LETTER TO JMG

Inadvertent diagnosis of male infertility through genealogical DNA testing T E King, E Bosch*, S M Adams, E J Parkin, Z H Rosser, M A Jobling

The potentially informative relationship between Y chromosomes and patrilinearly 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 g3⁵ 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.



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Key points

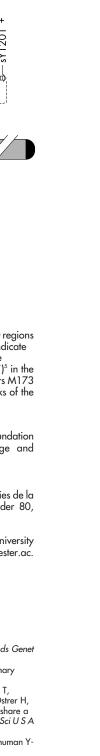
- Commercial Y chromosome testing for genealogical purposes is increasing in popularity and is employing an increasing number of polymorphic markers, raising the possibility of the detection of Y chromosomal deletions in clients.
- Here we show that commercially used markers detect *AZFa* and *AZFc* 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 al13 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



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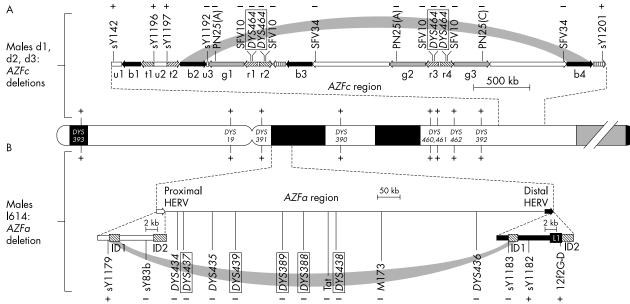


Figure 1 Detection of AZFc and AZFa deletions. The Y chromosome idiogram (centre) shows the approximate positions of the AZFc and AZFa regions and of eight Y-specific microsatellites.⁶ Throughout, "+" indicates marker present, "-" marker absent, and boxes around marker names indicate deleted markers 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 1614. Binary markers M173 and Tat and a set of eight microsatellites (italics) are absent, as are STss.⁷⁸ 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 children¹⁶⁻¹⁸). 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.

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ELECTRONIC-DATABASE INFORMATION



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

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REFERENCES

- Jobling MA. In the name of the father: surnames and genetics. Trends Genet 2001;17:353-7
- Jobling MA, Tyler-Smith C. The human Y chromosome: an evolutionary 2 marker comes of age. Nat Rev Genet 2003;4:598-612.
- 3 Hammer MF, Redd AJ, Wood ET, Bonner MR, Jarjanazi H, Karafet T, Santachiara-Benerecetti S, Oppenheim A, Jobling MA, Jenkins T, Ostrer H, Bonne-Tamir B. Jewish and Middle Eastern non-Jewish populations share a common pool of Y-chromosome biallelic haplotypes. Proc Natl Acad Sci USA 2000:97:6769-74.
- 4 Y Chromosome Consortium. A nomenclature system for the tree of human Y-
- chromosomal binary haplogroups. *Genome Res* 2002;**12**:339–48. **Kuroda-Kawaguchi T**, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S, Page DC. The AZFc 5 region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet* 2001;**29**:279–86.
- 6 Bosch E, Lee AC, Calafell F, Arroyo E, Henneman P, de Knijff P, Jobling MA. High resolution Y chromosome typing: 19 STRs amplified in three multiplex reactions. Forensic Sci Int 2002;125:42–51.
- Sun C, Skaletsky H, Rozen S, Gromoll J, Nieschlag E, Oates R, Page DC. Deletion of *azoospermia factor a* (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. *Hum Mol Genet* 2000.9.2291-6
- 8 Bosch E, Jobling MA. Duplications of the AZFa region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. *Hum Mol Genet* 2003;**12**:341–7.
- Bosch E, Calafell F, Rosser ZH, Norby S, Lynnerup N, Hurles ME, Jobling MA. High level of male-biased Scandinavian admixture in Greenlandic Inuit shown by Y-chromosomal analysis. *Hum Genet* 2003;**112**:353–63.

- 10 Blanco P, Shlumukova M, Sargent CA, Jobling MA, Affara N, Hurles ME. Divergent outcomes of intra-chromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. J Med Genet 2000;37:752–8.
- 11 Kamp C, Hirschmann P, Voss H, Huellen K, Vogt PH. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet* 2000;9:2563–72.
- 12 Affara NA. The role of the Y chromosome in male infertility. Exp Rev Mol Med 2001;3:1–16.
- 2 Soor, S. Ho. Roewer L, Krawczak M, Willuweit S, Nagy M, Alves C, Amorim A, Anslinger K, Augustin C, Betz A, Bosch E, Cagliá A, Carracedo A, Corach D, Dekairelle AF, Dobosz T, Dupuy BM, Füredi S, Gehrig C, Gusmaö L, Henke J, Henke L, Hidding M, Hohoff C, Hoste B, Jobling MA, Kärgel HJ, de Knijff P, Lessig R, Liebeherr E, Lorente M, Martínez-Jarreta B, Nievas P, Nowak M, Parson W, Pascali VL, Penacino G, Ploski R, Rolf B, Sala A, Schmidt U, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Kayser M. Online reference database of Y-chromosomal short tandem repeat (STR) haplotypes. Forensic Sci Int 2001;118:103–11.
- 14 Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, Page DC, Rozen S. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 2002;**71**:906–22.
- Redd AJ, Agellon AB, Kearney VA, Contreras VA, Karafet T, Park H, de Knijff P, Butler JM, Hammer MF. Forensic value of 14 novel STRs on the human Y chromosome. *Forensic Sci Int* 2002;**130**:97–111.
- 16 Chang PL, Sauer MV, Brown S. Y chromosome microdeletion in a father and his four infertile sons. Hum Reprod 1999; 14:2689–694.
- 17 Gatta V, Stuppia L, Calabrese G, Morizio E, Guanciali-Franchi P, Palka G. A new case of Yq microdeletion transmitted from a normal father to two infertile sons. J Med Genet 2002;39:e27.
- 18 Kühnert B, Gromoll J, Kostova E, Tschanter P, Luetjens CM, Simoni M, Nieschlag E. Case report: natural transmission of an AZFc Y-chromosomal microdeletion from father to his sons. *Hum Reprod* 2004;19:886–8.
- 19 Kayser M, Kittler R, Erler A, Hedman M, Lee AC, Mohyuddin A, Mehdi SQ, Rosser Z, Stoneking M, Jobling MA, Sajantila A, Tyler-Smith C. A comprehensive survey of human Y-chromosomal microsatellites. Am J Hum Genet 2004;74:1183–97.

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