Testicular cancer susceptibility in the 129.MOLF-Chr19 mouse strain: additive effects, gene interactions and epigenetic modifications

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Testicular germ cell tumors (TGCTs) are the most common solid cancers affecting young men. Although the evidence for genetic predisposition to TGCTs in humans is compelling, the genetic control of susceptibility is poorly understood. The 129S1/SvImJ (129/Sv) inbred strain of mice is an excellent model for studying TGCT susceptibility. We previously reported a new mouse strain, the 129.MOLF-Chr19 chromosome substitution strain, which develops spontaneous TGCTs at a high frequency (70–80%) as compared with the much lower rate in the 129/Sv strain (5%). To characterize the genetic control of TGCT susceptibility, we created a panel of single- and double-congenic strains derived from 129.MOLF-Chr19. The frequency of TGCTs in these strains suggests that several genes with additive and epistatic effects located at distinct sites on chromosome 19 control susceptibility. However, an alternative interpretation involving epigenesis is based on a striking correlation between TGCT frequency and the length of the MOLF-derived congenic segment, regardless of their chromosomal location on Chr 19 in each congenic strain. We also show that bilateral TGCT cases result from the coincidental co-occurrence of unilateral TGCTs rather than from the action of distinct genes that control susceptibility to bilateral versus unilateral TGCT cases. Finally, we propose that these TGCTs result from disrupted testicular and spermatogenic developmental programs.

INTRODUCTION

Although testicular germ cell tumors (TGCTs) are rare, comprising only 1–2% of all cancers in men, they are the most common malignancies affecting young men 15–35 years of age (1,2). Family history is a significant risk factor (3–5); the risk is increased 8–10 fold among brothers and 4-fold in sons of affected individuals, implying a strong genetic component (1–3). Although linkage to Xq27 for bilateral but not unilateral cases has been reported in humans (6), many linkages have probably escaped detection and TGCT susceptibility genes have not yet been identified. Environmental factors also affect susceptibility. The dramatic increase in the incidence of TGCTs, especially in Caucasian populations of Northern and Eastern Europe, indicates strong environmental influences (7,8). Although exposure to estrogenic agents in utero has been proposed as a contributing factor for the increased TGCT incidence (9–13), the nature of these factors and their interactions with susceptibility genes is not known. To date, the genes and molecular mechanisms controlling inherited susceptibility to TGCTs have not been identified.

Three classes of TGCTs occur in humans. These classifications are based on age of occurrence, tissue composition and probable cell of origin (1,2,14). Pediatric teratomas, teratocarcinomas and yolk sac tumors occur before puberty and are composed of various cell and tissue types at diverse stages of differentiation. Seminomas and non-seminomas occur primarily in males who are 10–34 years of age and they are often malignant. Finally, spermatocytic seminomas occur in males over 50 years of age. These various TGCTs appear to originate from primordial germ cells (PGCs) at different stages of development and differentiation, which give rise to carcinoma-in-situ (15), the precursor of all types of TGCTs except spermatocytic seminomas.

Various attributes suggest that TGCTs in the 129 family of inbred strains is a model for pediatric TGCTs in humans (16–18).
129/Sv mice have a tumor frequency of 1–10% depending on the subline and most cases of tumors are unilateral. TGCTs in mice originate from PGCs around embryonic day 12.5 (E12.5) (19,20). PGCs are then transformed by unknown mechanisms to give rise to embryonal carcinoma (EC) cells. At E15, clusters of foci derived from these EC cells rupture the semiferous tubules in which they originated and invade into the interstitial spaces. Embryonal-like cells form vesicles that resemble normal embryonic ectoderm, mesoderm and endoderm. Soon after birth, the vesicles disorganize and differentiate into a variety of embryonic and adult cells and tissues that can be readily detected macroscopically at postmortem examination of 3–4-week-old mice. Histological analysis of TGCTs in adult mice reveals a variety of differentiated tissues such as cartilage, bone, muscle, neuroepithelium and many other cell and tissue types (16). Although the precise developmental stage during which TGCTs originate and the identity and anatomical location of the TGCT stem cell are known in mice, neither the genetic nor molecular mechanisms that are responsible for the initiation or progression of TGCTs have been identified.

The genetic control of TGCT susceptibility in 129/Sv mice is complex. Crosses between 129/Sv and other inbred strains revealed only one affected individual in ∼11,000 males (16), suggesting that many genes with low penetrance are involved in TGCT development. Recently, a 129/Sv-derived TGCT susceptibility locus was mapped to mouse chromosome (Chr) 13 (21). Spontaneous and engineered mutations in genes such as Ter, Mgfr, and Trp53 on the 129/Sv inbred background increase the frequency of spontaneous TGCTs (16–18,22–26), either by increasing the penetrance of TGCT susceptibility genes, or by reducing the number of 129-derived alleles required for tumorigenesis, or both (27).

To map TGCT susceptibility loci as a prelude to gene discovery, backcrosses between 129/Sv-Ter/+ and the MOLF/Ei inbred strain showed that TGCT susceptibility is under multigenic control and that genes from the MOLF strain contribute to susceptibility when combined with 129/Sv-derived genes (24,28). Although MOLF/Ei is a fully fertile, inbred mouse strain in which spontaneous TGCTs have not been observed, it may nevertheless harbor susceptibility genes, but not in the right number or combination to lead to TGCT development. To verify the linkage results, a chromosome substitution strain (CSS), 129.MOLF-Chr 19, was made by replacing Chr 19 in 129/Sv with its homolog from MOLF/Ei (29). Males of this engineered strain have a high frequency of spontaneous TGCTs as predicted by the linkage analysis. Among 129.MOLF-Chr 19 males, 70–82% have TGCTs, ~57% of which are bilateral. These mice also have a modest frequency of testicular abnormalities affecting many aspects of germ cell development and spermatogenesis. Despite the frequent tumors and testicular abnormalities, this strain is fully fertile.

Results from linkage studies and the 129.MOLF-Chr19 CSS raise several questions: (1) what is the number and location of TGCT susceptibility genes on Chr 19; (2) what is their nature of action and interaction; and (3) is the development of unilateral and bilateral tumors under distinct genetic control? To answer these questions, we created a panel of single- and double-congenic strains, all derived from the 129.MOLF-Chr19 CSS, on the 129 inbred background. (A congenic strain has a chromosome segment from a donor strain that has been transferred to a host strain background by repeated backcrossing and selection.) Results from these congenic strains show that multiple genes, alone and in combination with conventional additive or epistatic effects, control susceptibility to unilateral and bilateral TGCTs. An alternative interpretation of these results is that epigenetic modification of the substituted chromosome by the host strain background contributed to susceptibility. Finally, the relative frequencies of unilateral versus bilateral TGCTs suggested that these tumors share a common genetic control.

RESULTS

TGCT frequencies in congenic strains

Testes from at least 100 males from each single-congenic strain were examined for unilateral and bilateral TGCTs (Fig. 1). TGCT frequencies ranged from a high of 56% in the A-2 congenic strain to baseline values of 4% in the B-81 strain. TGCT frequencies in the various congenic strains were reduced relative to the frequency in the 129.MOLF-Chr 19 strain, e.g. 129.MOLF-Chr19 versus the A-2 strain (χ² = 6.27, P < 0.05). All but two strains had frequencies that were higher than the baseline rate in 129/Sv, e.g. 129/Sv versus the C-2 and C-1 strains (goodness-of-fit test of the pooled data for these two strains versus the 5% baseline rate in 129/Sv; χ² = 72.87, P < 0.05). Only the B-20 and B-81 strains had TGCT frequencies that were similar to the baseline rate in the 129/Sv strain (goodness-of-fit test of the pooled data for these two strains versus the frequency in 129/Sv; χ² = 3.87; not significant, after correction for multiple testing). This baseline rate suggests that genes in these congenic segments did not have independent or additive effects on TGCT susceptibility.

For the three double-congenic strains, 44–107 males were examined. All three strains had a high frequency of TGCTs and the frequency in the A-1/C-1 double-congenic strain was indistinguishable from that in the 129.MOLF-Chr19 CSS (χ² = 0.24, not significant), demonstrating that the TGCT frequency in the original CSS could be reconstituted in strains with multiple congenic segments.

The combination of TGCT frequency and the location of the MOLF-derived segment that was present in each of the 13 congenic strains defined five regions (regions I–V) in which TGCT genes are located (Fig. 1). The genetic boundaries of each region are described in Figure 1.

Location and action of TGCT genes

The range of TGCT frequencies in the panel of strains that are congenic for one or two MOLF-derived chromosome segments argues that several genes control susceptibility in additive and epistatic manners. Instead of describing each of the several equally likely alternative interpretations that account for the location and action of the susceptibility genes, we highlight examples of additive and epistatic effects.

The two strains with region V (strains C-1 and C-2) provide examples of additive effects. Both strains had a significantly higher TGCT frequency than the baseline rate in 129/Sv (Fig. 1, and see above). Region IV did not affect the TGCT frequency.
when combined with region V, e.g. compare B-20 and B-81 with C-1 and C-2. Thus, at least one gene in region V was sufficient to increase the TGCT frequency above the baseline rate in 129/Sv, regardless of the presence of region IV.

The gene or genes in region V contributed 11.5% to the TGCT frequency; this estimate was based on taking the average TGCT frequency in the C-1 and C-2 strains (average = 16.5%) and subtracting from this average the 5% TGCT frequency that is attributable to the 129/Sv background. Similar calculations show that other segments contribute 25–30% to the overall TGCT frequency. Attributing quantitative effects to genetic background and to specific loci in this manner is an established principle in quantitative genetics (30–32, cf. 33).

Although region IV did not affect TGCT frequency by itself or when combined with region V, it had a profound effect when combined with regions I, II and III. For example, the A-1 strain, which does not have region IV, had a significantly higher TGCT frequency than the B-20 and B-81 strains (A-1 versus the combined results for the B-20 and B-81 strains: \( \chi^2 = 41.0, P < 0.05 \)), demonstrating that at least one TGCT gene is located in regions I, II or III. However, adding region IV to the A-1 congenic strain, which is the genetic constitution of the A-2 congenic strain, resulted in a significant increase in the TGCT frequency from 33 to 56% (A-2 versus A-1; \( \chi^2 = 13.4, P < 0.05 \)), demonstrating epistasis between region IV, which has no effect on its own, and regions I, II or III. Additional evidence that region IV affects tumorigenesis epistatically is that it lies at the peak of the likelihood curve for TGCT susceptibility in segregating crosses between 129.MOLF-Chr19 and 129/Sv (23).

Region IV can interact with any of three regions (regions I, II or III) and we can postulate at least three equally likely alternative models to account for these results. In these models, one of three regions (regions I, II or III) is postulated to interact
with region IV while the remaining two regions are postulated to have additive effects. For example, regions I and IV interact epistatically while regions II and III have additive effects; alternatively, regions II and IV interact epistatically while regions I and III have additive effects, and so on.

Results for the double-congenic strains complicate the evidence for additive and epistatic effects in two important ways. For example, the A-1/C-1 double-congenic strain provides an example of epistasis that increases susceptibility. The TGCT frequency that is attributable to the A-1 and C-1 strains was 28% (=33 – 5%) and 11% (=16 – 5%), respectively. Therefore, the expected frequency in the double-congenic strains was 44% (=28 + 11 + 5%). The observed frequency (75%) was significantly higher than expectations ($\chi^2 = 67.5, P < 0.05$), raising the possibility of epistasis between regions I, II or III and region V, but not region IV, because it is not present in the A-1/C-1 double-congenic strain. Similar arguments apply to the two other double-congenic strains.

The second complication is that the double-congenic strains also provide evidence for a TGCT suppressor. For example, the TGCT frequency in A-1/C-1 mice is expected to be higher if region IV is added because this region acts epistatically with regions I, II or III to increase TGCT frequency. However, the progenitor 129.MOLF-Chr19 CSS contains all five regions (regions I–V), but the TGCT frequency (71%) in the CSS was not higher than the mice with regions I–III and V but not IV. Thus the lower than expected TGCT frequency in the 129.MOLF-Chr19 strain suggests that a TGCT suppressor was active in these mice.

### Length of congenic segments

We next examined the relationship between TGCT frequency and the total length of the congenic segment in each strain (Fig. 2). The results suggest another interpretation of variation in TGCT frequencies among the panel of strains. In general, the TGCT frequency for each congenic strain increased with the genetic length (cM) of each congenic segment, regardless of the location of the donor chromosome segment (Fig. 2), suggesting that the total length of congenic segments may be more important than the genetic content of particular segments. The only exceptions were the C-1 and C-2 strains, perhaps because they were the only two strains not implicated in epistatic interactions with region IV. It is possible that conventional genetic variants, i.e. DNA sequence variants, are less important than chromosome-specific effects such as epigenesis involving a substituted chromosome on the 129/Sv background. Although this is a more conservative explanation than proposing numerous genes with additive and epistatic effects, we are unaware of any other examples of this kind of relationship in complex trait studies.

### Testicular and spermatogenic abnormalities

Testicular abnormalities are occasionally found in many inbred strains, but occur at an appreciable frequency in 129/Sv (15 of 83 mice examined) and at a significantly reduced frequency in 129.MOLF-Chr19 mice (5% of 119 mice examined; $\chi^2 = 5.3, P < 0.03$). By contrast, the MOLF/Ei strain does not have many

![Figure 2](https://academic.oup.com/hmg/article-abstract/12/4/389/584497)
abnormalities; testes from 20 males were examined for macroscopic abnormalities and two histological sections from each testis of eight mice were examined histologically for abnormalities, but none were found. We then examined testes from the congenic strains to determine the number and location of genes that control the high frequency of morphologically abnormal testes.

Testes in males from many of the congenic strains often showed TGCTs, seminiferous tubules with various defects affecting many aspects of spermatogenesis and spermiogenesis, and tubules with normal spermatogenesis (not shown). Despite these tumors and abnormalities, most males were fully fertile. Histologically, some seminiferous tubules appeared to contain Sertoli cells only, other tubules appeared to have spermatogonia but not spermatocytes, and still others appeared to have spermatogonia and spermatocytes. The abnormalities ranged from subtle, involving a modest number of seminiferous tubules, to extensive, involving a wide range of abnormalities in many tubules within a single testis. These assessments were based only on a histological examination of a small number of sections from each testis; a more thorough analysis involving markers for specific cell and tissue types is needed to fully characterize the nature of these abnormalities.

The frequency of abnormalities was high in the B-30, B-2, B-81, B-20, C-1 and C-2 congenic strains and was similar to the 15% frequency observed in 129/Sv; the frequencies of abnormalities in the A-2, A-1, B-3MM and B-3II were considerably lower and similar to the 5% frequency observed in 129.MOLF-Chr19 (Table 1, Fig. 1). Except for two strains (B-3MM and B-3II), the results are consistent with localization of at least one 129-derived gene contributing to testicular abnormalities in the 13.1 cM interval centromeric to D19Mit28. The anomalous results for the B-3MM and B-3II strains raise the possibility that additional regions of 129/Sv-derived Chr 19 contribute to these abnormalities.

### Unilateral versus bilateral TGCTs

The next step in the analysis involved testing whether a simple relationship accounts for unilateral and bilateral TGCTs in affected individuals. We analyzed the proportion of cases that involve bilateral versus unilateral TGCTs in a panel of inbred, chromosome substitution, congenic and mutant strains. The goal was to determine whether genes cause unilateral or bilateral TGCTs primarily, or whether TGCT genes lead to both kinds of TGCTs with bilateral cases resulting from the independent occurrence of TGCTs in the left and right testes.

The first step was to calculate the frequency of TGCTs in the left testis, and separately in the right testis, in each strain. Each testis was counted as an independent event, regardless of the status of the contralateral testis. If one testis was abnormal, the results for that mouse were discarded. These frequencies were calculated separately for each inbred, substitution, congenic and mutant strain (Fig. 3). By using the formulations described in the Appendix, the expected frequencies of unaffected, unilateral and bilateral cases were estimated. Comparison of the expected and

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<th>Uni-right</th>
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<th>n</th>
<th>Test score</th>
<th>Left/right</th>
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<td>109</td>
<td>1.67</td>
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</table>

*Abnormalities include hypomorphism, primitive, missing testis, germ cell deficiency and absence of testis cords and spermatogenic abnormalities including multinucleated giant cells.

bP < 0.01.
observed numbers tested whether the bilateral TGCTs occur more (or less) often than expected and therefore whether unilateral and bilateral cases might have distinct genetic controls.

We measured the frequency of left testes with a tumor \( f(L) \), the frequency of left testes that were tumor free \( 1 - f(L) \), the frequency of right testes with a tumor \( f(R) \), and the frequency of right testes that were tumor free \( 1 - f(R) \) for 129S1/SvImJ, 129.Molf-Chr19 homosomic and heterosomic mice, each of the congenic strains, and selected mutant strains, e.g. 129-Ter/+ and 129-Ter/Ter mice (12,16,19) and 129-Trp53/+ mice (13,14). The expected and observed numbers were then compared. In all but one case (B-3MM), the difference between the expected and observed numbers was insignificant, suggesting that bilateral cases usually result from the independent action of genes that control susceptibility to unilateral TGCTs. Only the B-3MM congenic strain had significantly more bilateral cases than expected. Although the sample size was large \( n = 107 \), relatively few bilateral cases were observed and a few more (or less) cases could affect the outcome and conclusion. In general however, the agreement between the expected and observed numbers, suggests that bilateral cases result from the independent action of factors that act in a unilateral manner and therefore that unilateral and bilateral cases have similar genetic control.

We next examined the relationship between the proportion of bilateral tumors as a function of the combined frequency of unilateral and bilateral tumors in these strains (Fig. 3). The proportion of cases that were bilateral increased non-linearly as the overall tumor frequency increased in the various strains and mutants. The expected frequency of bilateral cases was also calculated assuming that bilateral and unilateral cases have similar genetic controls and that the ratio of TGCTs in the left versus right testis was 2:1 (10–12). The good fit between the observed and expected relationship suggests that bilateral cases result from the coincidental occurrence of TGCTs in both the left and right testes. According to this model, as TGCTs become more frequent, the proportion of bilateral cases increases. Thus, bilateral cases probably result from independent events in each testis and involving the action of genes with strong effects on TGCT frequency.

Figure 3. Relation between the observed and predicted bilateral TGCTs and overall TGCTs frequencies in inbred, chromosome substitution, congenic and mutant strains. The data points correspond to the TGCT frequencies documented in Table 1 as well as data for homosomic and heterosomic 129.MOLF-Chr19 mice (9) (unpublished data), 129-Ter/+ and 129-Ter/Ter mice (12,16,19) and 129-Trp53/+ mice (13,14). The line shows the predicted proportion of bilateral TGCTs as a function of TGCT frequency (unilateral and bilateral TGCTs combined); the predictions are based on the model described in the text.
DISCUSSION

Quantitative genetics of TGCTs

Phenotypic characterization of these congenic strains revealed a striking phenotypic feature; each congenic strain displayed a highly quantitative frequency of TGCTs. Excluding the A-2 strain, the two strains that carried only region II (i.e. A-1 and B-30) had an average tumor frequency of 32% with a range of 31–33%, the three strains with only regions III and IV had an average tumor frequency of 29% (range 28–31%), the two strains with only region IV had an average tumor frequency of 6.5% (range 4–9%), and the two strains with only region V had an average tumor frequency of 16.5% (range 16–17%). Finally, in the various models (Fig. 1), genes in the five regions together yield a combined TGCT frequency of 68%, which is remarkably close to the 71% TGCT frequency observed in 129.MOLF-Chr19 (Fig. 1). The biological basis for these highly quantitative effects is unclear but has not been previously reported in other tumor models.

These quantitative effects may result from the unique genetic resources used to study TGCT susceptibility. CSSs are inbred strains composed of a single chromosome substitution on a defined and inbred genetic background where large numbers of genetically identical individuals can be evaluated (29,34). We intentionally studied a large number of mice for each strain to minimize stochastic fluctuations and thereby gain a more reliable measure of the magnitude of the trait effects on each chromosome and chromosome segment (Fig. 1). Perhaps these highly quantitative results illustrate the considerable power of CSSs and congenic strains derived from them to resolve the genetics of complex traits.

Additive, epistatic and epigenetic effects

The various TGCT frequencies in this panel of single- and double-congenic strains suggest that TGCT genes are widely distributed on Chr 19 in MOLF/Ei mice. The data are consistent with as many as five genes, some with additive effects and others are involved in epistasis. The location of these genes depends on the particular model and several alternative models are equally likely. Further dissection of these congenic strains and analysis of additional double-congenic strains may resolve the location, identity and action of the TGCT susceptibility genes.

It is striking, and perhaps unexpected, that so many MOLF-derived genes located at diverse sites on Chr 19 affect tumorigenesis, especially because TGCTs have not been described in the MOLF/Ei strain and because it is unlikely that a gene family accounts for susceptibility. Complex trait genes are typically located at specific sites along the chromosome and individuals with chromosome segments of different lengths tend to have similar trait values because they share the same trait-controlling genes. We found a striking and strong correlation between TGCT frequency and the length of congenic segments (Fig. 2). If TGCT susceptibility is genetically complex and many trait-controlling genes are located at distinct sites on many chromosomes, then a correlation might be expected because longer segments would have more susceptibility genes. CSS strains may therefore enable discovery of genes with modest phenotypic effects that are difficult to detect in other ways (29,34–36).

An alternative explanation for the unexpected relation between TGCT frequency and length of congenic segments involves epigenetic modifications imposed by the 129/Sv background on the MOLF-derived chromosome. Epigenesis could make any gene on the MOLF-derived chromosome 19 that is involved in development, differentiation, migration and proliferation of primordial germ cells or in testicular determination and development. If epigenesis is involved, other kinds of phenotypic variation such as other cancers, birth defects, neurological anomalies or pigmentation defects might be expected, but none have been found (unpublished), suggesting that the epigenetic effect is confined to the germ line. In early development, the imprint of the parental genome is erased in the germ line and replaced with the imprint of the sex (37,38). Genes such as H19 and IGF2 are imprinted differently in the germ cells and somatic cells (39,40). It has been proposed that imprinting is involved in the etiology of TGCTs (41). Perhaps the 129/S strain has an anomaly in the imprinting program in the male germ line and that genes on the substituted chromosomes are targets for epigenetic modification.

Laterality

Reports of bilateral TGCTs are frequent in humans (42,43). Some are metachronous, occasionally separated by years, and others are synchronous. Linkage for a gene controlling susceptibility to bilateral but not unilateral TGCTs was recently reported (6). In the mouse, circumstantial evidence suggests that distinct genes might control susceptibility to unilateral and bilateral cases in linkage crosses (28). In addition, several mouse models of TGCTs, including Ter and 129.MOLF-Chr19 mice, have a high frequency of bilateral TGCTs (23,29,44). Together these observations raise the possibility that unilateral and bilateral TGCTs have distinct genetic controls. An alternative hypothesis is that bilateral cases result from the coincidental co-occurrence of unilateral cases and that co-occurrence is more likely with increasing strength of the TGCT genes (Fig. 3). According to this hypothesis, the frequencies of unilateral and bilateral cases can be predicted from the overall frequencies of TGCTs in each testis. Analysis of TGCT laterality in 129/Sv, 129.MOLF-Chr19, congenic strains and mutant mice was consistent with the hypothesis that unilateral and bilateral cases have a common genetic control. An important implication of this hypothesis is that it is easier to detect linkage for bilateral cases than for unilateral cases because bilateral cases result from genes with strong effects that produce a high frequency of TGCTs.

Implications for TGCT genetics in humans

TGCT susceptibility is a highly multigenic trait in mice (16–18,22). Although TGCTs have not been observed in MOLF/Ei mice, its Chr 19 has several susceptibility genes (29). Extrapolating these results to the entire genome suggests that as many as 100 genes may affect susceptibility. Segregation models in humans suggest that relatively few genes control susceptibility (3) and only one linkage has been conclusively established (6). However, family studies typically have limited
power to detect linkage for complex trait genes in humans, numerous candidate linkages that have been suggested (4,6), raising the possibility that additional linkages remain to be discovered.

Based on the principles of comparative mapping, linkages in these mouse models predict the location of TGCT genes in humans. The corresponding portions of the human genome are 4q22–4q23, 9p24, 9q11–9q24, 10q23–10q26 and 11q12–11q13, none of which have been implicated in linkage studies (6) or in chromosomal aberrations (45,46) in TGCT cases.

Developmental abnormalities and TGCT susceptibility

It has been proposed that disruptions in the developmental programs controlling testicular differentiation lead to TGCTs (10,11,47,48). In humans, important factors for TGCT susceptibility involve a variety of urogenital abnormalities such as inguinal hernia, cryptorchism, testicular dysgenesis, infertility, androgen insensitivity, carcinoma-in-situ, Klinefelter’s syndrome, trisomy 21, prior history of TGCTs, and family history of TGCTs (1,2,10,47). It is unlikely however that these urogenital abnormalities lead directly to TGCTs; a more likely interpretation is that susceptibility genes have various phenotypic consequences including TGCTs and urogenital abnormalities. It is striking that 129/Sv and many of the congenic strains have a significant frequency of testicular and spermatogenic abnormalities (Table 1).

TGCTs in mice and pediatric teratomas, teratocarcinomas and perhaps other kinds of TGCTs in humans arise from PGCs during fetal development. PGCs undergo major changes during the critical developmental period between E11.5 and E13.5, including the arrival of migrating PGCs in the urogenital ridge, sex determination, changes in the activation of the X-chromosome, and mitotic arrest of germ cells until after birth in males (50–52). During this period, mesenchymal cells migrate from the mesonephros into the adjacent developing testes. Around E12.5, Sry expression initiates testes development by signaling Sertoli progenitor cells to differentiate thereby inducing other somatic cells to differentiate into testicular cell types (53). Coordinated action among various cell types is closely regulated during development and slight variation in timing can have profound effects on testicular development (54–57).

A possible explanation for TGCT susceptibility involves genetic differences in developmental programs of the testis in the MOLF/Ei and 129/Sv strains that leads to the increase in tumor incidence and testicular abnormalities. The MOLF/Ei strain is derived from a different subspecies of mice and is evolutionarily separated from 129/Sv and other inbred strains by 100 000 years (58). MOLF/Ei is a tumor-free strain, and therefore unlikely to contribute to the increased tumor incidence because of a single genetic mutation. Because testicular development probably requires coordinated action of a large number of genes, this hypothesis involving disrupted developmental programs may account for the five regions that we discovered and the larger number of TGCT susceptibility genes that are suggested in this study. It is possible that instead of a sterility phenotype resulting from adverse interactions in different species and subspecies (59–61), tumors result from disrupted programs for testicular development. The goal will be to identify these genes that are critical for normal germ cell development and characterize their functions whose disruption leads to TGCT development.

MATERIALS AND METHODS

Mice

MOLF/Ei (JR000550) and 129S1/SvImJ (JR002448, previously known as 129/SvJ and 129S3/SvImJ) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). The nomenclature for 129 substrains has been revised by the Jackson Laboratory (www.informatics.jax.org/mgihome/nomen/strain_129.shtml) and the recommended designations have been used in this paper. The 129.MOLF-Chr19 CSS strain (N15F2+) was described previously (29) and was obtained from our research colony. Mice were maintained in the CWRU Animal Resource Center on a 12:12 h light/dark cycle, and fed Lab Diet 5010 ad libitum.

Genotyping

DNA for PCR genotyping was obtained from samples of tail tissue. Each tail sample was digested in 89 µl water, 10 µl 10× PCR buffer, and 1 µl Protease K (10 mg/ml). PCR amplification of genomic DNA was carried out as described (24) in a 96-well block MJ Research PTC-200 thermal cycler. PCR conditions were as follows: initial denaturation step of 94°C for 2 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, final extension of 72°C for 5 min and then 4°C for 15 min. PCR products were resolved on a 6% acrylamide gel and visualized with ethidium bromide.

Construction of congenic strains from 129.MOLF-Chr19

Congenic strains were derived from heterosomic 129.MOLF-Chr19 intercrosses (N15F1 × N15F1). Intercross 129.MOLF-Chr19 progeny (N15F2) were initially typed for eight SSLPs spanning the 56 cM length of Chr 19 (Fig. 2). The SSLPs with the cM position from the centromere indicated in parentheses are: D19Mit32 (0 cM), D19Mit28 (12 cM), D19Mit30 (20 cM), D19Mit40 (24 cM), D19Mit81 (28 cM), D19Mit20 (36 cM), D19Mit17 (46 cM) and D19Mit71 (54 cM). Founder parents of each congenic line were then typed for the following SSLPs to further define recombinant breakpoints: D19Mit110 (15 cM), D19Mit60 (15 cM), D19Mit111 (15 cM), D19Mit132 (25 cM), D19Mit46 (28 cM), D19Mit64 (28 cM), D19Mit5 (32 cM), D19Mit133 (32 cM), D19Mit19 (36 cM), D19Mit118 (36 cM). These map locations were obtained in part from the consensus Whitehead map (www-genome.wi.mit.edu/cgi-bin/mouse/index) and chromosome 19 Committee Report 2000 (www.informatics.jax.org) maps and adjusted with localizations (crossovers) obtained during construction of the congenic strains. Mice with crossovers in the desired interval were intercrossed and homozygotes selected. Each congenic strain was then maintained with systematic brother–sister mating.
Tumor characterization

Males from each congenic strain were autopsied at 4–5 weeks of age and testes examined visually for evidence of a tumor, which are readily recognized with visual inspection in susceptible mice of this age (16,19,20,24,29).

Histology

Testes were examined histologically for evidence of tumors and spermatogenic abnormalities. Specimens were fixed in 10% phosphate-buffered formalin (Fisher) for at least 48 h. Sections (5 μm) were stained with hematoxylin and eosin. Histological analysis of all testes showed that tumors rarely escape visual detection.

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APPENDIX

Estimating the expected frequencies of unilateral and bilateral TGCTs

Let \( f(L) \) be the frequency of left testes with a TGCT, regardless of the presence of a TGCT in the right testis, or (number left tumors)/\( n \).

Let \( 1 - f(L) \) be the frequency of left testes that did not have a TGCT, regardless of the presence of a TGCT in the right testis.

Let \( f(R) \) be the frequency of right testes with a TGCT, regardless of presence of a TGCT in the left testis.

Let \( 1 - f(R) \) be the frequency of right testes that did not have a TGCT, regardless of presence of a TGCT in the left testis.

The expected frequencies of mice with unilateral-left, unilateral-right, bilateral and unaffected testes is

\[
[f(L) + (1 - f(L))] [f(R) + (1 - f(R))] = 1
\]

The frequency of mice that did not have a TGCT \( [f(NT)] \) is the product of the frequency of mice that did not have TGCT in the left testis \([1 - f(L)]\) and the frequency of mice that did not have a TGCT in the right testis, i.e. \([1 - f(R)]\), or

\[
f(NT) = [1 - f(L)] \times [1 - f(R)]
\]

The frequency of mice with a TGCT in the left but not the right testis \( [f(UL)] \) is

\[
f(UL) = f(L) \times [1 - f(R)]
\]

The frequency of mice with a TGCT in the right but not the left in \([f(UR)]\) is

\[
f(UR) = [1 - f(L)] \times f(R)
\]

And the frequency of mice with bilateral TGCTs \( [f(B)] \) is

\[
f(B) = f(L) \times f(R)
\]

REFERENCES


