Predisposition alleles for testicular germ cell tumour
Elizabeth A Rapley¹ and Katherine L Nathanson²

For some time, it has been known that there is a substantial genetic component to testicular germ cell tumour susceptibility, supported by several pieces of evidence, including the significantly increased familial risk and differential risk among races. However, despite extensive linkage searches on available families, no high penetrance genes have been identified. Recently genome-wide association studies have revealed three candidate loci, which confer up to a four-fold risk of developing TGCT. The genome-wide association studies for this cancer are noteworthy, because of the high effect sizes demonstrated at each loci and the biological plausibility of the genes at or near the associated SNPs, particularly KITLG.

Introduction
Although testicular germ cell tumour (TGCT) is a rare cancer affecting approximately 2000 [1] men in the UK and 8400 [2] men in the US each year, it is the most common cancer in men aged 15–45 years. The worldwide age-standardised incidence of the disease is 1.5/100,000 [3], but rates vary considerably between countries and ethnic groups. TGCT is 4.5-fold more common in white non-Hispanic Caucasian populations than in black populations, with intermediate incidence rates reported for Asian populations [4]. The risk of TGCT has increased significantly over the past 30 years, increasing from 3.3/100,000 in 1975 to 6.9/100,000 in 2006 in the UK [1] and 4.1/100,000 in 1975 to 6.6/100,000 in the US [5] (Figure 1). Similar trends are noted across most European countries [6]. The reasons for the increasing incidence remain undefined.

There are two histological types of TGCT, seminomas and non-seminomatous germ cell tumours (NSGCT), which differentiate towards spermatocytic, and embryonal and extraembryonal structures, respectively. Some TGCTs show features of both histological types. TCGT are believed to arise from primordial germ cells (PGCs) through a preinvasive phase of intratubular germ cell neoplasia (ITGCN) [7]. The peak in incidence for non-seminoma occurs between the ages of 20 and 30 with seminomas manifesting a decade later [8]. Most adult TGCT are malignant tumours with a strong propensity to metastasize, yet are remarkably sensitive to radiotherapy and/or chemotherapy with a five-year survival rate exceeding 95% [9].

Conclusive risk factors for TGCT include family history [10–14], previous germ cell tumour [15], subfertility [16,17], undescended testis (UDT) [18] and testicular microlithiasis [19]. Several studies have estimated that the increased risk to a brother or father of a patient with TGCT as 8–10- and 4–6-fold, respectively [10,12,20], much higher than for most other cancer types which are generally no more than two-fold [21]. Apart from infertility [22] and perhaps TM [23,24], there is no supporting evidence for increased incidence of TGCT risk factors among other male family members of TGCT cases [25,26].

Whereas environmental factors must be contributing to the increasing incidence of the disease, TGCT is highly heritable, with the proportion of TGCT susceptibility accounted for by genetic effects estimated to be 25% from a Swedish Family Cancer database of 9.6 million individuals, the third highest among all cancers [27]. Despite the high heritability, large multiple generational pedigrees for TGCT are largely unknown and the majority of families are relative pairs, usually brothers [28]. A genome-wide linkage study failed to provide evidence for the location of a gene predisposing to TGCT and suggested that susceptibility was likely to be due to genes with small or moderate effects on risk [28]. Although the gr/gr deletion on the Y chromosome, investigated as a candidate region, increases the risk of TGCT two- to three-fold the frequency of this variant is low (2–3%), suggesting that it only accounts for a small component of risk [29].

Genome-wide association studies
Two genome-wide association studies (GWAS) have been published for TGCT within the last year [30**,31**]. Compared to GWAS for other cancers the sample sizes of both studies are relatively small (Table 1). The UK study discovered five loci, with SNPs on chromosomes 1, 4, 5q31.3, 6 and 12p22, which were analysed further in a replication phase. The US study identified three loci on

addresses
¹ Testicular Cancer Genetics Team, Section of Cancer Genetics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK
² Department of Medicine, Medical Genetics, Abramson Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

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DOI 10.1016/j.gde.2010.02.006

This review comes from a themed issue on Molecular and genetic bases of disease
Edited by Ian Tomlinson and William Foulkes

Available online 19th March 2010

0959-437X/$ – see front matter

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Corresponding author: Rapley, Elizabeth A (Liz.Rapley@icr.ac.uk)


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chromosomes 2p14, 5q31.3 and 12p22 and took these forward to a replication phase. Both studies confirmed associations on chromosomes 5 and 12. The UK study also confirmed the association at the chromosome 6 loci (Table 2). The loci on chromosomes 1 and 4 were not convincingly replicated and these remain candidates for TGCT susceptibility but require further validation. The chromosome 2p14 locus in the US study failed to show an association upon case–control replication, but was replicated in parent–child triad and dyads (unpublished data).

Risk alleles
The strongest association identified was for SNPs at 12q22 within KITLG with both studies identifying a greater than 2.5-fold increased risk of disease per major allele. For the chromosome 5 and 6 loci, a 1.5-fold increased risk was identified per major allele. The risks to homozygotes with the high risk alleles on chromosome 12 is greater than four-fold and is approximately two-fold for the loci at chromosomes 5 and 6. There is some evidence to suggest a positive interaction between the loci on chromosomes 5, 6 and 12 [30**, with the combined risk greater than the sum of the individual risks. The highest risk individuals – that is males who are homozygous for all four high risk alleles (~0.7% of the population) – have a predicted risk approximately 40 times that of men who are homozygote for the lowest risk alleles [30**].

The power to detect the loci at chromosome 12 was approximately 96% indicating that additional studies are unlikely to reveal loci with similar effect. The power to detect the loci at chromosome 5 and 6 was approximately 65% and 80%, respectively, indicating that there may be more loci of similar or lower effects to be detected with additional GWAS using new sample sets and meta-analysis of the current GWAS.

Both studies investigated if the loci were associated with different subgroups of TGCT by specific phenotypes or risk factors. There was some evidence of age of diagnosis effect for the chromosome 5 SNP, rs4624820, with a higher OR in early onset cases [30**]. Neither the UK series with 220 (17.8%) cases with a family history of disease nor US series showed a difference between cases with and without a family history, with or without a history of UDT and with or without bilateral disease. However, the power to detect a difference in cases with maldescent or bilateral disease in both studies is limited owing to the small number of cases positive for bilateral disease or UDT.

None of the loci showed associations with histological subtype in either study. The absence of a difference between seminoma and non-seminoma may not be that
surprising. Both, despite their distinct biological and histological features, arise from the same cell of origin (primordial germ cells). Additionally, one-third of tumours have mixed pathology [8] and patients with bilateral disease do not have a histological concordance greater than that expected by chance [10]. Furthermore, there is no evidence of clustering of histological type within families with multiple cases of TGCT [32].

It would have been expected that there would be some degree of familial enrichment at the loci identified and it is surprising that a higher OR was not demonstrated in familial TGCT cases compared to non-familial. The reasons for this remain unclear and additional larger studies are needed.

**Genes implicated in TGCT susceptibility**

While the location of a disease associated SNP near or within a gene does not necessarily implicate that gene in disease susceptibility, all three loci identified suggest that the \textit{KITLG–KIT} pathway, which regulates the survival, proliferation and migration of PGCs, is central to the molecular pathology of TGCT [33] (Figure 2).

The SNPs at 12p22 implicate \textit{KITLG}, the only annotated protein coding gene within the linkage disequilibrium block of associated SNPs at chromosome 12p22. The \textit{KITLG} (also known as stem-cell factor or Steel in mice) encodes the ligand for the membrane-bound receptor tyrosine kinase \textit{KIT}. Several lines of biological evidence implicate the \textit{KITLG} in TGCT susceptibility.

In mouse models, \textit{Kit} (encoded at the Steel (Sl) locus) has been shown to be required for multiple aspects of PGC development. Kit plays a crucial role in the migration of PGCs from the hindgut and subsequent targeting to the genital ridges; downregulation of Kit in the midline triggers localised apoptosis of PGCs [34]. In the mouse, germline homozygous null mutations of either \textit{Kit} or \textit{Kitl} lead to infertility in male mice as a result of a failure of PGC development. Furthermore, in the 129/Sv mouse (the mouse model for TGCT) loss of the transmembrane form of \textit{Kitl} leads to decreased germ cell number and has been identified as the TGCT susceptibility allele, conferring a two-fold increased risk [35*].

In humans, multiple studies have suggested that TGCT arises from PGCs, which share numerous features in common with intratubular germ cell neoplasia (ITGCN), the precursor to invasive disease [36]. Delayed differentiation of PGCs has been associated with the development of ITGCN in individuals with intersex conditions and chromosomal anomalies [37]. Thus, downregulation of the \textit{KITLG–KIT} pathway may lead to a delayed differentiation of PGC and subsequent development of TGCT (Figure 1).

Somatic alterations in \textit{KIT} have been described for TGCT, particularly seminomas. Overexpression of \textit{KIT} is characteristic of seminomas [38,39] and increased copy number of the \textit{KIT} gene has been observed in 21% of seminomas and 9% of non-seminomas [40]. The COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) reports an overall somatic mutation rate in \textit{KIT} of 9% for all TGCT, 20% in seminomas. Paradoxically the somatic changes in \textit{KIT} are predicted to upregulate pathway activity, whereas germline deletions of \textit{KITLG} that modify increased susceptibility in mice are predicted to reduce it [41].

The involvement of the \textit{KITLG} in TGCT may provide an explanation for the observed difference in the incidence of TGCT between whites and blacks. \textit{KITLG} has a role in determining levels of pigmentation [42]. \textit{KITLG} has undergone strong selection in European and East Asian populations [43] and the data from Hapmap show significant difference between the frequency of the risk alleles of \textit{KITLG} between European (all ~0.80) and African populations (~0.25) [44]. This difference may in part explain the lower incidence of TGCT in men of African descent.

The biological observations support the hypothesis that disruption of the \textit{KIT–KITLG} pathway in involved in TGCT development. Interestingly, disruption of this pathway in the mouse leads to infertility and suggests a potential explanation for the epidemiological association between TGCT and infertility. Indeed a small
Migration of germ cells from hindgut to genital ridge and into gonads during which time PGC proliferate and differentiate. The KITLG/KIT pathway is central to this process. Germ Cells also undergo apoptosis during migration and in the gonad.

1. Signalling through the KITLG–KIT pathway induces the expression of SPRY4, which acts as an inhibitor of the mitogen-activating protein kinase (MAPK) pathway.
2. Expression of BAK1 in testicular germ cells is repressed by the KIT–KITLG pathway and interaction of BAK1 with antiapoptotic proteins is implicated in germ cell apoptosis that occurs in response to this pathway.
3. A disruption of the germ cell development pathway could cause TGCT, perhaps through delayed differentiation of PGC and development of ITGCN.
ease susceptibility. All encode proteins that are either within the KITLG–KIT pathway, the downstream MAPK pathway or interact with these pathways. Signalling through the KITLG–KIT pathway is central to the proliferation, maturation and migration of germ cells. It is interesting to note that g/g deletion on the Y chromosome, identified by candidate gene studies, also contains genes that are exclusively expressed in the testis and are involved in germ cell development, suggesting that any disruption of the germ cell development pathway could cause TGCT.

The effect sizes for the alleles identified for TGCT susceptibility are higher than those observed for any cancer phenotype studies to date. It is unlikely that further studies will reveal variation with a similar risk to that associated with KITLG; however additional studies may reveal loci with similar or smaller effects on risk as the loci on chromosome 5 and 6.

The results of the GWAS raise the possibility that these, and other variants identified in the future, could be used in conjunction with other risk factors for future risk prediction. However further studies are needed to refine risk estimates and to establish any interaction with known risk factors such as UDT, infertility or TM before consideration in clinical practice. While testicular cancer is successfully treated in most cases, tumours diagnosed early are almost always cured. Furthermore early diagnosis should lead to a decrease in the number of patients receiving chemotherapy and thus those suffering late effects from cisplatin-based chemotherapeutic regimes [50,51].

Identifying TGCT genes has not only allowed an improved understanding of the biology of the disease, but also may help to unravel the mystery of increasing incidence of TGCT and potentially provide targets for as effective but less toxic treatments for TGCT.

Acknowledgements
K.L.N. would like to acknowledge the support of US National Institutes of Health grant R01CA114478. E.A.R. would like to acknowledge the support of the Institute of Cancer Research Cancer Research UK and the Wellcome Trust.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Examines the evidence to support the notion that ITGCGN and subsequent TGCT arise from delayed development of primordial germ cells.  


