Inadvertent diagnosis of male infertility through genealogical DNA testing

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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 PN25 which defines an important haplogroup, R1b, common in Western Europe. The PN25 polymorphism is an A to C transversion in one of three copies of the PN25 sequence, which lie in the three ampiclonic repeat units g1, g2, and g3 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 three 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. 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, and have a likely population frequency of less than 1 in 100 000. 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 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, in our population studies (n = 5374): these would be expected to lack several microsatellites, including the widely typed DYS389 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, lying within the r1–r4 ampiclonic 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 chromosome testing for genealogical purposes.

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.

Abbreviations: NAHR, non-allelic homologous recombination; STS, sequence-tagged site
chromosome testing (fig 1B). Such testing will therefore lead to the detection of AZF deletions and thus an inadvertent diagnosis of likely infertility (some AZF deleted males have been reported to father children\(^{16–18}\)). Recent identification\(^{8}\) 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 AZF 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-paternity; while they continue to type the current set of markers, they should also warn that these deleted markers that are typed by commercial companies. (A) Deletion analysis of males d1, d2, and d3. The binary marker PN25 and the satellite DYS464 are absent, as are a set of sequence-tagged sites (STSs, prefixed ‘‘sY’’) and sequence family variants (prefix ‘‘SFV’’) in the region consistent with deletion by NAHR between repeat units b2 and b4 (curved grey bar). (B) Deletion analysis of male I614. Binary markers M173 and Tat and a set of eight microsatellites (italics) are absent, as are STSs, 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.

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.\(^{10}\) 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’’) and sequence family variants (prefix ‘‘SFV’’) in the region consistent with deletion by NAHR between repeat units b2 and b4 (curved grey bar). (B) Deletion analysis of male I614. Binary markers M173 and Tat and a set of eight microsatellites (italics) are absent, as are STSs, 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.

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

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