# A male germ cell tumor-susceptibility-determining locus, *pgct1*, identified on murine chromosome 13

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**Inbred 129 strain mice are predisposed to developing male germ cell tumors (GCTs) of the testes. The inherent genetic defects that underlie male GCT susceptibility in the 129 mouse strain are unknown. GCT incidence is increased in 129 strain males that lack functional p53 protein, and we have used this finding to facilitate the generation of panels of GCT-bearing intercross and backcross mice for genetic mapping analysis. A 129 strain locus, designated *pgct1*, that segregates with the male GCT phenotype has been identified on chromosome 13 near *D13Mit188*. This region of murine chromosome 13 may be syntenic to a portion of human chromosome 5q that is implicated in male GCT susceptibility in humans.**

Germ cell tumors (GCTs) of the testis develop from primordial germ cells (PGCs), normally the precursors of the gametes in the developing embryo. GCTs in humans are the most common malignancy among young adult males between the ages of 20 and 34 years (1). In contrast, GCTs are exceedingly rare in mice with the exception of the 129/Sv mouse strain, which is predisposed to developing these tumors at a 1–2% frequency (2). The etiology of GCT development in mice has been well characterized as the result of studies using the 129 mouse strain. Neoplastic foci are evident within the seminiferous tubules by days 15–17 of gestation (3). The frequency with which these foci occur corresponds to the incidence of male GCTs in adults, indicating that GCTs are congenital in nature. Consistent with the pluripotent nature of the PGC precursor (4), GCTs in 129 strain mice typically present as a histological polyglot of differentiated cell types among which undifferentiated embryonal carcinoma cells may or may not be present (5). The undifferentiated embryonal carcinoma cells are the malignant component of male GCTs, and fully differentiated tumors are typically benign.

Embryonic genital ridges, grafted to ectopic sites in adult hosts, have been demonstrated to have the capacity to form tumors that closely resemble spontaneously occurring GCTs with PGCs being the tumorigenic component (6, 7). The 66% incidence with which 129/Sv strain genital ridge grafts can form tumors is much greater than the 1–2% incidence of spontaneous tumor formation. This finding suggests that external factors within the normal developmental milieu may have an important restraining influence on the formation of PGC dysplasia. The tumorigenicity of transplanted genital ridges is strain-dependent, with the most aggressive tumors being formed by genital ridges obtained from 129 strain embryos (8, 9). Reaggregation experiments have indicated that strain-dependent tumorigenicity in genital ridge transplants is determined not by the genetic context of the gonadal environment, but rather is an inherent characteristic of the PGCs (10). This indication suggests that the underlying 129 strain defect is in the capacity of the PGCs to respond to environmental cues.

The GCT phenotype of 129 strain males behaves as a recessive trait with an estimated six to eight contributing loci. This estimation is based on experimental crosses in which $F_1$ hybrids between the 129 strain and several different inbred strains were backcrossed to the 129 strain. Only one GCT was observed among >11,000 backcross males (11, 12). This tumor incidence is much too low for sufficient numbers of affected mice to be feasibly generated for genetic linkage analysis. Mutations at modifier loci have been identified that increase the penetrance of the male GCT phenotype in the 129 strain. These mutations include disruption of either the mast cell growth factor ($Mgf^{Sl}$) gene (11) or the *Trp53* gene (13), as well as a spontaneous mutation at the *Ter* locus, which has been mapped to chromosome 18 (14–16) and a recently reported MOLF strain allele on chromosome 19 termed *Tgct1* (17). By increasing the penetrance of the male GCT phenotype in the 129 strain, such mutations might facilitate a genetic approach to identifying the underlying defects responsible for predisposing the 129 strain to male GCT development.

The p53 protein product of the *Trp53* gene plays a central role in suppressing neoplastic progression, with ≈50% of all solid tumors exhibiting direct loss of functional p53 (reviewed in ref. 18). Often this loss occurs as the result of point mutations within the *Trp53* gene, which disrupt the wild-type conformation and stabilize the mutated p53 protein. Male germ cell tumors have high levels of p53 protein, however, mutations in the *Trp53* gene are rare in these tumors (19–24), and the transcriptional activity of wild-type p53, in the absence of genotoxic stress, seems to be low (25, 26). These observations suggest that mutations that disrupt *Trp53* are not selected for during development of male GCTs. It therefore is surprising that homozygous disruption of the *Trp53* gene can dramatically increase the incidence of male GCTs in 129 strain mice (13). We have confirmed that the 129 strain genetic context is important for high frequency development of male GCT in *Trp53*-null mice and have used this finding to facilitate the generation of GCT-bearing intercross and backcross panels of mice to genetically map 129 strain loci that determine male GCT susceptibility. We report the identification of a 129 strain locus on chromosome 13, designated *pgct1* for *p*rimordial *g*erm *c*ell *t*umor locus *1*, that segregates with the male GCT phenotype in these mice.

## Materials and Methods

**Mice.** C57BL/6J (000664) and 129/S3/SvImJ (JR2448) strain mice were purchased from the Jackson Laboratory. Mice harboring disrupted copies of the *Trp53* gene were generously provided by Tyler Jacks (Massachusetts Institute of Technology, Cambridge, MA; ref. 27). The nomenclature for 129 substrains recently has been revised by the Jackson Laboratories (http://www.informatics.jax.org/mgihome/nomen/strain_129.shtml), and the recommended designations have been used in this manuscript.

Abbreviations: GCT, germ cell tumor; PGC, primordial germ cell; SSLP, simple sequence length polymorphism; cM, centimorgan.

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**PCR Analysis of *Trp53* Status.** Tissue samples were digested to extract genomic DNA as described previously (28). PCR was performed by using either crude extract diluted 1:3,000 in the final reaction or on purified genomic DNA at 1 ng/$\mu$l prepared as described previously (28). Three primers were used to distinguish between wild-type and null *Trp53* alleles, a forward primer located within p53 exon 6 (5′-GTATCCCGAGTATCTGGAAGACAG-3′), a second forward primer located within the aminoglycoside 3′-phosphotransferase gene of the knockout cassette (5′-GCCTTCTATCGCCTTCTTGACG-3′), and a reverse primer located within p53 exon 7 (5′-AAGGATAGGTCGGCGGTTCATGC-3′). PCR amplification of genomic DNA was carried out in 25-$\mu$l reactions. To analyze multiple DNA samples, a mixture of the following components was set up with the volumes multiplied by the number of reactions: 21 $\mu$l of $H_2O$, 2.5 $\mu$l of 10× plaque-forming units DNA polymerase buffer (Stratagene), 0.25 $\mu$l of dNTP mixture (dATP, dTTP, dGTP, and dCTP at 25 $\mu$M each; Amersham Pharmacia), 0.25 $\mu$l each of p53 primer, and 0.125 $\mu$l of plaque-forming units DNA polymerase (2.5 units/$\mu$l; Stratagene). A 22.5-$\mu$l sample of the reaction mixture was combined with 2.5 $\mu$l of genomic DNA. PCR reactions were carried out in a 96-well block of an Ericomp (San Diego) EZCycler TwinBlock System with an initial denaturation step of 96°C for 5 min followed by 30 cycles of 96°C for 15 sec, 50°C for 30 sec, 72°C for 30 sec, and a final extension of 72°C for 5 min. PCR products were resolved on 2% NuSieve agarose (FMC)/1% agarose (GIBCO/BRL) gels and visualized by staining for 5 min with 0.5 $\mu$g/ml ethidium bromide.

**Simple Sequence Length Polymorphism (SSLP) Genotyping.** Primer pairs for PCR amplification of simple sequence repeats were purchased from Research Genetics. Analyses were carried out as described previously (28). Haplotype distributions were generated with the program MAPMANAGER Version 2.6.5 (29) (http://mcbio.med.buffalo.edu/mapmgr.html).

**Statistical Analysis.** Segregation data for markers on chromosome 13 were analyzed by using the $\chi^2$ test for backcross data (30) [$\chi^2 = (obs_p - obs_r)^2/(obs_p + obs_r)$]. To determine $P$ values, an Internet-accessible statistical program $\chi^2$ calculator was used. This Internet site is accessible at http://www.fourmilab.ch/rpkp/experiments/analysis/chiCalc.html.

## Results

**Increased Incidence of Male GCT in Mice Lacking Functional p53.** We observed initially that the male progeny of mice lacking functional copies the *Trp53* gene (27) exhibited an unusually high incidence of GCT development. This mouse colony had a mixed genetic background segregating in an undefined manner, with 50% of the alleles derived from the 129 substrain 129S2/SvPas (129S2) and the other 50% derived from the C57BL/6 strain (B6). Among the first 78 *Trp53* homozygous null (*Trp53*−/−) males generated in the colony, 16 (≈20%) exhibited testicular abnormalities characteristic of GCTs. Eight of these abnormal testes were evaluated histologically and all were diagnosed as malignant teratoma (data not shown).

These observations indicated that the absence of functional p53 results in a marked increase in the occurrence of GCTs above the 1–2% incidence observed in 129 strain males and the negligible incidence observed in B6 strain males. Subsequently, it was reported that disruption of the *Trp53* gene in a pure 129 background increases the incidence of GCTs in males to ≈50% (13). In comparison, the incidence of GCTs in males with a mixed genetic background of 75% B6 and 25% 129 was reported to be much lower (13). This finding suggested to us that disruption of *Trp53* might facilitate genetic linkage analysis by increasing the penetrance of mutations that underlie the GCT predisposition of 129 strain males.

**Intercross Strategy to Generate Mice for Genetic Analysis.** The studies reported in this paper were initiated with *Trp53* knockout mice from a mixed genetic background comprised of 50% 129 substrain 129S2 and 50% strain B6. Previous studies had concluded that multiple mutations in the 129 strain appear to underlie male GCT susceptibility and that these predisposing mutations behave in a recessive manner (11, 12). Based on these determinations, we initiated breeding between testicular tumor-bearing males from the *Trp53* knockout colony and B6 strain females. Recessive GCT susceptibility alleles should be 129 homozygous in GCT-positive males of the *Trp53* knockout colony. Based on this assumption, the progeny produced by crossing GCT-positive, *Trp53*−/− males with B6 females should be heterozygous at these GCT susceptibility loci even though their genomes will be 50% homozygous B6. Mice produced in this manner were intercrossed, and males were evaluated for evidence of GCTs at autopsy. Males that exhibited obvious indications of GCT development while still alive were further backcrossed to B6 females, yielding backcross progeny with even greater percentages of B6 alleles in their genomes. Intercross males derived from backcross generations 1–4 were analyzed.

A total of 1,245 intercross males were autopsied, 271 of which were *Trp53*−/−. Twelve of the intercross males autopsied showed evidence of GCTs (≈1%), and all 12 of the GCT-positive males were *Trp53*−/−. Previously, it was reported that the penetrance of GCTs among *Trp53*−/− males in a pure 129 strain background is ≈50% (13). Assuming that purely recessive, 129 strain alleles are necessary for male GCT development in *Trp53*−/− mice, the ≈4% testicular tumor frequency observed among the p53−/− intercross males suggests that two independent 129 strain alleles might be anticipated to determine the development of the GCT phenotype.

To search for susceptibility alleles among GCT-bearing intercross males, SSLPs between the B6 strain and the 129 substrain 129S3/SvImJ (129S3), which is very closely related to the 129S2 substrain (31), were used. Because very few simple sequence repeats had been characterized in the 129S3 strain at this time, we first identified 155 simple sequence repeats with distinguishable polymorphic size differences between the B6 and 129S3 strains. Fig. 1 shows a schematic representation of the murine autosomal chromosome complement together with the X chromosome. The 155 SSLPs are arranged along the chromosomes according to their relative genetic map positions as posted on the Jackson Laboratory web site (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/).

A total of 15 *Trp53*−/−, GCT-bearing intercross mice (with 3 additional mice obtained from breedings in which the other offspring were not evaluated for *Trp53* status) were analyzed individually with this set of 155 SSLPs. As expected, homozygous 129S3 strain alleles were observed in all 15 mice at SSLPs that flank the reported map position of the *Trp53* gene on chromosome 11 (*D11Mit193*, *D11Mit4*, and *D11Mit30*). No evidence was found for exclusive association of homozygous 129 strain alleles segregating with the testicular tumor phenotype at any other locus. This finding suggests that the 129 strain alleles that contribute to male GCT susceptibility in these mice do not behave in a simple recessive manner. Four regions located on chromosomes 6, 9, 12, and 13 were found to have higher frequencies of homozygous 129 alleles than was observed throughout the rest of the genome. The frequencies of homozygous 129 strain alleles in these four regions were 0.53 between *D6Mit135* and *D6Mit199*, 0.47 between *D9Mit297* and *D9Mit67*, 0.47 between *D12Mit85* and *D12Mit64*, and 0.60 between *D13Mit283* and *D13Mit231* (Fig. 2). The frequency of homozygous 129 strain alleles throughout the rest of the genome, excluding the four candidate chromosomes and chromosome 11 where p53 is located, was on average 0.075 and not higher than 0.27 at any single SSLP. Subsequent attention was focused on

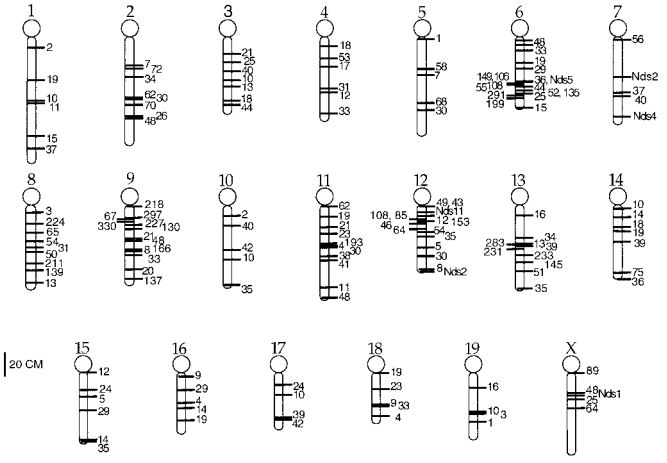


**Fig. 1.** Chromosomal distribution of SSLPs used to screen intercross mice. Here, 155 SSLPs that distinguish between the B6 and 129S3 strain mice are arranged on this schematic of 19 autologous and X mouse chromosomes based on their published map positions (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/). The chromosomes are oriented with the centromeres at the top (represented by open circles) above which are printed the numerical designations for each chromosome. Markers identified at Massachusetts Institute of Technology are designated by using the final numerical digits of their names only. Markers from other sources are designated by additionally including the middle three letters of their names. A vertical line representing a span of 20 cM is shown. From the published map positions (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/), the average spacing between SSLPs was calculated to be 9.3 cM, with the longest uncovered span being 51.5 cM at the telomere of the X chromosome.

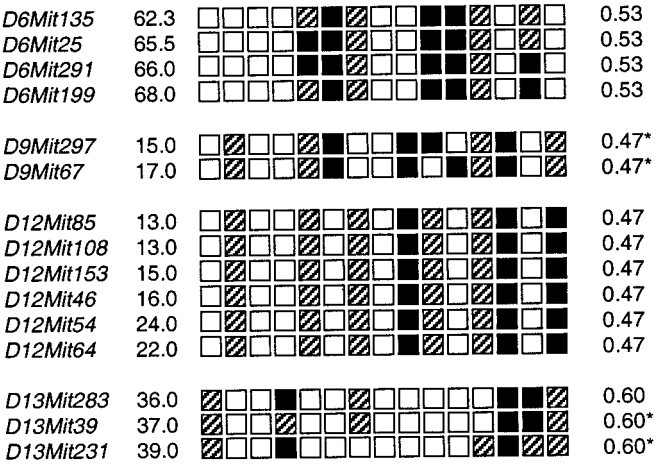


**Fig. 2.** Individual haplotypes of 15 GCT-positive intercross mice at SSLPs spanning four candidate regions potentially associated with the GCT-susceptibility phenotype. Open boxes represent homozygous 129S2 alleles, filled boxes represent homozygous B6 alleles, and hatched boxes represent heterozygous alleles. The SSLP used to generate each row of the haplotype data set is indicated on the far left followed by the published map position of each SSLP (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/). On the right is shown the frequency of homozygous 129S2 alleles at each SSLP. An * indicates possible overlapping recombinants between two SSLPs that could potentially raise the frequency of homozygous 129S2 alleles within the interval (possibly 0.67 between *D9Mit297* and *D9Mit67* and 0.53 between *D13Mit39* and *D13Mit231*). The frequency of 129S2 homozygous alleles over the rest of the genome, excluding the four candidate chromosomes and chromosome 11, was 0.075 (100/1,340) on average and was not greater than 0.27 (4/15) at any individual SSLP.

evaluating these four regions for potential linkage to the male GCT phenotype.

**Backcross Strategy to Evaluate Candidate Regions.** Concurrent with generating intercross mice, successive backcrosses were performed to generate congenic lines with the *Trp53*-null allele in the background of the B6 strain and in the background of the 129 substrain 129S3SvImJ (129S3), which is closely related to the 129S2 substrain (31). Mice carrying the *Trp53*-null allele in the B6 strain background (>10 backcross generations) and in the 129S3 strain background (8, 9, or 10 backcross generations) were then mated to generate $F_1$ intercross offspring. Backcrossing to the 129S3 strain subsequently was performed either between *Trp53*+/− and *Trp53*−/− mice or between *Trp53*−/− mice. From these breedings, 1,307 backcross males were autopsied, 730 of which were *Trp53*−/−. The total number of testicular tumors

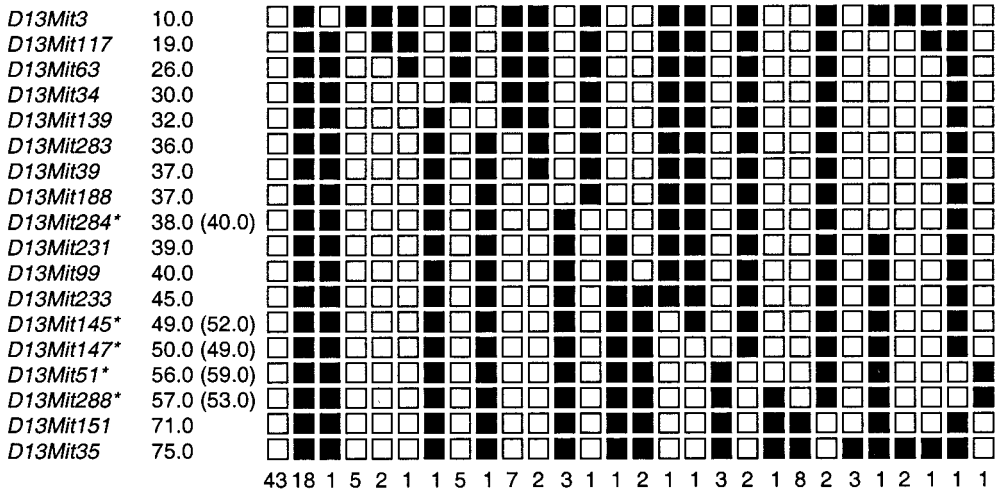


**Fig. 3.** Combined haplotype data from 120 GCT-positive backcross mice analyzed with 18 SSLPs that span chromosome 13. Open boxes represent homozygous 129S3 alleles and filled boxes represent heterozygous alleles. The SSLP used to generate each row of the haplotype data set is indicated on the far left, and the number of mice with each haplotype combination represented is indicated along the bottom. The SSLPs are ordered according to their published map positions (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/) (indicated to the right) from the most centromeric (top) to the most telomeric (bottom). The map order for five SSLPs, denoted with an *, was changed to minimize the frequency of double recombinants in the data set. These reassigned map positions are listed first with the published map positions listed in parentheses.

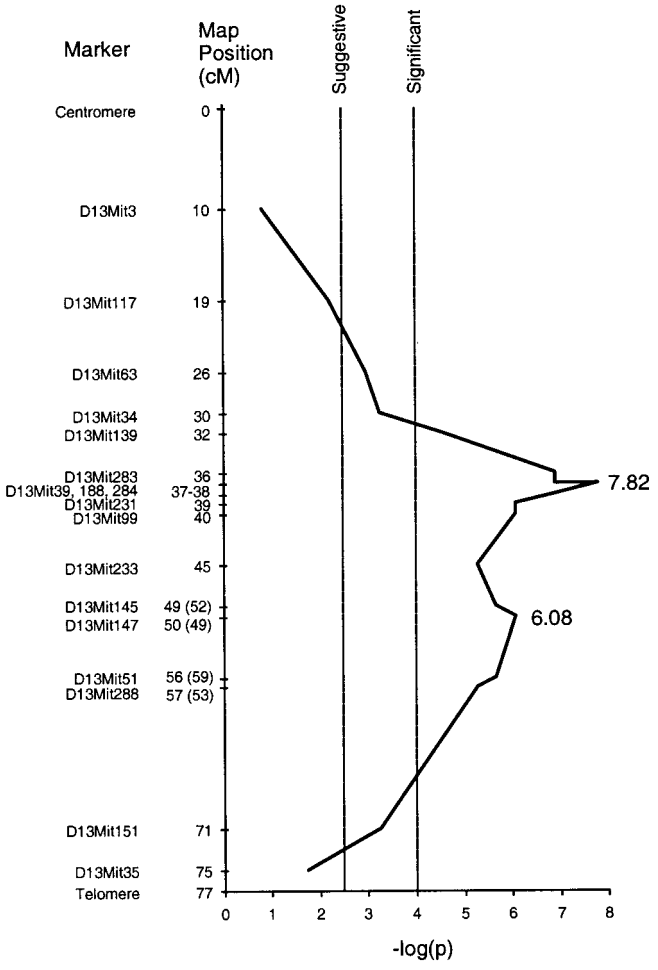


**Fig. 4.** Association of 129 strain homozygosity across chromosome 13 with the GCT phenotype. The 18 chromosome 13 SSLPs used in this analysis are plotted along the *y* axis with the marker names listed to the far left followed by the map position of each SSLP (with positional adjustments made as described in Fig. 3). Centromere and telomere positions also are indicated. The significance of preferential segregation of 129S2 homozygous alleles with the GCT phenotype, which was determined by $\chi^2$ analysis of SSLP data from 120 resistant GCT-positive backcross mice, is plotted on the *x* axis as $-\log(p)$. Vertical dashed lines indicate recommended thresholds for assigning suggestive linkage ($P = 2.4 \times 10^{-3}$ plotted as 2.620) and significant linkage ($P = 7.2 \times 10^{-5}$ plotted as 4.143) to a recessive trait in a backcross mouse population (32).

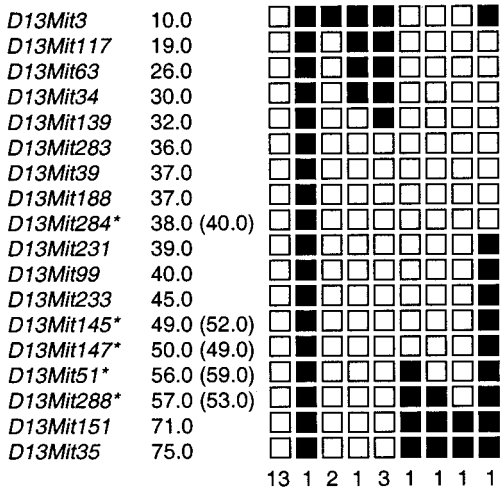


**Fig. 5.** Combined haplotype data from 24 backcross mice with bilateral GCT analyzed with 18 SSLPs that span chromosome 13. Open boxes represent homozygous 129S3 alleles and filled boxes represent heterozygous alleles. The SSLP used to generate each row of the haplotype data set is indicated on the far left followed by the map position of each SSLP (with positional adjustments made as described in Fig. 3). The number of mice with each haplotype combination represented is indicated along the bottom.

observed was 120 (9.2% incidence in total population), 119 of which occurred in *Trp53*−/− males (16% incidence among *Trp53*−/− mice) and 1 occurred in a *Trp53*+/− male (0.17% incidence among *Trp53*+/− mice). Twenty-four mice, all *Trp53*−/−, had bilateral tumors (20% of total). Among unilateral tumors, 61 (51% of total) occurred on the left side and 35 (29% of total) occurred on the right side. Left-sided skewing of the distribution has been reported previously for testicular tumors that arise spontaneously in 129 strain mice with wild-type *Trp53* (2, 5).

Preparations of genomic DNA from 100 testicular tumor-bearing mice were analyzed individually with 18 SSLPs spanning the four chromosomes (6, 9, 12, and 13) where candidate regions were identified. No compelling evidence was found for segregation of 129 homozygous alleles with the GCT phenotype on chromosomes 6, 9, or 12 (data not shown). In contrast, results highly suggestive of a 129 strain locus segregating with the male GCT phenotype were obtained with SSLPs on chromosome 13, and the number of SSLPs analyzed on chromosome 13 was expanded.

Haplotype distributions obtained from analyzing 120 GCT-bearing backcross mice with 18 chromosome 13 SSLPs are presented in Fig. 3. The calculated average spacing between these 18 markers is ≈4 centimorgans (cM). To determine the statistical significance of the overrepresentation of homozygous 129 strain alleles at each marker among the resistant mice, $\chi^2$ analysis was performed by using as the null hypothesis a 50:50 ratio of homozygous 129 strain alleles to heterozygous alleles (30). The results are presented as a graph of SSLP chromosomal map position vs. $-\log(p)$ for each SSLP (Fig. 4). Homozygous 129 strain alleles segregate disproportionately with the resistance phenotype across the medial to distal regions of chromosome 13. The predominant linkage peak is associated with the SSLP *D13Mit188* (91/120; $\chi^2 = 32.0$; $P = 1.52 \times 10^{-8}$; $-\log(p) = 7.82$), which is located at a reported distance of 37.0 cM from the centromere (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/). This peak is coincident with the candidate region on chromosome 13 previously identified on the intercross GCT mouse panel (Fig. 2). A *P* value of $\leq 1.0 \times 10^{-4}$ has been recommended for establishing significant linkage on a backcross mouse panel (32). By this criterion, the linkage established at the peak marker *D13Mit188* is highly significant. This locus has been designated *pgct1* for *p*rimordial *g*erm *c*ell *t*umor locus *1*.

To assess the impact of segregation distortion within the general backcross population on the significance of the reported linkage data, a randomly selected group of 90 *Trp53*−/− male mice was analyzed with the markers *D13Mit188* and *D13Mit147*. In this random population, both markers appeared to segregate in a normal Mendelian fashion with 43% (39/90) 129S3 homozygous *D13Mit188* alleles and 51% (46/90) 129S3 homozygous *D13Mit147* alleles. Actually, segregation was slightly distorted in the opposite direction, toward heterozygous alleles, at the *D13Mit188* locus, although the sample size was not large enough to demonstrate this segregation to be statistically significant. This finding suggests, however, that the significance of the *pgct1* linkage data may have been underestimated.

The effect of the loss of p53 on male GCT development

completely outside the context of the 129 strain background has not been reported. To more thoroughly understand the role of p53 in suppressing the outgrowth of male GCTs, the incidence of male GCTs in p53−/−, B6 strain mice was examined. One testicular tumor was observed in a group of 63 B6 strain, p53−/− male mice. Thus, outside the context of the 129 strain background, the absence of functional p53 resulted in a male GCT frequency of ≈1–2%. By comparison, 43% of p53−/−, 129S3 strain males autopsied (10/26) had testicular tumors. These results suggest that the p53-null mutation, like the endogenous male GCT-predisposing mutations of the 129 strain, can cause low testicular tumor incidence. However, the combination of the p53-null mutation on the 129 strain genetic background results in a synergistic increase in male GCT penetrance. Among p53−/− (B6 × 129S3) $F_1$ hybrid males, the frequency of testicular tumors observed was ≈6.7% (2/30), which suggests that the 129 alleles that cooperate with the p53-null mutation to cause male GCT may not behave in a purely recessive manner. However, homozygous 129 strain alleles enhance the penetrance in much more than an additive fashion.

Twenty-four mice presented with bilateral tumors. Chromosome 13 haplotype distributions for this group of mice are shown (Fig. 5). Among these mice, the incidence of homozygous 129 strain alleles at the *D13Mit188* SSLP was 96% (23/24). By comparison, only 71% (68/96) of the mice with unilateral tumors had homozygous 129 strain alleles at the *D13Mit188* SSLP. Bilateral testicular tumors probably arise as the result of two independent transformation events, and the higher relative frequency of homozygous 129 strain alleles in mice with bilateral tumors provides additional support for the conclusion that the *pgct1* locus contributes to the male GCT predisposition.

Approximately 5% of 129 strain males have been reported to exhibit testicular degeneration, again predominantly affecting the left side, resulting in testes that are abnormally small (2). It was conjectured that degeneration of the testis in the 129 strain might have etiological factors in common with the development of male GCT. In the course of autopsying 1,307 backcross mice, 17 mice with strikingly small to nearly absent testes were observed. The status of the *Trp53* gene had no apparent influence on the development of this phenotype, because 9 of these 17 mice were *Trp53*−/−. Genotype analysis with the *D13Mit188* SSLP also provided no evidence for association of the *pgct1* locus with the degenerative testis phenotype, with 7 of 16 mice analyzed carrying homozygous 129S3 alleles at *D13Mit188*. Thus, mutation at the *pgct1* locus does not seem to be a common etiological factor that can connect testicular degeneration with GCT predisposition in the 129 strain.

## Discussion

In this study we have identified a locus on chromosome 13 of 129S3 strain mice, designated *pgct1*, that shows highly significant association with GCT susceptibility in *Trp53*−/− mice. The low 1–2% penetrance of the testicular tumor phenotype observed in 129 strain males coupled with evidence implicating the involvement of multiple recessive GCT-susceptibility alleles precluded genetic mapping directly in the 129 strain. The *Trp53*-null mutation, which previously has been reported to increase the incidence of testicular tumors in the 129 strain males to nearly 50% (13), was used to facilitate genetic mapping of underlying GCT-susceptibility mutations in the 129 strain. The absence of p53 itself was not sufficient to account for high male GCT incidence, as evidenced by the much lower GCT frequency that we observed among B6 *Trp53*−/− males compared with 129S3 *Trp53*−/− males. Instead, within the context of the 129 strain genetic background, the absence of p53 caused a synergistic increase in male GCT incidence. Male GCTs that occur spontaneously do not appear to be under selective pressure to acquire inactivating mutations within the *Trp53* gene, suggesting that endogenous susceptibility factors within the 129 strain may be of primary etiological significance.

The *Ter* allele, which also increases the incidence of male GCTs in 129 strain mice (14–16), has been used to generate testicular tumors for genetic linkage analysis, but no evidence of linkage to chromosome 13 was reported (33). Linkage data were obtained for two chromosome 19 loci associated with the SSLPs *D19Mit1* and *D19mit28*. Alleles of the 129 strain preferentially segregated with the distal *D19Mit1* marker in unilateral tumors, although a recent study failed to confirm this finding (17). MOLF/Ei strain alleles, the other strain used in the cross, preferentially segregated with the proximal *D19Mit28* marker in bilateral tumors. We analyzed a panel of 88 GCT-positive backcross mice with the markers *D19Mit16* (near the marker *D19Mit28*, which itself is not informative between 129S3 and B6) and *D19Mit1*. The results obtained with *D19Mit16* did not deviate sufficiently from a standard Mendelian distribution even to be considered weakly suggestive. This finding is consistent with the previously reported linkage being associated with a MOLF strain-specific allele. On the other hand, homozygous 129 strain alleles at *D19Mit1* were overrepresented, albeit weakly, in the GCT-positive population (53/88; $\chi^2 = 3.68$; $P = 5.51 \times 10^{-2}$). The significance of this association increased when only the mice with unilateral tumors were considered (45/71; $\chi^2 = 5.08$; $P = 2.42 \times 10^{-2}$), whereas homozygous 129 strain alleles were observed in nearly 50% (8/17) of the mice with bilateral tumors. These data are consistent with a very weak male GCT predisposing allele that is associated with unilateral tumors being present in the vicinity. However, we observed a much lower frequency of 129 strain homozygous alleles segregating with unilateral tumors (45/71; 0.63) than was previously reported (11/13; 0.85) (33). Two important differences between the studies are the mutated modifier genes used to increase GCT penetrance (*Trp53* vs. *Ter*) and the outcross strains used (C57BL/6 vs. MOLF/Ei). Either one of these factors could alter the relative importance of different 129 strain susceptibility alleles to the development of male GCT. Complications of this sort also may have an impact on the degree to which the *pgct1* locus is associated with the male GCT phenotype in different experimental models.

Studies have been conducted to search for male GCT susceptibility factors in humans. By pooling patient samples, The International Testicular Cancer Linkage Consortium (34) has identified four candidate regions. However, these linkage data are still very tenuous with *P* values no lower than 0.05 (34). One of the candidate regions spans a rather large area between 5q12 and 5q21. Other studies have been conducted to identify frequently deleted chromosomal regions in male GCT. Again, 5q has been implicated, with three potential hotspot regions in the vicinity of 5q21, 5q14, and 5q34-ter (35, 36). Two of these three regions fall within the large area of potential linkage identified by the International Consortium.

The region to which the *pgct1* locus has been mapped on chromosome 13 is reported to be within 2 cM of a region that is syntenic to 5q22–5q32 in humans (The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/). Thus, the *pgct1* locus may be syntenic to a region on human chromosome 5q that is potentially associated with male GCT susceptibility and also is a hotspot for deletion in male GCT. A shoulder, which may be indicative of a second linkage peak, is evident >10 cM distal to the *pgct1* locus in a region that is coincident with genes that map to human 5q13.3–5q14, which would put it in the vicinity of the second deletional hotspot region. Finally, the *Ter* locus on mouse chromosome 18 is reported to be coincident with genes that map to human 5q31–5q32, which would put the *Ter* locus in the vicinity of the third deletional hotspot region. These mapping data are still very imprecise, but they do suggest the intriguing possibility that

mutations that render 129 strain mice susceptible to male GCT development may have relevance to susceptibility in humans as well.

GCT development in 129 strain males is a congenital disease that seems to arise no later than 15–17 days postcoitum (dpc) (3). Coincidentally, genital ridges form transplantable tumors most effectively when obtained from 129 strain embryos at 11–12 dpc, and no longer form tumors effectively when obtained from embryos beyond 13 dpc (6, 37). These observations suggest that the susceptibility of PGCs from 129 strain males to undergo spontaneous neoplastic transformation is restricted to a limited temporal window. PGCs between 6–13 dpc proliferate rapidly (38) and are actively motile and invasive (39, 40). Uncontrolled, these are hallmark characteristics of malignancy. After colonization of the gonadal anlagen, PGCs normally become quiescent and stationary (39, 41). This activity coincides with the time that sex-specific differentiation of the gonads is first observed (42). Furthermore, GCT susceptibility factors appear to be sex-specific, with GCT development restricted to males of the 129 strain (2) and to females of the LT/SvA strain (43). These observations suggest that signals associated with the process of sexual differentiation might play a key role in transitioning the PGC population to a quiescent state and that these signals may differ significantly between males and females. The male GCT susceptibly gene at the *pgct1* locus might be involved in mediating responsiveness of the PGC population to such signals in the male, and identifying the mutation at the *pgct1* locus in the 129 strain may have important implications for understanding how PGCs are developmentally regulated.

What is the role of p53 in suppressing the frequency of GCTs? PGCs that are refractory to antiproliferative signals may still be subject to clearance by a mechanism such as apoptosis. Those PGCs that escape this clearance mechanism could then go on to form tumors. If p53 is involved in the clearance of refractory PGCs, then its absence would not affect the frequency of tumor initiation but would affect the number of tumors that develop. Observations in mice with the *Ter* mutation appear to be consistent with such a model. Examination of developing gonads has revealed that *Ter* embryos exhibit PGC hypoplasia even though the *Ter* mutation causes PGCs to undergo an extended proliferative period (44). This observation is consistent with the *Ter* mutation contributing to a lack of responsiveness to antiproliferative signals among the PGC population, with most of the cells being subject to clearance but a few escaping to form tumors. If *Ter* enhances PGC proliferation and *Trp53*−/− impedes clearance in the 129 strain, then combining these two mutations should synergistically increase male GCT susceptibility. Identifying the underlying 129 strain mutation at the *pgct1* locus should help to elucidate how the tumorigenic characteristics of PGCs are normally held in check, which in turn might have broad ramifications for understanding the molecular basis of neoplastic progression in other forms of malignancy.

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