and quantitative DNA blot experiments.
***Results*:** Using semi-quantitative Southern blot, no partial *DAZ* deletion was detected in fertile men. In many cases, the results were discordant with the PCR assays and qualitative *DYS*1-*blot* experiments suggesting that the molecular events detected by the later methods could reflect gene conversion events. Many fertile men present four copies of the *DAZ* genes but an atypical organization of this *DAZ* locus. No difference in sperm concentration and motility in the fertile men were observed according to the different *DAZ*-haplotypes.
***Conclusion*:** The different *DAZ*-haplotypes are compatible with normal spermatogenesis.

KEY WORDS: DAZ gene; male infertility; PCR; Southern blot; Y chromosome.

INTRODUCTION

Infertility occurs in approximately 14% of couples (1) and abnormalities in the male partner are estimated to be present in upto half of the cases. Efforts to evaluate the causes of azoospermia have shown that after exclusion of traditionally recognizable causes, most cases are unexplained and are termed idiopathic. In 1992, microdeletions on the long arm of the Y chromosome (Yq) were found in men with azoospermia (2). Several studies have been published indicating that Yq microdeletions, especially in the Azoospermia Factor c (AZFc) region including the four nearly identical Deleted in AZoospermia (*DAZ*) gene copies, occur in 3–15% of azoospermic or oligozoospermic men (2–5). The b2/b4 deletion, which spans 3.5 Mb and eliminates the entire AZFc region is the most common known genetic cause of spermatogenic failure (6).

The four *DAZ* copies were mapped as *DAZ*1/*DAZ*2 and *DAZ*3/*DAZ*4 doublets in the middle of the 4.94 Mb AZFc BAC contig with a distance of 1.47 Mb between them (6,7). Two of the *DAZ* genes are located in the palindrome P1 and two in palindrome P2 (8). Three different molecular approaches have been developed to analyse the *DAZ* gene copies number. The first method is based on the PCR detection of sequence family variants (i.e. single nucleotide variants between the different *DAZ* copies) (9,10) . The second approach is to perform *DNA-DYS1* blot experiments after restriction digest of genomic DNA samples with *EcoRV* and *TaqI* (9,11). By using both approaches, it is possible to differentiate the individual *DAZ* genes and to determine the number of *DAZ* gene copies. The third method is using fluorescence in situ hybridization (FISH) with specific *DAZ* cosmids (12)

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which is a reliable method to identify the number of *DAZ* gene clusters but not the *DAZ* gene copies number (13).

Recently, a reduced number of *DAZ* gene copies has been described in subfertile and infertile men (9–14). However, as these studies did not include a well-defined and studied control group of proven fertile men, the clinical relevance of these partial deletions is still unknown.

The aim of our study was to determine the *DAZ* gene copies number in a well-defined population of 47 fertile men, using both gene specific PCR digestion assays and qualitative and quantitative blot experiments and to assess the relation between the *DAZ*-haplotypes and the sperm parameters of these men.

MATERIALS AND METHODS

Selection of the Fertile Men and Semen Analysis

The 47 participating fertile men were included in the study, while their partners were pregnant. No pregnancy was obtained by assisted reproductive techniques. The mean age of the men was 32.2 ± 5.0 years. The mean time necessary to get the pregnancy (TTP) could be precisely defined in 39 couples, it was 5.9 ± 0.9 months (median: 3 months). Five men had an history of testicular disease: three cryptorchidisms, one seminoma and one orchitis. The testicular volume could be measured in 37 men using a calli per Lambert (15) and the semen of 46 men was collected by masturbation in the laboratory and analysed according to World Heath Organization recommendations (16). The modified classification of David *et al.* was used to analyse the sperm morphology (17,18). Informed consent was obtained from each subject who was included in a larger study on time to pregnancy and sperm characteristics of fertile men approved by the local ethical committee.

SNV/STSs PCR Assays for Partial *DAZ* Deletions

The *DAZ* gene copies number was determined using nine gene-specific sequence PCRs: seven *DAZ*-single nucleotide variants (SNVs): sY581, SNV I, II, III, IV, V and VI and two *DAZ*-sequence tagged sites (STSs): *DAZ*-RRM3 and *Y-DAZ3* (Fig. 1). Analysis of SNV and STSs was performed according to de Vries *et al.,* 2002 and Fernandes *et al.*, 2002 (9,12).

Southern Blot Analysis

A total of 6.4 μg of purified genomic DNA were digested with 2.5 IU/μg of the restriction enzyme EcoRV or TaqI (Ozyme, St-Quentin en Yvelines,

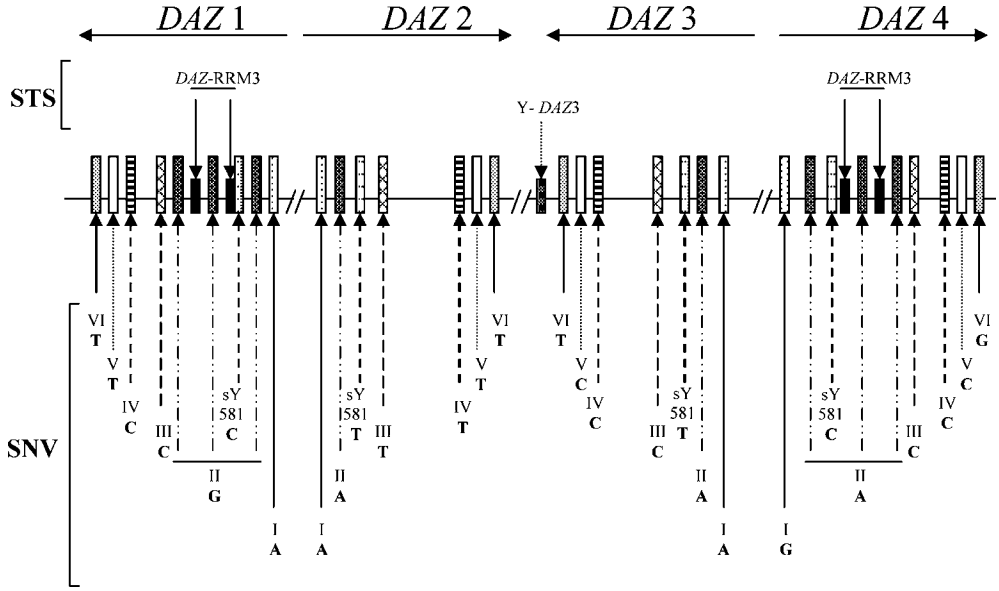

Fig. 1. Localization of SNV and STS- PCR markers on the different copies of *DAZ* gene. SNVs (SNV I, SNV II, SNV III, SNV IV, SNV V, SNV VI, sY 581) and STSs localization *(DAZ-RRM*3 on *DAZ*1 and *DAZ*4, *Y-DAZ*3 on *DAZ*3) are indicated by arrows. Nucleotide variant studied by each SNV marker is indicated in bold, under each marker number.

France). The fragments were separated by electrophoresis on 0.8% agarose gels (Invitrogen, Cergy Pontoise, France) and transferred to nylon membranes by vacuum. Membranes were pre-hybridized for 1 h and hybridized overnight at 65°C. The probe used was the 2.8 kb *EcoRI* fragment of plasmid p49f (kindly provided by S. Fernandes), gel-purified and labelled with ^{32}P dCTP by random priming (Amersham, Orsay, France). After overnight hybridization, membranes were washed for 15 min at room temperature in two Standard Saline Citrate/0.1% SDS and exposed for 24 h at −80°C with Kodak XAR films (Kodak, Châlons/Saône, France).

Semi Quantitative Southern Blot

Southern blot of EcoRI-digested genomic DNA hybridized with a *DAZ* probe was performed as already described (19). The *DAZ* probe is a 434 bp fragment derived from the 5′ half of the coding region of the gene, outside the 72 bp *DAZ* repeat, by PCR from the *DAZ* cDNA pDP1577 as previously described (20).

RESULTS

Incidence of DAZ Gene Partial Deletions in Fertile Men

The four *DAZ* copies gene structure previously detected on the Y chromosome of the RPCI-11 donor was found in 28 fertile men (20/47; 59.6%) both by PCR and qualitative southern blot assays (Table I) (Fig. 2). PCR and qualitative southern blot assays suggested that four fertile men (8.5%) presented *DAZ* deletions: Either a single *DAZ* copies missing *(DAZ4)* in 3 men (ID25, ID40, ID37), or two *DAZ* copies missing *(DAZ2 and DAZ4)* in one man (ID6) (Table I). In the remaining 15 fertile men (31.9%), the partial *DAZ* deletions suspected after PCR assays were not always confirmed using qualitative southern blot. In two cases, the *DAZ2* or *DAZ*4 deletions suspected by the absence of SNV IV (ID11) or SNV VI (ID27) analysis respectively were not found using qualitative southern blot experiments (Fig. 2 A,B,C).

In 12 men, where both *DAZ2* and *DAZ*4 copies were not observed using PCR assays, the *DAZ2* deletion was not confirmed by *DYS1—TaqI* blot experiment in five patients (ID 41, 34, 31, 28, 12) and the *DAZ*4 deletion not confirmed by *DYS1—EcoRV* DNA blot in the 12 patients (Table I). At least, in one man, the deletion associating *DAZ1, DAZ2* and *DAZ*4 by PCR/SNV assays was only confirmed for the *DAZ*4 copies by southern blot (ID 23, Table I).

To confirm or contradict the PCR and *DYS*1 blot experiment's results suggesting a partial deletion of *DAZ*2 or *DAZ*4 in some fertile men, semi-quantitative southern blot analysis was performed using a *DAZ* probe encompassing exon 2 to exon 6 (RBM region). Genomic DNA from the twelve subjects suspected to have a *DAZ* partial deletion (ID13, 25, 40, 2, 5, 7, 44, 6, 8, 15, 23 and 37) using qualitative southern blot and from four subjects without suspected *DAZ* partial deletion (ID 27, 30, 3, 43) was digested with *Eco*RI and Southern blotted with the *DAZ* probe. We observed that the two fragments (1.8 and 3 kb) corresponding to the *DAZ* copies presented the same intensity in the fertile subjects without suspected partial *DAZ* deletion (ID46, ID3; Fig. 3) and in the fertile men with partial deletion *of DAZ2* detected by *DYS*1 *TaqI*/DNA blot experiment (ID15 and ID44; Fig. 3) or with partial deletion of *DAZ*4 detected by *DYS*1*EcoRV* DNA blot experiment (ID40, Fig. 3). In contrast to the large number of aberrations found with PCR digest assays and *DYS*1 Southern blot, our semi-quantitative blot results suggested the presence of four *DAZ* genes in all cases.

Relation Between the *DAZ*-Haplotypes and the Seminal and Clinical Characteristics of Fertile Men

Among the 47 men studied, five (ID 5, 20, 31, 42, 45) had a previous history of testicular disease which could alter their spermatogenesis. Therefore they were not included in the analysis even if their sperm characteristics were in the normal range. In one more case (ID 40), the semen analysis could not be done.

With our marker set, the presence of absence of an STS or SNV in the genomic DNA samples identified nine different patterns (*DAZ*-haplotypes 1–9) (Table I). No significant difference of semen characteristics and testis volume were found according to the *DAZ*-haplotypes in the fertile men (Table II). Similarly no difference in total sperm count, sperm motility and morphology were observed between the various groups.

Table I. Results from PCR Digest Assays and Qualitative Southern Blot Analysis in Fertile Men

	SNV[a] and STSs[b] PCR									Qualitative southern blot	
Subject	SNV I	SNV II	SNV III	SNV IV	SNV V	SNV VI	sY 581	STS[d]	*DAZ* copies deletions	*DAZ* copies deletions	*DAZ* haplotype[c]
ID 1	AG	GA	CT	TC	CT	TG	CT	+			1
ID 3	AG	GA	CT	TC	CT	TG	CT	+			1
ID 4	AG	GA	CT	TC	CT	TG	CT	+			1
ID 9	AG	GA	CT	TC	CT	TG	CT	+			1
ID 10	AG	GA	CT	TC	CT	TG	CT	+			1
ID 14	AG	GA	CT	TC	CT	TG	CT	+			1
ID 16	AG	GA	CT	TC	CT	TG	CT	+			1
ID 17	AG	GA	CT	TC	CT	TG	CT	+			1
ID 18	AG	GA	CT	TC	CT	TG	CT	+			1
ID 19	AG	GA	CT	TC	CT	TG	CT	+			1
ID 20	AG	GA	CT	TC	CT	TG	CT	+			1
ID 21	AG	GA	CT	TC	CT	TG	CT	+			1
ID 22	AG	GA	CT	TC	CT	TG	CT	+			1
ID 24	AG	GA	CT	TC	CT	TG	CT	+			1
ID 26	AG	GA	CT	TC	CT	TG	CT	+			1
ID 29	AG	GA	CT	TC	CT	TG	CT	+			1
ID 30	AG	GA	CT	TC	CT	TG	CT	+			1
ID 32	AG	GA	CT	TC	CT	TG	CT	+			1
ID 33	AG	GA	CT	TC	CT	TG	CT	+			1
ID 35	AG	GA	CT	TC	CT	TG	CT	+			1
ID 36	AG	GA	CT	TC	CT	TG	CT	+			1
ID 38	AG	GA	CT	TC	CT	TG	CT	+			1
ID 39	AG	GA	CT	TC	CT	TG	CT	+			1
ID 42	AG	GA	CT	TC	CT	TG	CT	+			1
ID 43	AG	GA	CT	TC	CT	TG	CT	+			1
ID 45	AG	GA	CT	TC	CT	TG	CT	+			1
ID 46	AG	GA	CT	TC	CT	TG	CT	+			1
ID 47	AG	GA	CT	TC	CT	TG	CT	+			1
ID 13	**A**	GA	**C**	TC	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	2
ID 25	**A**	GA	CT	TC	CT	**T**	CT	+	*DAZ*4	*DAZ*4	3
ID 40	**A**	GA	CT	TC	CT	**T**	CT	+	*DAZ*4	*DAZ*4	3
ID 2	AG	GA	**C**	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	4
ID 5	AG	GA	**C**	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	4
ID 7	AG	GA	**C**	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	4
ID 44	AG	GA	**C**	**C**	CT	T	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	4
ID 6	AG	GA	**C**	TC	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2 + *DAZ*4	5
ID 8	AG	GA	**C**	TC	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	5
ID 15	AG	GA	**C**	TC	CT	**T**	CT	+	*DAZ*2 + *DAZ*4	*DAZ*2	5
ID 11	AG	GA	CT	**C**	CT	TG	CT	+	*DAZ*2		6
ID 12	AG	GA	CT	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4		7
ID 28	AG	GA	CT	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4		7
ID 31	AG	GA	CT	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4		7
ID 34	AG	GA	CT	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4		7
ID 41	AG	GA	CT	**C**	CT	**T**	CT	+	*DAZ*2 + *DAZ*4		7
ID 23	AG	GA	CT	**C**	**C**	**T**	CT	+	*DAZ*1 + *DAZ*2 + *DAZ*4	*DAZ*4	8
ID 27	AG	GA	CT	TC	CT	**T**	CT	+	*DAZ*4		9
ID 37	AG	GA	CT	TC	CT	**T**	CT	+	*DAT*4	*DAZ*4	9

Note. SNV: Single Nucleotide Variant; STS: Sequence Tagged Site. Discordant results between SNV-PCR and Southern blot analysis for detection of *DAZ* gene partial deletions: ▭ Deletions identified by SNV-PCR but not confirmed by TaqI or EcoRV Southern blot analysis. Aberrations are indicated in bold.

[a]In *DAZ*1, 2 and 3, SNV I has the A variant. In *DAZ*4, SNV I has the G variant. In *DAZ*2, SNV III and SNV IV have the T variant. In *DAZ*1, 3 and 4, SNV III and SNV IV have the C variant. In *DAZ*1 and 2, SNV V has the T variant. In *DAZ*3 and 4, SNV V has the C variant. In *DAZ*4, SNV VI has the G variant, and in the *DAZ*1, 2 and 3, SNV VI has the T variant. In *DAZ*1, and 4, sY581 has the C variant, and in the *DAZ*2 and 3, sY581 has the T variant.

[b](+) presence of sequence tagged sites detected by PCR and (−) absence of sequence tagged site.

[c]The presence or absence of an STS or SNV in the genomic DNA samples analysed could distinguish nine different patterns (*DAZ*-haplotypes 1 to 9).

[d]*Y-DAZ*-3 and DAZ-RRM3.

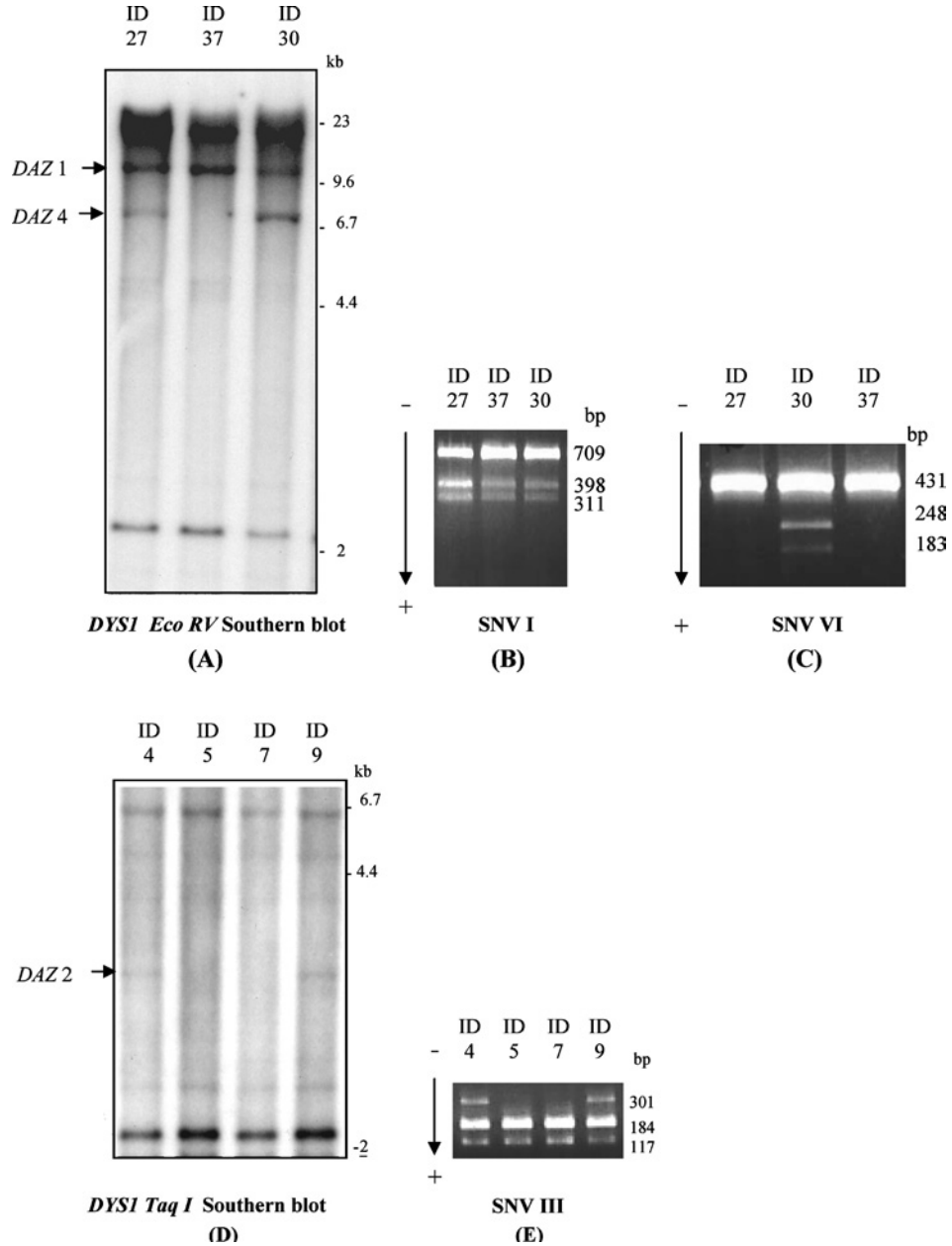

Fig. 2. Detection of *DAZ*2 or *DAZ*4 deletion by Southern blot and PCR assays in fertile men (A) *DYS1 EcoRV DNA* blot *DAZ* deletion analysis with the *DYS*1 probe 49f. A deletion of *DAZ*4 is indicated by absence of the 7.3 kb *DYS*1 *Eco*RV fragment. The 4.5 kb cross hybridization genomic *DYS*1 fragment is present in all lanes, but with low intensity. (B) Digestion of SNV I-PCR with *Fsp1* produces two fragments of 398 and 311 base pairs (bp) in *DAZ*4. *DAZ*1, 2 and 3 SNV I-PCR remain undigested, resulting in one 709 bp fragment. (C) Digestion of SNV VI-PCR with *Afl*III produces two fragments of 248 and 183 base pairs in *DAZ*4. *DAZ*1, 2 and 3 SNV VI-PCR remain undigested, resulting in one 431 bp fragment. ID 27: Fertile man with a suspected deletion of *DAZ*4 using SNV VI marker, not confirmed by *DYS1 Eco*RV Southern blot analysis. ID 37: Fertile man with a suspected deletion of *DAZ*4 using SNV VI marker, confirmed by *DYS*1 *Eco*RV Southern blot analysis. ID 30: Fertile man without deletion of *DAZ*4 detected using SNV I and SNV VI markers, confirmed by *DYS*1 *EcoRV* Southern blot analysis. (D) *DYS*1 *TaqI* DNA blot *DAZ* deletion analysis with the *DYS*1 probe 49f. A deletion of *DAZ*2 is indicated by absence of the 3.1 kb *DYS1 TaqI* fragment. The 4 and 5 kb cross hybridization genomic *DYS1* fragments are not intense but are present in all DNA samples. (E) Digestion of SNV III-PCR with *TaqI* produces two fragments of 184 and 117 base pairs (bp) in *DAZ*1 *DAZ*3 and 4. *DAZ*2 SNV III-PCR remain undigested, resulting in one 301 bp fragment. ID 5, ID 7: Fertile men with a suspected deletion of *DAZ*2 using SNV III marker, confirmed by *DYS*1 TaqI Southern blot analysis. ID 4, ID 9: Fertile men without deletion of *DAZ*2 detected using SNV III marker, confirmed by *DYS*1 TaqI Southern blot analysis.

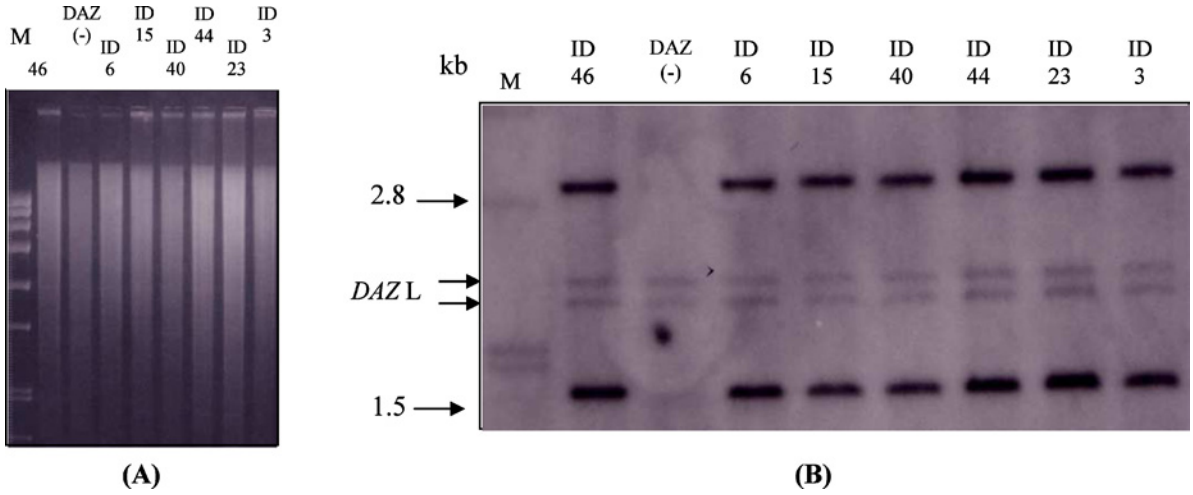

Fig. 3. Semi quantitative Southern blot analysis in fertile men. (A) 0.8% agarose gel UV transillumination after *Eco*RI digestion of genomic DNA samples and migration over night in BET. (B) DNA Blot analysis after *Eco*RI digestion and revelation by *DAZ* probe. ID 3, ID 46: Fertile subject without suspected *DAZ* partial deletion. *DAZ* −: Infertile man with a complete AZFc region deletion. ID 6: Fertile man with a partial deletion of *DAZ*2 and *DAZ*4 copies detected by *DYS*1 *Taq*I and *DYS1 Eco*RV DNA blot analysis. ID 15, ID 44: Fertile men with a partial deletion of *DAZ*2 copy detected by *DYS*1 *TaqI* DNA blot analysis. ID 23, ID 40: Fertile men with a partial deletion of *DAZ*4 copy detected by *DYS1 Eco*RV DNA blot analysis. *DAZ* L: Autosomal *DAZ*-like 1 gene.

DISCUSSION

The number of *DAZ* gene copies on the human Y chromosome has been assumed to be variable in a range between three and seven (7,21,22). Sequences analysis obtained from the RPCI-11 man (BAC donor for GenBank BAC sequences) and the CTA/CTB men (BAC donors for CTA and CTAB BAC librairies) suggest that the *DAZ* copies are four and mapped as *DAZ*1/*DAZ*2 and *DAZ*3/*DAZ*4 doublets with a distance of 1.47 Mb between them (7). The four *DAZ* gene copies have a high sequence homology (>99%) which could be distinguished by small sequence nucleotide variants (SNVs) and some sequence tagged sites (STSs). Using 7 *DAZ*-single-nucleotide-variants (SNV I-VI and sY581) spanning the 5′ end till the 3′ end of the *DAZ* gene sequences, 2 *DAZ* gene copy specific STSs *(DAZ-RRM*3, *Y-DAZ*3) and *DYS*1-*EcoRV* and TaqI blot experiments, we found that the four copy gene structure represents the most common *DAZ* gene structure. Using PCR and qualitative southern blot, partial *DAZ* deletions were suspected in 25.4% of fertile men. However, using semi-quantitative Southern blot, we were unable to confirm these partial *DAZ* deletions suggesting the presence of four *DAZ* copies in all tested fertile men. The molecular events detected by PCR assays and qualitative DYS1-Southern blot are probably the results of gene conversion events. Similarly, using FISH to analyse spermatozoa and leukocytes of 56 semen donors, de Vries and collegues did not detect partial deletions of the

Table II. Spermatic Characteristics of Fertile Men

DAZ haplotype	1 ($n = 25$)	2 ($n = 1$)	3 ($n = 1$)	4 ($n = 3$)	5 ($n = 3$)	6 ($n = 1$)	7 ($n = 4$)	8 ($n = 1$)	9 ($n = 2$)
Semen volume[a] (mL)	4.1 ± 1.8	1.5	3.4	3.7 ± 0.6	3.9 ± 2.1	3.1	3.3 ± 2.3	5.1	2.3 ± 0.2
Sperm concentration[a] (10^6/mL)	93 ± 75	35	66	49 ± 43	129 ± 38	19	54 ± 33	108	181 ± 4
Total sperm number per ejaculate[a] (10^6)	391 ± 392	55	222	197 ± 200	542 ± 433	59	188 ± 176	554	422 ± 26
Progressive motility[a](a + b)(%)	40.0 ± 10.6	40	50	51.6 ± 7.6	31.6 ± 16.0	40	45.0 ± 4.0	50	40

[a]Values are mean ± SD.

DAZ gene (13). Unfortunately, we could not perform FISH experiment with our DNA samples, because a request for a second blood sample was not possible. In a previous report, using only SNV-PCR techniques in 107 men with proven fertility, similar results to our PCR assays were obtained: deletion of *DAZ*2 was found in 26 subjects (24%), deletion of *DAZ*4 in 10 subjects (9.3%) and deletion of *DAZ*2 and *DAZ*4 in 3 subjects (2.8%) (9). However, it appears clear now that this method does not distinguish the absence of a variant from a true deletion. Previous reports suggesting partial *DAZ* deletions in fertile individuals detected using only PCR assays should be reconsidered (9,10). The recent observation of no gr/gr deletion in 148 men with normal spermatogenesis confirms our data showing that partial *DAZ* deletion appears to be rare in fertile men (23).

Recently, several authors have reported that the complete AZFc sequence consists of a series of homologous large repetitive blocks or amplicons. The high sequence homology (>99.9%) of these homologous AZFc amplicons suggests frequent sequence alignments and gene conversion between them (6,8,24). Discordant results between qualitative and quantitative southern blot could only be the result of gene conversion between the rl/r2 or r3/r4 amplicons (25) or recombination within the RBM or the *DAZ* repeat region of a *DAZ* gene. In these cases, many fertile men should present four *DAZ* copies but an atypical organization of the *DAZ* locus (for example *DAZ*1-*DAZ*2-*DAZ*3-*DAZ*1 in ID25, ID40; *DAZ*1-*DAZ*3-*DAZ*3-*DAZ*4 in ID2, ID5, ID7, ID44; *DAZ*1-*DAZ*3-*DAZ*3-*DAZ*1 in ID 6). Moreover, comparison of molecular results obtained with SNV/STS markers and *DYS*1—*EcoRV* and *TaqI* blot experiments showed discordant results in several cases (Table I). In one subject (ID13) with a suspected *DAZ*2 deletion, absence of the G allele for SNV VI suggested also a deletion of the *DAZ*4 copy. However, this last deletion was not confirmed by *DYS*1 blot experiment. Similar discrepancies were observed in six other men with a suspected *DAZ*2 deletion (ID2, 5, 7, 8, 15 and 44) where the absence of the T allele for SNV III was always associated with absence of G allele for SNV VI. In five other men (ID 12, 28, 31, 34, 41), the lack of the T allele for SNV IV and absence of the G allele for SNV VI suggested a deletion of *DAZ*2 and *DAZ*4 but the deletion of both *DAZ* copies was not confirmed by *DYS*1 blot experiments. Moreover, the absence of the G allele for SNV VI suggested a deletion of *DAZ*4 in subject ID27, the absence of T allele for SNV IV suggested a deletion of *DAZ*2 in subject ID11, and the absence of G allele for SNV IV and T allele for SNV VI associated with absence of T allele for SNV V suggested a deletion of *DAZ*1, 2 and 4 in subject ID23. In these last three cases, *DYS*1 blot experiments did not confirm the deletion or the exact nature of the deletion suspected by the PCR assays. We conclude from these results that SNVs markers are polymorphic as previously observed (de Vries *et al.,* 2002b; Fernandes *et al.,* 2002). In particular, SNV I, SNV IV, SNV V and SNV VI have been shown to be polymorphic suggesting that they are not conclusive in the detection of the number of *DAZ* gene copies. However, the simple and relatively rapid molecular approach using PCR could be used in a first step to eliminate a partial deletion of *DAZ* gene. If a deletion is suspected by PCR or PCR digest assays, semi quantitative Southern blot, or the recently described sperm HALO—FISH method (26) should be used in a second step to confirm *DAZ* gene deletions. Sperm HALO—FISH method is not easy to develop and needs to analyse a high number of spermatozoa, this can be a limit to determine the *DAZ* gene copy number in infertile men. We conclude that in DNA samples where the deletion of *DAZ*2 or *DAZ*4 could not be confirmed by appropriate blot experiments, most probably gene conversion events occurred between the highly homologous *DAZ* gene copies in the *DAZ* locus.

The human spermatogenesis efficiency varies in a large range, so we compared the semen characteristics of men with the different *DAZ*-haplotypes. No difference was observed and no relation was found between the *DAZ*-haplotype and sperm concentration, motility and morphology in the studied fertile men. This suggests that the different sequence organizations of the *DAZ* locus are compatible with normal spermatogenesis.

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