Environmental agents and genetic variants can induce heritable epigenetic changes that affect phenotypic variation and disease risk in many species. These transgenerational effects challenge conventional understanding about the modes and mechanisms of inheritance, but their molecular basis is poorly understood. The Deadend1 (Dnd1) gene enhances susceptibility to testicular germ cell tumors (TGCTs) in mice, in part by interacting epigenetically with other TGCT modifier genes in previous generations. Sequence homology to Atcf, the RNA-binding subunit of the ApoB editing complex, raises the possibility that the function of Dnd1 is related to Apobec1 activity as a cytidine deaminase. We conducted a series of experiments with a genetically engineered deficiency of Apobec1 on the TGCT-susceptible 129/Sv inbred background to determine whether dosage of Apobec1 modifies susceptibility, either alone or in combination with Dnd1, and either in a conventional or a transgenerational manner. In the paternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. By contrast, partial deficiency in the maternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. By contrast, partial deficiency in the maternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. By contrast, partial deficiency in the maternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. By contrast, partial deficiency in the maternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. By contrast, partial deficiency in the maternal germ-lineage, Apobec1 deficiency significantly increased susceptibility among heterozygous but not wild-type male offspring, without subsequent transgenerational effects, showing that increased TGCT risk resulting from partial loss of Apobec1 function is inherited in a conventional manner. We conclude that heritable epigenetic changes are transgenerational, and that transgenerational epigenetic effects are related to the ability of a cytidine deaminase to catalyze inactivating lesions in messenger RNAs that influence male and female fertility.
suggested that several deaminases in the Apobec1 family also have DNA demethylase activity (39–41). Targeted deletion of Apobec1 (abbreviated ko hereafter) results both in homozygous mice that are healthy and fertile and in knockout heterozygotes that show tissue-specific abnormalities in ApoB RNA editing (42). The present study focused on tests to determine whether Apobec1 deficiency affects TGCT susceptibility in a conventional or transgenerational manner, either alone or in combination with Dmd1ter.

**Results**

**Effects of Complete and Partial Apobec1 Deficiency on TGCTs.** The first task was to test the impact of complete and partial APOBEC1 deficiency on TGCT susceptibility with respect to both conventional inheritance and transgenerational effects. We began with a test for effects of ko homozygosity on ko/ko male offspring in a pure-breeding strain (test 1). Then we tested the effects of parental ko/+ heterozygosity on ko/+ and +/+ male offspring (test 2), parental homozygosity versus heterozygosity on genetically identical ko/+ heterozygous sons (test 3), ancestral genotype and switching the parent of origin (test 4), and finally long-term epigenetic effects of parental APOBEC1 deficiency on wild-type progeny (test 5).

**Test 1: Parental and offspring ko/ko homozygosity.** To test whether complete deficiency of Apobec1 affects TGCT susceptibility, we surveyed homozygous ko/ko males that were derived from at least two generations of ko/ko intercrosses (Fig. 1). Results were compared with both the current wild-type 129 control [(+/-)ko] and published reports for wild-type [(+/-)p] mice. For these two control populations, the proportion of affected (+/-)ko and (+/-)p males did not differ significantly (Table 1, cross 4), suggesting that current results reflect the long-term average TGCT prevalence in 129 males (19–22, 36, 43–46). Interestingly, affected Apobec1-deficient males occurred at a frequency (4.1%) that represents an ~43% reduction (P < 0.05) compared with that for the (+/-)ko, and (+/-)p control populations [Table 1, cross 1 and (+/-)p]. The finding of reduced testicular cancer risk is reminiscent of the effects of Apobec1 deficiency on small intestinal adenoma formation in ApcMin/+ mice (47). Moreover, the magnitude of the reduction is similar to that reported for males that are partially deficient for the Eif2s2 factor, which is an established TGCT suppressor (48).

**Test 2: Parental ko/+ heterozygosity.** Because partial Apobec1 deficiency can lead to tissue-specific dysfunction (42, 47), we asked whether parental ko/+ heterozygosity affected TGCT susceptibility (cross 2). For this test, we measured the frequency of affected ko/+ (test) wild type and (+/-)p (control) male offspring from reciprocal crosses between ko/+ heterozygotes and strain 129 wild-type (+/-)ko homozygotes. The direction of ko inheritance was maintained independently through the female and male lineages for at least three generations before the test crosses, and results were analyzed separately (Fig. 2A). Surprisingly, with maternal heterozygosity, TGCTs were absent in ko/+ male offspring, a highly significant reduction relative to (+/-)p males (Table 1, cross 2a). Interestingly, the 1.6% prevalence in (+/+)p male offspring also was significantly reduced relative to wild-type controls (Table 1, cross 2a), even though these wild-type (+/+)p males were genetically identical to strain 129 (+/+)p control males. By contrast, with paternal heterozygosity, 14% of heterozygous ko/+ male offspring were affected, which represented a twofold increase relative to (+/-)p controls (Table 1, cross 2b), an increase that was similar to published values for established heterozygous TGCT enhancers including Dmd1ter (17%), Kiltgsl (14%), and Trp53mt1J (15%) (27, 31, 36, 43–46, 49, 50). In contrast, (+/+)p male offspring resulting from paternal heterozygosity showed the baseline prevalence typical of strain 129 males (Table 1, cross 2b). Thus, paternal partial deficiency reduced risk in a parent-of-origin manner among both heterozygous and wild-type sons, whereas paternal partial deficiency enhanced tumorigenesis only in ko/+ sons in a manner that showed conventional inheritance.

**Test 3: Parental homozygosity versus heterozygosity effects on ko/+ offspring.** Next we tested whether TGCT prevalence among ko/+ male offspring depended on parental homozygosity (ko/ko) versus heterozygosity (ko/+). Occurrence of TGCT-affected males among genetically identical heterozygous (ko/+) progeny derived from reciprocal ko/ko × (+/-)p intercrosses (cross 3) was compared with those described above for ko/+ × (+/-)p backcrosses (Table 1, crosses 2 and 3, respectively). To control for lineage-specific effects, parents were obtained from lineages in which the parental germ-lineage had been maintained consistently for at least three prior generations.

When the ko mutation was inherited through the female germ-lineage, heterozygous ko/+ sons of ko/ko females were fully protected (0% affected) from TGCTs (Table 1, crosses 2a and 3a). By contrast, when the ko mutation was inherited through the male germ-lineage, the prevalence of affected males (7.9%) did not differ significantly from the (+/-)p baseline (Table 1, cross 3b) but was significantly less than the 14% prevalence found in genetically identical ko/+ offspring of ko/+ heterozygous males (Table 1, cross 2h = Efe2s2, TGCT controls) (Table 1, cross 2h). Thus, the 129 males that inherited this effect differed dramatically among genetically ko/+ identical male offspring depending on parental sex and genotype, with complete protection among sons of ko/+ heterozygous and ko/ko homozygous females and with increased risk in ko/+ heterozygous sons of ko/+ heterozygous but not ko/ko homozygous males.

**Test 4: Ancestral genotype and switching parent of origin.** The next test involved determining whether the special physiological relationship between mother and fetus during the critical developmental period for PGC transformation to TGCTs contributed to the transgenerational genetic effects observed in cross 2a (Table 1). In the first cross, ko/+ male offspring that inherited the ko deletion through the female germ-lineage for at least two previous generations were mated to wild-type females, thereby switching the ancestral-female mode of inheritance to transmission through males (Fig. 3A). Heterozygous ko/+ sons that inherited this ancestral-female allele from their mother instead of their mother were screened for TGCTs, and the prevalence was compared with that observed for ko/+ males that inherited the deficiency through the female germ-lineage. Surprisingly, none of the ko/+ males from this switched mating were affected with TGCTs, and this prevalence was significantly reduced relative to both the increased TGCT prevalence (14%) observed in ko/+ males inheriting the ancestral-male ko allele through the male germ-lineage and to the baseline rates in (+/-)p, and (+/-)p controls (Fig. 3A). In the reciprocal cross, we tested the effect of transmitting the ancestral-male ko allele through females

![Fig. 1. Effects of Apobec1 deficiency on TGCTs. Parental and offspring Apobec1ko/ko homozygosity (test 1). *P < 0.05.](image-url)
Table 1. Effects of complete and partial Apobec1 deficiency on occurrence of TGCT-affected males

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parental cross</th>
<th>Offspring genotype</th>
<th>N</th>
<th>No. affected</th>
<th>Percent affected</th>
<th>$\chi^2$</th>
<th>P value</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ko/ko x ko/ko</td>
<td>ko/ko</td>
<td>338</td>
<td>14</td>
<td>4.1</td>
<td>4.3</td>
<td>&lt;0.05</td>
<td>Complete deficiency reduces susceptibility</td>
</tr>
<tr>
<td>2a</td>
<td>ko/+ x +/+</td>
<td>ko/+</td>
<td>186</td>
<td>0</td>
<td>0</td>
<td>14.0</td>
<td>&lt;0.0002</td>
<td>Maternal heterozygosity provides complete protection</td>
</tr>
<tr>
<td>2b</td>
<td>+/+ x ko/+</td>
<td>ko/+</td>
<td>132</td>
<td>9</td>
<td>14.0</td>
<td>10.2</td>
<td>&lt;0.001</td>
<td>Paternal heterozygosity reduces susceptibility</td>
</tr>
<tr>
<td>3a</td>
<td>ko/ko x +/+</td>
<td>ko/+</td>
<td>179</td>
<td>0</td>
<td>0</td>
<td>13.9</td>
<td>&lt;0.0002</td>
<td>Paternal heterozygosity does not affect susceptibility</td>
</tr>
<tr>
<td>3b</td>
<td>+/+ x ko/ko</td>
<td>ko/+</td>
<td>189</td>
<td>15</td>
<td>7.9</td>
<td>&lt;0.1</td>
<td>Ns</td>
<td>Paternal homozygosity provides complete protection</td>
</tr>
<tr>
<td>4</td>
<td>+/+ x +/+</td>
<td>(+/+)$_w$</td>
<td>208</td>
<td>15</td>
<td>7.2</td>
<td>0.01</td>
<td>Ns</td>
<td>Contemporaneous control was not different from published results for (+/+)$_w$</td>
</tr>
</tbody>
</table>

$\chi^2$ Goodness-of-fit tests were used to compare the prevalence of TGCT-affected test and concurrent wild-type (+/+), control males. N is the number of males examined; no. affected is the number of males with at least one TGCT; significance was determined as $P < 0.05$; and Ns indicates results that did not pass the threshold of statistical significance. Because (+/+)$_w$ and (+/+)$_f$, were not different, and both were consistent with long-term rates in the 129 family of inbred strains, the 7% prevalence in (+/+)$_w$ was used as expectation for comparisons with +/+ and ko/+ male offspring.

In this case, the 13% TGCT prevalence was not significantly different from direct inheritance through the male lineage but was significantly increased over the expected rate based on direct maternal effects. Together these reciprocal crosses showed that ancestral rather than parental genotype underlies the lineage-dependent effects of partial Apobec1 deficiency on TGCT prevalence.

We then asked whether maintaining the new direction of inheritance of the ko allele for another generation was sufficient to reverse lineage-specific effects to reflect the grandparental direction of inheritance. In the first cross, the ancestral-female allele was inherited for a second generation through the male germ-lineage (Fig. 3A), and in the reciprocal cross, the ancestral-male allele was inherited for a second generation through the female germ-lineage (Fig. 3B). In contrast to results for the ancestral-female allele in the previous generation, we found that the prevalence of affected ko/+ males now was switched in the second generation, showing a significant increase over the absence of affected males in the previous generation and a non-significant difference from the expected prevalence of 7% based on their genotype and the direction of the cross (Fig. 3A). Similarly, the occurrence of affected ko/+ males with the ancestral-male allele also was switched in the second generation, with a significant decrease from the previous generation and a nonsignificant difference from the rate expected with maternal inheritance (Fig. 3B). Thus, lineage-specific factors that control the TGCT phenotype were reset after transmission for two consecutive generations through the alternative germ-lineage and corresponded with the grandparental rather than the parental direction of inheritance.

Test 5: Persistence of transgenerational effects in wild-type (+/+)$_w$ males.

Finally, we asked whether the transgenerational effects of Apobec1 on wild-type progeny persisted to subsequent generations, independently of maternal ko/+ heterozygosity. If the observed effect among wild-type (N1) sons resulted from in utero exposure to the consequences of partial deficiency of maternal Apobec1, reduced prevalence would be found among sons (N1) and perhaps grandsons (N2), but not among great-grandsons (N3), where prevalence should return to baseline values. Alternatively, if TGCT prevalence found among N1 males resulted from heritable epigenetic changes in N0 females, a similarly reduced prevalence might be expected among N1, N2, and N3 male offspring.

For this test, we backcrossed wild-type (+/+)$_w$, N1 offspring from reciprocal ko/+ × (+/+)$_w$, N0 crosses (Table 1, with cross 2a as the test and cross 2b as the control) to 129 (+/+)$_w$, mice (Fig. 4). We then surveyed (+/+)$_w$, N2 males and, after an additional backcross, (+/+)$_w$, N3 males for TGCTs. In these tests, the ko allele was present only in the N0 parents and N1 littermates. The N2 and N3 males that were surveyed for TGCTs did not inherit the ko/+ allele and were genetically, but perhaps not epigenetically, identical to the 129 (+/+)$_w$ and (+/+)$_w$ controls.

Remarkably, maternal Apobec1 deficiency significantly reduced TGCT prevalence, not only among N1 males but also among N2 and N3 (+/+)$_w$ males relative to (+/+)$_w$ controls (Fig. 4). Inheritance of this suppressive effect from the ancestral female lineage was independent of parental direction in subsequent generations and did not require the ko allele to maintain the phenotype, suggesting that the observed TGCT suppression in sons of Apobec1-deficient females results from a stable, inherited epigenetic factor rather than direct exposure to the maternal deficiency in utero. For partial Apobec1 deficiency in males, TGCT prevalence in N1 (+/+)$_w$ sons did not differ from the expected baseline prevalence in wild-type sons (Fig. 4B). As expected, with continued backcrossing, TGCTs were observed in wild-type sons at the baseline rate regardless of whether the ancestral-male allele was transmitted through a male or female wild-type parent in subsequent generations.

Transgenerational Interactions Between Apobec1 and Dnd1 Ter

Previous studies revealed transgenerational genetic interactions that led to both an increased prevalence of affected Dnd1 Ter (Ter) males and an increased proportion of bilateral cases in Ter/+ males when any of six TGCT modifier genes were present in the parental generation, compared with results for Ter/+ and these modifiers in separate single-gene control crosses (29). We therefore sought to test whether ko interacts with Ter in a similar manner. For this study, we focused on three specific ko and Ter interaction effects. We also asked whether ko and Ter have additive effects in double-heterozygous males. The study design of Lam et al. (29) was used for these tests (Fig. S1), with the expected frequency of affected males based on results from corresponding single-modifier tests (Table S1).
To determine whether partial deficiency of Apobec1 in the parental generation affected TGCT risk in Ter/+ sons, we compared the TGCT prevalence in Ter/+ control males (31.5%) from a separate Ter-only colony. Male offspring were affected with TGCTs at a rate of 21.0%, which represents a significant 40% reduction relative to the prevalence observed in Ter/+ controls (Table 2).

These results with ko differed from those with all six previously tested modifiers in that the parental modifier, in this case Apobec1 ko, reduced rather than increased prevalence in Ter/+ sons (cf ref. 29).

Because partial Apobec1 deficiency acted as a TGCT suppressor in the female germ-lineage and as an enhancer in the male germ-lineage (Table 1, crosses 2a and 2b), we tested whether the sex of the Apobec1-deicient parent in the interaction test-cross affected TGCT prevalence in Ter/+ sons by comparing the prevalence in sons of partially deficient mothers (22.0%) and partially deficient fathers (19.1%) with the expected prevalence based on a separate ko-only colony. Because partial Apobec1 deficiency had lineage-specific effects on the frequency of TGCT-affected males in separate crosses (Table 1), sons inher-
3a), an appreciable number of affected males was observed among those that inherited the ko allele maternally when Ter also was present in the parental generation [Table 2, test 2, +/+ ko/+(m)]. In the reciprocal cross, in contrast, the prevalence of affected ko/+ males did not differ significantly from wild-type strain 129 controls [Table 2, test 2, +/+ ko/+(p)], suggesting that presence of Ter in the parental generation negated the protective parent-of-origin effect of Apobec1 deficiency in the female lineage.

Test 3: Wild-type sons. Although wild-type offspring from Apobec1-deficient females showed a significant reduction in TGCT prevalence in separate crosses (Table 1, cross 2a), wild-type males in the interaction test crosses were affected at the expected baseline rate (6.6%) (Table 2), suggesting that the increased prevalence in Ter/+ sons and the reversal of the protective effect of maternal Apobec1 deficiency in ko/+ sons result from specific interactions between the parental and inherited mutations rather than being attributable solely to premeiotic effects of the parental mutations that would manifest in all offspring regardless of their genotype.

**Test 4: Apobec1<sup>ko</sup> and Dnd1<sup>ter</sup> sons.** To test for traditional genetic interactions between Apobec1 and Dnd1, double-mutant male offspring (ko/+, Ter/+ ) were surveyed for TGCTs with the expected prevalence (29.9%) based on an additive model of gene interaction and calculated using the observed frequencies for single-mutant and wild-type littermate controls (29). The observed TGCT prevalence in double-mutants (27.5%) did not differ significantly from the predicted prevalence and therefore fits an additive model of gene interaction. Apobec1<sup>ko</sup> therefore is the only genetic variant that does not interact with Ter to modulate TGCT susceptibility.

**Lineage-specific ko-Ter interactions and embryonic viability.** In the ko-Ter interaction test, we noted that segregation ratios for single- and double-mutant males did not appear to be normal (Table 2). We therefore examined this question directly (Table 3). In fact, these ratios deviated dramatically from expectations, depending on parental and progeny genotypes. When ko was inherited

---

**Table 2. Occurrence of TGCT-affected males in transgenerational interaction tests between Apobec1<sup>ko</sup> and Dnd1<sup>ter</sup>**

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Test</th>
<th>Offspring genotype</th>
<th>N</th>
<th>No. affected</th>
<th>No. expected</th>
<th>Percent affected</th>
<th>Percent expected</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for transgenerational effects</td>
<td>1</td>
<td>Ter/+ (+/+)</td>
<td>124</td>
<td>26</td>
<td>39.0</td>
<td>21.0</td>
<td>31.5</td>
<td>6.3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+/+ ko/+ (m)</td>
<td>163</td>
<td>14</td>
<td>0</td>
<td>8.6</td>
<td>12.6</td>
<td>&lt;0.0004</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/+ ko/+ (f)</td>
<td>159</td>
<td>17</td>
<td>15.1</td>
<td>10.7</td>
<td>9.5</td>
<td>0.3</td>
<td>Ns</td>
</tr>
<tr>
<td>Internal wild-type (w) control</td>
<td>3</td>
<td>+/+ (+/+</td>
<td>213</td>
<td>14</td>
<td>15.4</td>
<td>6.6</td>
<td>7.2</td>
<td>0.1</td>
<td>Ns</td>
</tr>
<tr>
<td>Test for epistasis</td>
<td>4</td>
<td>Ter/+ (+/)</td>
<td>189</td>
<td>52</td>
<td>56.6</td>
<td>27.5</td>
<td>29.0</td>
<td>0.5</td>
<td>Ns</td>
</tr>
</tbody>
</table>

Mice in the contrast group were obtained from contemporaneous crosses in which only the designated genetic variant was segregating. The expected effects for the additive model were obtained by summing the independent effects for ko/ and Ter/. (m) and (p) refer to maternal and paternal ko/+ heterozygosity, respectively. Several considerations are relevant: We found a TGCT prevalence of 31.5% in Ter/+ control males on the 129S1/SvImJ background, which represents a significant increase (\( P < 10^{-5} \)) compared with the published values (30, 38, 39). However, the previous studies were performed with a different 129 substrain (129T1/Sv). Therefore, throughout the present study, we used the control values observed in the concurrent surveys because these mice were bred on the same 129 background, screening was performed concurrently with test males, and mice used in the interaction test and in control crosses were on the same inbred genetic background.
through the paternal germ-lineage, segregation ratios at 3–4 wk of age did not differ significantly from Mendelian expectations ($\chi^2 = 1.0$, not significant). However, when ko was inherited through the maternal germ-lineage, these ratios showed a highly significant deviation ($\chi^2 = 58.5, P < 0.0001$). If we accept that the observed number of wild-type males (n = 64) was the number expected in each genotypic class, then a total of 192 (3 × 64) males would be expected across the three other genotypes. However, with a total of only 51 observed, $\sim 75\% (n = 141)$ of the single- and double-heterozygotes were missing. We next asked when these deviations arose, focusing first at ∼E12.5 and then at E3.5 (Table S2). Interestingly, segregation ratios were already biased at ∼E3.5 in maternal ko– paternal Ter crosses but not in the reciprocal cross. Litter sizes did not differ significantly across the entire study (Table S3).

**Discussion**

Many environmental factors and genetic variants are known to induce heritable epigenetic changes that can persist for multiple generations, affecting a broad range of traits, and that often are as frequent and strong as direct environmental exposures and conventional genetic inheritance (18, 51–57). These transgenerational effects challenge our understanding of the modes and mechanisms for inherited phenotypic variation and disease risk, as well as the premise of most genetic studies in which causal DNA sequence variants are sought within the genome of affected individuals. Several molecular mechanisms have been implicated, ranging from inherited RNAs to chemically modified DNA and proteins (51–61). These transgenerational effects have important implications for our understanding of adaptation and evolution, the origins of phenotypic variation and disease risk, and the molecules in addition to DNA that can be the basis for inheritance (18, 62, 63).

Studies of transgenerational genetic effects on testicular cancer in mice show that Dnd1 is an essential component of a cross-generation system, with genes such as Trp53, Kitl, and Eif2s2 in the parental generation acting with Dnd1 in male offspring to increase significantly the number of TGCT-affected males and the proportion of bilateral cases (29). Dnd1 encodes an RNA-binding protein that controls access of specific microRNAs to their mRNA targets in testicular cancer cell lines (64) and also transports mRNAs from the nucleus to perinuclear RNA granules—binding protein that controls access of specific microRNAs to their mRNA targets in testicular cancer cell lines (64) and also transports mRNAs from the nucleus to perinuclear RNA granules—

| $\chi^2$ | Nexp | %obs | 27 | 32 | 24 | 13 | 4 | 11 |
| 30 | 32 | 26 | $<+/-$, Ter/+ | 19 | 64 | 17 |
| 25 | 32 | 22 | ko/+, +/+ | 19 | 64 | 17 |
| 32 | 32 | 28 | +/+, +/+ | 64 | 64 | 56 |

Table 3: Non-Mendelian segregation in ko–Ter interaction tests

| Ter/+ $\times$ ko/+ $\times$ Ter/+ Offspring genotype (ko/+, Ter/+), Nexp | Nobs | %obs |
| 27 | 32 | 24 | 13 | 4 | 11 |
| 30 | 32 | 26 | 19 | 64 | 17 |
| 25 | 32 | 22 | 19 | 64 | 17 |
| 32 | 32 | 28 | 64 | 64 | 56 |

$N^{\text{obs}}$ is the number of males of each genotype observed, and $N^{\text{exp}}$ is the number of males of each genotype expected based on Mendelian expectations, i.e., 25% of the total number of males in each genotypic class of the reciprocal crosses; %obs is the percent observed; and %exp is the percent expected based on Mendelian expectations, i.e., 25% of the total number of males in each genotypic class.

Several findings in this study document the conventional and transgenerational effects of Apobec1 deficiency on TGCT susceptibility, both alone and in combination with Dnd1. In the paternal germ-lineage, the increased number of affected ko/+ but not +/+ sons (Table 1, crosses 2b and 3b) shows that Apobec1 acts as TGCT enhancer and that this effect is inherited in a conventional manner. By contrast, ko/+ sons of ko/+ and ko/ko females were not affected with a TGCT (Table 1, crosses 2a and 3a; cf. refs. 28, 66). In addition, maternal ko/+ heterozygosity significantly reduced the prevalence of affected +/+ wild-type sons, not only in the first generation (Table 1, cross 2a) but also for at least two subsequent generations (Fig. 4), suggesting both that reduced dosage of maternal Apobec1 alone was sufficient to induce a heritable epigenetic change that persisted for multiple generations and that direct maternal–fetal interactions were not essential. Interestingly, parental Dnd1$^{+/−}$/+ heterozygosity does not induce transgenerational effects (29). Finally, these lineage-specific effects were fully reversed after transmission for two consecutive generations through the alternative parental germ-lineage (Fig. 3), suggesting that the heritable epigenetic change was not permanent and could be reversed under specific conditions. The ability of Apobec1 to modulate TGCT risk in both a conventional and a transgenerational manner, depending on parental germ-lineage, raises important questions concerning the mechanisms by which Apobec1 may function in PGC biology, testicular cancer etiology, and, most importantly, in heritable epigenetic changes.

Tests for interactions between Apobec1ko and Dnd1$^{+/−}$ provided contrasting results, depending on parental genotypes. The combination of maternal ko/+ and paternal Ter/+ heterozygosity neutralized each other’s effects on TGCTs among male offspring, significantly increasing or reducing risk in the direction of the baseline rate that is characteristic of the 129 strain (Table 2). In contrast, in the reciprocal cross with maternal Ter/+ heterozygosity and paternal ko/+ heterozygosity, a striking deviation from Mendelian expectations was found at embryonic day E3.5 (Table S2), with substantial loss of both double-heterozygous and single-heterozygous mutant mice (Table 3). Thus, Apobec1 and Dnd1 interact, albeit in unconventional and complex manners, to modulate TGCT risk and also, unexpectedly, to control the occurrence of genotypic classes among early embryos.

The strong but unexpected bias in segregation ratios in maternal ko/−– paternal Ter/+ heterozygosity also has several unusual features. The effects depended completely on the direction of the cross (Table 3), with Apobec1 acting in the female germ-lineage, and Dnd1 acting in the male germ-lineages. Loss of substantial numbers of single and double heterozygotes in the interaction test is remarkable, given that both ko/ko and Ter/Ter homozygotes are fully viable in separate crosses (36, 42). Normal segregation also is found in mutant heterozygotes in separate crosses to wild-type mice (29; also see Table S3), showing that gametes are produced in normal ratios and functionality. Moreover, modest differences in litter size do not account for the substantial genotypic bias (Table S3). Together these observations suggest that strong segregation bias results from a preference for specific combinations of gametes at fertilization rather than selective embryo loss after conception. Interestingly, segregation ratios for heterozygous Aicf knockout mice also are strongly skewed (10:1) toward mutant heterozygotes versus wild type (67).

These results are reminiscent of non-Mendelian segregation in mice that are heterozygous for t-haplotype in mice (68, 69) but differ in that both maternal and paternal factors are required. Together these observations suggest that Apobec1, Dnd1, and perhaps Aicf have important but as yet unknown functions during gametogenesis and fertilization.

Questions that emerge from this work include whether these genetic effects act directly on the germ-line or indirectly through the soma to the germ-line, perhaps through signaling pathways such as Kit ligand from somatic cells and Kit receptor on germ
cells (18, 31); whether heritable epigenetic changes in the germ-line involve methylation, histone modifications, RNAs, or perhaps other molecules (18, 51–61); whether the relevant APOBEC1 functions involve RNA editing or DNA demethylation (37–41, 60, 61); and whether the same epigenetic changes are transmitted to subsequent generations or are responsible for reversing heritable epigenetic changes.

Materials and Methods

Mice. Mice from the 129S1/SvImJ inbred strain (“129”, previously known as 129/SvJ and 129S3/SvImJ; stock number JR02448) were obtained from the Jackson Laboratory. A targeted deletion of APOBEC1 (herein abbreviated ko) was made with the RF8 ES cells (cell line from 129/SvImJ mice) and then maintained on the 129/SvImJ inbred genetic background (42 and this study). Mice were genotyped using a PCR-based assay as described previously (42), with wild-type homoygotes, heterozygotes, and mutant homoygotes designated as (+/+), +/ko, and ko/ko, respectively. Two control groups were included, (+/+), and +/+, which correspond respectively to a contemporaneous (c) survey of wild-type 129/SvImJ mice from an independent colony and to a review of TGCT incidence in wild-type 129/SvImJ reported in the literature (j) (19–22, 36, 43–46). All mice in this study were maintained on a standard 50% rodent chow diet (PMI Nutrition International), housed in the Case Western Reserve University Animal Resources Center and maintained on a 12/12-h light/dark cycle. The Case Western Reserve Institutional Animal Care and Use Committee approved all mouse work described in this report.

TGCT Survey. Male mice (3- to 5-wk-old) were killed, and incisions were made in the abdomen to expose the testes. TGCTs were identified through macroscopic inspection of the tests for abnormalities in size, color, shape, or texture (19–22, 36, 43–46). Mice in the various test and control groups were scored for the presence of unilateral or bilateral TGCTs. The frequency of affected males with either unilateral or bilateral TGCT was used as a measure of prevalence. x2 tests were used to test for significant differences in the frequency of affected males.

ACKNOWLEDGMENTS. We thank members of the J.H.N. group for ideas, comments, and discussions. This work was supported by National Institutes of Health (NIH)/National Cancer Institute Grant CA75556 and NIH Pioneer Award DP1OD006911 (to J.H.N.) and by NIH/National Heart, Lung and Blood Institute Grant HL-38180, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant DK-56260, and NIDDK Grant DK-52574 (to N.O.D.). P.J.T. is funded as a Robertson Investigator of the New York Stem Cell Foundation.