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ELECTRONIC LETTER

Y chromosome haplotypes and testicular cancer in the English population

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Testicular cancer (TC) affects 1 in 500 males and is the most common malignancy among young men in western European populations; for example, in Denmark 1% of all men now develop TC.¹⁻⁴ More than 97% of testes cancers are germ cell tumours.⁴ Although the aetiology of these malignancies is unknown, there is accumulating evidence of an intrauterine stage of TC development that may involve both environmental and genetic factors acting on the primordial gonocyte.⁴ Although many tumour suppressor genes have been studied, there is little evidence supporting a role for these factors in the pathogenesis of TC.⁵ A number of epidemiological studies suggest that hypospadias, cryptorchidism, testicular cancer, and male infertility may share, in part, a common aetiology and this has given rise to the term "testicular dysgenesis syndrome".⁴ TC is associated with poor spermatogenic function and recent data indicate that this dysfunction is associated with impaired infertility several years before diagnosis.⁶ Spermatogenic dysfunction is more common than can be explained by either local tumour or general cancer effect, since patients with other malignant diseases have normal, or only slightly decreased, semen quality.⁷

Human Y chromosome loss and rearrangements have been associated with specific types of cancer, such as bladder cancer, male sex cord stromal tumours, lung cancer, and oesophageal carcinoma,⁸⁻¹¹ suggesting that both oncogenes and tumour suppressor genes exist on this chromosome. A number of Y chromosome genes, or gene families, appear to be necessary for male germ cell development and maintenance¹²⁻¹³ and are candidates for involvement in oncogenesis of male specific cancers. Indeed, a cancer predisposition locus has been assigned to this chromosome, the gonadoblastoma locus (*GBY*).¹⁴ The location, function, and expression profile of the testis specific protein Y gene (*TSPY*) in germ cell tumours, prostate tumours, and normal tissue suggest that it is an excellent candidate for the *GBY* gene.¹⁵ Also, the loss and gain of Y chromosome material and differential expression of some Y genes was reported in prostate cancers,¹⁶⁻²⁰ reinforcing a role for the Y chromosome in malignancy and cancer progression.

Y chromosome lineages are highly geographically stratified among human populations. The variation of the non-recombining region of the Y chromosome (NRY) has been successfully used to study human origins and population histories,²¹⁻²² based on the assumption that Y chromosome variation is selectively neutral. However, if selection occurs, the majority of the Y chromosome will be affected, and not just single genes, given the absence of recombination. An increased (or decreased) frequency of a particular Y lineage in the affected population may unmask the presence of a functional variant, in linkage with the neutral mutation defining the haplogroup. In this context, the choice of the control population is critical, since statistical differences in haplogroup frequencies between the affected and control populations may be caused by population related factors (for example, population substructure) and not by an actual

Key points

- The incidence of testicular cancer has increased world wide by a factor of 3-4 during the last decades. The observations of both an apparent decrease in semen quality in the general population and a reduced spermatogenic function in males presenting with testicular cancer suggest that both disorders may share a common aetiology.
- Since the human Y chromosome encodes several genes necessary for germ cell development and maintenance, it is possible that Y encoded factors are also involved in testicular cancer formation or development.
- To test this hypothesis, we have analysed a set of 13 Y linked polymorphisms (seven biallelic and six microsatellite markers) to defined Y chromosome haplotypes in a group of 43 English patients presenting with testicular cancer. Their haplotype profile was compared with a selected population of 179 controls to determine if there is an association between Y chromosome background and a predisposition to this cancer.
- When using three different levels of marker resolution, the results of the exact test of population differentiation indicated no significant differences between the case and control groups. Although our data do not show a correlation between a particular Y chromosome lineage and a higher predisposition to testicular tumour formation, a direct role of Y chromosome genes or gene families in tumour formation or progression cannot be excluded.

association with the phenotype under study. Several associations have already been reported between Y chromosome lineages and various phenotypes, such as protection against Y chromosome transfer to the X chromosome leading to XX-Y⁺ maleness,²³ high blood pressure,²⁴ and alcoholism.²⁵ Conversely, absence of correlation has been reported between Y variation and some behavioural disorders, such as autism²⁶ and personality traits related to alcohol dependence.²⁵ Recently, an association between Y background and reduced sperm counts has been reported in Japanese²⁷ and Danish populations.²⁸ In Denmark, a specific class of Y chromosomes, hg26+, is associated with reduced sperm counts (<20 × 10⁶/ml), although the underlying molecular cause of this reduction remains undefined.

It is conceivable that Y encoded factors, involved in germ cell maturation, are implicated in the progression of TC. The purpose of this study was to analyse the effects of the Y chromosome background in testicular tumour formation. To assess Y chromosome differences, seven unique event polymorphism (UEP) markers and six microsatellites were used to construct

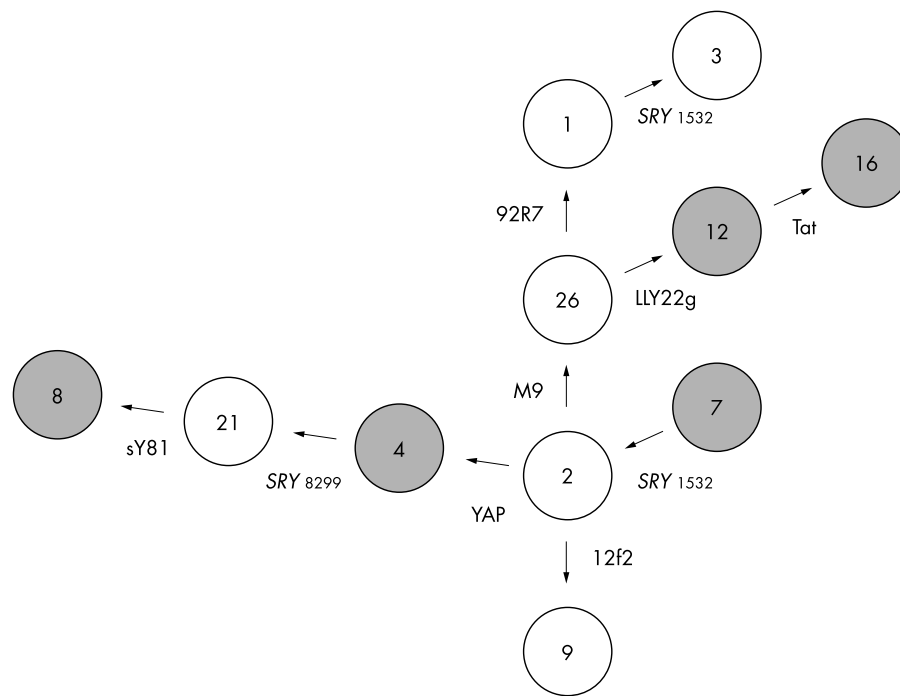


Figure 1 Schematic representation of the phylogenetic relationships among the Y chromosomal haplogroups. The markers that defined each mutational step are reported and arrow orientation defines the ancestral and derivative state of each marker. Filled circles indicate haplogroups not detected in either the control or the testicular cancer population.

haplotypes from a sample of 43 patients presenting with testicular cancer. Their Y haplogroup profile was compared with a matched control population of English origin.

MATERIALS AND METHODS

Patients and controls

A total of 43 cases of testicular cancer patients of white English origin were recruited for the study from the general Manchester area. Out of the 43 cases, 30 presented with classical seminoma, three with malignant undifferentiated teratoma plus seminoma, three with malignant undifferentiated teratoma plus yolk sac elements, three with malignant intermediate teratoma, two with malignant intermediate teratoma plus seminoma, one with malignant undifferentiated teratoma, and one with combined germ cell tumour with malignant teratoma plus seminoma. The control population consisted of 179 apparently healthy, unaffected subjects taken from a larger data set described in Weale *et al.*²⁹ They were sampled from three Midlands towns (Ashbourne, Southwell, and Bourne), each separated from the other by at least 30 miles, and all had paternal grandparents who were born in England.

Molecular analysis

To define Y chromosome lineages in these subjects, we analysed seven of the biallelic unique event polymorphism (UEPs) markers (SRY-1532, 92R7, YAP, M9, LLY22g, Tat, 12f2)

and six microsatellites (DYS19, DYS388, DYS390, DYS391, DYS392, DYS393). The seven biallelic markers, which are known to be polymorphic in European populations and define the main Y chromosomal haplogroups (fig 1), were analysed as described in Rosser *et al.*³⁶ The deep rooting markers SRY1532, M9, 92R7, and YAP were typed in all samples and, in some cases, the remaining markers were typed hierarchically, for example, LLY22g and Tat only on those chromosomes M9 derived and 92R7 ancestral. The microsatellite typing was performed as described in Thomas *et al.*³⁷

Statistical analysis

To test for significant population differentiation between testicular cancer patients and the control population, we performed the exact test of population differentiation,³⁰ using the ARLEQUIN package version 2.0.³⁸ This test assays significant departures from the null hypothesis of random distribution of alleles (or haplotypes) among population pairs. A Markov chain of 10 000 steps was used and the significance level of the test was set at 5%. Genetic diversity and its standard error were estimated using the unbiased formulae described by Nei.³⁹

RESULTS AND DISCUSSION

The UEP markers defined four different Y chromosome lineages, or haplogroups (Hgs), in the testicular cancer sample. Frequency distribution of Y chromosome lineages in

Table 1 Y chromosome haplogroup (hg) distribution in testicular cancer patients and in the relevant English control population

Population	N	Hg 1	Hg 2	Hg 3	Hg 9	Hg 21	Hg 26
Testicular cancer	43	32	8	2	–	–	1
(%)		74.4	18.6	4.7	–	–	2.3
English population	179	117	39	8	8	7	–
(%)		65.4	21.8	4.4	4.4	3.9	–

Table 2 UEP and microsatellite combined haplotype frequencies in the testicular cancer (TC) and English control population

Haplogroup	Microsatellite haplotype*	TC (n=43)	%	Control (c=179)	%	
Hg 1	12 12 23 11 13 13	–	–	2	1.12	
	12 12 24 10 13 13	–	–	1	0.56	
	12 12 24 11 13 13	–	–	1	0.56	
	13 12 23 10 13 13	–	–	1	0.56	
	13 12 24 10 13 14	–	–	1	0.56	
	13 12 24 11 13 13	–	–	2	1.12	
	14 10 23 11 13 13	–	–	1	0.56	
	14 11 23 10 12 13	–	–	1	0.56	
	14 12 23 10 13 13	–	–	7	3.91	
	14 12 23 10 14 13	1	2.33	–	–	
	14 12 23 11 13 13	5	11.63	19	10.61	
	14 12 23 11 13 14	–	–	1	0.56	
	14 12 24 10 12 13	1	2.33	–	–	
	14 12 24 10 13 12	–	–	1	0.56	
	14 12 24 10 13 13	3	6.98	20	11.17	
	14 12 24 10 13 14	2	4.65	1	0.56	
	14 12 24 10 14 13	–	–	2	1.12	
	14 12 24 11 12 13	–	–	1	0.56	
	14 12 24 11 13 12	1	2.33	1	0.56	
	14 12 24 11 13 13	10	23.26	23	12.85	
	14 12 24 11 13 14	–	–	1	0.56	
	14 12 24 12 13 13	–	–	2	1.12	
	14 12 24 13 13 13	–	–	1	0.56	
	14 12 25 10 13 13	–	–	3	1.68	
	14 12 25 11 13 12	–	–	4	2.23	
	14 12 25 11 13 13	1	2.33	5	2.79	
	14 12 25 11 14 13	2	4.65	–	–	
	14 13 23 11 13 13	–	–	1	0.56	
	14 13 24 10 12 13	–	–	1	0.56	
	15 12 23 10 13 13	1	2.33	2	1.12	
	15 12 23 11 13 13	–	–	4	2.23	
	15 12 24 10 13 13	2	4.65	2	1.12	
	15 12 24 11 13 13	1	2.33	3	1.68	
	15 12 24 11 13 14	–	–	1	0.56	
	15 12 24 11 15 13	–	–	1	0.56	
	16 12 24 10 13 13	1	2.33	–	–	
	16 12 25 11 14 14	1	2.33	–	–	
	Hg 2	14 12 21 10 11 14	–	–	1	0.56
		14 12 22 10 11 13	–	–	1	0.56
		14 14 22 10 11 13	2	4.65	8	4.47
14 14 22 10 11 14		2	4.65	–	–	
14 14 22 11 11 12		–	–	1	0.56	
14 14 23 10 11 13		1	2.33	4	2.23	
14 14 23 11 11 13		1	2.33	–	–	
14 16 22 09 11 14		–	–	1	0.56	
14 16 22 10 11 13		–	–	1	0.56	
15 12 21 10 11 14		–	–	1	0.56	
15 12 22 11 11 14		–	–	1	0.56	
15 13 22 10 11 14		–	–	1	0.56	
15 13 23 10 12 14		–	–	3	1.68	
15 13 23 10 12 15		–	–	3	1.68	
15 13 23 11 12 14		–	–	1	0.56	
15 13 24 10 12 15		–	–	1	0.56	
15 14 22 10 11 12		1	2.33	–	–	
15 14 22 10 11 13		1	2.33	3	1.68	
15 14 22 11 11 13		–	–	1	0.56	
15 15 23 10 11 13		–	–	1	0.56	
16 13 23 10 12 13		–	–	1	0.56	
16 13 23 10 13 13		–	–	1	0.56	
16 13 24 10 12 14		–	–	1	0.56	
16 14 22 10 11 13		–	–	2	1.12	
17 13 23 11 12 14		–	–	1	0.56	
Hg 3		15 12 23 10 11 12	–	–	1	0.56
		15 12 25 11 11 13	1	2.33	–	–
	16 12 24 10 11 13	–	–	1	0.56	
	16 12 25 10 11 13	1	2.33	2	1.12	
	16 12 25 10 11 14	–	–	1	0.56	
	16 12 25 11 11 13	–	–	2	1.12	
17 12 25 10 11 13	–	–	1	0.56		

contd

Table 2 *continued*

Haplogroup	Microsatellite haplotype*	TC (n=43)	%	Control (n=179)	%
Hg 9	14 14 23 10 11 12	–	–	1	0.56
	14 16 23 10 11 12	–	–	3	1.68
	14 16 24 10 11 12	–	–	1	0.56
	15 15 23 10 11 12	–	–	1	0.56
	15 15 24 10 11 12	–	–	1	0.56
	16 14 24 10 11 12	–	–	1	0.56
Hg 21	13 12 22 10 11 13	–	–	1	0.56
	13 12 23 10 11 13	–	–	1	0.56
	13 12 24 10 11 13	–	–	3	1.68
	13 12 25 10 11 13	–	–	1	0.56
	14 12 24 10 11 13	–	–	1	0.56
Hg 26	16 12 26 10 13 12	1	2.33	–	–

*Microsatellite haplotypes are defined by six numbers giving the repeat size at loci DYS19, DYS388, DYS390, DYS391, DYS392, and DYS393.

TC patients is indicated in table 1, together with that of the English control population. Because the control group comprised three samples from separate towns in central England, and because a previous study on these samples has found no significant differences in Y chromosome frequencies among these towns,²⁹ the chances of geographical substructuring in the control group is minimised. The most frequent Hgs in the TC patients are Hg 1 (74%) and Hg 2 (19%) and, at lower frequencies, Hg 3 (5%) and Hg 26 (2%). The haplogroup profile observed in the TC sample is consistent with that observed in the control English population, where Hgs 1 and 2 are the most representative lineages. To compare haplogroup distribution between the affected and control populations, an exact test of population differentiation was performed.³⁰ The results of this test, based on frequency distribution, indicate no significant differences in haplogroup distribution between the TC and control groups ($p=0.23 \pm 0.008$). However, the failure to find any statistical association between a Y chromosome haplogroup and incidence of testicular cancer may be because of an insufficient definition of the Y chromosome haplogroups, solely based on UEP markers. Indeed, some of these lineages are paraphyletic groups that show a high within haplogroup diversity. Thus, it is plausible that a particular sublineage within a haplogroup may show an association with a Y chromosome phenotype but not the whole lineage. Therefore, we sought to refine further the Y chromosome lineages in the testicular cancer subjects, by analysing a set of six microsatellite markers.

Among the 43 subjects studied, 23 distinct six locus haplotypes were observed (table 2). Genetic diversity, which estimates the probability that two randomly chosen haplotypes are different in the sample, was high ($h=0.93 \pm 0.027$), and comparable to that found in the control sample ($h=0.95 \pm 0.008$). This argues against a strong association between testicular cancer and a recent causative mutation, as this would result in a lower diversity in the TC sample. A comparison of microsatellite haplotype distribution between the TC sample and the matched control population also failed to show any statistical difference between the two groups ($p=0.57 \pm 0.027$). Because there might be an intermediate level of resolution between the full microsatellite haplotype and the UEP based haplogroup where a difference between cases and controls could reveal itself, we also split the two main haplogroups (Hg 1 and Hg 2) into two subcategories according to whether or not the microsatellite haplotype belonged to the modal cluster (defined as haplotypes belonging to or one step removed from the modal haplotypes in each haplogroup; note both cases and controls had the same the modal haplotype for Hg 1 and Hg 2). However, we again failed to find a significant

difference at this level of resolution between cases and controls ($p=0.16 \pm 0.008$).

Although we did not detect an association between Y chromosome background and testicular tumour formation, this does not exclude a Y chromosome contribution to the aetiopathogenesis of this cancer. For example, Hg 1 has the highest observed haplogroup association with testicular cancer, with an estimated odds ratio of 1.54 (95% CI 0.74-3.22). Because of the wide confidence intervals, it is possible that a high association with a particular Y chromosome type exists (for example, the real odds ratio with Hg 1 could be as high as 3.22) but remained hidden in our study. An alternative approach to uncover an association between the Y chromosome and TC may be to examine directly Y chromosome gene copy number in populations with distinct ethnic backgrounds and to compare their profiles with relevant samples of patients presenting with testicular dysfunction. In this context, the Y located *TSPY* gene family may play a role in oncogenesis because of its expression profile (male germ cells and neoplasia of the testis), and its homology to the *SET* oncogene, a cyclin B binding protein.³¹ *TSPY* is polymorphic in copy number (20-40 copies) and is arranged in a tandemly organised cluster,³² which exhibits interperson polymorphism in gene copy number³³ that may be associated with phenotype variation. In addition, the *DAZ* and *RBM Y* genes are also present in multiple copies along the Y chromosome and have a germ cell specific expression profile.³⁴ Indeed, the autosomal homologue of *DAZ*, *DAZL1*, is expressed in testicular germ cell tumours.³⁵

In conclusion, although our data do not show an effect of the Y chromosome background in testicular cancer in a cohort of English patients, it is necessary to study other populations where the incidence of TC is rising dramatically and where a Y chromosome haplogroup has been found to be associated with reduced testicular function, such as in Denmark.²⁸

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