Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours

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Testicular germ-cell tumours (TGCT) affect 1 in 500 men and are the most common cancer in males aged 15–40 in Western European populations[1]. The incidence of TGCT has risen dramatically over the last century[2–5]. Known risk factors for TGCT include a history of undescended testis (UDT), testicular dysgenesis, infertility[6], previously diagnosed TGCT (ref. 7) and a family history of the disease[8–10]. Brothers of men with TGCT have an 8–10-fold risk of developing TGCT (refs 8,9), whereas the relative risk to fathers and sons is fourfold (ref. 9). This familial relative risk is much higher than that for most other types of cancer. We have collected samples from 134 families with two or more cases of TGCT, 87 of which are affected sibpairs. A genome-wide linkage search yielded a heterogeneity lod (hlod) score of 2.01 on chromosome Xq27 using all families compatible with X inheritance. We obtained a hlod score of 4.7 from families with at least one bilateral case, corresponding to a genome-wide significance level of *P*=0.034. The proportion of families with UDT linked to this locus was 73% compared with 26% of families without UDT (*P*=0.03). Our results provide evidence for a TGCT susceptibility gene on chromosome Xq27 that may also predispose to UDT.

Localization of a TGCT predisposition gene has been hampered by the relative rarity of multigenerational pedigrees with several affected cases, which are most informative for genetic linkage analysis. Genetic linkage analysis of the International Testicular Cancer Linkage Consortium set of 134 families (Table 1) using polymorphic markers has excluded the possibility that susceptibility to TGCT is due to a single autosomal gene that accounts for all the familial risk (data not shown). We have previously reported regions with suggestive evidence in favour of linkage identified in our autosomal genome search[11–12].

The increased risk of TGCT to fathers or sons of cases has been reported to be less than the increased risk to brothers of cases[8,9]. As this could be interpreted as evidence of X inheritance, we extended the linkage search to include the X chromosome. In these analyses we excluded from genotyping the 35 (26% of all families) families which show male-to-male transmission and hence, a priori, are inconsistent with X linkage. Linkage analysis of the set of families compatible with X linkage, 80% of which are sibpairs, provided preliminary evidence for a TGCT predisposition locus at Xq27–28 (maximum hlod score=2.01, α=0.32, maximum multipoint lod score under homogeneity of −19.92; Table 2, Figs 1 and 2). Evidence in favour of linkage was observed in families from all contributing groups (data not shown).

We subsequently stratified families according to the presence of at least one bilateral case, the presence of UDT, histology and age (Table 2). Families with at least one case of bilateral disease, all of which are sibpairs except for one maternal cousins pedigree and one of three sibs, showed strong evidence of linkage to the locus on Xq27 (hlod score=4.76, α=1.00; Fig. 2) and were more likely to be linked to the X chromosome than families without a

Table 1 • Breakdown of all families in consortium set

Family type	Australia	Canada	Germany	Ireland	Norway	UK	Total
sibpair	6	3	12		13	53	87
sibtrio						4[a]	4
father/son[b]	2				2	6	10
cousins	1		1			2	4
maternal cousins	1				1[c]	2	4
paternal cousins	1					4	5
paternal uncle/nephew					3	1	4
maternal uncle/nephew			1		2	4	7
other	3[d]	1[e]		1[f]	2[g]	2[h]	9
total	14	4	14	1	23	78	134[i]

[a]Pedigree 341; third affected sib deceased and no sample exists. [b]Father/son pedigrees were only used in the genome wide linkage search if another unaffected male sibling was available for genotyping. [c]Pedigree 155; two brothers married two sisters. [d]Four cousins, uncle/two nephews and second cousins. [e]Four sibs and second cousins once removed. [f]Father/son and cousin. [g]Father/two sons; three cousins. [h]Father/son and cousin; sibs and cousin. [i]There are 103 potentially X-linked families in this set. However, DNA from four families was unavailable for typing of X chromosome markers.

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Table 2 • Hlod score results by model

Data set		Location (cM)	Maximum hlod score	Proportion of families linked (α)
all families	(n=99)	183.04	2.01	0.32
bilaterality	positive (n=15)	175.28	4.76	1.00
	negative (n=75)	190.19	1.32	0.32
	unknown (n=9)	35.28	0.69	1.00
UDT status	positive (n=19)	184.74	2.50	0.74
	negative (n=56)	162.39	0.56	0.27
	unknown (n=24)	8.2	0.76	1.00
mean age	≤30 (n=39)	183.61	2.09	0.49
	>30(n=35)	189.53	0.58	0.29
tumour type	seminoma (n=17)	174.94	1.33	0.60
	non-seminoma (n=17)	190.99	1.54	0.64

bilateral case (hlod score=1.20, α=0.33; *P*=0.0002). When sibpairs only were considered, the hlod score was 4.25 with α=1.00. The difference in proportion of linked families when dichotomizing by presence/absence of bilateral disease is statistically significant after taking into account multiple testing (*P*=0.001, allowing for five tests).

The probability of obtaining a hlod score of 4.76 or greater by chance in a genome-wide search in at least one of the subgroups examined was estimated by 1,000 simulations to be 0.034, equivalent to a lod score of 3.78 in a genome-wide linkage search using affected sibpairs without subgrouping[13]. This result provides significant[13] evidence for a TGCT susceptibility gene on chromosome Xq27. We have named this gene *TGCT1*. Our results suggest that about one-third of the excess familial TGCT risk to brothers is due to *TGCT1*, with little difference in the residual risks to brothers and sons after this locus has been accounted for.

Families with one or more cases of UDT, three of which are non-sibpairs (two uncle nephew and one cousin pedigree), show evidence of linkage to the same region on Xq27 (hlod score=2.52, α=0.73, multipoint lod score=1.58 under homogeneity) and are more likely to be linked than families without a case of UDT (hlod score=0.56, α=0.26; *P*=0.03 for the comparison). Age of onset (*P*=0.15 for age-of-onset less than or equal to 30 years versus. greater than 30 years) and histopathology did not discriminate those families which were linked to the X chromosome from those that did not (Table 2).

Haplotypes were constructed for the 15 families with bilateral tumours (Fig. 3). Two informative recombinants appear to limit the size of the common interval to *DXS8043–FMR1Di* (although the small size of these two families and the consequent weakness of the linkage information from each indicate that both could be linked by chance), a distance of approximately 7 cM. There is no segregating haplotype throughout the *DXS8043–FMR1Di* interval that is common to all these families (Fig. 3). Although there are haplotype similarities between families in the region *DXS548–FMR1di*, the haplotype frequencies are not significantly different from those obtained by genotyping of 762 control males (data not shown).

The only gene so far characterized in this region is *FMR1*, which is responsible for fragile-X syndrome. Individuals with fragile-X syndrome frequently exhibit macroorchidism, although histologically the only abnormality is the presence of edema (MIM 309550). There is no evidence for an excess risk of TGCT in fragile-X cases[14].

In Klinefelters syndrome (47, XXY), there is an elevated risk of extragonadal GCT (ref. 15). The relative risk of mediastinal GCT in Klinefelters syndrome is 67 (ref. 15), and 8% of males with mediastinal GCT have Klinefelters syndrome[16]. This raises the possibility that two active normal copies of *TGCT1* may be responsible for the increased risk of GCT in Klinefelters syndrome; however, the incidence of TGCT in Klinefelters syndrome is low[15,17–19] (although this may be attributed to the fact that adults with Klinefelters syndrome have few residual testicular germ cells[20]).

These results provide the first evidence for the location of a familial TGCT-susceptibility gene. Our data suggest that *TGCT1* is associated with a higher risk of bilateral TGCT and perhaps UDT than other TGCT-susceptibility genes. It seems unlikely, however, that *TGCT1* is the only TGCT-susceptibility gene that predisposes to this syndrome because there are other families with both bilateral disease and UDT which are incompatible with X linkage. *TGCT1* is the first cancer-susceptibility gene to be mapped in a genome-wide search predominantly using sibpairs and the third cancer-predisposing gene to be mapped to the X chromosome following the report of a prostate-cancer-susceptibility gene[21] and the association of mutations in the gene encoding the androgen receptor with familial male breast cancer[22].

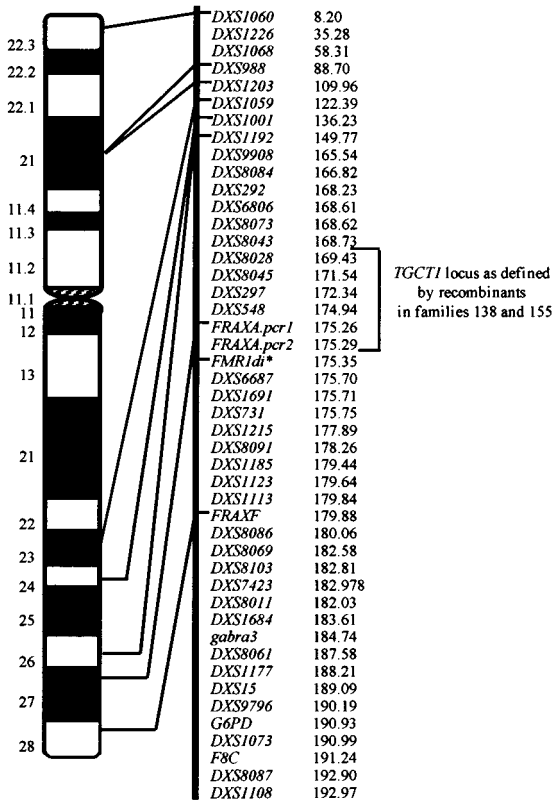

Fig. 1 Ideogram of the X chromosome showing positions of markers used in the analysis. Genetic locations are given by estimated female recombination (fcM) value based on the LDB map[27]. **FMR1Di* is a new microsatellite repeat derived from sequence information around *FMR1* (forward, 5′–ACCTGCCTTTTCTACT TTTTCT–3′; reverse, 5′–GGAGTAATGACCCTGTAGTAGCA–3′) *FMR1Di* is ~120,000 bp from *FRAXA.pcr2*.

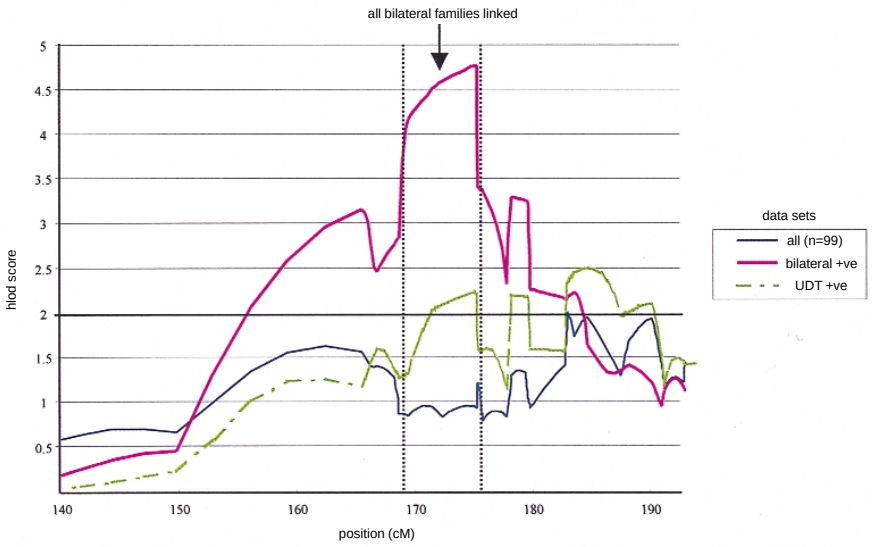

Fig. 2 Plots of hlod score for the X chromosome.

placed on the map additional information was sought from other genome maps (for example Genethon[28]). For most markers genotyped on the X chromosome, only the LDB composite score was available. Therefore the LDB map was used to determine marker order and, where there was no value for fcM or the interval between the markers was negative (that is, fcM was not in line with LDB location), the cM distance was determined using flanking markers for which fcM was known. The map shows the 46 markers that were typed over the length of the X chromosome and the relative distances (cM) between them.

Previous research suggests that there is no single major locus for familial testicular cancer (data not shown). Any analysis of the set of testicular cancer families must therefore be examined under heterogeneity. As such we report lod scores under heterogeneity (hlod) and the proportion of families linked (α). In general, the NPL scores show the same pattern of linkage and significance as do the hlod scores, with a *P* value of 0.05 or less from non-parametric analysis corresponding to a hlod score of 1.3 or more. For brevity and consistency of result we only reported hlod scores.

Methods

Families. A total of 134 families with two or more cases of testicular cancer have been ascertained in the UK, Norway, Germany, Australia, Ireland and Canada. Patients donated samples and information with full informed consent and with local Ethical Review Board Approval. Information on clinical status, including type of TGCT, age of diagnosis, presence of UDT and laterality of disease, was confirmed by reviewing histological reports and clinical notes.

Genotyping. We prepared genomic DNA from whole blood, immortalized lymphoblastoid cell lines and formalin-fixed, paraffin-embedded tumour sections using standard techniques. Microsatellite markers were amplified in PCR reactions with one primer 5′ labelled with a fluorochrome and electrophoresed on ABI377 DNA sequencers (Applied Biosystems). Depending on the amount of PCR product and the fluorescent label of the marker, we combined 2–10 μl of each sample with up to nine other markers. Gels were analysed using the ABI Genescan and Genotyper software. In addition, some markers were end-labelled with [γ-^{32}P] ATP using T4 polynucleotide kinase, electrophoresed on standard denaturing polyacrylamide gels, dried and exposed to X-ray film.

Statistical analysis. Linkage analysis was performed with the GENEHUNTER software[23] using non-parametric and a variety of formal linkage models reflecting the concerns about the underlying mode of inheritance. Linkage models were based on described segregation analyses[24,25]. A lifetime penetrance of 0.45 and a gene frequency of 0.03 for a recessive model and a lifetime penetrance of 0.14 and gene frequency of 0.003 for the dominant model were assumed. As there has not been a formal segregation analysis involving a sex-linked locus for testicular cancer, the autosomal recessive values were assumed for the X-chromosome model for this analysis. We carried out multipoint analyses based on the Marshfield chromosome maps[26] and/or the location database[27] (LDB). Some conflict exists between these two maps; in some cases (in particular on X) markers used could not be found on the Marshfield map, we therefore used the LDB map for that chromosome. In cases where multiple double recombinants indicated a marker had been incorrectly

As familial testicular cancer is heterogeneous, we analysed the families under a number of subgroups based on histology, UDT status, age of onset and bilaterality of cancer. Families were coded as follows: bilateral if there was one or more cases of bilateral disease or CIS in a contralateral testis within the family; UDT if at least one of the affecteds had a history of UDT (retractile testis or hernia were not counted as UDT); seminoma if at least two affected had seminoma; non-seminoma if at least two affecteds had non-seminoma (that is, sibs are concordant for histology); ≤30 if the mean age of diagnosis of the affecteds in the family is less than 30 years and >30 if the mean age at diagnosis is greater than 30 years.

We assessed the genome-wide significance level of the maximum heterogeneity lod scores by simulation. For each of the 134 families, haplotype sharing was simulated throughout the genome assuming no linkage, recombinant events were assumed to occur without interference. The genetic lengths of each chromosome were defined by the map distance between the most telom-

			Family numbers														
Interval	Location	Marker	129	138	155	162	169	172	176	177	232	233	277	326	329	335	343
122.39	122.39	*DXS1059*	0			0	0	0	0	0			0	0		8	5
13.84	136.23	*DXS1001*	0			0	0	0	0	0			0	0		9	0
13.54	149.77	*DXS1192*	0			0	0	0	0	0	4		0	2	0	6	8
15.77	165.54	*DXS9908*	3	5		0	0	0	0	0	3		3	5	3	5	0
1.28	166.82	*DXS8084*	8	8		0	0	0	0	0	6	6	6	10	6	6	0
1.41	168.23	*DXS292*	5	5		0	0	0	0	0	5	5	5	0	5	6	0
0.38	168.61	*DXS6806*	1	5		0	0	0	0	0	2	0	1	3	3	2	2
0.01	168.62	*DXS8073*	7	7		0	0	0	0	0	3	7	6	0	7	6	0
0.11	168.73	*DXS8043*	2	8		0	0	0	0	0	2	2	9	2	2	8	9
0.70	169.43	*DXS8028*	4	12	14	2	1	1	1	9	0	0	1	15	13	1	9
2.11	171.54	*DXS8045*	4	7	7	7	7	7	3	6	4	7	7	4	7	7	0
0.80	172.34	*DXS297*	11	11	11	9	9	10	11	7	9	11	10	11	10	11	11
2.60	174.94	*DXS548*	5	5	5	5	7	5	4	3	5	6	15	5	6	5	5
0.32	175.26	*FRAXA.pcr1*	8	8	8	7	10	8	8	8	8	7	8	8	8	8	8
0.03	175.29	*FRAXA.pcr2*	7	7	7	3	8	9	6	7	7	3	6	6	7	7	6
0.06	175.35	*FMR1di*	4		5	5	5	5	4	5	4	0	4	4	5	5	4
0.35	175.70	*DXS6687*	0		12	0	10	0	12	0	12	12	12	0	11	0	0
0.01	175.71	*DXS1691*	4		4	4	3	3	4	4	4	4	5	3	4	4	0
0.04	175.75	*DXS731*	10		5	0	0	0	0	0	9	0	9	10	5	5	1
2.14	177.89	*DXS1215*	6		5	0	0	0	0	0	5		6	6	6	5	5
0.37	178.26	*DXS8091*	1		9	0	0	0	0	0	9		9	5	8	1	10
1.18	179.44	*DXS1185*	5		4	0	0	0	0	0	4		4	4	4	4	4
0.20	179.64	*DXS1123*	5		4	0	0	0	0	0	4		6	4	4	7	4
0.20	179.84	*DXS1113*	3		3	0	0	0	0	0	12		3	5	9	3	3
0.04	179.88	*FRAXF*	6		6	0	0	0	0	0	4		6	6	4	6	0
0.18	180.06	*DXS8086*	6		6	0	0	0	0	0	6		6	0	7	8	7
2.52	182.58	*DXS8069*	4		4	0	0	0	0	0	4		4	3	3	3	3
0.23	182.81	*DXS8103*	11		0	0	0	0	0	0	4		4	4	0	4	2

Fig. 3 Segregating haplotypes of TGCT families with a history of bilateral disease, showing recombinants in families 138 and 155.

eric markers on the p and q arms used in our genome search. We computed at each point the maximum hlod score assuming a completely informative marker, for the whole data set and the eight subgroups defined by bilaterality (+/–), UDT status (+/–), age (≤30 or >30) and histology, separately for the dominant and recessive models used in the actual analysis. For the X chromosome we included only the 99 families compatible with X linkage (that is, no male-to-male transmission) and computed the corresponding heterogeneity lod scores under the X-linked model. An empirical significance level was then calculated as the proportion of replicates for which the maximum heterogeneity lod score achieved at any location for any model in any subgroup was greater than the maximum achieved in the real data set.

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1. Oliver, R.T. Atrophy, hormones, genes and viruses in aetiology germ cell tumours. *Cancer Surv.* **9**, 263–286 (1990).
2. Zheng, T. *et al*. Continuing increase in incidence of germ-cell testis cancer in young adults: experience from Connecticut, USA, 1935–1992. *Int. J. Cancer* **65**, 723–729 (1996).
3. Adami, H.O. *et al*. Testicular cancer in nine northern European countries. *Int. J. Cancer* **59**, 33–38 (1994).
4. Bergstrom, R. *et al*. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon *J. Natl Cancer Inst.* **88**, 727–733 (1996).
5. Wanderas, H.E., Tretli, S. & Fossa, S.D. Trends in incidence of testicular cancer in Norway 1955–1992. *Eur. J. Cancer* **31A**, 2044–2048 (1995).
6. United Kingdom Testicular Cancer Study Group. Aetiology of testicular cancer, association with congenital abnormalities, age at puberty, infertility and exercise. *BMJ* **308**, 1393–1339 (1994).
7. Bokemeyer, C. *et al*. Bilateral testicular tumours prevalence and clinical implications. *Eur. J. Cancer* **29A**, 874–876 (1993).
8. Heimdal, K. *et al*. Familial testicular cancer in Norway and southern Sweden. *Br. J. Cancer* **73**, 964–969 (1996).
9. Forman, D. *et al*. Familial testicular cancer: a report of the UK family register estimation of risk and HLA class I sib-pair analysis. *Br. J. Cancer* **65**, 255–262 (1992).
10. Tollerud, D.J. *et al*. Familial testicular cancer and urogenital developmental anomalies. *Cancer* **55**, 1849–1854 (1985).
11. The International Testicular Cancer Linkage Consortium. Candidate regions for testicular cancer susceptibility genes. *APMIS* **106**, 64–70 (1998).
12. Leahy, M.G. *et al*. Candidate regions for a testicular cancer susceptibility gene. *Hum. Mol. Genet.* **4**, 1551–1555 (1995).
13. Lander, E. & Kruglyak, L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet.* **11**, 241–247 (1995).
14. Phelan, M.C., Stevenson, R.E., Collins, J.L. & Trent, H.E. Fragile X syndrome and neoplasia. *Am. J. Med. Genet.* **30**, 77–82 (1988).
15. Hasle, H., Mellemgaard, A., Nielsen, J. & Hansen, J. Cancer incidence in men with Klinefelter syndrome. *Br. J. Cancer* **71**, 416–420 (1995).
16. Hasle, H., Jacobsen, B.B., Asschenfeldt, P. & Andersen, K. Mediastinal germ cell tumour associated with Klinefelter syndrome. A report of case and review of the literature. *Eur. J. Pediatr.* **151**, 735–739 (1992).
17. Okada, H. *et al*. Klinefelter's syndrome in the male infertility clinic. *Hum. Reprod.* **14**, 946–952 (1999).
18. Reddy, S.R., Svec, F. & Richardson, P. Seminoma of the testis in a patient with 48,XXYY variant of Klinefelter's syndrome. *South Med. J.* **84**, 773–775 (1991).
19. Carroll, P.R., Morse, M.J., Koduru, P.P. & Chaganti, R.S. Testicular germ cell tumor in patient with Klinefelter syndrome. *Urology* **31**, 72–74 (1988).
20. Muller, J., Skakkebaek, N.E. & Ratcliffe, S.G. Quantified testicular histology in boys with sex chromosome *Int. J. Androl.* **18**, 57–62 (1995).
21. Kruglyak, L., Daly, M.J., Reeve-Daly, M.P. & Lander, E.S. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am. J. Hum. Genet.* **58**, 1347–1363 (1996).
22. Xu, J. *et al*. Evidence for a prostate cancer susceptibility locus on the X chromosome. *Nature Genet.* **20**, 175–179 (1998).
23. Wooster, R. *et al*. A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. *Nature Genet.* **2**, 132–134 (1992).
24. Heimdal, K. *et al*. A segregation analysis of testicular cancer based on Norwegian and Swedish families. *Br. J. Cancer* **75**, 1084–1087 (1997).
25. Nicholson, P.W. & Harland, S.J. Inheritance and testicular cancer. *Br. J. Cancer* **71**, 421–426 (1995).
26. Broman, K.W., Murray, J.C., Sheffield, V.C., White, R.L. & Weber, J.L. Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am. J. Hum. Genet.* **63**, 861–869 (1998).
27. Morton, N.E., Collins, A., Lawrence, S. & Shields, D.C. Algorithms for a location database. *Ann. Hum. Genet.* **56**, 223–232 (1992).
28. Gyapay, G. *et al*. The 1993–94 Genethon human genetic linkage map. *Nature Genet.* **7**, 246–339 (1994).