Chapter 2

Genetic Predisposition to Testicular Germ-Cell Tumours

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Introduction
The term testicular cancer encompasses a group of neoplasms that occur from childhood through to old age. This review focuses on germ-cell tumours of adolescents and adults, specifically seminomas and non-seminomas, which are thought to arise from carcinoma-in-situ. We do not discuss paediatric germ-cell tumours or spermatocytic seminomas which do not arise from carcinoma-in-situ and probably have a different cause from germ cell tumours in adolescents and adults. We focus mainly on testicular germ-cell tumours (TGCT). However extragonadal germ-cell tumours of adolescents and adults have a very similar cause and arise from carcinoma-in-situ; they can therefore be thought of as a part of the same disorder.

TGCT is the most common type of malignant disorder in men aged 15 - 40 years. The yearly incidence in this age group is about 7.5 per 100,000 people, but the incidence varies substantially between countries. TGCT can be classified histologically as seminoma (about 55%), which is most commonly diagnosed during the fourth decade of life, and non-seminoma (about 45%), which is generally diagnosed in the third decade of life. Both subtypes probably arise from preinvasive carcinoma-in-situ. The strongest risk factors for TGCT are a family history of the disorder, a previously diagnosed TGCT, and cryptorchism (undescended testis). In addition, patients with Klinefelter's syndrome or XY gonadal dysgenesis have a high risk of developing germ-cell tumours. Other less strong risk factors documented and confirmed in several studies include: testicular atrophy, inguinal hernia, infertility, hydrocele, previously diagnosed extragonadal germ-cell tumour and other disorders of male sexual differentiation. Many of the risk factors for TGCT include disorders of male urogenital differentiation. This feature has led to the term testicular dysgenesis syndrome (Figure 1), and both environmental and genetic factors are probably involved. In this review, we summarise the current knowledge of genetic predisposition to TGCT.

Clues to the existence of genetic predisposition
Family history
1 – 3% of patients with TGCT report an affected first-degree relative, a proportion higher than would be expected by chance (see Table 1). The largest number of reported cases in a family is five, but most familial clustering consists of relative pairs such as two affected brothers and to a smaller extent an affected father and son. Figure 2 gives examples of familial clustering in TGCT. Brothers of patients with TGCT have a relative risk of TGCT of 8-10, and for father-son the relative risk is 4 – 6. These relative risks for TGCT are higher than for most other cancer types, which rarely exceed 4. The high relative risk for TGCT is difficult to attribute only to a shared environmental component. To account for a relative risk greater than 3 without some form of genetic predisposition requires that the environmental risk factor or factors are extremely potent. Khoury and colleagues calculated that in this situation the risks
to exposed people are 50-100 times those of unexposed individuals. Such potent risk factors have not yet been identified for TGCT.

Racial differences and geographic clustering

Geographic clustering of TGCT and racial differences in the incidence of this disease could indicate a genetic component in the cause of the disease. The highest incidence is seen in white people of northern European descent, whereas people of African or Asian descent seem to have a universally low incidence. Differences in incidence persisting after migration argue in favour of genetic rather than exogenous risk factors. This maintenance of risk contrasts sharply with the situation for cancers of breast, stomach, colon and ovaries, for which the incidence in immigrant populations tends rapidly towards that of the host population.

### Table 1: First-degree familial TGCT and estimates of relative risk in male first-degree relatives of patients with TGCT 1985-2002

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study</th>
<th>No. FTC / total group</th>
<th>% FTC</th>
<th>Sibs / Fath -son</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tollerud</td>
<td>1985</td>
<td>Multicentre hospital-based, Retrospective</td>
<td>6/269</td>
<td>2.2</td>
<td>2/4</td>
<td>5.9</td>
</tr>
<tr>
<td>Kruse</td>
<td>1987</td>
<td>Single-centre hospital based, Retrospective</td>
<td>3/255</td>
<td>1.2</td>
<td>3/0</td>
<td>NC</td>
</tr>
<tr>
<td>Patel</td>
<td>1990</td>
<td>Multicentre hospital-based, Retrospective</td>
<td>5/500</td>
<td>1.0</td>
<td>1/4</td>
<td>NC</td>
</tr>
<tr>
<td>Forman</td>
<td>1992</td>
<td>Multicentre hospital-based and national population based, Retrospective</td>
<td>12/794</td>
<td>1.5</td>
<td>8/4</td>
<td>9.8</td>
</tr>
<tr>
<td>Westergaard</td>
<td>1996</td>
<td>Data from Danish cancer registry, Retrospective</td>
<td>22/2261</td>
<td>1.0</td>
<td>10/12</td>
<td>12.3</td>
</tr>
<tr>
<td>Polednak</td>
<td>1996</td>
<td>Population-based Connecticut Tumor Registry</td>
<td>12/1395</td>
<td>0.86</td>
<td>8/4</td>
<td>7.6</td>
</tr>
<tr>
<td>Heimdal</td>
<td>1996</td>
<td>Multicentre hospital-based, Retrospective</td>
<td>32/1159</td>
<td>2.8</td>
<td>NC</td>
<td>7.6</td>
</tr>
<tr>
<td>Dieckmann</td>
<td>1997</td>
<td>Multicentre hospital-based, Retrospective and Prospective</td>
<td>18/1692</td>
<td>1.1/1.7</td>
<td>9/9</td>
<td>7/2</td>
</tr>
<tr>
<td>Ondrus</td>
<td>1997</td>
<td>Single-centre hospital based, Retrospective</td>
<td>2/650</td>
<td>0.3</td>
<td>3/0</td>
<td>NC</td>
</tr>
<tr>
<td>Sonneveld</td>
<td>1999</td>
<td>Single-centre hospital based, Retrospective</td>
<td>17/686</td>
<td>2.5</td>
<td>11/6</td>
<td>8.5-12.7/1.7</td>
</tr>
<tr>
<td>Dong et al</td>
<td>2001</td>
<td>Data from Swedish family-cancer database, Retrospective</td>
<td>62/4650</td>
<td>1.3%</td>
<td>38/24</td>
<td>8.3-3.9</td>
</tr>
</tbody>
</table>

The incidence of this disorder in African Americans is a quarter of that among white Americans, and is similar to that of native African populations; thus the risk has not changed by much with migration to a new environment.

# Figure 1: The testicular dysgenesis syndrome. The asterisk indicates the possibility that cryptorchidism (testicular maldescent) acts as a causal risk factor. CIS: carcinoma in situ. Modified frame from: Skakkebaek NE, Raipert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16:972–978. © European Society of Human Reproduction and Embryology.

**FTC** = familial testicular cancer

**NC** = not calculated

**RR** = Relative Risk for all male first-degree relatives

= RR for brothers / RR for fathers

= RR only for brothers

= In a prospective multi centre study 18 of the 1692 patients had a first-degree relative with TGCT. Also a selection of patients from the Berlin (Germany) hospital were investigated: 518 patients, 9 had a first-degree relative with TGCT.
Bilateral TGCT and risk of other tumours

A consistent risk factor for the development of TGCT is having a previous testicular tumour. For patients who have had a first primary TGCT, the relative risk of developing a second is 25.1,2,3 The prevalence of bilateral TGCT ranges between 1.0% and 5.8% and studies have even shown an increase.4,5 Bilateral involvement of paired organs (breast, retina, and kidney) has proved to be a clinical marker of hereditary cancer. Bilateral cases are commonly associated with a positive family history for the same tumours.5,6 Heimdal and co-workers5,6 found that 2.8% of patients with TGCT who did not have a family history had bilateral disease compared with 9.8% of those with a positive family history. Bilateral disease in paired organs could also be the result of a very early somatic mutation (in embryogenesis), and evidence suggests that a proportion of bilateral TGCT could indeed be accounted for such a mechanism. Although mutations in the KIT (tyrosine kinase receptor) gene are rare in TGCT and mediastinal germ-cell tumours7,8, mutations of this gene occur in a very high proportion (95%) of tumours from patients with bilateral disease compared with a smaller proportion (3%) of tumours from those with unilateral disease.8,9 When both tumours from bilateral cases could be assessed, the same mutation was present in both. Together these results suggest that somatic KIT mutations occur early in embryogenesis, before the primordial germ cells have divided and migrated to the gonads. Primordial germ cells with KIT mutations are therefore distributed to both testes; hence KIT mutations are associated with bilateral disease.2,10 In patients with a family history of TGCT, the frequency of KIT mutations in unilateral TGCT was similar to that detected previously by Looijenga and colleagues.2,10 However, in patients with bilateral disease and a family history of TGCT, only 28% of tumours had mutations of KIT.2,10 Although the reason for this difference is unclear, the evidence suggests that bilateral disease in the context of familial TGCT has a different pathogenesis from sporadic bilateral cases and that most familial bilateral cases are explained by the high risk conferred by the underlying inherited genes.2,10

A genetic predisposition to TGCT does not necessarily have to be site specific; in analogy with the situation with many proven hereditary tumour predispositions, patients with TGCT and their relatives could have an increased risk of developing non-TGCT tumours. Dong and co-workers10,11 investigated this trend in 4650 patients with TGCT and with a mean follow-up of 11 years. They calculated a significantly increased standardised incidence ratio for various second primary tumours after testicular seminomas: TGCT 11.6 (95% CI 7.0-18.1), colorectal cancer 1.9 (1.1-3.1), pancreatic cancer 3.8 (2.1-6.4), renal cancer 2.2 (1.2-4.0), bladder cancer 2.4 (1.5-3.7), thyroid cancer 5.4 (1.4-14.2) and malignant lymphomas 2.5 (1.5-4.1). Some of the second primary tumours could be associated with treatment, since seminomas (mostly treated by radiotherapy) were associated with a higher frequency of second malignant disorders than non-seminomas (mostly treated with chemotherapy). Furthermore, an analysis of the prevalence of tumours in relatives also showed an increased risk of TGCT in male relatives of patients with TGCT (standardised incidence ratio 8.3 for brothers and 3.8 for fathers and sons).2,5,10 For all relatives together there was no excess risk of other cancer types in first-degree relatives of patients with
TGCT. However, mothers of patients with TGCT had significantly increased standardised incidence ratios for lung cancer (1.9; 95% CI 1.4-2.6), non-endometrial uterine cancer (2.6; 1.2-4.7), soft-tissue tumours (2.5; 1.1-4.9) and malignant melanoma (1.8; 1.1-2.6). Other studies\(^\text{(7-9)}\) have shown similar trends (although some not significant) of other cancers in mothers. However, all studies\(^\text{(6,7,9)}\) show a high frequency of TGCT in relatives of patients with TGCT. These findings generally suggest that relatives of patients with TGCT have a greatly increased risk of developing TGCT but may not be at risk of developing other types of cancers.

**Syndromic characteristics**

Two constitutional chromosomal abnormalities are clearly associated with an increased risk of TGCT. Patients with Klinefelter’s syndrome (47 XXY) have a very high risk of mediastinal (extragonadal) germ-cell tumours.\(^\text{1-4}\) About 8% of patients with such tumours have Klinefelter’s syndrome.\(^\text{14}\) Patients with this syndrome rarely develop TGCT, probably because they do not have germ cells in the testis from shortly after birth; however, about a third of patients with extragonadal germ-cell tumours present with testicular carcinoma-in-situ and these patients have a substantial risk of developing TGCT (see later).\(^\text{14,17-20}\) Patients with XY gonadal dysgenesis have a greatly increase risk of germ-cell tumours.\(^\text{19,21}\) This increased risk is seen only in patients with Y chromosome material; those with gonadal dysgenesis and XX or 45X karyotype do not have an increased risk of TGCT.\(^\text{11,19,21}\) Patients with Down’s syndrome (trisomy 21) might also be at increased risk of TGCT but the numbers are too small for firm conclusions to be drawn.\(^\text{10,22}\)

The presence of TGCT in a hereditary syndrome might be an indication of a hereditary predisposition to TGCT. We have described the prevalence of TGCT in patients with various hereditary disorders.\(^\text{24}\) Owing to the rarity of most of these disorders and the scarce reports of their occurrence in combination with TGCT, at present there is no statistical proof of an association of TGCT with these disorders. An important clinical issue is that in a proportion of these disorders there is also a range of defects in urogenital differentiation, which suggests that TGCT in these disorders is indeed a further complication of such a differentiation defect, as postulated in the model of Skakkebaek and colleagues (figure 1).\(^\text{24}\)

**Cryptorchism and other disorders of testicular differentiation in patients and relatives**

Cryptorchism and other testicular abnormalities such as atrophy, infertility, hydrocele and inguinal hernia are risk factors for TGCT.\(^\text{5,6,14,22,68,80}\) As regards family history, Forman and co-workers\(^\text{58}\) showed that the frequency of cryptorchism in patients with TGCT did not differ between those with or without a positive family history. However, very few studies have looked at the frequencies of these risk factors in male relatives of patients with TGCT. A small study by Tollerud and colleagues\(^\text{35}\) showed that cryptorchism occurred in a significantly greater proportions of first-degree male relatives of patients with a family history of TGCT (27%) than of relatives of patients with TGCT who did not have such a family history (5.3%) or of controls (2.7%). Their study also showed that 50% of patients who had TGCT and a family history reported a first-degree relative with inguinal hernia, compared with 10.3% of those without a family history and with 12.7% of controls. The high prevalence of cryptorchism, inguinal hernias, and hydrocele among men in these families suggests that an underlying alteration in urogenital embryogenesis could be associated with the familial predisposition to testicular neoplasia.

**Studies on Twins**

Studies on twins with cancer have been used to address two general issues. First, are there any carcinogenic effects of twinning? This question can be answered by comparison of the occurrence of a cancer in twins and in singletons. The second issue, what the heritable components are to that cancer, can be addressed by a proband-wise analysis (proband = the affected individual through whom a family with a genetic disorder is ascertained) of monozygotic twins compared with dizygotic twins or siblings. If there is a heritable component the relative risk should be higher for monozygotic than for dizygotic twins.

In a cohort of 14326 elderly twins aged 66 – 77 years, Braun and co-workers\(^\text{59}\) noted that a personal history of TGCT was reported by five (0.08%) of 5951 monozygotic twins and 11 (0.16%) of 6992 dizygotic twins. Swerdlow and colleagues\(^\text{60}\) also found a significantly higher risk of TGCT in dizygotic than in monozygotic twins (odds ratio 1.5; 95% CI 1.1-2.2).\(^\text{60}\) These finding suggest that an environmental component was acting in utero to cause TGCT. To find a possible genetic effect, twin studies must assess the risk of TGCT in the twin brothers of affected patients; however, the numbers are too small in many studies for any conclusions to be drawn. Swerdlow and colleagues\(^\text{60}\) identified six pairs of concordant (both affected) twins. The risk of TGCT was raised in twin brothers of patients with TGCT (relative risk 37.5; 95% CI 12.3 – 115.6) and was greater in monozygotic (26.5) than dizygotic (35.7) twins, which would be expected if there is a heritable part to TGCT. This relative risk is several times that found in non-twin brothers (although the confidence limits are wide because of small numbers) but it does imply that the genetic element for risk is far larger for TGCT than for most other cancers. Other twin studies on TGCT have not had sufficient numbers of concordant twin pairs and have been unable to determine zygosity so could not confirm this result.

**Environmental components**

The worldwide incidence of TGCT has more than doubled over the past 40 years. The increase follows a birth-cohort effect, and a probable explanation is that factors in embryogenesis or early life are contributing to the development of TGCT.\(^\text{20,35,84}\) The rapid increase highlights the importance of environmental factors in this disease, because the genetic composition of a population simply cannot change in the course of one or two generations. Why this increase is occurring is unclear, but one theory is that an increase in endogenous oestrogens is contributing to increase in TGCT in addition to risk factors for the disorder such as the increase in incidence of cryptorchism and the decrease in fertility and sperm quality.\(^\text{36,88}\) Another hypothesis is that abnormally high oestrogen concentrations in pregnancy predispose the developing gonad to
TGCT in adulthood. Direct investigation of this idea is difficult, and many studies have looked at surrogate features that could reflect high oestrogen concentrations in pregnancy.\(^{(90)}\) According to this hypothesis, a high rate of TGCT in dizygotic twins (because uterine oestrogen concentration during pregnancy are higher for dizygotic than for monozygotic twins), as well as older ages of mothers and a higher rate of oestrogen related cancers (eg, breast cancer) in mothers and sisters would be expected.\(^{(88)}\) Overall published reports\(^{(87;88)}\) provide conflicting results on the analysis of these variables. These features are merely weak indicators of high oestrogens concentrations, so the sample size and statistical power of many of these case-control studies might have been too low to show any significant association. Although hormonal factors could be causally involved in the development of TGCT, mothers of patients with TGCT do not have an increased risk of oestrogen related cancer and the risks of breast cancers does not seem to be increased in the sisters of these patients.\(^{(91;92)}\) Even though many studies have showed similar results, this hypothesis remains unconfirmed.

Several studies have confirmed a higher frequency of TGCT in dizygotic twins than in singletons and monozygotic twins. This difference suggests that the concentration of circulating oestrogen, which is significantly higher in pregnancies with dizygotic than in those with monozygotic twins or singletons, is a contributory causative factor.\(^{(88;89;93)}\) However, more research is needed into the pathogenetic mechanisms that could cause the increase risk of TGCT in dizygotic twins. Twinning itself is to a substantial extent genetically determined, and co-inherited genetic factors could conceivably be contributing to this increase rather than circulating oestrogens.

**Modelling studies**

Two studies so far have tried to identify the mode of inheritance of TGCT susceptibility genes. One was based on the frequency of bilateral TGCT,\(^{(90)}\) and the other was a segregation analysis on a group of Norwegian and Swedish families.\(^{(95)}\) In a segregation analysis, a disease model is sought for familial aggregation of a disease by fitting the patients and non-affected family members in pedigrees to, for example, a dominant or recessive disease model. Heimdal and colleagues\(^{(90)}\) did such an analysis on all available patients with TGCT treated at a Norwegian hospital and a Swedish hospital between 1981 and 1991 (n=978). For 30 patients, a first-degree relative also had TGCT; there were no families with more than two affected members. The investigators concluded that the familial clustering was best accounted for by a major gene for TGCT with a recessive mode of inheritance. Their analysis took into account that the incidence of TGCT has increased substantially over the past few years and that the treatment for TGCT has improved greatly and led to better maintenance of fertility and longer survival. The study showed that time trends in TGCT make little difference to the evidence for the recessive mode of inheritance.\(^{(90)}\) Under this recessive model, the estimated gene frequency was 3.8%. Thus 7.3% (according to the Hardy-Weinberg equilibrium) of men in the population carry the mutant allele and that 0.1% are homozygous. According to the calculations by Heimdal and colleagues, the life-time risk of development of TGCT in homozygous men is 43%.\(^{(89)}\)

Nicholson and Harland\(^{(91)}\) aimed to define the incidence of genetically determined TGCT in the general population. They analysed published data on the age of onset of TGCT and the prevalence of bilateral TGCT in familial and unselected general cases according to the arguments used by Knudson’s two-hit model for tumorgenesis in patients with a familial predisposition to a certain cancer.\(^{(90)}\) In the general population bilateral TGCT occurs much more frequently than could be attributed to chance. On the basis of the comparison of the distribution of age at diagnosis between patients with bilateral TGCT and familial cases (ie, those likely to be genetically determined), the investigators assumed in their model that the increased risk was due solely to genetic susceptibility. Thus patients with bilateral TGCT probably carry the same susceptibility genotype as that causing familial TGCT. Nicholson and Harland\(^{(91)}\) estimated that about a third of the general patients with TGCT carry the susceptibility genotype and that the penetrance is 0.45. Calculations with these values showed that a recessive disease model showed a better fit with the observed risks for brothers (2.2%) and fathers (0.5%) of patients with TGCT than a dominant disease model. The frequency of the susceptibility allele in the recessive model was estimated to be 5%. Although this analysis, based on the simplest set of assumptions, fitted the data reasonably well, Nicholson and Harland conceded that it was based on data obtained under diverse conditions and could be subject to several unknown biases. Therefore, the assumptions that underlie the model might be incorrect. They also conceded that some unilateral tumours may be predisposed to a contralateral tumour by some as yet unknown biological mechanism (indeed, such a mechanism was later identified as early somatic KIT mutations, as discussed above) or that there is more than one predisposition gene and that the mode of inheritance is more complex.

X-linkage was not specifically addressed by the two studies mentioned. The higher relative risk for brothers than for father-son pairs is compatible with a recessive mode of inheritance, but since the early 1990s the incidence of TGCT has increased sharply, maintenance of fertility after treatment for TGCT has improved greatly, and the introduction of cisplatin-based chemotherapy has substantially lowered the number of deaths from TGCT.\(^{(92-94)}\) These changes might lead to a situation in which the relative risk for father-son is higher than was believed initially. A further possibility is that because of shorter survival (ie, a lower chance that a TGCT patient will father a child) and abolition of fertility in the past (before the 1990s), less transmission of TGCT from father to son was seen. Although the study by Heimdal and colleagues\(^{(90)}\) tried to take this factor into account, the investigators admitted that to do so is very difficult and that more complex assumptions are involved. The study by Nicholson and Harland\(^{(91)}\) made allowance for the time trends, but partly because only two studies have attempted to define the mode of inheritance, there is a risk that the conclusion of inheritance according to a recessive model is incorrect. A dominant form of inheritance should therefore not be totally excluded. Because a proportion of bilateral cases are caused by early somatic mutations in KIT and because there is clearly more than one TGCT susceptibility gene, susceptibility to TGCT is probably more complex than suggested by either of these models.
**Association studies and haplotype analyses**

Association studies try to find statistical evidence of an association between polymorphic loci on, or closely linked to, candidate genes (eg, genes involved in the metabolism of mutagenic agents, known tumour-suppressor genes or oncogenes) and a particular phenotype (eg, TGCT) by comparing frequencies between cases and controls. Because TGCT is a rare disease and mapping of the causal genes is difficult, TGCT patients and families from founder populations are very suitable people in whom to detect TGCT predisposition genes with the aid of association or haplotype analyses. Patients in founder populations are expected to share a high number of mutations predisposing to TGCT from recent common ancestors. They will also share segments of DNA surrounding the disease mutations. Haplotypes consisting of conserved, ancestral alleles at genetic markers covering the region of a disease mutation will therefore be more frequent among patients than among controls.

Several association studies have been done on TGCT. In Table 2, an overview is given of these studies. Much attention has been paid to HLA genes and TGCT. The HLA region is thought to be interesting for such studies, because differences in immune response based on HLA variation might have a role in the development of cancer and metastases. Hodgkin’s lymphoma, Kaposi’s sarcoma, colorectal cancer and breast cancer are associated with this genomic region. The effects (based on HLA variation) of the immune response to carcinogenic factors such as viruses that might be associated with TGCT, could contribute to the development of the disorder.

Previous studies have shown that HLA factors might be associated with the development of TGCT. In particular, consistent associations were found with the HLA class II antigens. No association was found with the HLA A or C regions, and inconsistent associations were found with the HLA B region. The method used in most studies was serotyping, which is much less accurate and less efficient than the more recent genotyping studies. DNA typing is far more sensitive and can identify larger numbers of alleles. The first HLA genotyping study by Ozdemir and colleagues in 55 Japanese patients, showed two associations: one HLA susceptibility allele and one HLA protective allele (relative risk 3.26 and 0.26 respectively). A much larger genotyping study on the HLA class II region of chromosome 6p21 in 151 patients from the northern part of the Netherlands could not replicate this association. The association between TGCT and HLA class II alleles either does not exist or is much weaker than the earlier report suggested.

The other association studies in Table 2 give an overview of the diverse associations that researchers have attempted to show with TGCT. In many cases, only one study has investigated a certain region. To date, no convincing associations has been shown between TGCT and a genetic polymorphism.

**Linkage analysis and genome-wide screens**

Linkage analysis relies on the fact that during the formation of gametes through meiosis chromosomal material is exchanged between homologous chromosomes. This recombination of genes means that were once on the same homologue of a particular chromosome pair become separated and those once on different homologues are brought together. The process of recombination is achieved by crossing-over of the chromosomes during meiosis. The probability that a cross-over will occur and two loci on the same chromosome will randomly segregate increases with the distance between the genetic loci. For two genes close together on a chromosome, a cross-over is unlikely to occur and the two loci will be inherited together.

Linkage analysis uses this feature to map genes by means of a series of polymorphic genetic markers (microsatelite markers or single-nucleotide polymorphisms) along the chromosomes to follow the inheritance pattern of marker loci and the disease locus through a family. If a particular polymorphism or marker is inherited by a higher proportion of patients than would be expected by chance, the locus is said to be linked to the disease and is in fact probably very closely located to the disease locus on the DNA strand. Since the position of the marker locus is known, the location of the unknown disease locus is then found. Linkage analysis in a set of pedigrees with many cases of a cancer has identified genes associated with breast cancer, colon cancer, familial melanoma, and others. For many of these cancers the pedigrees used had many affected cases spread over several generations. In many cases, a single pedigree provides sufficient numbers of affected cases to generate a statistically significant LOD score (logarithm of odds, a statistic that indicates whether a locus is inherited by a higher proportion of patients than expected by chance). The search for a TGCT susceptibility gene has been hampered by a lack of these large multigenerational pedigrees. Most families identified are relative pairs, generally siblings. Larger pedigrees with three, four or five affected cases have been reported but these rarely extend beyond two generations.

The International Testicular Cancer Linkage Consortium (ITCLC) has the largest collection of TGCT pedigrees with two or more cases of TGCT in a family. An analysis by this group has shown that no single autosomal gene accounts for all TGCT pedigrees. The analysis of 160 TGCT pedigrees consisted of calculations under the best autosomal dominant and autosomal recessive models given by the segregation analysis of Heimdal and colleagues, but allowing for the possibility of genetic heterogeneity (ie, several genes are involved separately or in interaction with each other). An X-linked component to susceptibility was not considered. The analysis suggests that this set of pedigrees has sufficient power to detect two TGCT genes each contributing to 50% of the set with a statistically significant LOD score greater than 3 under both a dominant and a recessive model. The analysis also investigated the power to detect four TGCT susceptibility genes each contributing to a quarter of the family set. In this case, significant LOD scores would not be possible unless the number of families in the set approached 500 pedigrees. The last report by the ITCLC was on a total of 179 pedigrees and the failure to find LOD scores greater than 3 in any autosomal locus suggests that no single locus can explain occurrence of TGCT in at least 50% of the families. It also suggests that there are more than two TGCT susceptibility genes. The power to detect some or all of the susceptibility genes
### Table 2: Testicular Cancer Association Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of study</th>
<th>Gene</th>
<th>Association</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>DeWolf et al. 1979</td>
<td>Investigated genomic DNA of 61 TGCT patients for HLA A, B, C specificities.</td>
<td>HLA A, B, C, D</td>
<td>Increased antigen frequency of DW7 insubgroup with teratocarcinoma</td>
<td>Possible association between HLA DW7 antigens and TGCT</td>
</tr>
<tr>
<td>Carr et al. 1979</td>
<td>Assessed 20 patients with teratocarcinoma</td>
<td>HLA antigens</td>
<td>Increased antigen frequency of AW24 in patients with metastatic disease</td>
<td>Possible association between HLA AW24 and metastatic TGCT</td>
</tr>
<tr>
<td>Majsky et al. 1979</td>
<td>Tested 23 HLA antigens of A and B loci in 62 patients with testicular germinative tumours and 301 healthy unrelated subjects.</td>
<td>HLA A, B</td>
<td>No significant result</td>
<td>No association between TGCT and HLA A or B antigens</td>
</tr>
<tr>
<td>Pollack et al. 1982</td>
<td>Assessed expression of HLA antigens in 145 unrelated white patients with TGCT</td>
<td>HLA A, B, C, D</td>
<td>Low frequency of DR3 in all patients, although no difference was significant</td>
<td>Causal and prognostic importance of overall decreases in HLA DR3 remains to be determined</td>
</tr>
<tr>
<td>Oliver et al. 1986</td>
<td>114 TGCT patients</td>
<td>HLA A, B, C, D</td>
<td>Increased DR5 in seminoma patients, (p&lt;0.04) and increased HLA-DR7 in stage IV disease TGCT (p&lt;0.05)</td>
<td>Possible association between HLA DR5 and DR7 antigens and TGCT</td>
</tr>
<tr>
<td>Aliginger et al. 1987</td>
<td>1: Investigated 132 TGCT patients 2: completed joint calculation of published data on HLA antigens in 351 TGCT patients</td>
<td>HLA B13, DR</td>
<td>1: Increased frequencies of HLA B13 in TGCT with haematogeneous metastases (p&lt;0.01), of DR2 in TGCT without metastases (p&lt;0.001), and of DR1 in seminoma (p&lt;0.035) 2: Increased frequencies of DR1 (p&lt;0.025) and DR5 (p&lt;0.015) in seminoma; increased frequency of DR5 (p&lt;0.05) and DR7 (p&lt;0.05) in non-seminoma with haematogeneous metastases</td>
<td>Possible association between HLA antigens and TGCT</td>
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<td>Dieckmann et al. 1989</td>
<td>Reviewed 119 families in published reports with aggregation of TGCT</td>
<td>HLA antigens</td>
<td>Increased frequency of HLA antigens HLA A3 and B7 (p&lt;0.02)</td>
<td>Haplotype sharing in all (61% of) TGCT cases that is more than expected. Additional evidence for theory of genetic influence in the cause of TGCT</td>
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<td>Ryberg et al. 1993</td>
<td>Assessed restriction-fragment-length polymorphism of the VNTR region flanking the HRAS gene in genomic DNA from 348 TGCT patients and 343 healthy controls</td>
<td>HRAS</td>
<td>Higher frequency of rare alleles in TGCT patients (23/696) than in controls; (p&lt;0.004). Higher frequency of rare alleles in bilateral TGCT (p&lt;0.001)</td>
<td>Patients with bilateral cancer or unilateral cancer younger than 20 years of age had significantly higher incidence of rare HRAS alleles than patients with unilateral cancer older than 20 years</td>
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<td>Dieckmann et al. 1993</td>
<td>Investigated blood groups of 577 patients treated for TGCT and controls; Lewis antigens of 143 patients treated for TGCT, and controls; and HLA antigens of 215 patients treated for TGCT and controls</td>
<td>Lewis antigen and HLA Bw41 antigens</td>
<td>More frequent Lewis antigen Le(a-b-) in patients than in controls; (p&lt;0.046; relative risk 2.38). HLA Bw41 associated with seminoma (p&lt;0.00001; relative risk 8.2)</td>
<td>Associations of Le(a-b-) with TGCT and of HLA Bw41 with seminoma support contention that genetic factors are involved in the cause and pathogenesis of TGCT. HLA Bw41 could be used as risk marker for seminoma</td>
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<tr>
<td>Heimdal et al. 1994</td>
<td>Compared TGCT (n = 442) and control (n = 384) population for the allele frequencies of two polymorphic loci located at chromosome band 11p13</td>
<td>WT1 (Wilm’s tumor); One of the polymorphisms (WT) was located within and the other (D15S25) in close proximity to WT1</td>
<td>A1 allele of WT1 polymorphism associated with bilateral TGCT (p&lt;0.03) and disseminated TGCT (p&lt;0.03)</td>
<td>Findings might indicate an involvement of the WT1 both in susceptibility to TGCT and in progression of the disease</td>
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for TGCT will depend greatly on the degree of genetic heterogeneity of this disease. Continued identification and recruitment of families into this linkage study will be crucial to the efforts to identify these genes.

The first evidence for a TGCT susceptibility gene was for the X chromosome. The analysis on 99 pedigrees compatible with X linkage (no male-to-male transmission) yielded a heterogeneity LOD score of 2.01 on chromosome Xq27. The data were subsequently stratified according to the presence of at least one bilateral case, the presence of undescended testis, histology, and age. Families with at least one case of bilateral disease showed strong evidence of linkage to a locus on Xq27 (heterogeneity LOD score 4.76) and were more likely to show linkage to the X chromosome than were families without a bilateral case. This score corresponds to a genome-wide significance of p=0.034, equivalent to a LOD score of 3.78 in a genome-wide linkage search of affected sibling pairs without subgrouping. This proposed susceptibility gene on chromosome Xq27 was called TGCT1. In Klinefelter’s syndrome, the increased risk of extradermal germ-cell tumours is associated with an extra copy of the X chromosome which further suggests X-linked involvement in TGCT. In addition, the subset analysis provided evidence that the TGCT1 might also predispose to cryptorchism. Linkage to this locus was found in 73% of families with cryptorchism compared with 26% of families without cryptorchism (p=0.03). The results also suggest that about a third of the excess familial TGCT risk to brothers is from TGCT1, with little difference in the residual risks to brothers and sons after this locus has been accounted for. This is the first cancer gene to be mapped in a genome wide search of predominantly sibling pairs and was the third male cancer gene mapped to the X chromosome.

Conventionally, such a result would be ratified in another set of families; however no other set of TGCT pedigrees of similar size is known. These pedigrees are rare and collection of a similarly-sized set may take several years. The ITCLC reported preliminary results on an additional 25 pedigrees compatible with X linkage but the set was too small for any firm conclusions to be drawn. The gene in this region has not been identified.

TGCTs is unlikely to be the only TGCT susceptibility gene and it could account for as few as 25% of all TGCT pedigrees. Several genome searches have now been done on the ITCLC set, and suggestive evidence for linkage has been obtained for several autosomal regions including 3q27, 12q12-q13, 16p13, 18q22 – qter. Many more pedigrees need to be assessed before any of these regions can be conclusively identified as including a TGCT susceptibility gene.

Discussion
There is evidence that TGCT susceptibility genes exist and are important in this disease. This evidence includes increased TGCT risks associated with a positive family history, the higher frequency of bilaterality in familial cases and the ethnic and racial differences that do not change with migration, as well as the mathematical modelling of observed familial and non-
familial cases, and possible associations with known hereditary syndromes and constitutional chromosomal anomalies; the latter commonly seen within the setting of defects in urogenital differentiation.

Human TGCT susceptibility genes have not yet been identified. The putative gene mapped to Xq27 is postulated to confer an increased risk of TGCT as well as cryptorchism. Completion of the human gene map, further studies on animal models, the arrival of advanced gene arrays (chips), genome-wide single-nucleotide polymorphism technology, and applied bioinformatics are expected to facilitate further exploration of genetic predisposition to TGCT. One point of interest is whether such predisposition can be linked to a genetic contribution from increased intrauterine oestrogen concentrations and susceptibility to disruption of usual urogenital differentiation or to an environmental factor that would account for the increasing incidence of TGCT. Insight into genetic features of TGCT not only might contribute to the identification of individuals at increased risk of developing the disorder, but also is likely to increase our understanding of normal urogenital differentiation and non-hereditary TGCT. This knowledge will contribute to improving the diagnosis and treatment of TGCT in the general population. From a practical clinical perspective, identification of men with an increased risk of TGCT depends on the presence of known risk factors, including the family history of cancer. Clinicians should therefore record the family history of cancer and urogenital differentiation defects as part of their routine clinical practice in patients with TGCT or urogenital differentiation defects. Brothers of patients with TGCT should be informed about their risk of developing the disorder and should be encouraged to examine their testicles regularly. The US National Cancer Institute and the ITCLC are doing a genetic and causal multidisciplinary study of familial TGCT. (http://familial-testicular-cancer.cancer.gov/). However, unlike some other increased risk situations determined by positive family histories (eg, those of colorectal cancer), the effects of preventive measures, including testis self-examination, in terms of tumour risk as well as psychosocial effects remain to be investigated.

Search strategy and selection criteria
Articles and studies included in this review were identified by searches of titles, abstracts and keywords of reports included in PubMed and through the list of references cited by the papers found by those PubMed searches. Searches were done with combinations of the search terms: "testicular", "seminoma", "non-seminoma", "familial", "risk factors", "genetic", "tumo(u)r", "gene(s)", "linkage" and "association". Papers published before 1970 and those published in languages other than English, French and German were excluded.