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 patrilineally inherited surnames 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, 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 Leicester 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 ampliconic 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 ~3 Mb deletions caused by non-allelic homologous recombination (NAHR) between the b2 and b4 repeats, previously observed in 47 of 48 infertile AZFa deletion patients.

In a population study of 69 Greenlandic Inuit males, 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 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome. In one male nine of 19 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome. In one male nine of 19 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome.

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. 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 DYS392, 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 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, 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

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 patrilineally inherited surnames 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, 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 Leicester 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 ampliconic 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 ~3 Mb deletions caused by non-allelic homologous recombination (NAHR) between the b2 and b4 repeats, previously observed in 47 of 48 infertile AZFa deletion patients.

In a population study of 69 Greenlandic Inuit males, 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 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome. In one male nine of 19 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome. In one male nine of 19 typed a set of 19 Y-specific microsatellites and a number of binary markers on the Y chromosome.

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. 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 DYS392, 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 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, 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
Achromosome testing (fig 1B). Such testing will therefore lead to the detection of \textit{AZF} deletions and thus an inadvertent diagnosis of likely infertility (some \textit{AZF} deleted males have been reported to father children\textsuperscript{12–14}). Recent identification\textsuperscript{16} of 166 new \textit{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 \textit{AZF} intervals of the chromosome. There certainly seems no good reason for continued commercial typing of the \textit{AZF} marker \textit{DYS464}, which in any case offers problems of interpretation because of its multilocal nature. Markers within the \textit{AZFa} and \textit{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 markers are not neutral with respect to fertility.

**ACKNOWLEDGEMENTS**

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

**ELECTRONIC-DATABASE INFORMATION**

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

**Authors' affiliations**

T E King, E Bosch, S M Adams, E J Parkin, Z H Rosser, M A Jobling, Department of Genetics, University of Leicester, Leicester Road, Leicester LE1 7RH, UK

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

*Current address: Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Doctor Aiguader 80, 08003 Barcelona, Catalonia, Spain

Correspondence to: Mark A Jobling, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK; maj4@leicester.ac.uk

Received 8 June 2004

Revised version received 23 June 2004

**REFERENCES**

1. Jobling MA. In the name of the father: surnames and genetics. Trends Genet 2001;17:353–7
8. Bosch E, Jobling MA. Duplications of the \textit{AZF} region of the human \textit{Y} chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. Hum Mol Genet 2003;12:341–7


Clinical Evidence—Call for contributors

Clinical Evidence is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. Clinical Evidence needs to recruit a number of new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

Areas for which we are currently seeking authors:

- **Child health:** nocturnal enuresis
- **Eye disorders:** bacterial conjunctivitis
- **Male health:** prostate cancer (metastatic)
- **Women’s health:** pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

Being a contributor involves:

- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.

If you would like to become a contributor for Clinical Evidence or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Klara Brunnhuber (kbrunnhuber@bmjgroup.com).

Call for peer reviewers

Clinical Evidence also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge). Topics are usually 1500–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for Clinical Evidence, please complete the peer review questionnaire at www.clinicalevidence.com or contact Klara Brunnhuber (kbrunnhuber@bmjgroup.com).
Inadvertent diagnosis of male infertility through genealogical DNA testing

T E King, E Bosch, S M Adams, E J Parkin, Z H Rosser and M A Jobling

doi: 10.1136/jmg.2004.023796