



SHORT REPORT

Y-chromosomal DNA haplotype differences in control and infertile Italian subpopulations

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Y-chromosomal DNA haplotypes were determined in 74 infertile and 216 control Italian males using eight biallelic markers. A significant difference in haplotype frequency was found, but could be explained by the geographical origins of the samples. The Y chromosome is thus a sensitive marker for population substructuring and may be useful for determining whether two population samples come from a single population, for example in association studies.

Keywords: Y-chromosomal DNA haplotype; biallelic marker; SNP; male infertility; population substructure

Introduction

Infertility is estimated to affect 2–7%¹ or 10%² of males. In over 10% of infertile patients, representing almost 1% of the population, there is oligozoospermia or azoospermia with no obvious cause, described as 'idiopathic'. The Y chromosome carries several genes required for male fertility. *De novo* deletions of the *AZFa*, *AZFb* or *AZFc* loci on Yq together account for a proportion of idiopathic infertility cases, estimated at 3% (12/370) in one survey³ and 18% (6/33) in another,⁴ but the cause of infertility in most of the remaining cases is unknown. It is possible that other mutations or

genes on the Y chromosome are involved. Although mutations on the Y leading to infertility would not be transmitted to the next generation, a *predisposition* to such a mutation could be transmitted. Such a predisposition might be recognised directly through an understanding of its molecular mechanism, or indirectly through its occurrence on a particular haplotypic background. We have previously identified an association between one rare class of infertile males, *PRKX/PRKY* translocation XX males, and a particular Y haplotype.⁵ In this case, it was suggested that an inversion polymorphism on Yp which includes the *PRKY* gene allows efficient alignment and ectopic recombination with *PRKX* in one orientation but not in the other. We therefore set out to look for more general associations between Y haplotypes and infertility in a set of Italian patients and controls. Such associations would be important because they would indicate that novel Y defects or genes contribute to infertility, and also because they might reveal differential selective forces acting on Y haplotypes.

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Materials and Methods

DNA Samples

The infertile males were 74 patients with idiopathic infertility presenting at the University of Chieti. All patients were carefully examined in order to rule out any known cause of infertility and classified according to WHO criteria.⁶ Fifteen patients were azoospermic, 52 were oligozoospermic and the remaining seven had abnormal sperm morphology and/or motility. Deletions of Yq loci within interval 6 including the *DAZ* gene cluster⁷ were detected in two of the 55 individuals tested. 65 of the patients (88%) were from central Italy. Unrelated normal males used as controls were a set with proven fertility from central Italy ($n = 59$), and random males (> 90% expected to be fertile) from northwestern ($n = 99$) and northeastern ($n = 58$) Italy.

Markers

The biallelic Y markers used were the presence or absence of the Alu insertion YAP,⁸ and seven base substitution polymorphisms: *SRY*-8299 (G → A; 4064⁹), sY81 (A → G),¹⁰ M9 (C → G),¹¹ 92R7 (C → T)¹² in PCR format, *SRY*-1532 (A → G → A),¹³ *SRY*-2627 (C → T; also called *SRY*-2628¹⁴) and LLY22g/*Hind*III (C → A).¹⁵ All markers are located in the Y-specific portion of the chromosome. Those designated *SRY*-X are located 'X' bp upstream of the *SRY* gene on Yp; 92R7 is also on Yp.¹⁶ The other markers are located in Yq in intervals 5A (sY81), 5O (YAP, M9)¹⁷ or 17 (LLY22g/*Hind*III).¹⁶

Results

Y-chromosomal DNA haplotypes were determined for 290 Italian males. In a worldwide survey, the eight markers used could together distinguish ten different haplotypes, referred to as 'haplogroups', (¹⁸ and results not shown). A parsimony network illustrating the relationships between these haplogroups is shown in Figure 1a. Only six haplogroups (numbered in Figure 1a) were found in Italy. The two commonest were haplogroups 1 and 2, which differ at the loci M9 and 92R7. Overall, 57% (165/290) of the chromosomes tested carried the M9 C allele, while the remaining 43% (125/290) carried the G allele; with 92R7, 62% (180/290) carried the C allele, and the remaining 38% (110/290) carried the T allele (Table 1, Figure 1b). These frequencies are not, of course, independent. For this reason, we consider only the 92R7 results, but the same conclusions were reached using M9. There were statistically significant differences between the frequencies of the 92R7 alleles in the infertile males and three normal control populations grouped together; 73% (54/74) of the infertile males carried the 92R7 C, whilst only 58% (126/216) of the pooled controls carried the C. This difference is significant at the 5% level ($P = 0.027$, χ^2 or G-test).

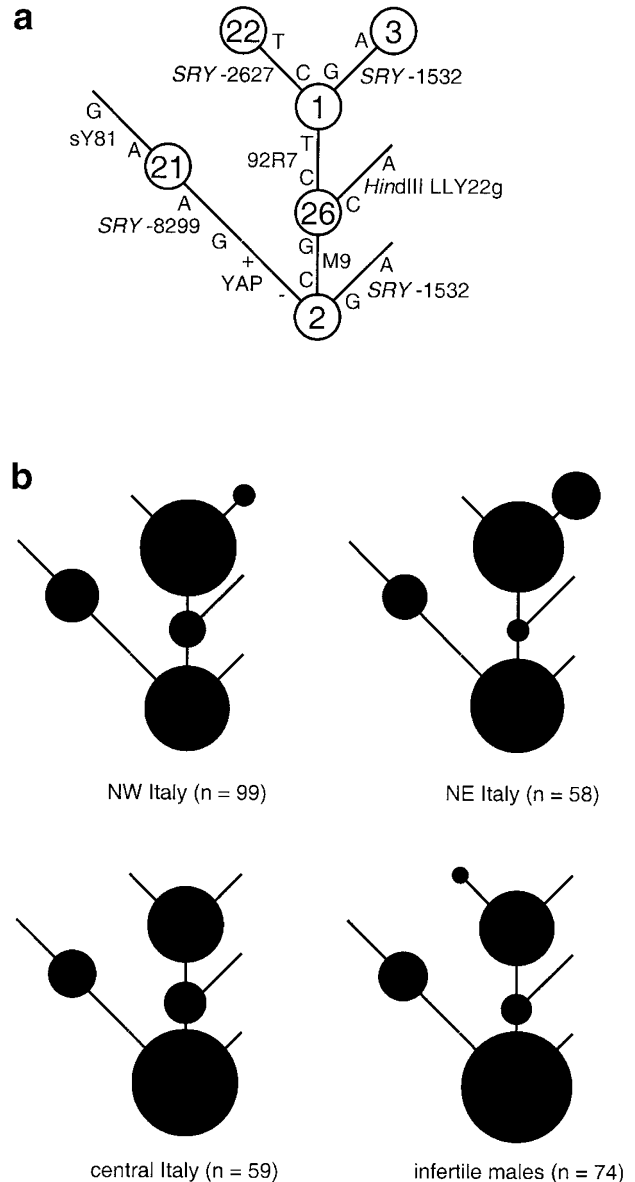


Figure 1 (a) Markers and the haplogroups they define. In the phylogenetic tree, the markers used are indicated along the lines, together with the two bases found, or the absence (–) or presence (+) of the retroposon insertion YAP. The haplogroups present in the Italian samples are indicated by numbers inside circles. (b) Relative frequencies of haplogroups in the Italian population samples. Frequency is proportional to the area of the circle.

Nevertheless, further examination of the data suggests that the frequency differences are due to geographical variation rather than a link between the C allele chromosomes and infertility. Most (65/74, 88%) of the infertile males came from central Italy. Among these, 48 (74%) carried the 92R7 C allele; among the control males from central Italy, 73% (43/59) carried the C. The difference is not statistically significant.

Table 1 Y-chromosomal DNA haplotypes of infertile and control males

Sample	n	Haplogroup					
		1	2	3	21	22	26
Infertile	74	19	43	–	8	1	3
Controls							
NW Italy	99	44	34	2	13	–	6
NE Italy	58	22	24	6	5	–	1
C Italy	59	16	32	–	6	–	5

Differences between the central Italian controls and either of the northern control populations were significant ($P = 0.014$, northwest; $P = 0.022$, northeast). The sample of infertile males from outside central Italy ($n = 9$) was too small for separate statistical analysis.

Discussion

The Y chromosome is known to show relatively high levels of geographical specificity compared with autosomal or mitochondrial DNA sequences, probably as a result of its small effective population size and/or a lower geographical mobility of males.^{11,19,20} Nevertheless, it is surprising to find such striking differences within the Italian population. A possible explanation could be that there are higher levels of inbreeding and, consequently, drift within the central Italian population sampled. Overall, these results suggest that the Y chromosome is the most sensitive locus in the genome to genetic differentiation, and could provide the best internal control to test whether two population samples, such as affected and control individuals in an association study, are well matched. Indeed, a comparison of Y haplotypes could be a useful initial step in any association study. A consequence of these findings is that associations between Y haplotypes and phenotypes will be particularly difficult to distinguish from population differences. Despite the lack of a significant difference between our samples of infertile and control males from central Italy, it remains possible that some Y haplotypes do predispose to infertility. However, a different strategy, such as comparing Y haplogroups among different molecular classes of Y deletion, may be required to reveal such predispositions.

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