



## Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection

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Many human Y-chromosomal deletions are thought to severely impair reproductive fitness, which precludes their transmission to the next generation and thus ensures their rarity in the population. Here we report a 1.6-Mb deletion that persists over generations and is sufficiently common to be considered a polymorphism. We hypothesized that this deletion might affect spermatogenesis because it removes almost half of the Y chromosome's *AZFc* region, a gene-rich segment that is critical for sperm production<sup>1,2</sup>. An association study established that this deletion, called *gr/gr*, is a significant risk factor for spermatogenic failure. The *gr/gr* deletion has far lower penetrance with respect to spermatogenic failure than previously characterized Y-chromosomal deletions; it is often transmitted from father to son. By studying the distribution of *gr/gr*-deleted chromosomes across the branches of the Y chromosome's genealogical tree, we determined that this deletion arose independently at least 14 times in human history. We suggest that the existence of this deletion as a polymorphism reflects a balance between haploid selection, which culls *gr/gr*-deleted Y chromosomes from the population, and homologous recombination, which continues to generate new *gr/gr* deletions.

Much of the human Y chromosome consists of long, Y-specific repeats called amplicons<sup>1,3</sup>. Homologous recombination between amplicons has been shown to generate deletions, commonly resulting in spermatogenic failure<sup>1,4-7</sup>. The *AZFc* region is comprised completely of amplicons (Fig. 1a) and is particularly susceptible to deletions. For example, the *b2/b4* deletion, which spans 3.5 Mb and eliminates the entire *AZFc* region (Fig. 1a), is the most common known genetic cause of spermatogenic failure<sup>1</sup>. Inspection of the ampliconic structure of *AZFc* led to the prediction that two other deletions could arise there by homologous recombination<sup>2</sup>.

Consistent with this prediction, we and others have previously reported evidence that some men have partial deletions of *AZFc*, although the precise nature and size of these deletions was not determined and their possible impact on spermatogenesis was unclear<sup>8-12</sup>. Here we report the identification of both predicted deletions<sup>2</sup>: the *gr/gr* deletion (1.6 Mb; Fig. 1) and the *b1/b3* deletion (also 1.6 Mb; Fig. 2). Using sequence-tagged sites (STSs) to screen specifically for these deletions in 689 men, we found 22 apparent cases of the predicted *gr/gr* deletion and a single instance of the predicted *b1/b3* deletion.

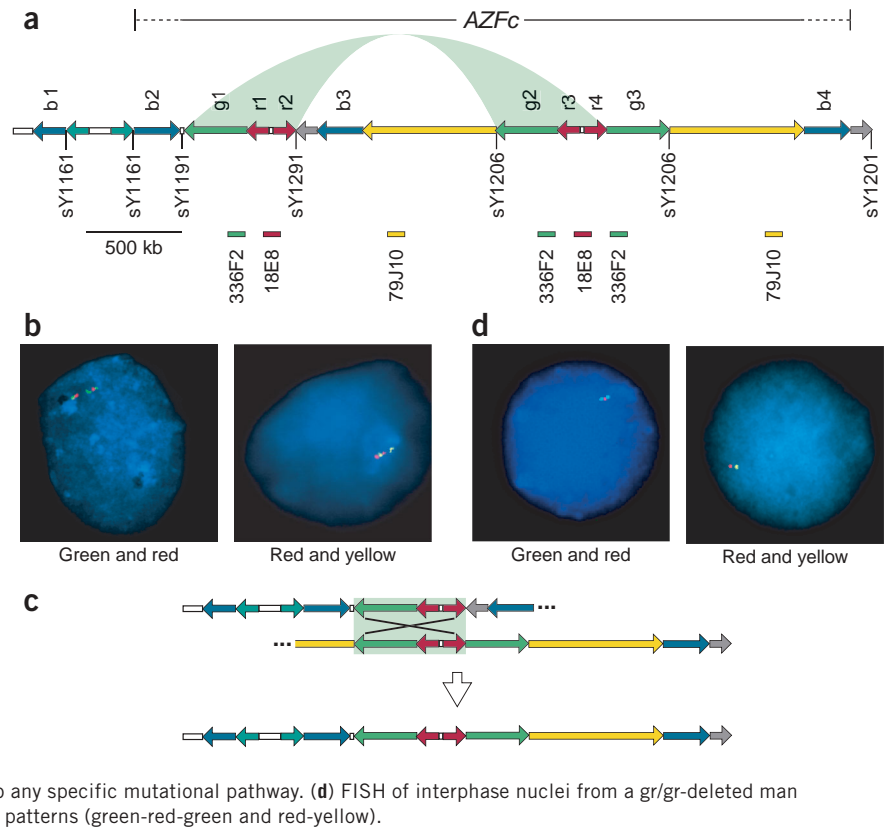
In light of the structural complexity and repetitive nature of the *AZFc* region, we sought to confirm these putative *gr/gr* and *b1/b3* deletions using fluorescence *in situ* hybridization (FISH). The single *b1/b3* deletion was confirmed (Fig. 2). Of the 22 *gr/gr* deletions, we were able to test 20 cases by FISH, and all were confirmed (Fig. 1 and Supplementary Table 1 online). In two of these 20 cases, the FISH studies identified a secondary duplication (Fig. 3).

It seemed likely that the *gr/gr* deletion would predispose men to spermatogenic failure, because it removes nine transcription units with testis-specific expression (Table 1) and part of *AZFc*, a region essential for normal spermatogenesis (Fig. 1). A reanalysis of the 689 men originally screened for the *gr/gr* deletion, which included 473 individuals known to have spermatogenic failure, provided preliminary support for this hypothesis. We sorted each of the 689 men into one of ten Y haplotypes to minimize the effects of stratification. In this sample, the *gr/gr* deletion was consistently more common among men with spermatogenic failure (Supplementary Table 2 online).

Encouraged by these preliminary results, we then carried out a formal association study in a separate population. Here we compared the frequency of *gr/gr* deletions in men with spermatogenic failure to that in controls known to have normal spermatogenesis. Both the affected and unaffected men were drawn from the same clinic population, and

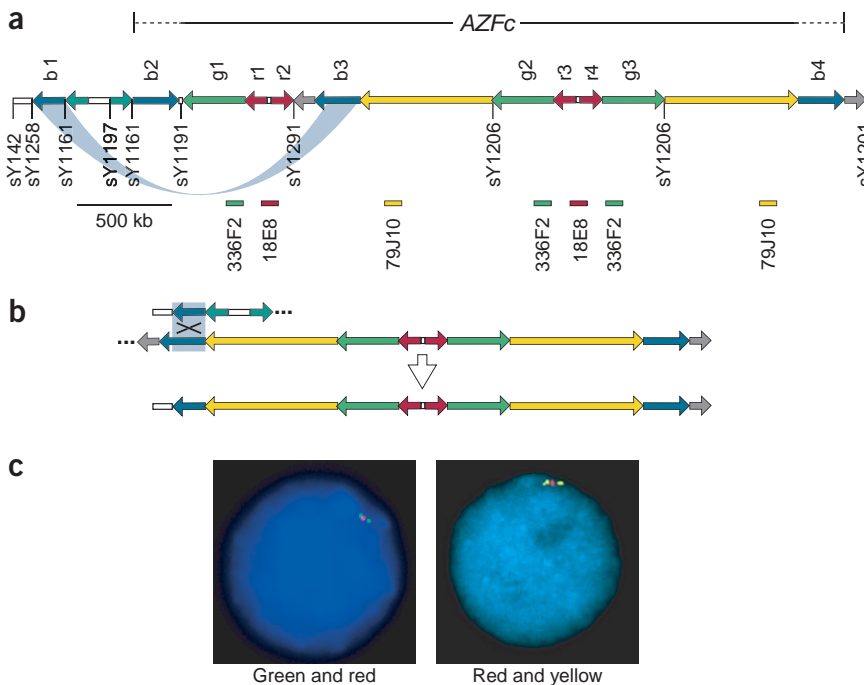
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**Figure 1** The *gr/gr* deletion. (a) The *AZFc* region of the Y chromosome<sup>1</sup>. The central bar depicts the organization of amplicons, including those labeled b1 through b4 (blue), g1 through g3 (green) and r1 through r4 (red). The green arch highlights the regions demarcating the *gr/gr* deletion (an abbreviation of Yen's designation g1/g2,r1/r3,r2/r4; ref. 2). Positions of STSs used to detect this deletion are indicated immediately below the central bar. Shown further below are hybridization sites for the following FISH probes: BAC RP11-336F2 (green, ref. 28), cosmid 18E8 (red, ref. 27) and BAC RP11-79J10 (yellow, ref. 28). At top is shown the extent of the recurrent, 3.5-Mb *AZFc* (b2/b4) deletion<sup>1</sup>. (b) FISH of interphase nuclei from a man (GM10470) in whom there is no *gr/gr* deletion. Hybridizations with red and green probes (left panel) and red and yellow probes (right panel) produced the expected patterns (green-red-green-red-green and red-yellow-red-yellow, respectively). (c) Simplest model of homologous recombination generating the *gr/gr* deletion. The green shaded box highlights the recombination targets. Recombination could be between sister chromatids or within a chromatid. Theoretically, more complex mutational pathways, each consisting of a series of homologous recombination events, could also generate the *gr/gr* deletion. The term '*gr/gr* deletion' refers to the resulting organization of *AZFc* amplicons, not to any specific mutational pathway. (d) FISH of interphase nuclei from a *gr/gr*-deleted man (GM04535). Hybridizations produced the expected patterns (green-red-green and red-yellow).



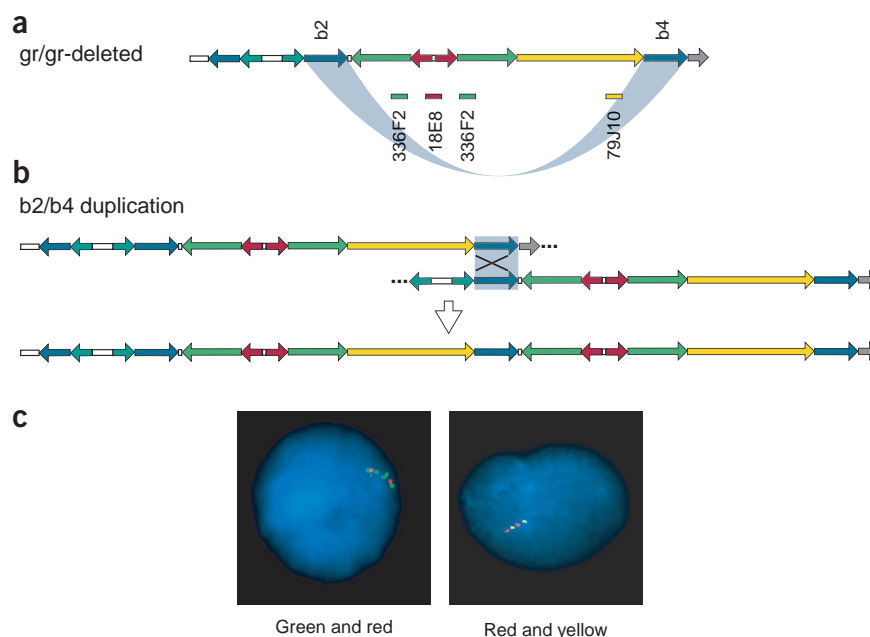
the distribution of Y-chromosome haplotypes was similar in both groups (**Supplementary Table 3** online). This study identified a significant association between the *gr/gr* deletion and spermatogenic failure ( $P < 0.014$ ; **Table 2**). As in our preliminary study, the prevalence of the *gr/gr* deletion was elevated consistently among men with

spermatogenic failure (**Supplementary Table 4** online). This was true regardless of Y haplotype (as in our preliminary study), suggesting that the observed association is not due to stratification (**Supplementary Note** online). We conclude that the *gr/gr* deletion is a risk factor for spermatogenic failure.



**Figure 2** The b1/b3 deletion. (a) The *AZFc* region of the Y chromosome, as in **Figure 1a**. The blue arch highlights the b1 and b3 amplicons, which demarcate the deleted region. Positions of STSs used to detect this deletion are indicated immediately below the central bar. Shown further below are hybridization sites for FISH probes, as in **Figure 1a**. (b) Model of homologous recombination generating the b1/b3 deletion. The blue shaded box highlights the recombination targets. Recombination could be between sister chromatids or within a chromatid. (c) FISH of interphase nuclei from a man (WHT3453) with STSs results suggesting a b1/b3 deletion: he lacked sY1161, sY1197, sY1191 and sY1291 but possessed sY142, sY1258, sY1206 and sY1201. Hybridizations produced the patterns expected for a b1/b3-deleted chromosome (green-red-green and yellow-red-yellow). (In the case of a *gr/gr* deletion, the red and yellow probes would instead have produced the pattern red-yellow, as in **Figure 1**.)

**Figure 3** b2/b4 duplication arising in a *gr/gr*-deleted chromosome. (a) Organization of amplicons in a *gr/gr*-deleted chromosome. The blue arch highlights the b2 and b4 amplicons, which demarcate the duplicated segment. (b) Model of homologous recombination creating the b2/b4 duplication in a *gr/gr*-deleted chromosome. The blue shaded box highlights the recombination targets. This duplication is presumably the result of recombination between sister chromatids. (c) FISH of interphase nuclei from a man (WHT3173) with both a *gr/gr* deletion and a b2/b4 duplication. FISH probes are as in Figure 1a. Hybridization with green and red probes (left) produced the pattern green-red-green-red-green, and hybridization with red and yellow probes (right) produced the pattern red-yellow-red-yellow.



The *gr/gr* deletion does not completely eliminate any of the many testis-specific gene and transcription unit families on the Y chromosome. Instead, it reduces the copy number of eight such families (Table 1). We speculated that the dosage of one or more of these families affects the quantity of sperm produced. If so, then the secondary duplication observed in a few *gr/gr*-deleted chromosomes (Fig. 3) may act as a compensatory mutation by restoring gene copy number (Table 1).

Taking advantage of the clonal transmission of the Y chromosome (that is, without sexual recombination) and our knowledge of its genealogy<sup>13–15</sup>, we examined the origins and dynamics of *gr/gr* deletions across the course of human history. We determined both the *gr/gr* status and the high-resolution Y haplotype of 368 men who collectively represent the full breadth of the chromosome's genealogical tree (43 branches; Fig. 4). We found no *gr/gr*-deleted chromosomes in 29 branches and found 13 branches in which these chromosomes were in the minority. Because 42 of 43 branches, including the deepest branches, consist partly or entirely of chromosomes that were not *gr/gr*-deleted, we concluded that the last common ancestor of modern human Y chromosomes was not *gr/gr*-deleted. Conversely, we found *gr/gr*-deleted chromosomes in 14 branches, including 1 that consisted entirely of these chromosomes. Examination of the tree indicates that the *gr/gr* deletion arose independently, one or more times, in each of these 14 branches.

One branch, D2b (Fig. 4), contained only *gr/gr*-deleted chromosomes. If the *gr/gr* deletion is a risk factor for spermatogenic failure, then men with D2b chromosomes should be at increased risk. Indeed, this probably accounts at a molecular level for an association between Y haplotype and spermatogenic failure observed in Japan, where D2b chromosomes are common. (They are rare in other populations, including the European and American populations that we studied<sup>13</sup>.) Men with Y chromosomes roughly equivalent to branch D2b (Supplementary Note online) were found to be more likely than other Japanese men to have spermatogenic failure<sup>16</sup>. The *gr/gr* deletion that we have shown to be characteristic of branch D2b provides an explanation for this observation.

The present results have implications for the use of Y-chromosomal polymorphism as a tool for reconstructing the phylogeographic origins of modern human populations<sup>13,14,17–19</sup>. These reconstructions have routinely relied on the simplifying assumption that all Y polymorphisms are selectively neutral. This assumption should be reconsidered in light of the present evidence that a common Y-chromosomal variant can affect fertility and reproductive fitness.

Because the *gr/gr* deletion increases the risk of infertility, one might expect a substantial fraction of *gr/gr* deletions to have arisen *de novo*. Previously reported Y-chromosome deletions causing infertility, including deletions of the entire *AZFc* region, were almost always *de novo*<sup>1,4,6,7,20–22</sup>. In all four instances in which the father of an infertile, *gr/gr*-deleted man was available for testing, however, we found that the father's Y chromosome was also *gr/gr*-deleted. We conclude that the penetrance of the spermatogenic failure caused by the *gr/gr* deletion is much lower than that of previously characterized Y-chromosomal deletions.

**Table 1** Genes and transcription units affected by deletions in the *AZFc* region

Gene or transcription unit	Reference Y chromosome	Numbers of copies present in:			
		<i>AZFc</i> (b2/b4)-deleted	<i>gr/gr</i> -deleted	<i>gr/gr</i> -deleted with b2/b4 duplication	b1/b3-deleted
<i>RBMY</i>	6	6	6	6	4
<i>BPY2</i>	3	0	2	4	2
<i>DAZ</i>	4	0	2	4	2
<i>CDY1</i>	2	0	1	2	2
<i>PRY</i>	2	2	2	2	0
<i>CSPG4LY</i>	2	0	1	2	2
<i>GOLGA2LY</i>	2	0	1	2	2
<i>TTY3</i>	2	0	1	2	2
<i>TTY4</i>	3	0	2	4	2
<i>TTY5</i>	1	1	1	1	0
<i>TTY6</i>	2	2	2	2	0
<i>TTY17</i>	3	0	2	4	2
Total	32	11	23	35	20

Modified from ref. 2.



**Y-chromosome haplotyping.** Individuals were haplotyped using the Y-linked polymorphisms listed in **Supplementary Table 6** and **Supplementary Table 7** online.

**GenBank accession numbers for STSs.** sY142, G38345; sY1161, G66148; sY1191, G73809; sY1197, G67168; sY1201, G67170; sY1206, G67171; sY1258, G75499; sY1291, G72340.

*Note: Supplementary information is available on the Nature Genetics website.*

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#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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