

## RESEARCH COMMUNICATION

# Linkage between Prostate Cancer Occurrence and Y-Chromosomal DYS Loci in Malaysian Subjects

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### Abstract

**Purpose:** Prostate cancer differs markedly in incidence across ethnic groups. Since this disease is influenced by complex genetics, it is many genetic factors may affect the level of susceptibility to development of the disease. In this study, four Y-linked short tandem repeats (STRs), DYS388, DYS435, DYS437, and DYS439, were genotyped to compare Malaysian prostate cancer patients and normal control males. **Materials and methods:** A total of 175 subjects comprising 84 patients and 91 healthy individuals were recruited. Multiplex PCR was optimized to co-amplify DYS388, DYS435, DYS437, and DYS439 loci. All samples were genotyped for alleles of four DYS loci using a Genetic Analysis System. **Results:** Of all DYS loci, allele 10 (A) of DYS388 had a significantly lower incidence of disease in compare with other alleles of this locus, while a higher incidence of disease was found among males who had either allele 12 (C) of DYS388 or allele 14 (E) of DYS439. Moreover, a total of 47 different haplotypes comprising different alleles of four DYS loci were found among the whole study samples, of which haplotypes AABC and CAAA showed a lower and higher frequency among cases than controls, respectively. **Conclusions:** It is likely that Malaysian males who belong to Y-lineages with either allele 12 of DYS388, allele 14 of DYS439, or haplotype CAAA are more susceptible to develop prostate cancer, while those belonging to lineages with allele 10 of DYS388 or haplotype AABC are more resistant to the disease.

**Keywords:** Prostate cancer - microsatellite loci - Y chromosome - haplotypes

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### Introduction

Based on the annual world cancer report, prostate cancer has become the second leading cancer among men in the world with more than half a million new cases each year (World Cancer Report, 2008). Epidemiological data suggest that prostate cancer is a multi-factorial disease influenced by both genetic and environmental components (Nwosu et al., 2001; Larson et al., 2005). Hence, deciphering the genetic factors that play a role in this disease would provide us with the ability for screening, prevention, and even diagnosis and treatment (Larson et al., 2005). Attempts have been done to find different genes that influence prostate cancer (Latil et al., 1995; Witte et al., 2000; Xu, 2000; Tavtigian et al., 2001; Carpten et al., 2002; Xu et al., 2002; Dong et al., 2003; Edwards et al., 2003; Ntais et al., 2003a; Ntais et al., 2003b; Paiss et al., 2003; Huang et al., 2004; Camp et al., 2005; Larson et al., 2005; Camp et al., 2007; Chun et al., 2009). However, because of the multi-factorial nature of this disease and the strong evidences regarding its different incidences among different racial groups, it is supposed that there

are some underlying genetic factors in some lineages that would potentially increase the susceptibility or resistance to develop this cancer (Kehinde et al., 2005).

Microsatellite loci are Short Tandem Repeats (STRs) mostly located on non-coding parts of genome (Chambers and MacAvoy, 2000). They have extensively been used in population genetic studies due to their high level of polymorphism, which render them as a powerful tool to test the variation among different populations (Kayser et al., 2003). DYS (DNA Y-chromosome Segments) loci, STRs on Y-chromosome, have extensively been used as genetic markers to investigate human evolution, forensic and many other population genetic studies (Underhill et al., 2000). Studies conducted on these markers have shown that men from different racial backgrounds have different Y-chromosomal characteristics that may affect their susceptibility or resistance to different diseases (Ewis et al., 2006).

In this study, we compared the allele and haplotype frequencies of four Y-chromosomal STRs comprising DYS388, DYS435, DYS437, and DYS439 among prostate cancer patient and healthy control groups in Malaysian

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population. We hypothesized that there are some DYS lineages among Malaysian populations with significant different frequencies between prostate cancer and healthy control people, indicating that belonging to these lineages would potentially increase the level of susceptibility or resistance to prostate cancer. The hypothesis was preliminary based on consistent evidences obtained from Japanese populations in Japan (Ewis et al., 2002) and the United States (Paracchini et al., 2003).

## Materials and Methods

The study group included a total of 175 subjects comprising 84 men with prostate cancer (36 Malays, 44 Chinese, and 4 Indians) and 91 healthy male individuals as controls (40 Malaysian-Malays, 47 Malaysian-Chinese, and 4 Malaysian-Indians). Samples were collected through Clinic of Surgery, Hospital University of Malaya (HUM), Kuala Lumpur, Malaysia. All patients participated in the study were males over 40 years old who had been referred to the hospital for treatment because of advanced level of cancer. For the controls, males over 40 years old who were healthy with a PSA level <2 ng/ml were recruited. The Ethic clearance for this study was acquired from Medical Ethics Committee, School of Medicine, University of Malaya (Reference number: 703.55); and informed consent was obtained from all participants.

A total of 2 ml of peripheral blood samples into EDTA anticoagulant were collected from all participants. DNAs were extracted using Genomic DNA extraction kit (Bioneer, South Korea). All DNA samples were genotyped for alleles of Y-chromosomal specific microsatellite markers DYS388, DYS435, DYS437, and DYS439 by a Genetic Analysis System CEQ8000 (Beckman Coulter, USA). A multiplex PCR was optimized to co-amplify four above mentioned DYS loci. The PCR was carried out in a volume of 15  $\mu$ l including 50-100 ng of genomic DNA, 15 pmol of DYS388 F and R primers, 10 pmol of DYS435 F

and R primers, 15 pmol of DYS437 F and R primers, 25 pmol of DYS439 F and R primers, and 4  $\mu$ l of i-PCR 5X Master Mix (iDNA, Singapore) (in which containing of 0.1 unit/ $\mu$ l of Taq DNA Polymerase, 1 mM of dNTP's, 10% of glycerol and 7.5 mM of MgCl<sub>2</sub>) in each single reaction tube. The PCR cycling was performed on iCycler thermal cycler (BioRad Laboratories, Hercules, California, USA). Cycling conditions was set up as follows: 95°C for 10 min as initial denaturation, 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min for 30 cycles, and 65 °C for 30 min as final extension. For fragment analysis (genotyping), a mixture of 1.5  $\mu$ l PCR product, 38  $\mu$ l Sample Loading Solution, SLS (Beckman Coulter, PN 608087) and 0.5  $\mu$ l DNA size Standard (Beckman Coulter, PN 608098) was loaded into the instrument (Genetic Analysis System CEQ8000, Beckman Coulter). Raw genotype data were collected using GenomeLab software (Beckman Coulter, USA), and gel files were analyzed with the GenomeLab GeXP software package (Beckman Coulter, USA).

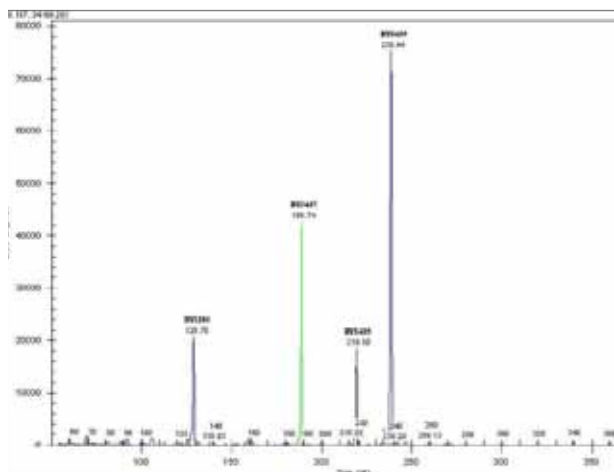
The following primer sets were employed for PCR. The forward primer for each set was labeled with a WellRED Dye-labeled Phosphoramidite (D2, Black; D3, Green; D4, Blue). DYS388: F, (D4)5'-GTG AGT TAG CCG TTT AGC GA-3'; R, 5'-CAG ATC GCA ACC ACT GCG-3'. DYS435: F, (D2)5'-AGC ATC TCC ACA CAG CAC AC-3'; R, 5'-TTC TCT CTC CCC CTC CTC TC-3'. DYS437: F, (D3)5'-GAC TAT GGG CGT GAG TGC AT-3'; R, 5'-AGA CCC TGT CAT TCA CAG ATG A-3'. DYS439: F, (D4)5'-TCC TGA ATG GTA CTT CCT AGG TTT-3'; R, 5'-GCC TGG CTT GGA ATT CTT TT-3'.

Statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 16.0 for Microsoft Windows®. The results were processed statistically using the Chi square test to assess differences in genotype prevalence and association between case and control groups. The Odds Ratio (OR) and its 95% Confidence Interval (CI) were used to illustrate the association, with p < 0.05 considered in all tests to be statistically significant.

**Table 1. Frequencies of Alleles of DYS388, DYS435, DYS437, and DYS439 Loci among Malaysian Patients and Controls and their Comparison based on the Chi-square Test**

DYS388 Loci	Alleles	Patients		Controls		$\chi^2$	P value	Odds Ratio	95% Confidence Interval
		N	F	N	F				
DYS388	10 (A)	5	0.060	30	0.330	19.923	< 0.01*	0.129	0.047 – 0.357
	11 (B)	0	0.000	1	0.011	0.928	0.335	-	-
	12 (C)	66	0.786	46	0.505	14.887	< 0.01*	3.587	1.847 – 6.966
	13 (D)	7	0.083	5	0.055	0.551	0.458	1.564	0.477 – 5.130
	14 (E)	2	0.024	4	0.044	0.535	0.464	0.530	0.095 – 2.974
	15 (F)	3	0.036	4	0.044	0.077	0.781	0.806	0.175 – 3.710
	16 (G)	1	0.012	0	0.000	1.090	0.297	-	-
	17 (H)	0	0.000	1	0.011	0.928	0.335	-	-
DYS435	11 (A)	65	0.774	69	0.758	0.059	0.808	1.091	0.541 – 2.199
	12 (B)	19	0.226	22	0.242	0.059	0.808	0.917	0.455 – 1.848
DYS437	14 (A)	57	0.679	53	0.582	1.730	0.188	1.514	0.815 – 2.811
	15 (B)	24	0.286	34	0.374	1.523	0.217	0.671	0.355 – 1.267
	16 (C)	3	0.035	4	0.044	0.077	0.781	0.806	0.175 – 3.710
DYS439	10 (A)	4	0.048	1	0.011	2.112	0.146	4.500	0.493 – 41.10
	11 (B)	30	0.357	32	0.352	0.006	0.939	1.024	0.551 – 1.904
	12 (C)	36	0.429	45	0.495	0.764	0.382	0.767	0.422 – 1.392
	13 (D)	9	0.107	13	0.143	0.507	0.476	0.720	0.291 – 1.783
	14 (E)	5	0.060	0	0.000	5.576	0.018*	-	-

N, Number; F, Frequency; \*Statistically significant



**Figure 1. Capillary Electrophoresis of DYS388, DYS435, DYS437, and DYS439 (Genetic Analysis System CEQ8000, Beckman Coulter, USA)**

## Results

Capillary electrophoresis of DYS388, DYS435, DYS437, and DYS439 loci amplified by a multiplex PCR is shown in Figure 1. In this study, eight alleles for DYS388, two alleles for DYS435, three allele for DYS437, and five alleles for DYS439 were found among the whole Malaysian subjects examined. The frequency distributions of alleles of all DYS loci among Malaysian patients and controls regardless of their ethnic group and their comparison based on Chi-square test are presented in Table 1.

A sum of 47 haplotypes was found among Malaysian population, of which 20 haplotypes were shared between Malaysian patient and healthy control groups. Ten haplotypes were found only among patients, while the number of haplotypes specific to healthy controls was 17. Comparison of the frequencies of different DYS-haplotypes among Malaysian patient and healthy control groups based on Chi-square test showed significant differences for haplogroups AABC ( $P=0.024$ ) and CAAA ( $P=0.035$ ).

## Discussion

In this study, alleles 10 - 17 of DYS388, alleles 11 - 12 of DYS435, alleles 14 - 16 of DYS437, and alleles 10 - 14 of DYS439 were found among the whole Malaysian subjects. Based on Y-chromosome database (<http://www.smfg.org>, Sorenson Molecular Genealogy foundation), DYS388 is a tri-nucleotide STR locus consisting 12 alleles with 7 - 18 repeats of ATT motif (Butler et al., 2002; Gusmao et al., 2006), DYS435 is a tetra-nucleotide STR consisting 5 alleles with 9 - 13 repeats of TGGA motif (Kayser et al., 2004), DYS437 is a tetra-nucleotide STR consisting 8 alleles with 11 - 18 repeats of TCTA motif, and DYS439 is a tetra-nucleotide STR consisting 9 alleles with 8 - 16 repeats of AGAT motif (Butler et al., 2002; Gusmao et al., 2006). Alleles 7, 8, 9, and 18 of DYS388, alleles 9, 10, and 13 of DYS435, alleles 11, 12, 13, 17 and 18 of DYS437, and alleles 9, 15, and 16 of DYS439

were not found among the subjects examined in this study.

As shown in Table 1, no significant differences were observed between frequency distributions of different alleles of DYS435 and DYS437 loci among cases and controls. However, on comparing DYS388 and DYS439 allele frequencies between prostate cancer patients and those of normal controls, it was found that Malaysian males with allele 10 (A, 123 bp) of DYS388 had a significantly lower incidence of prostate cancer than males with other alleles of this locus (OR, 0.129; 95% CI, 0.047 - 0.357;  $P < 0.01$ ). On the other hand, males who had either allele 12 (C, 129 bp) of DYS388 or allele 14 (E, 254 bp) of DYS439 showed a significantly higher risk to develop prostate cancer, compared to males having other alleles of these loci (OR, 3.576; 95% CI, 1.847 - 6.966;  $P < 0.01$  for DYS388 allele 12, and  $P = 0.018$  for DYS439 allele 14). These findings are consistent with those reported by Ewis et al. (2002) and Paracchini et al. (2003) and support the hypothesis that males from different Y-chromosomal origins are different concerning their susceptibility or resistance to develop prostate cancer. Ewis et al. (2002) compared allele frequency distribution of DYS19 in Japanese prostate cancer patients and healthy controls. Based on their findings, males with allele C (194 bp) of DYS19 were more susceptible to develop prostate cancer, while those with allele D (198 bp) were less exposed to prostate cancer than men with other alleles. Also, in a cohort study done by Paracchini et al. (2003), Y-lineages of prostate cancer patients and healthy control individuals were determined for four ethnic groups living in Hawaii and California. They found one lineage, belonging to the Japanese group in the study, associated with a statistically significant predisposition to develop prostate cancer. In another study done on Iranian population by the current study group, it was found that Iranian males with alleles 12 (129 bp) and 13 (132 bp) of DYS388 had a significantly higher and lower incidence of prostate cancer, respectively, in compare to males with other alleles.

The comparison between DYS-haplotype frequencies between Malaysian patient and healthy control groups revealed that the frequency of AABC was significantly higher in control than patient group (OR, 0.198; 95% CI, 0.042 - 0.930;  $P = 0.024$ ), while haplotype CAAA showed a significantly higher frequency in patients than controls ( $P=0.035$ ). Based on this result, it is likely that Malaysian men who belong to the lineages with AABC and CAAA haplotypes have a lower and higher risk to develop prostate cancer, respectively, in compare with those belonging to other haplotypes. However, considering 47 haplotypes observed among 175 subjects, there is a high chance that some haplotypes show pseudo-significant difference between cases and controls due to random chance. Therefore, the hypothesis is not strongly supported by results obtained for Haplotypes. In another study conducted by the current study group on Iranian population regarding comparison of Y-haplotype lineages of prostate cancer patients and healthy control individuals comprising DYS388, DYS435, DYS437, and DYS439 loci, it was revealed that some haplotypes had higher frequency among Iranian patients than controls (unpublished data). However, the evidence did not

strongly support the hypothesis in that population as well. Moreover, in a study done by Kim et al. (2007) on Korean populations of prostate cancer patients and healthy controls using Y-chromosomal binary loci, no significant difference was observed in distribution of Y-haplogroup frequencies among Korean case and control groups.

In summary, we found significant differences in frequency of alleles 10 and 12 of DYS388, allele 14 of DYS439, as well as haplotypes AABC and CAAA between prostate cancer patient and healthy control groups in Malaysian population. These results are consistent with the hypothesis that male descendants from different Y-chromosomal origins are different in susceptibility or resistance to develop prostate cancer. As a conclusion, results of current study show the influence of genetic elements on prostate cancer, and it seems that DYS388 locus and less likely DYS439 locus as well as haplotypes comprising DYS388, DYS435, DYS437, and DYS439 have the potential to be used as a screening method for prediction of susceptibility to prostate cancer in Malaysian population. Nonetheless, further study on larger number of samples from different Malaysian ethnic groups is suggested to confirm the results obtained by this study.

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