

Inadvertent diagnosis of male infertility through genealogical DNA testing

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J Med Genet 2005;42:366–368. doi: 10.1136/jmg.2004.023796

The potentially informative relationship between Y chromosomes and patrilineally inherited surnames¹ has led to a major expansion in the number of commercial companies offering Y chromosomal DNA polymorphism analysis to members of the public; because of the geographical specificity of Y chromosomal types,² many companies also offer to deduce “ancestry”. As the number of markers used in these tests increases, so does the probability of inadvertently diagnosing male infertility through the detection of Y chromosomal deletions. Using commercially typed Y markers, we here report the ascertainment of such deletions in general population samples.

METHODS

Samples were collected with informed consent and relevant ethical approval from the Leicestershire Research Ethics Committee (ref. 5796) and the Committee for Scientific Investigations in Greenland (ref. 505-16).

Deletion analysis was carried out using standard PCR techniques; primer sequences and conditions are given in original references cited for markers in the text below.

RESULTS

As part of a Y chromosomal haplotyping study of 2574 English males ascertained on the basis of surname and geographical origin, we included the binary marker PN25³ which defines an important haplogroup, R1b, common in Western Europe.⁴ The PN25 polymorphism is an A to C transversion in one of three copies of the PN25 sequence, which lie in the three ampliconic repeat units g1, g2, and g3³ of the *AZFc* region on Yq (fig 1A). In three unrelated males PN25 sequences were absent, and analysis of markers across the *AZFc* region showed a pattern of presence or absence consistent with these males carrying ~3 Mb deletions caused by non-allelic homologous recombination (NAHR) between the b2 and b4 repeats, previously observed in 47 of 48 infertile *AZFc* deletion patients.⁵

In a population study of 69 Greenlandic Inuit males,⁹ we typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome. In one male nine of 19 microsatellites and a single binary marker, M173, failed to amplify (fig 1B). Since all of these markers lie in the *AZFa* region on Yq, this is consistent with this male carrying an *AZFa* deletion. Testing of further loci in and around the region confirmed this, and showed that the deletion has arisen through a mechanism observed in the majority of *AZFa* cases,^{7 10 11} that is, by NAHR between directly repeated HERVs 780 kb apart and flanking the *AZFa* region.

DISCUSSION

AZF deletions are normally ascertained by testing the DNA of men with idiopathic infertility, and estimates of their frequencies are derived from clinical data. Here, we have ascertained deletions in an unbiased way.

Key points

- Commercial Y chromosome testing for genealogical purposes is increasing in popularity and is employing an increasing number of polymorphic markers, raising the possibility of the detection of Y chromosomal deletions in clients.
- Here we show that commercially used markers detect *AZFa* and *AZFc* deletions associated with male infertility in general population samples.
- Companies should avoid markers in the commonly deleted regions of the chromosome, and meanwhile their clients should be warned of the possibility and implications of the inadvertent diagnosis of infertility.

AZFc deletions are the commonest of the classes found in infertile men, with a frequency estimated to be 1 in 4000.⁵ We found three deletions in 2574 English males, and can add to these an additional 681 males (mostly from the Iberian peninsula) typed for PN25 in whom we would have expected to detect some deletions had there been any. The frequency we find, three in 3255, is not significantly different from 1 in 4000 ($p = 0.20$, Fisher exact test).

AZFa deletions are particularly rare, constituting 1–2% of all pathogenic Y chromosomal deletions,¹² and have a likely population frequency of less than 1 in 100 000.^{5 12} We found one deletion in 69 Inuit males, but including 5303 additional undeleted chromosomes from many, mostly Eurasian, populations typed with *AZFa* region microsatellites in our laboratory, the observed incidence is one case in 5374. A large database (see Roewer *et al*¹³ and <http://www.yhrd.org>) of ~23 000 Y chromosomal microsatellite haplotypes includes *DYS389*, and contributors would therefore be expected to detect *AZFa* deletions, although it is possible that such “incomplete” haplotypes would not be submitted. The database contains no examples with null alleles at this locus. Notably, we have found no examples of *AZFb* deletions, intermediate in frequency between *AZFa* and *c* deletions,¹⁴ in our population studies ($n = 5374$): these¹⁴ would be expected to lack several microsatellites, including the widely typed *DYS385* and *DYS392*.

While the typing of the binary marker PN25, in the *AZFc* region, is not being offered commercially, at least one major testing company types the highly informative multi-locus microsatellite, *DYS464*,¹⁵ lying within the r1–r4 ampliconic repeats, and also absent in the three *AZFc* males we have identified (fig 1A). Microsatellites within the *AZFa* and *b* regions are typed by all companies carrying out commercial Y

Abbreviations: NAHR, non-allelic homologous recombination; STS, sequence-tagged site

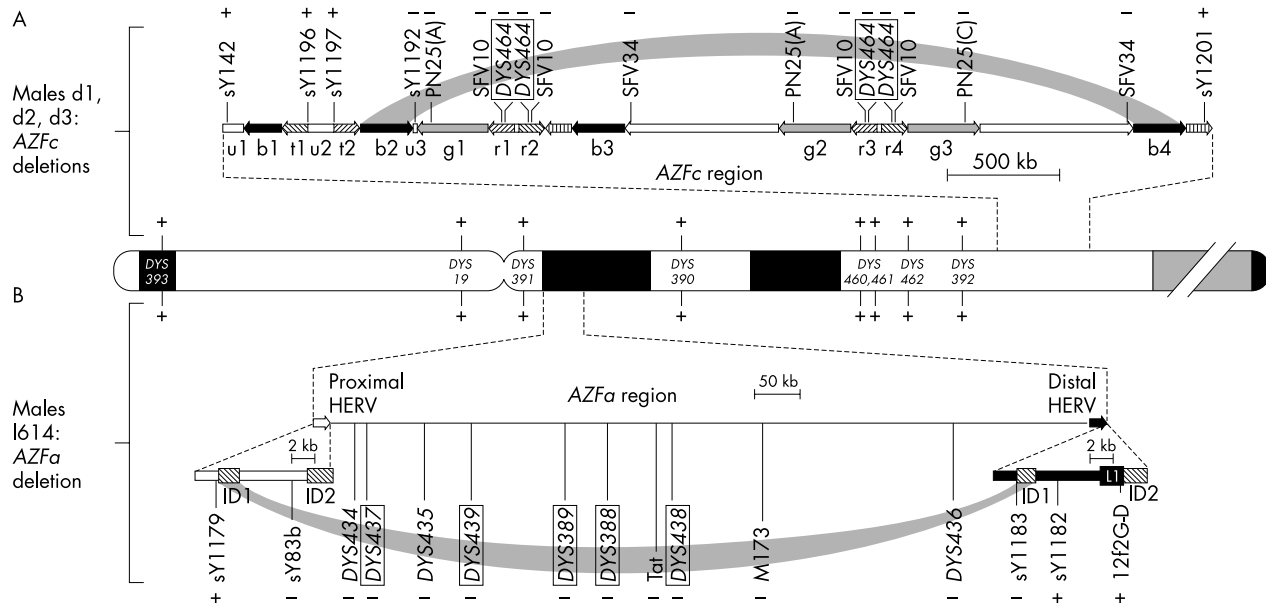


Figure 1 Detection of *AZFc* and *AZFa* deletions. The Y chromosome idiogram (centre) shows the approximate positions of the *AZFc* and *AZFa* regions and of eight Y-specific microsatellites.⁶ Throughout, “+” indicates marker present, “-” marker absent, and boxes around marker names indicate deleted markers that are typed by commercial companies. (A) Deletion analysis of males d1, d2, and d3. The binary marker PN25 and the microsatellite *DYS464* are absent, as are a set of sequence-tagged sites (STSs, prefixed “sY”)⁵ and sequence family variants (prefixed “SFV”)⁵ in the region consistent with deletion by NAHR between repeat units b2 and b4 (curved grey bar). (B) Deletion analysis of male l614. Binary markers M173 and Tat and a set of eight microsatellites (italics) are absent, as are STSs,^{7,8} consistent with deletion by NAHR between the ID1 identity blocks of the flanking human endogenous retroviral sequence (HERVs; curved grey bar). L1 indicates insertion of L1 material into distal HERV.

chromosome testing (fig 1B). Such testing will therefore lead to the detection of *AZF* deletions and thus an inadvertent diagnosis of likely infertility (some *AZFc* deleted males have been reported to father children^{16–18}). Recent identification¹⁹ of 166 new Y-specific microsatellites brings the total number known to over 200, and with so many to choose from it would be easy to avoid markers within the *AZF* intervals of the chromosome. There certainly seems no good reason for continued commercial typing of the *AZFc* marker *DYS464*, which in any case offers problems of interpretation because of its multilocal nature. Markers within the *AZFa* and *b* regions are so well established, however, that it is unlikely that they will be abandoned—a problem mitigated by the comparative rarity of these classes of deletions. Testing companies routinely inform their customers of the possibility of detecting non-paternities; while they continue to type the current set of markers, they should also warn that these markers are not neutral with respect to fertility.

ACKNOWLEDGEMENTS

We thank all DNA donors, and Søren Nørby for assistance.

ELECTRONIC-DATABASE INFORMATION



The URL of the Y-STR Haplotype Reference Database is <http://www.yhrd.org>.

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MAJ was supported by a Wellcome Trust Senior Fellowship in Basic Biomedical Science (grant no. 057559), TEK by a Wellcome Prize Studentship (grant no. 061129), and EB, SMA, and ZHR by the Wellcome Trust. EJP was supported by the Arts and Humanities Research

Board within the framework of the European Science Foundation EUROCORES programme “The Origin of Man, Language and Languages”.

Competing interests: none declared

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Received 8 June 2004

Revised version received 23 June 2004

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