

## ORIGINAL ARTICLE

# Partial deletions are associated with an increased risk of complete deletion in *AZFc*: a new insight into the role of partial *AZFc* deletions in male infertility

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**Background:** The *AZFc* region on the human Y chromosome has been found to be functionally important in spermatogenesis. Complete *AZFc* deletion is one of the most frequent causes of male infertility and the roles of partial *AZFc* deletions (gr/gr and b2/b3 deletions) in spermatogenesis are controversial.

**Methods:** To further study the roles of partial *AZFc* deletions in spermatogenic impairment and the relationship between complete and partial *AZFc* deletions, these deletions were typed and quantitative analysis of *DAZ* gene copies and Y chromosome haplogrouping were performed for seven pedigrees of complete *AZFc* deletion carriers, comprising 296 infertile and 280 healthy Chinese men.

**Results:** Neither the gr/gr nor the b2/b3 deletion was found to be associated with spermatogenic failure. In one pedigree, a complete *AZFc* deletion was observed to result from the gr/gr deletion, suggesting that complete deletions of *AZFc* can be preceded by partial deletions. In addition, a new gr/gr-deleted Y haplogroup Q1 was identified and the reported fixation of the b2/b3 deletion in haplogroup N confirmed. The frequency of complete *AZFc* deletion in haplogroups Q1 and N was significantly higher than that in the other haplogroups with fewer partial deletions. Duplications of *DAZ* gene copies were also observed in this study.

**Conclusions:** To date, these observations comprise the first evidence showing that partial *AZFc* deletions can increase the risk of complete *AZFc* deletion. The susceptibility of partial *AZFc* deletions to complete *AZFc* deletion deserves further examination, especially in the populations or Y haplogroups abundant in partial *AZFc* deletions.

Deletions on the human Y chromosome are one of the main causes of male infertility.<sup>1–2</sup> Three azoospermia factors (*AZFa*, *AZFb* and *AZFc*) have been mapped to Yq11,<sup>3</sup> and of these, *AZFc* is the region most often involved in deletions.<sup>4–5</sup> As *AZFc* is composed of several distinct families of long repeats (amplicons) (fig 1A), it is susceptible to non-allelic homologous recombination between amplicons, which induces the recurrence of various deletions in the gene.<sup>6–11, 12</sup>

Recently, two types of partial *AZFc* deletions were identified. One partial deletion is the gr/gr deletion, which is caused by homologous recombination between two *g* or two *r* amplicons (fig 1B).<sup>8</sup> In particular, a 1.6 Mb DNA segment was excised from the *AZFc* region, which was considered as a significant risk factor for spermatogenic failure in Dutch, Spanish, Italian and Australian studies.<sup>8–13–16</sup> However, the association of this deletion with spermatogenic failure was not confirmed in French, German, Brazilian, Japanese or Sri Lankan men, or in our previous study in Chinese men.<sup>17–24</sup> Furthermore, this association was also contradicted by the fact that all the tested men of a common Y haplogroup, D2b, presumably fertile, carry the gr/gr deletion.<sup>8</sup>

The other partial deletion is the b2/b3 deletion (also known as the g1/g3 deletion or u3-gr/gr deletion), which removes a 1.8 Mb DNA segment from the b2/b3-inversed or gr/rg-inversed *AZFc* (fig 1C, D).<sup>9–10, 17</sup> This deletion is found to be fixed (100%) in N, a haplogroup widely distributed in northern Eurasia.<sup>25–26</sup> The association of the b2/b3 deletion with male infertility was recently reported in Chinese men, whereas no predisposition was detected in other populations.<sup>9–10, 16–18, 24</sup>

In contrast, complete *AZFc* deletion (referred to as the b2/b4 deletion), which removes all eight testis-specific expressed gene

families in *AZFc* (fig 1A), has long been known to cause azoospermia or oligozoospermia with few exceptions.<sup>6–27</sup> In fact, it accounts for over half of the Y chromosome deletions causing male infertility.<sup>4–5</sup>

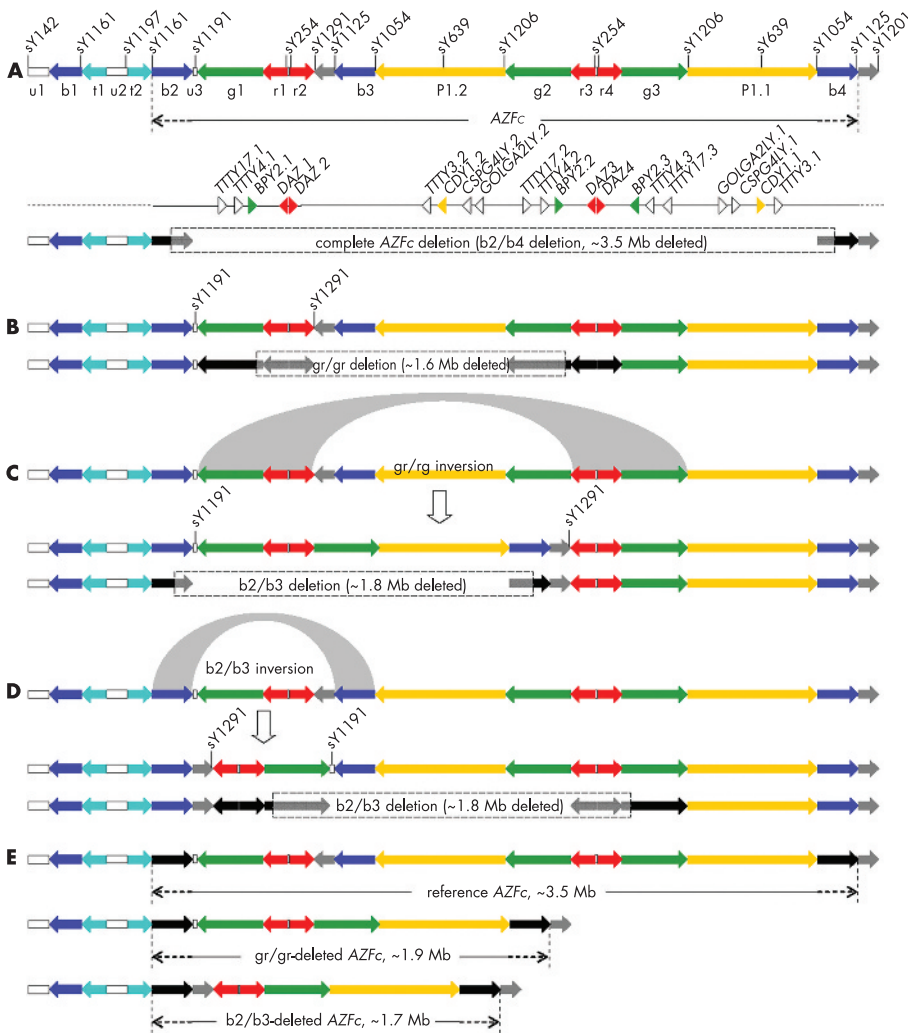
In this study, we typed complete and partial *AZFc* deletions in 296 patients with spermatogenic impairment and 280 healthy donors (controls) in a Chinese population to study the roles of these deletions in spermatogenesis and male infertility. We also typed 19 binary markers on the Y chromosome and subdivided patients and controls into different haplogroups. Studying the deletion distribution in Y haplogroups could help uncover the effect of the genetic background of each Y haplogroup in determining the incidence of deletions, which may account for the diverse frequency of partial *AZFc* deletions and their inconsistent association with spermatogenic impairment across populations. In addition, we performed quantitative analysis of *DAZ* gene copy in *AZFc* to further characterise partial *AZFc* deletions in this study.

## METHODS

### Subjects

This study was approved by the institutional ethics committees of Nanjing Medical University and Renji Hospital, and informed consent was obtained from all participants. In total, 296 unrelated patients with non-obstructive azoospermia (216 men) or oligozoospermia (<20 million sperm per mL; 80 men) were sampled from the infertility clinic at the First

**Abbreviations:** *AZFc*, azoospermia factor c; STS, sequence tagged site; SFV, sequence family variant; SNV, single nucleotide variant; YCC, Y Chromosome Consortium



**Figure 1** Structures and rearrangements of amplicons in the AZFc region. (A) The amplicon structure, sequence-tagged sites, three protein-coding gene families (solid triangles) and five non-coding gene families (open triangles) in AZFc of the GenBank reference sequence.<sup>6,7</sup> The recombination between two *b* amplicons (shown in black) can lead to complete deletion of AZFc. (B) The gr/gr deletion removed the part of AZFc that included sY1291.<sup>8</sup> (C) The b2/b3 deletion removed the part of the gr/rg-inverted AZFc that included sY1191.<sup>9</sup> (D) The b2/b3 deletion removed the part of the b2/b3-inverted AZFc that included sY1191.<sup>9,10</sup> (E) Comparison of the distances between the *b* amplicons (the recombination targets of complete AZFc deletion) of reference AZFc, gr/gr-deleted AZFc and b2/b3-deleted AZFc.

Affiliated Hospital with Nanjing Medical University at Jiangsu and Renji Hospital, Shanghai. As controls, 280 healthy donors, who had normal sperm concentration, motility and morphology (90 men), or had fathered one or more healthy children (190 men), were also sampled from these two hospitals. In addition, seven pedigrees with infertile patients carrying complete AZFc deletion were sampled at Renji Hospital. The semen analysis for sperm concentration, motility and morphology was performed following World Health Organization criteria.<sup>28</sup> These patients and controls were all Han Chinese from East China.

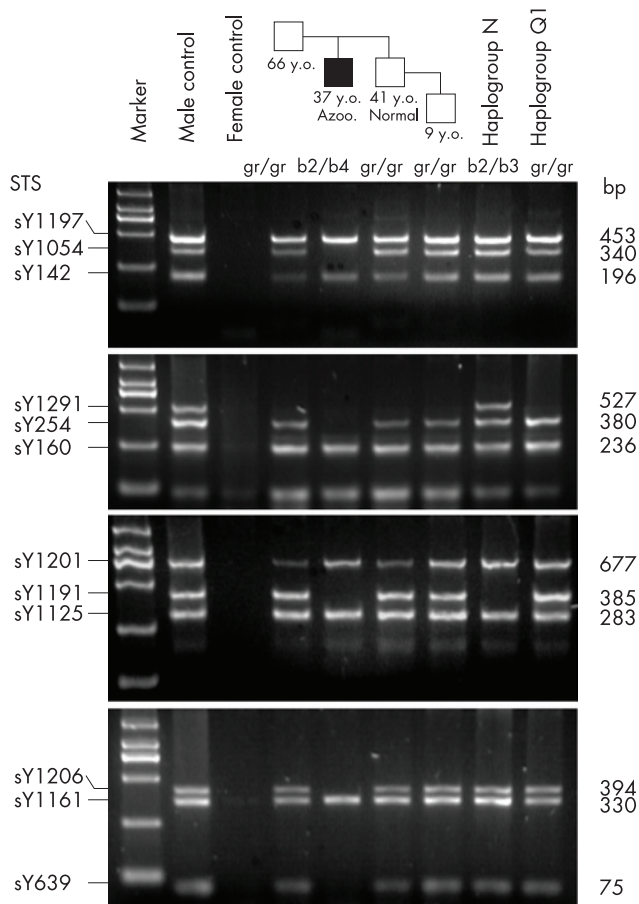
### Deletion typing

Details of the procedure were described in our previous study.<sup>23</sup> Briefly, genomic DNA was extracted from peripheral blood samples. Partial AZFc deletions were typed by the following sequence-tagged site (STS) pattern: gr/gr deletion (sY1161, sY1191, sY1201 and sY1206 are positive; sY1291 is negative) and b2/b3 deletion (sY1161, sY1201, sY1206 and sY1291 are positive; sY1191 is negative) (fig 2).<sup>8-9</sup> Complete AZFc deletions were typed by STS analysis as described previously<sup>6</sup> (fig 2). The locations of the STSs adopted are shown in fig 1A. Quantitative analyses of *DAZ* gene copies were performed to further characterise partial AZFc deletions. Using the method of Machev *et al*,<sup>17</sup> we typed the sequence family variant (SFV) at sY587—that is, the single nucleotide variant (SNV) II of *DAZ* (*DAZ*-SNV II), which can distinguish *DAZ1/2* from *DAZ3/4*.

Quantitative real-time PCR of the *DAZ* gene was also performed to measure *DAZ* copy number. M159, a Y chromosomal locus outside AZFc, was used as the reference locus to calibrate measurement. *DAZ*-SNV 1 and *DAZ*-SNV 5, which are located at either end of *DAZ*, were chosen as the test loci. Standard curves were generated by amplifying a standard DNA without AZFc deletions, and the relative *DAZ* copy number of the test to the standard were measured.

### Y chromosome haplogrouping

Y chromosome haplogroups were defined using 16 binary markers: M9, M89, M95, M117, M119, M120, M122, M130, M134, M175, M176 (SRY+465), M214, M231, M268, LLY22g and YAP (M1).<sup>29-31</sup> LLY22g was typed using the protocol kindly provided by Y. Xue and C. Tyler-Smith (Wellcome Trust Sanger Institute, personal communication). These 16 markers defined 16 Y haplogroups (fig 3A) following the nomenclature recommended by the Y Chromosome Consortium (YCC) and its update.<sup>31-33</sup> In contrast to the previous report that M231 was phylogenetically equivalent to LLY22g in defining haplogroup N,<sup>26</sup> we found that M231 defined a new haplogroup, N\*. Therefore, following the YCC naming rules, we renamed the haplogroup defined by LLY22g as N1.<sup>33</sup> Haplogroup N1 can be further divided into four sub-haplogroups by three additional markers: N1\*-LLY22g, N1a-M128, N1b-P43 and N1c-Tat.<sup>25, 26, 29, 34</sup>



**Figure 2** The +/- STS pattern to identify complete or partial deletions in AZFc.<sup>6-8,9</sup> The names (left) and product sizes in bp (right) of the adopted STSs are shown. The black box indicates the patient with azoospermia (Azoo.) in the pedigree. The age information (y.o., years old) has also been given under the box.

### Statistical analysis

Rousset's exact test of population differentiation between infertile patients and healthy controls was performed by using Arlequin software.<sup>35</sup> A Markov chain of 10 000 steps and the statistical significance level of  $p < 0.05$  were used. Differences in deletion frequencies between Y chromosome haplogroups or between infertile patients and healthy controls were examined using Fisher's exact test, with statistical significance set at of  $p < 0.05$ .

## RESULTS

### Genesis of complete AZFc deletions in pedigrees

Seven pedigrees of complete AZFc deletion were studied, in which the probands lacked AZFc (complete AZFc deletion), whereas their fathers and other paternal relatives do not. In one pedigree, we found that the paternal relatives of the patient were gr/gr-deleted; neither the gr/gr nor the b2/b3 deletion was found in the other six pedigrees. The patient in the gr/gr-deleted pedigree is azoospermic. His brother has normal spermatogenesis, and his nephew (9 years old) and father (66 years old, assumed healthy) did not attend semen analysis (fig 2). Y chromosome haplogrouping indicated that this pedigree belongs to haplogroup O1. SFV analysis of DAZ gene indicated the absence of DAZ1/2 in the gr/gr deletions of this pedigree. After the confirmation of paternity using Y chromosome markers, we concluded that the complete AZFc deletion

was derived from a gr/gr-deleted Y chromosome in this pedigree.

### Distribution of AZFc deletions in patients and controls

We investigated the distributions of partial AZFc deletions in 296 infertile patients with spermatogenesis failure and in 280 healthy controls (tables 1 and 2). In all, 24 (8.1%) gr/gr and 26 (8.8%) b2/b3 deletions were found in patients, and 20 (7.1%) gr/gr and 18 (6.4%) b2/b3 deletions were observed in controls. No significant difference in deletion frequency between patients and controls was found for either the gr/gr or b2/b3 deletion.

According to the published data of SFV or SNV analysis of the DAZ gene, it was proposed that only partial AZFc deletions with DAZ1/2 SFV absence seem to be associated with impairment of spermatogenesis, and absence of the DAZ3/4 SFV may have a limited effect on spermatogenesis.<sup>9,10,14,15</sup> To further characterise the partial deletion subtypes, we typed the SFV at sY587, which can distinguish DAZ1/2 from DAZ3/4 (table 2). In contrast to the gr/gr deletion, most b2/b3 deletions showed DAZ3/4 SFV absence, which was consistent with previous reports and the arrangement mechanism of the b2/b3 deletion (fig 1C, D).<sup>10</sup> Although there were more DAZ1/2 SFV absences in patients than in controls, no significant difference in the frequency of partial deletion subtypes was found between these two groups.

We also observed 14 (4.7%) complete AZFc deletions in patients, whereas none was found in healthy controls. This is consistent with previous reports that complete AZFc deletions lead to azoospermia or oligozoospermia with few exceptions.<sup>6,27</sup>

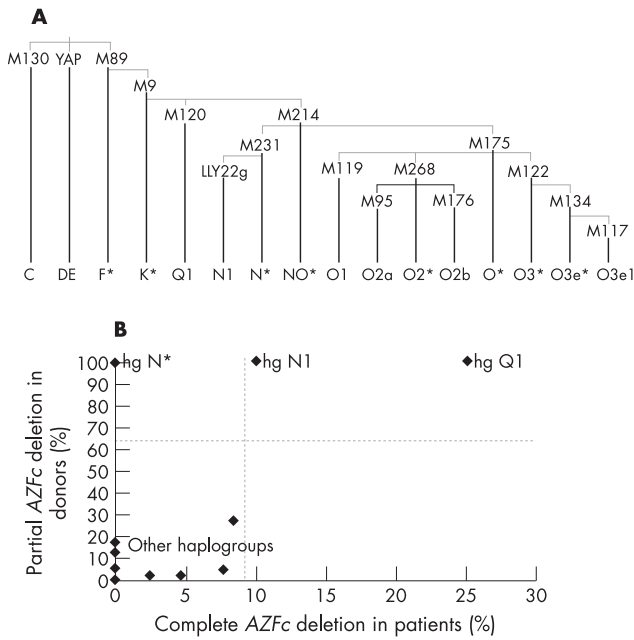
### Haplogroups with high frequency of partial AZFc deletions

To explore the relationship of deletions and Y haplogroups, we first typed 16 Y chromosomal markers to define 16 haplogroups in patients and controls. Deletion frequencies varied greatly among the Y haplogroups (table 1). High frequencies of partial AZFc deletions were found in haplogroups Q1, N1 and N\*.

Haplogroup Q1 was found to be gr/gr-deleted. All 10 controls carry gr/gr deletions. Six of eight (75%) patients are gr/gr-deleted, and the other two patients (25%) carry complete AZFc deletions. Therefore, we speculated that the ancestor of haplogroup Q1 was a gr/gr deletion carrier and that these two complete deletions were derived from gr/gr-deleted Y chromosomes of Q1.

In haplogroup N1, all the tested men carry AZFc deletions, complete or partial. All 13 controls and 18 of 20 (90%) patients carry b2/b3 deletions, and the other two patients (10%) carry a complete AZFc deletion, which is consistent with the reported fixation of b2/b3 deletion in this haplogroup.<sup>9,10</sup> Again, we speculated that these complete deletions were derived from b2/b3-deleted ancestors of haplogroup N1. In this study, we also typed three further markers (M128, P43 and Tat) to subdivide haplogroup N1. One N1a-M128 control and five N1c-Tat subjects (four patients and one control) were found. Although no N1b-P43 was detected in this study, it was found that both of two tested N1b-P43 men of unknown spermatogenic phenotype had b2/b3 deletions (P. A. Underhill, Stanford University, personal communication). The b2/b3 deletions in haplogroup N1 were shown to be widely distributed throughout all the N1 sub-haplogroups, which suggests that they have a common origin and are derived from the b2/b3-deleted ancestor of N1.

Similar to N1, all three patients and one control in haplogroup N\* have b2/b3 deletions. As N1 was phylogenetically derived from N\*, the b2/b3 deletions in these two haplogroups could share the same deletion ancestor.



**Figure 3** (A) Y chromosome phylogeny. The markers typed in this study are indicated in their defined branches. (B) Scatter plot of deletion frequencies in the Y haplogroups. Frequency of complete AZFc deletion in patients (X axis) and frequency of partial AZFc deletions in controls (Y axis). As there is no haplogroup K\* or haplogroup NO\* found in healthy controls, these two haplogroups are not shown in this plot.

In contrast to the 100% fixation of AZFc deletions in haplogroups N\*, N1 and Q1, the frequency of partial AZFc deletions was low (averaging 8.7% in patients and 5.5% in controls) in the other haplogroups.

**Copy number variation of the DAZ gene in partial AZFc deletions**

A previous report showed that there are four DAZ gene copies in AZFc on most human Y chromosomes.<sup>36</sup> The frequent rearrangements in AZFc can change DAZ copy numbers. The gr/gr deletion and b2/b3 deletion commonly reduce DAZ copy from four to two,<sup>8-10</sup> whereas partial duplication in AZFc was able to raise DAZ copy to >4 in non-deletions or compensate for DAZ copy reduction caused by partial deletion.<sup>36, 37</sup> DAZ copy numbers were examined using quantitative real-time PCR analysis of two loci in the DAZ gene (fig 4).

**Table 2** SFV absence of DAZ genes in partial AZFc deletions

Group	n	gr/gr deletion		b2/b3 deletion	
		DAZ1/2 absence	DAZ3/4 absence	DAZ1/2 absence	DAZ3/4 absence
Patient	50	17	7	1	25
Control	38	10	10	0	18

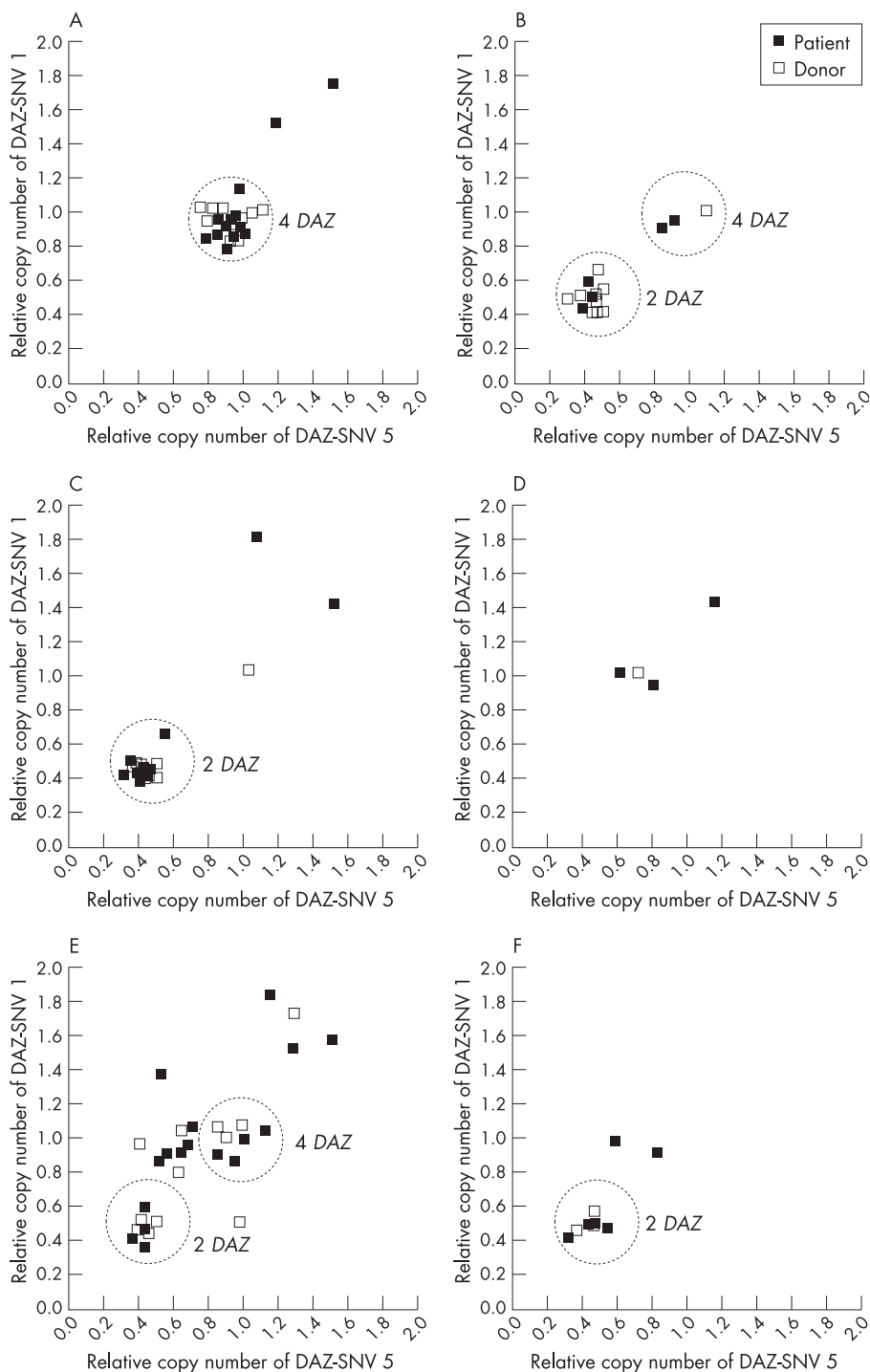
In total, 26 (13 patients and 13 controls) of 28 randomly selected non-deletions were shown to have the same DAZ copy number as the standard (fig 4A). Considering the previous observation that most Y chromosomes bear four DAZ copies,<sup>36</sup> these 26 non-deletions and the standard were assumed to have four DAZ copies. In addition, an increase in DAZ copies was found in two patients without deletions, but no increase was found in controls. This suggests that partial duplication of AZFc occurred in these two patients. No reduction in DAZ copy number was found in patients without deletions.

All 44 gr/gr deletions were examined. In haplogroup Q1, 13 (4 patients and 9 controls) of 16 men with gr/gr deletions bore two DAZ copies, which is consistent with the fact that commonly half of the four DAZ copies are removed by the gr/gr deletion (fig 4B).<sup>8</sup> The other two patients and one control carrying the gr/gr deletion were shown to have four DAZ copies, suggesting that partial duplications followed gr/gr deletion and compensated for the reduction in DAZ copy number caused by the gr/gr deletion.<sup>37</sup> There were 28 gr/gr deletions (18 patients and 10 controls) in non-Q1 haplogroups, 25 of which bore two DAZ copies (fig 4C). DAZ duplications were also found in three men, including one control with four DAZ copies, one patient with six DAZ copies and one patient with a complex DAZ substructure (four DAZ-SNV 1 copies but >4 DAZ-SNV 5 copies). As DAZ-SNV 1 and 5 are at opposite ends of DAZ genes, the unequal copy numbers of these two loci may indicate duplication of incomplete DAZ.

In contrast to the fact that DAZ copy numbers are reduced to two in most gr/gr deletions, partial duplication of DAZ copy may have occurred in many b2/b3 deletions. In haplogroup N\*, all the four men (three patients and one control) with b2/b3 deletions bore at least four DAZ copies, according to the DAZ-SNV 1 locus (fig 4D). In haplogroup N1, 10 (five patients and five controls) of 31 patients with deletions had two DAZ copies, and the other 21 subjects (13 patients and 8 controls) had >2 copies according to the DAZ-SNV 1 or 5 locus (fig 4E). Of the nine subjects with b2/b3 deletions in the non-N haplogroups, three patients and four

**Table 1** Distribution of the subjects and complete/partial AZFc deletions in Y chromosome haplogroups

Group	n	Y chromosome haplogroup															
		C	DE	F*	K*	Q1	N1	N*	NO*	O1	O2a	O2*	O2b	O*	O3*	O3e*	O3e1
All																	
Patient	296	25	3	2	1	8	20	3	1	52	5	12	3	1	82	35	43
Control	280	24	1	2	0	10	13	1	0	49	8	11	3	1	70	39	48
gr/gr deletion only																	
Patient	24	1	1			6				2		3			4	5	2
Control	20	1				10				2	1	3				2	1
b2/b3 deletion only																	
Patient	26	3					18	3									2
Control	18	3					13	1									1
Complete AZFc deletion only																	
Patient	14					2	2		1	4		1			2		2
Control	0																



**Figure 4** Scatter plot of the copy number of DAZ-SNVs in the test DNAs relative to the standard DNA. (A) Non-deleted samples; (B) gr/gr deletions in haplogroup Q1; (C) gr/gr deletions in non-Q1 haplogroups; (D) b2/b3 deletion in haplogroup N1; (E) b2/b3 deletion in non-N haplogroups. Filled box indicates patient and open box indicates healthy control.

controls had two *DAZ* copies and the other two patients had >2 copies of the *DAZ*-SNV 1 locus (fig 4F).

It has been recently reported in Han Chinese in Taiwan that partial duplication occurred in one-third of b2/b3 deletions in haplogroup N-LLY22g (corresponding to N1 in this study), a frequency that is less than our finding of two-thirds.<sup>37</sup> However, the frequencies of partial duplication are relatively high for in haplogroup N1 in both studies. There may be two reasons for the difference in frequency difference between the two studies. One is the different substructure of haplogroup N1 between Han Chinese in Taiwan and Han Chinese in East China. The other is

that imbalanced duplication between *DAZ*-SNV 1 and 5 loci may not be detectable by the Southern blot analysis used by Lin *et al.*<sup>37</sup> This possibility is also supported by our observation that partial duplications indicated by both *DAZ*-SNV 1 and 5 together were found in 11 of 31 (about one-third) b2/b3 deletions in haplogroup N1, which is consistent with the recent report.<sup>37</sup>

**Distribution of complete AZFc deletions in Y haplogroups**

In total, 14 complete *AZFc* deletions were found in seven haplogroups of patients, with frequencies of 2.4–100%, of

which the three highest were haplogroups NO\* (100%), Q1 (25.0%) and N1 (10.0%). Considering the fixation of partial *AZFc* deletions in haplogroups N\*, N1 and Q1, it was suggested that the haplogroups abundant in partial deletions showed a higher frequency of complete deletion than the other haplogroups (fig 3B). As haplogroup NO\* contained only one sample (complete *AZFc* deletion) and the frequency of partial deletion was undetermined for this haplogroup, haplogroup NO\* was not included in the following association study.

A significant difference ( $p < 0.04$ , one-sided; odds ratio (OR) = 4.20, 95% CI 1.21 to 14.5) in the frequency of complete *AZFc* deletion was found between N\*N1Q1 (fixation of partial *AZFc* deletions) and non-N\*N1Q1 haplogroups (non-fixation of partial *AZFc* deletions) (table 3). In contrast to haplogroups N1 and Q1, all the b2/b3 deletions in haplogroup N\* were followed by partial duplication. When partial *AZFc* deletions without duplication were accounted for, a more significant difference ( $p < 0.03$ , one-sided; OR = 4.78, 95% CI 1.37 to 16.7) in complete deletion frequency was found between haplogroups N1 and Q1 (high frequency of partial *AZFc* deletions without duplication) and the non-N1Q1 haplogroups (relatively low frequency of partial *AZFc* deletions without duplication) (table 3). The aforementioned observations suggest that complete *AZFc* deletions are more likely to occur in partially deleted than in non-deleted *AZFc* genes.

## DISCUSSION

### A new gr/gr-deleted haplogroup: Q1

The previous report of fixation of the gr/gr deletion in haplogroup D2b challenged its effect on male infertility.<sup>8</sup> It was suggested that the ancestor of haplogroup D2b was gr/gr-deleted and this deletion can be successfully transmitted from generation to generation, contradicting a direct association of gr/gr deletion and spermatogenic failure.

In this study, we found that a new haplogroup, Q1, is gr/gr-deleted. In this group, all the tested healthy controls carried the gr/gr deletion, and all the infertile patient carried either the gr/gr deletion or complete *AZFc* deletions that were speculated to derive from gr/gr-deleted ancestors.

As the STS absence of sY1291, which is characteristic of a gr/gr deletion, can also be caused by polymorphic loss of o1084/o1085 (sY1291) in haplogroup J,<sup>17</sup> we performed the quantitative *DAZ* copy assay to confirm the gr/gr deletions.<sup>23</sup> Most gr/gr deletions of haplogroup Q1 were found to bear two *DAZ* copies, which is consistent with the fact that two of the four *DAZ* gene copies are commonly removed by the gr/gr deletion.<sup>8</sup> Partial duplication occurred in the other three haplogroup Q1 gr/gr deletions and compensated for the *DAZ* copy reduction. Therefore, the polymorphic loss of sY1291 in haplogroup Q1 can be excluded, and haplogroup Q1 is a new haplogroup that is gr/gr-deleted.

It was recently reported that gr/gr deletion and later duplication has been found in seven Han Chinese of haplogroup Q (defined as P36) in Taiwan. Considering two

previous observations that non-deletions and b2/b3 deletions have been found in Q sub-haplogroups other than Q1 and that haplogroup Q1 is the dominant Q sub-haplogroup in Han Chinese,<sup>8,9,38</sup> it is possible that the gr/gr deletions of Q haplogroup detected by Lin *et al* in Taiwanese Han Chinese belong to haplogroup Q1, not to other Q sub-haplogroups.

Haplogroup Q1 is widely distributed in Sino-Tibetan populations, with a frequency of 1.8% to 7.1%.<sup>38</sup> No predisposition to male infertility has been reported in haplogroup Q1. Further examination of the spermatogenic ability of men in haplogroup Q1 is therefore imperative to uncover the role of the gr/gr deletion in spermatogenesis.

### A two-step process of complete *AZFc* deletion

Because of the rearrangement structure of *AZFc*, complete *AZFc* deletion (between amplicon b2 and b4) is assumed to be derived from two types of Y chromosome: a normal one or one with a partial *AZFc* deletion. In the pedigrees of this study, one of seven (14.3%) complete *AZFc* deletions was found to be derived from the gr/gr deletion, which suggested that some complete deletions of *AZFc* can be caused by a two-step process (partial *AZFc* deletion followed by deletion of the rest of the gene). However, it is unknown whether it is easier to generate complete *AZFc* deletion in a partially deleted *AZFc* than in a non-deleted one. If it is easier, there will be a higher frequency of complete *AZFc* deletions in haplogroups that have a high frequency of partial *AZFc* deletions than in haplogroups lacking these deletions.

### Association of partial *AZFc* deletions with increased incidence of complete *AZFc* deletion

In total, 14 (4.7%) complete *AZFc* deletions were found in our 296 patients with azoospermia or oligozoospermia, which is consistent with the frequency estimate (5–6%) in azoospermia or severe oligozoospermia.<sup>12</sup> The distribution of this deletion was found vary between the different haplogroups in this study. Significantly more complete *AZFc* deletions were found in the haplogroups (N1 and Q1) that had a high frequency of partial *AZFc* deletion (excluding partial duplication) than in the other haplogroups with fewer partial deletions. This observation suggests an association of partial *AZFc* deletions with an increased incidence of complete *AZFc* deletion.

Although the mechanism of this susceptibility of partial *AZFc* deletions to complete *AZFc* deletion requires further scrutiny, comparison of partially deleted *AZFc* with that of normal *AZFc* suggested a reason for this predisposition. Complete *AZFc* deletion has known to be caused by intrachromosomal homologous recombination between *b* amplicons at opposite ends of the *AZFc* region.<sup>6</sup> The distance between these two *b* amplicons (that is, the deletion length) is about 3.5 Mb in reference *AZFc*, 1.9 Mb in the gr/gr deletion and 1.7 Mb in the b2/b3 deletion (fig 1E). The recent genomic survey of deletion polymorphisms in humans reported that the deletion length followed a L-shape distribution for deletion frequency,

**Table 3** Distribution of the patients of complete *AZFc* deletion in Y chromosome haplogroups with different frequencies of partial *AZFc* deletions

Haplogroup	Complete <i>AZFc</i> deletion	No complete <i>AZFc</i> deletion	OR (95% CI)	p Value*
N1 and Q1 (high frequency of partial <i>AZFc</i> deletions†)	4 (14.3%)	24 (85.7%)	4.78 (1.37 to 16.7)	0.026
Non-N1Q1 (low frequency of partial <i>AZFc</i> deletions)	9 (3.4%)	258 (96.6%)		
N*, N1, Q1 (fixation of partial <i>AZFc</i> deletions‡)	4 (12.9%)	27 (87.1%)	4.20 (1.21 to 14.5)	0.036
Non-N*N1Q1 (non-fixation of partial <i>AZFc</i> deletions)	9 (3.4%)	255 (96.6%)		

\*Fisher's exact test, one-sided.

†Excluding the partial *AZFc* deletions followed by partial duplication.

‡Including the partial *AZFc* deletions followed by partial duplication.

suggesting that small deletions have a higher prevalence than larger ones.<sup>39</sup> Therefore, the reduction of nearly half in the distance between recombination targets may consequently increase the incidence of complete AZFc deletion and raise the risk of male infertility.

Recently, the susceptibility of haplogroup E to complete AZFc deletion was reported in northern Italian men,<sup>40</sup> although its mechanism was unknown. As higher frequencies of the gr/gr deletion (40% in haplogroup E3b2 and 20% in haplogroup E3b3) were reported in the sub-haplogroups of haplogroup E3b (the dominant E haplogroup in Europeans) than in other haplogroups of Europeans (<16.7%),<sup>8, 41</sup> the effects of partial AZFc deletions can be considered a candidate cause for this susceptibility.

### The possible susceptibility of overdosage of DAZ gene to spermatogenic impairment

It was recently reported that partial AZFc duplications with six DAZ copies was a risk factor of male infertility in Taiwanese Han Chinese.<sup>37</sup> In this study, most of the tested men without deletions normally bore four DAZ copies, whereas the non-deleted men with >4 copies were found only in patients. In contrast to the non-deleted men, there were commonly two DAZ copies left in partial AZFc deletions. DAZ copy duplication was also found in some partial deletions. Four gr/gr deletions (two patients and two controls) and 21 b2/b3 deletions (13 patients and 8 controls) were shown to have four copies of DAZ-SNV 1 or 5. No obvious difference was found between patients and controls, which suggested that the DAZ restoration to four copies caused by partial AZFc duplication after deletion had a limited effect on spermatogenesis. However, two gr/gr deletions (both patients) and six b2/b3 deletions (five patients and only one control) were shown to bear >4 copies of DAZ-SNV 1 or 5. The observation that high DAZ copy number (>4) was over-represented in patients suggested a possible susceptibility of DAZ overdosage to spermatogenic impairment.

### Variable spermatogenic effects of partial AZFc deletions

As described in the introduction, the spermatogenic effects of partial AZFc deletions vary among populations. In our previous study, the gr/gr deletion was not shown to render an increased risk of spermatogenic impairment in East Asians.<sup>23</sup> In this study, the sample size was enlarged and the b2/b3 deletion was also typed. We also performed Rousset's exact test of population differentiation,<sup>35</sup> and no significant difference in Y haplogroup distribution was found between patients and controls (table 1). Therefore, the genetic stratification of the Y chromosome was excluded. In this study, we showed that neither the gr/gr nor the b2/b3 deletion was found to be associated with spermatogenic impairment.

### CONCLUSION

In summary, by examining 296 patients with spermatogenic impairment and 280 healthy controls, we found that a new common haplogroup Q1 is gr/gr-deleted. In addition, no association of the gr/gr or b2/b3 deletion with spermatogenic impairment was found in the tested population. Both of these results suggest phenotypic variation of partial AZFc deletions across populations. As Y haplogroups are always region-characteristic,<sup>32</sup> the different genetic backgrounds between Y haplogroups may account for this variation among populations. Therefore, Y haplogrouping is strongly recommended for comprehensive analysis of the controversial spermatogenic effects of partial AZFc deletions. Furthermore, we identified a patient in whom further deletion in the gr/gr-deleted AZFc led to complete loss of AZFc, which suggested a two-step process of complete AZFc deletion other than de novo complete deletion.

### ELECTRONIC DATABASE INFORMATION

- Arlequin's Home on the Web, <http://lgb.unige.ch/arlequin/>
- GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for sY142, sY160, sY254, sY639, sY1054, sY1125, sY1161, sY1191, sY1197, sY1201, sY1206 and sY1291)

Although no significant frequency difference in duplication following partial deletion in AZFc was found between patients and controls, overdosage of DAZ loci was over-represented in patients, and its susceptibility to spermatogenic impairment can not be excluded. The association of partial AZFc deletions with increased incidence of complete AZFc deletion also provided a new insight into the role of partial AZFc deletions in male infertility. Further examination of this correlation in more populations will shed new light on the mechanism underlying complete deletion of AZFc on the human Y chromosome.

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