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ORIGINAL ARTICLE

The association of Y chromosome haplogroups with spermatogenic failure in the Han Chinese

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Abstract A significant proportion of male infertility is accompanied by an abnormal semen analysis, azoospermia or severe oligozoospermia, which is generally assumed to be the result of spermatogenic failure. The genetic contribution in the process of spermatogenesis, particularly the role of the Y chromosome in determination of semen quality, is still obscure. In order to explore the relationship between Y chromosome haplogroup and spermatogenic failure, we collected 285 idiopathic infertile males with azoo-/oligozoospermia and 515 fertile men, adopted 12 binary markers and recruited the subjects (cases and controls) in the same region to test whether there is a possible susceptibility of certain Y haplogroups to spermatogenic failure in the Han Chinese population. The results indicated that the prevalences of *hg* K* in the control and the case population were 0.78% (4/515) and 2.80% (8/285), respectively. The difference between the frequencies of the *hg* K* in the infertile males and the normal control population was significant [odds ratio (OR) = 3.69; 95% confidence interval (CI) = 1.10–12.36] (P = 0.028). However, in the other haplogroups no significant differences were found. In conclusion, Y haplogroup-K* might bear a risk factor of male infertility, and the individuals in the haplogroup need to be further examined.

Keywords Y chromosome · Haplogroup · Male infertility · Biallelic marker · Spermatogenesis · Spermatogenic failure

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Introduction

Infertility affects an estimated 10–15% of couples, and roughly half of these cases can be traced to the man (Okabe et al. 1998). The important role of the human Y chromosome in the causation of male infertility is increasingly recognized. The male-specific region of the Y chromosome (MSY) contains totally 156 known transcription units, including 78 protein-coding genes (Skaletsky et al. 2003). Among these genes, many are testis-specific expressed and thought to be important in spermatogenesis and male fertility. For instance, the sex-determining gene on the Y chromosome (*SRY*) is well known to be crucial for testis formation (Okabe et al. 1998). And the genes of *RBM* (RNA-binding motif), *DAZ* (deleted in azoospermia) and *SPGY* (spermatogenesis gene on the Y) have been identified to be the candidate genes for azoospermia factor (*AZF*) (Foresta et al. 2001; Krausz et al. 2003). Though the biological functions of these genes in spermatogenesis have been elucidated, the roles of many other genes on the Y

chromosome were unknown. The polymorphisms of the Y chromosome genes, which are strictly paternally inherited and at low rate of back mutation, are particularly useful in understanding the functions of these genes in spermatogenesis and the importance of genetic background in male infertility.

Due to the lack of recombination of the non-recombining portion of the Y-chromosome (NRY) and the discovery of biallelic markers by using the techniques of denaturing high performance liquid chromatography (DHPLC) and single-stranded conformation polymorphism (SSCP) (Underhill et al. 1997, 2000, 2001), it is possible now to categorize diverse Y chromosomes into different Y haplogroups, within which the Y chromosomes derive from the same ancestor and share a similar Y genetic background. Thus, the differences of spermatogenic ability between haplogroups should be useful for further studies on the functional polymorphisms.

Related reports have been presented on Italian and Danish populations (Previdere et al. 1999; Krausz et al. 2001). By performing haplogroup, Krausz et al. (2001) identified an European Y haplogroup associated with reduced sperm count and raised the possibility that selection may be indeed active on the Y chromosome in Danish males. Previdere et al. (1999) found a significant difference of Y haplotype distribution between infertile males and healthy controls in Italians, which could also be explained by the geographical origins of the subjects. Thus, the cases and the controls should be matched with population substructure; otherwise, false-positive results would be occur. In Asian populations, only the Japanese have been studied, but the results were inconsistent (Carvalho et al. 2003; Kuroki et al. 1999). In order to unmask the relationship between the spermatogenic failure and Y chromosome haplogroup in the population of East Asian, this research was performed in the Han Chinese population by using additional unique event polymorphisms (UEP), eliminating the influence of geographic substructure and augmenting the sample size.

Materials and methods

Sample collection

We sampled 428 infertile men and 515 fertile men in this study, who all came from Nanjing and the neighboring suburban area. The patients with infertility were candidates for seeking treatment at the Center of Clinical Reproductive Medicine from April 2004 to April 2006 (NJMU Infertile Study) based on a retrospective design. The controls were healthy young men who had fathered at least one healthy child without assisted reproductive measures during the same period as those of the cases recruited in the same hospital. A scheduled interview was arranged for each subject to collect demographic data, past medical and surgical history, genetic risk factors and sexual and procreate state. After the interview, a 5-ml venous blood sample was collected. Genomic DNA was prepared from the venous blood by the phenol–chloroform method. These patients and healthy donors were all ethnically Han Chinese from East China. Informed consent was obtained from each subject. The study was approved by the Institutional Ethics Committee of Nanjing Medical University.

Semen analysis

Semen samples were obtained in a private room by masturbation into a sterile wide-mouth and metal-free glass container after a recommended 2-day sexual abstinence. After liquefaction at 37°C for 30 min, conventional semen analysis was conducted in accordance with guidelines of the WHO Laboratory Manual for the Examination of Human Semen (World Health Organization, 1999) including semen volume, sperm number per ejaculum, sperm concentration, motility, progression and motion parameters by using Micro-cell slide and computer-aided semen analysis (CASA, WLJY 9000, Weili New Century Science & Tech Dev.). In total, 428 infertile patients underwent semen analyses, a series of physical examinations and serum determination by karyotyping, which helped us to exclude 62 individuals: 3 obstructive azoospermic cases, 8 cases with karyotype abnormality (half of them with Klinefelter's syndrome), 4 with cryptorchidism and 47 secondary sterility cases. The remaining 366 idiopathic infertility patients were divided into three groups according to the WHO semen parameters (World Health Organization, 1999): 187 with non-obstructive azoospermia (no sperm in ejaculate even after centrifugation), 98 with oligozoospermia (sperm count from 0.1 to $20 \times 10^6 ml^{-1}$) and 81 with normozoospermia (sperm count $\geq 20 \times 10^6/ml^{-1}$). In this study, we chose 285 infertile males who were idiopathic azoo-/oligozoospermic as the patient group.

Y haplogroup and molecular analysis

Y chromosome lineages in the subjects were defined by using 12 biallelic markers: M130 (C to T), YAP(M1) (Alu insertion), M89 (C to T), M9 (C to G), M175 (5 bp deletion), M119 (A to C), M122 (T to C), M134 (1 bp deletion), M117 (4 bp deletion), M120 (T to C), M268 (A to G) and LLy22g (C to A)(Underhill et al. 2000; Sengupta et al. 2006; Shinka et al.1999). LLY22g was typed using the protocol kindly provided by Y. Xue and C. Tyler-Smith

(the Wellcome Trust Sanger Institute, personal communication). These markers are highly informative in East Asians (Jin and Su 2000, Jobling and Tyler-Smith 2003) and could be typed as described previously (Bergen et al. 1999; Hammer and Horai 1995; Underhill et al. 2000; Shen et al. 2000; Sengupta et al. 2006). In total, 12 haplogroups (Fig. 1) were defined following the nomenclature recommended by the Y Chromosome Consortium (2002) and its update described by Sengupta et al. (2006).

Statistical analysis

The final analysis included 285 cases and 515 controls. The differences of haplogroup distribution between the cases and the controls were evaluated by using Fisher's exact test, and the statistical significance levels of $P \leq 0.05$ were set. The associations between the Y chromosome haplogroups and risk of idiopathic male infertility with azoo-/oligospermia were estimated by computing the OR and 95% CI.

Results

Characteristics of the study subjects

The patient group included 285 idiopathic infertile males with azoo-/oligozoospermia who were chosen for this study; the ages ranged from 25 to 38. The control group included 515 fertile men aged 26–40 who had fathered at least one child without assisted reproductive measures. The controls were frequency-matched to the cases on age.

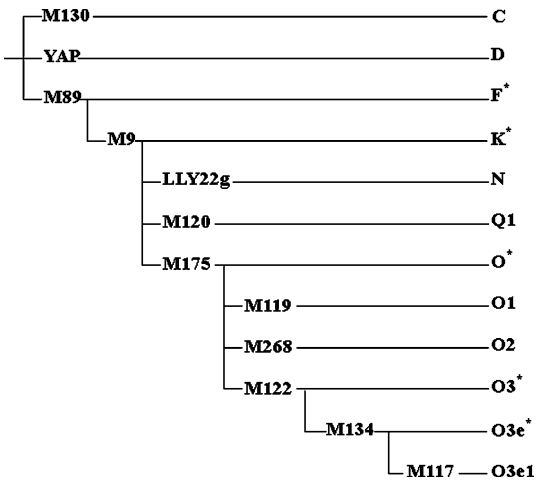

Fig. 1 The phylogenetic tree of Y-chromosomal haplogroups in the tested population. The phylogeny is based on that of the Y Chromosome Consortium (2002). The binary markers typed in this study are indicated in their defined branches. *Asterisks* indicate the paragroups that are not defined by the presence of a derived marker

Distributions of the Azoo-/oligozoospermic and the control population in Y chromosome haplogroups

To study the spermatogenic effects in different Y haplogroups in the Chinese, 285 idiopathic infertile males with azoo-/oligozoospermia and 515 fertile men were haplotyped by analysis 12 Y markers, which could classify 12 haplogroups. The prevalence of the respective haplogroup in the tested population is shown in Table 1. Since the Y haplogroup-O is predominant in the Asian population, we subdivided this haplogroup into more sub-haplogroups (Fig. 1). The prevalence of *hg* K* in the control population is 0.78% (4/515), while in the case population it is 2.80% (8/285). In *hg* K*, four controls (two with normozoospermia, two with semen parameters missing) were frequency-matched to the eight cases (five with azoospermia, three with oligozoospermia) on age. In addition, the difference between the frequencies of the *hg* K* in the infertile males and the normal control population is significant ($P = 0.028$, one-sided) (Table 2). The OR of the case group against the control group was 3.69 with a 95% CI of 1.10–12.36. In the other haplogroups, no significant differences of the haplogroup frequencies were detected between cases and controls.

Discussion

To detect the potential correlation between the Y haplogroup and spermatogenic failure, several factors must be taken into account: (1) the population stratification, (2) statistical analysis, (3) sample size and (4) the quantity of the sub-haplogroup. In an Italian population, Previdere et al. (1999) and his colleagues found a significant difference between the 92R7 alleles in the infertile males and the control population, but the difference can be attributed to the geographical origins of the samples. Considering the influence of population stratification, we collected the cases and controls from the same region. To date, in East Asia, only the Japanese have been studied. Kuroki et al. (1999) claimed to have found that the occurrence of azoospermia is related to one particular Y chromosome lineage, which is very common in Japan. However, there were only four sub-haplogroups (Shinka et al.1999). In this study, not only more sub-haplogroups were haplotyped, the majority, of which were located at the bottom of the phylogenetic tree, but also the samples size was increased to 800.

In human genetics, the individual of haplogroup K (M9) first appeared approximately 40,000 years ago in Iran or southern Central Asia. According to previous related reports, haplogroups K*(excluding N, Q1, O) were found only at low frequency in the Han Chinese population (Xue et al.2006). Though the sample size in this study was 800,

Table 1 Y haplogroup distributions in an unselected control Han Chinese population and the case population with azoo-/oligozoospermia

Group	No.	Y chromosome haplogroup										
		C	DE	F*	K*	N	O*	O1	O2*	O3*	O3e*	O3e1*	Q1
Azoo-/oligozoospermic population	285 (%)	18 (6.32)	2 (0.70)	2 (0.70)	8 (2.80)	19 (6.67)	3 (1.05)	50 (17.54)	22 (7.72)	80 (28.07)	31 (10.88)	42 (14.74)	8 (2.80)
Control population	515 (%)	41 (7.96)	8 (1.55)	2 (0.39)	4 (0.78)	30 (5.83)	8 (1.55)	89 (17.28)	41 (7.96)	135 (26.21)	69 (13.40)	72 (13.98)	16 (3.11)

Azoo-/oligozoospermia idiopathic infertile males with sperm count $<20 \times 10^6$ sperm/ml

Table 2 Distributions of the azoo-/oligozoospermic and the control population between *hg* K* and non-*hg* K* in Y chromosome haplogroups with different frequencies

Group	Case (n = 285)		Control (n = 515)		OR (95% CI)	P[a]
	Number	%	Number	%		
hg K*	8	2.80	4	0.78	3.69 (1.10–12.36)	0.028[b]
Non-*hg* K*	277	97.20	511	99.22		

[a] Fisher's exact test for the frequency of distribution between *hg* K*and non-*hg* K*

[b] $P \leq 0.05$, the difference of haplogroups distribution between *hg* K* and non-*hg* K* is significant

the individuals in *hg* K* were limited. If we perform the more detailed subdivision of the *hg* K* (excluding N, Q1, O), there will be few or even no individuals in each subgroup, and the results of statistical analysis will not be reliable. Overall, the difference between the frequencies of the *hg* K* in the idiopathic infertile males with azoo-/oligozoospermia and the normal control population has been detected in this study, while other haplogroups showed no significant differences. The results were consistent with previously studies in both Danish and Italian populations (Previdere et al. 1999; Krausz et al. 2001).

Although our data showed only Y haplogroup-K* to be associated with a higher predisposition to spermatogenic failure, much more importance should be attached to the role of a Y genetic background in spermatogenesis. Considering the probable heterogeneity of *hg* K*, we will subdivide the hg K* in the follow-up studies with augmented sample size, and the relationship will be further confirmed. Furthermore careful analysis with additional binary markers in multiple populations of different ethnic backgrounds, in conjunction with further association studies with augmented samples sizes and clinically well-defined individuals, could increase the statistical power of the tests, and then be helpful to reveal the potential association between Y chromosome haplogroups and male infertility.

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