GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association study between Y-chromosome haplogroups and susceptibility to spermatogenic impairment in Han People from southwest China

J. Ran, T.T. Han, X.P. Ding, X. Wei, L.Y. Zhang, Y.P. Zhang, T.J. Li, S.S. Nie and L. Chen

Institute of Medical Genetics,
Key Laboratory of Bio-Resources and Eco-Environment,
Ministry of Education, School of Life Sciences, Sichuan University,
Chengdu, P.R. China

Corresponding author: X.P. Ding
E-mail: brainding@scu.edu.cn

Genet. Mol. Res. 12 (1): 59-66 (2013)
Received April 13, 2012
Accepted June 24, 2012
Published January 22, 2013
DOI http://dx.doi.org/10.4238/2012.January.22.4

ABSTRACT. The non-recombining portion of the Y-chromosome contains numerous polymorphisms; therefore, it is now the most informative haplotyping system with wide-ranging applications. Idiopathic azoospermia and oligospermia are among the most important causes of male infertility. Different haplogroups may have different genetic backgrounds, which may be either susceptible or unsusceptible to idiopathic azoospermia or oligospermia. This study investigated the possible association between Y-chromosome haplogroup distribution and susceptibility to spermatogenic impairment. Peripheral blood was collected from 193 men with normozoospermia, 193 men with idiopathic azoospermia and 105 men with idiopathic oligospermia. All of the subjects underwent karyotyping, azoospermia factor (*AZF*) deletion analysis by 15 *AZF*-specific sequence-tagged sites and Y-chromosome haplotype analysis by 17 binary markers. Excluding men with *AZF*

deletions and abnormal karyotypes, the remainder of these 3 groups was named Group i, Group ii, and Group iii, respectively. The comparisons of 17 Y-haplogroup distributions between Group i and Group ii, Group iii or Group ii + iii were performed with the SPSS V.18.0 software. Significantly different Y-haplogroup distributions were observed between Group i and Group ii in N1* (P = 0.002), between Group i and Group iii in F*, K*, P*, and O3* (P = 0.002, 0.001, 0.004, and 0.007, respectively), and between Group i and Group ii + iii in K*, N1* and O3* (P = 0.008, 0.012, and 0.009, respectively). These results suggest that Y-chromosome haplogroups play a role in spermatogenic impairment.

Key words: Y-chromosome haplogroups; Spermatogenic impairment; *AZF* deletion; Idiopathic azoospermia/oligospermia

INTRODUCTION

Idiopathic azoospermia/oligospermia is a complicated, multigene-related and multicausal disease (Ferlin et al., 2006). Azoospermia factor (*AZF*) deletion is a dominant molecular etiology of idiopathic azoospermia/oligospermia. The non-recombining portion of the Y-chromosome (NRY) contains numerous polymorphisms, and some combination types of these normal mutations have been reported to be associated with partial *AZF* deletions (Yang et al., 2008). These findings suggest that variations in NRY may create a genetic background conferring susceptibility to idiopathic azoospermia/oligospermia.

In this study, to investigate the possible association between Y-chromosome genetic background and idiopathic azoospermia/oligospermia, we analyzed the distributions of 17 Y-haplogroups and 15 *AZF* deletions in 193 men with normozoospermia, 193 men with azoospermia and 105 men with oligospermia. All subjects were from southwest China and were of the same geographical origin.

MATERIAL AND METHODS

Subjects

Peripheral blood samples were collected from 193 men with normozoospermia (as controls), 193 men with idiopathic azoospermia and 105 men with oligospermia, which were named Group i, Group ii, and Group iii, respectively. The selection standard of subjects was conducted in accordance with the WHO manual for the standardized investigation, diagnosis and management of the infertile male. All 491 subjects were Han People recruited from southwest China. This research was approved by the Institutional Ethics Review Boards of Sichuan University, and all the subjects signed informed consent forms.

Karyotype analysis and *AZF* deletion detection

Karyotyping was performed on all the subjects to eliminate samples with chromosomal abnormalities.

Genomic DNA was extracted from peripheral blood samples using DNA isolation kits (Takara, Shiga, Japan). Fifteen *AZF*-specific sequence-tagged sites (STSs: sY84 and sY86 for *AZFa*; sY127, sY134, sY121, sY124, and sY133 for *AZFb*; sY254, sY255, sY157, sY242, and sY277 for *AZFc*, and sY145, sY152, and sY153 for *AZFd*) were used to detect *AZF* deletions. The PCR products were separated by electrophoresis on a 2% agarose gel. The primer sequences and PCR conditions have been described previously (Kent-First et al., 1999).

Y-chromosome haplogroup analysis

Seventeen highly informative NRY binary markers for East Asians were used to identify 17 haplogroups (Su et al., 1999; Jin and Su, 2000), and the phylogenetic tree named the Y-Chromosome Consortium (YCC, 2002) is shown in Figure 1. DNA segments containing the 17 markers (YAP, M15, M130, M89, M9, M45, M175, M95, M119, M120, M88, M110, M122, M7, M134, M117, and LLY22g) were amplified with the previously described primers (Karafet et al., 2008) shown in Table S1. PCR was carried out in a 25-µL reaction volume containing 50 ng genomic DNA, 1.5 U *Taq* DNA polymerase (Fermentas International Inc., Burlington, Canada), 1X PCR buffer (Fermentas International Inc.), 25 mM $MgCl_2$ (Fermentas International Inc.), 2 mM of each dNTP (TransGen, Beijing, China), and 1 mM forward and reverse primers (Invitrogen, Beijing, China). Samples were amplified on an MG96G thermocycler (LongGene Scientific Inc., Hangzhou, China) with the following conditions: 94°C for 5 min; 32 cycles of 94°C for 30 s, 56°C for 1 min, 72°C for 1 min, and a final 10-min extension at 72°C.

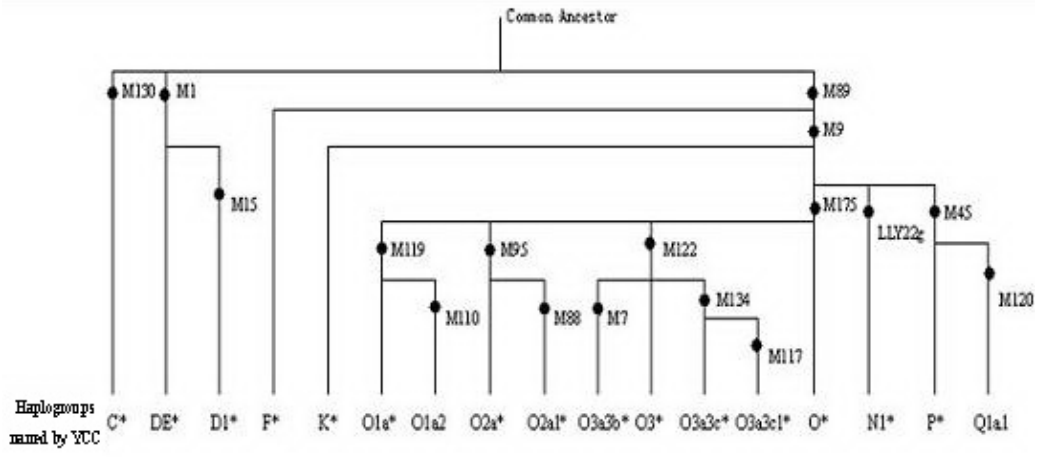

Figure 1. Phylogenetic tree of Y-chromosome haplogroups named by the Y-Chromosome Consortium (YCC). (*) = haplogroup is not monophyletic.

Fifteen binary markers (M15, M130, M89, M9, M45, M175, M95, M119, M120, M88, M110, M122, M7, M134, and M117) were detected by allele-specific primer extension (ASPE), and the segments containing these 15 markers were used for templates after treatment. The treatment reaction was carried out in a 7.5-µL reaction

volume containing 5 µL PCR product, 2 U shrimp alkaline phosphatase (Fermentas International Inc.), and 10 U exonuclease I (Fermentas International Inc). Samples were treated at 37°C for 15 min and 80°C for 15 min; then, 1 µL resulting product was diluted by 1:35 times.

The PCR products of the YAP marker were separated by electrophoresis on a 2% agarose gel, and the binary marker LLY22g was detected by sequencing the PCR products. The ASPE primer sequences are shown in Table 1. The ASPE was carried out in a 15-µL reaction volume containing 2 µL PCR product, 1.25 U TrueStart™ Hot Start *Taq* DNA Polymerase (Fermentas International Inc.), 1X TrueStart™ *Taq* buffer (Fermentas International Inc.), 25 mM $MgCl_2$ (Fermentas International Inc.), 2 mM of each dNTP (TransGen), and 0.8 mM primer A-1/A-2 and primer B, or primer A and primer B-1/B-2 (Invitrogen). Samples were amplified on an MG96G thermocycler (LongGene Scientific Inc.) with the following conditions: 94°C for 3 min; 10 cycles of 94°C for 30 s, 57°C for 1 min, and 72°C for 50 s; 20 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 50 s, and a final 8-min extension at 72°C. The products were separated by electrophoresis on a 2% agarose gel.

Table 1. Allele-specific primer extension sequences.

SNPs	Primers	SNPs	Primers
YAP	-	LLY22g	-
M7	Primer A: AATGCCTGATTTTCTCCCT	M117	Primer A-1: ACCAAAGGAATGCACATCTATCT
	Primer B-1: TGTAGTTGAGTTACTGTTCTTCTTG		Primer A-2: CCAAAGGAATGCACATCTGC
	Primer B-2: TGTAGTTGAGTTACTGTTCTTCTTC		Primer B: TGAAATTATTTTTTCAGAAAGCAGA
M9	Primer A-1: CGGCCTAAGATGGTTGAATC	M119	Primer A: GAATGCTTATGAATTTCCCAG
	Primer A-2: CGGCCTAAGATGGTTGAATG		Primer B-1: TCCAATTCACCATACAGGCT
	Primer B: AAAACCTAACTTTGCTCAAGC		Primer B-2: TCCAATTCACCATACAGGCG
M15	Primer A-1: CACCTAGGGAGACATTGTACAGAG	M120	Primer A: GAACTTGGACTTTAGGACGG
	Primer A-2: ACTGCACCTAGGGAGACTGGC		Primer B-1: TTCCCTTAAAAACAGCATGA
	Primer B: TGCATGTGGTTAAAATTTCC		Primer B-2: TTCCCTTAAAAACAGCATGG
M45	Primer A: CAACACAAGGATTCAGGCTC	M122	Primer A-1: AAAAGCAATTGAGATACTAATTCAT
	Primer B-1: ATTGGCAGTGAAAAATTATAGAGAA		Primer A-2: AAAAGCAATTGAGATACTAATTCAC
	Primer B-2: ATTGGCAGTGAAAAATTATAGAGAG		Primer B: CAGCGAATTAGATTTTCTTGC
M88	Primer A: GGTCAGGCAACTAGGGAATAC	M130	Primer A: GATTTTGTTGGGTGGTGGT
	Primer B-1: GAGGAAAACCTATCTTGGCTAT		Primer B-1: CACTTTACCCTGGGCTGG
	Primer B-2: GAGGAAAACCTATCTTGGCTAC		Primer B-2: CACTTTACCCTGGGCTGA
M89	Primer A: TCATCCCCTGGTCAGTTTT	M134	Primer A-1: AAAGGCCCAGGAAAGTATG
	Primer B-1: AAGTCAGGCAAAGTGAGAGATG		Primer A-2: AAAAGGCCCAGGAAAGTATA
	Primer B-2: CAAGTCAGGCAAAGTGAGAGATA		Primer B: TGGCTTCTCTTTGAACAGGT
M95	Primer A: AACCCCACTTTCACAACATT	M175	Primer A-2: CACATGCCTTCTCACTTCTCAA
	Primer B-1: GAAAGGCTAAGCCATCCAG		Primer A-2: CACATGCCTTCTCACTTCTCTTC
	Primer B-2: GAAAGGCTAAGCCATCCAA		Primer B: CTCCATTCTTAACTATCTCAGGGA
M110	Primer A: CAGGGAAGGACCGTAAAAG		
	Primer B-1: TGCAACAGCTTACAAGAACATA		
	Primer B-2: TGCAACAGCTTACAAGAACATG		

SNPs = single nucleotide polymorphims.

Statistical analysis

Data regarding the Y-chromosome haplogroup distribution of the experimental groups were compared with respect to the control group using an exact test. The comparison between the individual Y-haplogroup distribution of the control group and that of each experimental group was performed by the chi-square test with the SPSS V.18.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Karyotype analysis and *AZF* deletion detection

According to the karyotyping results, 20 abnormal cases were found, including 2 men (both with a karyotype of 47,XY) in Group iii and 18 men (1 with a karyotype of 45,XO/46,XY, 1 with a karyotype of 46,XY,t(Xp:16q), 1 with karyotype of a small Y and 15 with karyotypes of 47,XY) in Group ii.

None of the men had *AZF* deletions in Group i, 18 men had *AZF* deletions in Group ii, and 8 men had *AZF* deletions in Group iii. *AZF*-specific STSs, on which these *AZF* deletions were based, are shown in Table 2.

Table 2. Sequence-tagged sites and azoospermia factor (*AZF*) deletions.

Clinical situation	*AZFa*	*AZFb*	*AZFc*	*AZFd*	Karyotype	Count	Total
Azoospermia			sY254, sY255		46,XY	4 (2.07%)	18 (9.33%)
			sY157, sY242, sY254, sY255		46,XY	2 (1.04%)	
	sY86		sY157, sY242, sY254, sY255		46,XY	1 (.518%)	
	sY86				46,XY	1 (.518%)	
			sY157		46,XY	2 (1.04%)	
		sY121, sY127, sY134	sY157, sY242, sY254, sY255		46,XY	3 (1.55%)	
		sY134			46,XY	1 (.518%)	
		sY127, sY134			46,XY	2 (1.04%)	
		sY121, sY127, sY134	sY157, sY242, sY254, sY255	sY145	45,XO/46,XY	1 (.518%)	
		sY127, sY134	sY157, sY254, sY255		46,XY small Y	1 (.518%)	
Oligospermia			sY157, sY242, sY254, sY255		46,XY	4 (3.74%)	8 (7.48%)
			sY242		46,XY	1 (0.935%)	
			sY254, sY255		46,XY	2 (1.87%)	
			sY242, sY254, sY255		46,XY	1 (0.935%)	

Deletions based on sY84, sY124, sY133, sY277, sY152, and sY153 were not found. Single *AZFd* deletion was not found in the present study.

Y-haplogroup distribution analysis

Excluding the men with *AZF* deletion or abnormal karyotypes, there were 193 men with normozoospermia, 159 men with azoospermia and 97 men with oligospermia in our cohort. We named these three groups as Group i, Group ii, and Group iii, respectively. Group i was considered the control group. We observed population differences in Y-haplogroups between Group i and Group ii, Group iii and Group ii + iii (P = 0.012, 0.000, and 0.000, respectively). The comparison between the individual Y-haplogroup distribution in each experimental group and that of the control group are shown in Table 3. After Bonferroni's correction, results of the multiple significance test were as follows: 1) a significant difference in Y-haplogroup distribution was observed between Group i and Group ii in haplogroups N1* (P = 0.002), and the frequency of haplogroup N1* was higher in Group ii (10.7%) than that of Group i (2.6%); 2) a significant difference in Y-haplogroup distribution was found between Group i and Group iii in haplogroups F*, K*, P*, and O3* (P = 0.002, 0.001, 0.004, and 0.007, respectively), and the frequencies of F*, K*, and P* were higher in Group iii (9.3, 12.4, and 7.2%, respectively) than those of Group i (1.0, 2.6, and 0.5%, respectively), whereas the frequency of O3* was lower (13.4% in Group iii and 27.5% in Group i), and 3) a signifi-

cant difference was also observed between Group i and Group ii + iii in haplogroups K*, N1*, and O3* (P = 0.008, 0.012, and 0.009, respectively). In contrast to Group i, the frequencies of haplogroups K* and N1* were higher in Group ii + iii (8.6 and 8.2%, respectively, in Group ii, both 2.6% in Group i), whereas the frequency of haplogroup O3* was lower (17.2% in Group ii + iii and 27.5% in Group i).

Table 3. Y-haplogroup distribution.

Y-haplogroups	Groups studied				P		
	Group ii	Group iii	Group ii + iii	Group i	Group ii	Group iii	Group ii + iii
C*	3 (1.9%)	1 (1.0%)	4 (1.6%)	7 (3.6%)	0.512	0.372	0.275
DE*	3 (1.9%)	0	3 (1.2%)	1 (0.5%)	0.484	0.666	0.824
D1*	5 (3.1%)	2 (2.1%)	7 (2.7%)	0	0.018	0.111	0.019
F*	4 (2.5%)	9 (9.3%)	13 (5.1%)	2 (1.0%)	0.531	0.002[b]	0.018
K*	10 (6.3%)	12 (12.4%)	22 (8.6%)	5 (2.6%)	0.087	0.001[b]	0.008[c]
P*	0	7 (7.2%)	7 (2.7%)	1 (0.5%)	0.548	0.004[b]	0.184
N1*	17 (10.7%)	4 (4.1%)	21 (8.2%)	5 (2.6%)	0.002[a]	0.725	0.012[c]
Q1a1	1 (0.6%)	0	1 (0.4%)	2 (1.0%)	1.000	0.442	0.805
O*	15 (9.4%)	8 (8.2%)	23 (9.0%)	23 (11.9%)	0.455	0.340	0.310
O1a*	13 (8.2%)	11 (11.3%)	24 (9.4%)	20 (10.4%)	0.484	0.799	0.727
O1a2	6 (3.8%)	2 (2.1%)	8 (3.1%)	5 (2.6%)	0.744	1.000	0.738
O2a*	8 (5.0%)	4 (4.1%)	12 (4.7%)	11 (5.7%)	0.783	0.568	0.630
O2a1*	1 (0.6%)	0	1 (0.4%)	3 (1.6%)	0.757	0.293	0.428
O3*	31 (19.5)	13 (13.4%)	44 (17.2%)	53 (27.5%)	0.081	0.007[b]	0.009[c]
O3a3b*	12 (7.5%)	6 (6.2%)	18 (7.0%)	18 (9.3%)	0.552	0.360	0.375
O3a3c*	27 (17.0%)	15 (15.5%)	42 (16.4%)	29 (15.0%)	0.618	0.922	0.691
O3a3c1*	3 (1.9%)	1 (1.0%)	4 (1.6%)	8 (4.1%)	0.366	0.278	0.093
Total	159	95	254	193			

In the comparison of each individual Y-haplogroup, three groups were compared with the same control group respectively. Therefore, according to the formula $\alpha' = \alpha / (k - 1)$ ($k = 4$ in the present study), $\alpha' = 0.017$. After Bonferroni's correction, $P < 0.017$ was regarded as statistically significant in this study. [a]P = 0.002 *vs* Y-haplogroup N1* in Group i. [b]P = 0.002, 0.001, 0.004, and 0.007 *vs* Y-haplogroups F*, K*, P*, and O3* in Group i, respectively. [c]P = 0.008, 0.012 and 0.009 *vs* Y-haplogroups K*, N1* and O3* in Group i, respectively.

DISCUSSION

AZF, located on the long arm of the Y-chromosome, was divided into 3 regions: *AZFa*, *AZFb*, and *AZFc* in 1996 (Vog et al., 1996). Kent-First et al. (1999) found a 4th *AZF* region between *AZFb* and *AZFc* in 1999, and termed it *AZFd*. *AZF* deletions are an important etiology responsible for spermatogenic impairment (Krausz and Degl'Innocenti, 2006). The clinical symptoms caused by *AZFd* deletion(s) are less serious than those caused by *AZFa*, *AZFb*, or *AZFc* deletion(s). According to the European Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) guidelines, 6 STSs (sY84 and sY86 for *AZFa*, sY127 and sY134 for *AZFb*, and sY254 and sY255 for *AZFc*) are recommended for the detection of *AZF* deletions that are termed classical *AZF* deletions (Simoni et al., 2004). In this study, we added another 9 STSs (sY121, sY124, and sY133 for *AZFb*; sY157, sY242, and sY277 for *AZFc*, and sY145, sY152, and sY153 for *AZFd*) for *AZF* deletion detection, with the aim of further segregating subjects with *AZF* deletions. Individual *AZFd* deletion was not detected in any of these subjects, which might be caused by the choice of samples. The subjects we studied had idiopathic azoospermia and oligospermia, although the *AZFd* deletion is related to teratozoospermia and mild oligospermia (Maurer et al., 2001).

The frequency of Y-chromosome microdeletions in infertile men ranges from 5 to 20% in worldwide surveys (Vogt, 1998; Krausz and McElreavey, 1999; Krausz et al., 2003). In this study, the incidences observed in Group ii, Group iii and Group ii + iii were 9.33, 7.48, and 8.67%, respectively. In contrast to classical *AZF* deletions, the *AZFc* subdeletions appear to be more complicated and have regional differences. In the study conducted by Wu et al. (2007), the b1/b3 deletion was completely absent in the studied Han-Chinese population, whereas the gr/gr subdeletion and b2/b3 subdeletion displayed higher frequencies than those of other populations.

In the human genome, the Y-chromosome contains the longest non-recombining portion (Lahn et al., 2001). Since the polymorphisms in the NRY appeared in chronological order and had low rates of back mutation, they are suitable for identifying stable paternal lineages. The Y-chromosome haplotyping system is now the most informative haplotyping system, which has been used in evolutionary studies, forensics, medical genetics, and genealogical reconstruction (Y Chromosome Consortium, 2002). The YCC (http://ycc.biosci.arizona.edu/) published a phylogenetic tree with 153 haplogroups identified by 243 binary markers and devised a standardized nomenclature system for it in 2002. These 153 haplogroups were distributed into 18 major clades identified by the capital letters (A-R; Y Chromosome Consortium, 2002). In 2008, the YCC reshaped the tree; the revised tree showed the relationship between 311 haplogroups based on approximately 600 binary markers, and increased the size of 2 major clades (S and T; Karafet et al., 2008).

Different Y-haplogroups may create different genetic backgrounds, which may be associated with spermatogenic impairment. Some studies reported that haplogroups K* and D* might be linked with susceptibility to spermatogenic impairment (Kuroki et al., 1999; Krausz et al., 2001). In this study, a significant difference of Y-haplogroup distribution was observed between Group i and Group ii in haplogroup N1*, between Group i and Group iii in haplogroups F*, K*, P*, and O3*, and between Group i and Group ii + iii in haplogroups K*, N1*, and O3*. Among these 5 Y-haplogroups, the frequencies of haplogroups F*, K*, P*, and N1* were higher in experimental groups than those of the control group, whereas the frequency of haplogroup O3* was lower in experimental groups than that of the control group. These findings suggested that the Y-haplogroups F*, K*, P*, and N1* may confer Y-chromosome backgrounds that are susceptible to spermatogenic impairment. On the contrary, the Y-haplogroup O3* may create a Y-chromosome background that is less susceptible to spermatogenic impairment. The mechanism by which these Y-haplogroups function is not yet clear. We hypothesize that binary markers, which these Y-haplogroups based on, may cause some small differences in the Y-chromosome DNA structure from other Y-haplogroups. These small differences may promote some spermatogenesis-linked gene mutations or protect some spermatogenesis-linked genes from mutation during DNA replication. On the other hand, these small differences could regulate the expression of some spermatogenesis-linked genes, rendering them either overexpressed or underexpressed, or even completely inhibited. However, the molecular mechanism of this theory demands further studies.

ACKNOWLEDGMENTS

Research supported by the Institute of Medical Genetics, Key Laboratory of Bio-Resources and Eco-Environment, Ministry of Education. We are grateful to the volunteers who participated in this study.

Supplementary material

REFERENCES

Ferlin A, Arredi B and Foresta C (2006). Genetic causes of male infertility. *Reprod. Toxicol.* 22: 133-141.

Jin L and Su B (2000). Natives or immigrants: modern human origin in east Asia. *Nat. Rev. Genet.* 1: 126-133.

Karafet TM, Mendez FL, Meilerman MB, Underhill PA, et al. (2008). New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. *Genome Res.* 18: 830-838.

Kent-First M, Muallem A, Shultz J, Pryor J, et al. (1999). Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. *Mol. Reprod. Dev.* 53: 27-41.

Krausz C and McElreavey K (1999). Y chromosome and male infertility. *Front Biosci.* 4: E1-E8.

Krausz C and Degl'Innocenti S (2006). Y chromosome and male infertility: update, 2006. *Front Biosci.* 11: 3049-3061.

Krausz C, Quintana-Murci L, Rajpert-De ME, Jorgensen N, et al. (2001). Identification of a Y chromosome haplogroup associated with reduced sperm counts. *Hum. Mol. Genet.* 10: 1873-1877.

Krausz C, Forti G and McElreavey K (2003). The Y chromosome and male fertility and infertility. *Int. J. Androl.* 26: 70-75.

Kuroki Y, Iwamoto T, Lee J, Yoshiike M, et al. (1999). Spermatogenic ability is different among males in different Y chromosome lineage. *J. Hum. Genet.* 44: 289-292.

Lahn BT, Pearson NM and Jegalian K (2001). The human Y chromosome, in the light of evolution. *Nat. Rev. Genet.* 2: 207-216.

Maurer B, Gromoll J, Simoni M and Nieschlag E (2001). Prevalence of Y chromosome microdeletions in infertile men who consulted a tertiary care medical centre: the Munster experience. *Andrologia* 33: 27-33.

Simoni M, Bakker E and Krausz C (2004). EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int. J. Androl.* 27: 240-249.

Su B, Xiao J, Underhill P, Deka R, et al. (1999). Y-Chromosome evidence for a northward migration of modern humans into Eastern Asia during the last Ice Age. *Am. J. Hum. Genet.* 65: 1718-1724.

Vogt PH (1998). Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update. *Mol. Hum. Reprod.* 4: 739-744.

Vogt PH, Edelmann A, Kirsch S, Henegariu O, et al. (1996). Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet.* 5: 933-943.

Wu B, Lu NX, Xia YK, Gu AH, et al. (2007). A frequent Y chromosome b2/b3 subdeletion shows strong association with male infertility in Han-Chinese population. *Hum. Reprod.* 22: 1107-1113.

Y Chromosome Consortium (2002). A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res.* 12: 339-348.

Yang Y, Ma M, Li L, Zhang W, et al. (2008). Y chromosome haplogroups may confer susceptibility to partial AZFc deletions and deletion effect on spermatogenesis impairment. *Hum. Reprod.* 23: 2167-2172.