

Oligotriche and Quaking Gene Mutations

Phenotypic Effects on Mouse Spermatogenesis and Testicular Steroidogenesis

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ABSTRACT: The phenotypic actions of the oligotriche gene mutation on testicular function have not been elucidated, although it is known that male mice homozygous for the mutation are infertile. In the present study, the effect of the oligotriche gene mutation on mouse testicular function was analyzed by comparing normal and mutant mice. Spermatogenesis was analyzed by enumerating germ cells in seminiferous tubules at specific stages of spermatogenesis and by electron microscopy. Steroidogenic potential was estimated by radioimmunometric determination of testosterone secreted by testes perfused in vitro. Parallel studies were completed for male mice homozygous for the quaking gene mutation, a mutation known to cause male

mouse sterility by disrupting sperm tail development. The experimental results suggest that the oligotriche and quaking gene mutations interfere with sperm tail formation by different mechanisms. Testicular steroidogenesis was not affected by either gene mutation. The results provide the first evidence that the oligotriche gene mutation induces male mouse sterility by effecting the complete absence of a sperm tail. This phenotypic action is different from that of the quaking gene mutation.

Key words: Spermiogenesis, mutant mice, reproduction, testosterone, quaking gene mutation, oligotriche gene mutation.

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The factors involved in spermiogenesis have not been elucidated. One experimental approach to understanding factors controlling spermiogenesis is the study of mice bearing single-gene mutations that disrupt spermiogenesis. Genes and their products involved in spermiogenesis theoretically should be definable by comparing results from mutant and normal mice. The validity of this approach has been demonstrated for other gene mutations affecting male mouse reproductive function (Mason et al, 1986; Tan et al, 1990; Brilliant et al, 1991). Before these experiments can be initiated, the phenotypic effects of the gene mutations on spermiogenesis at the cellular level must be defined.

In this report, we compare the phenotypic effects of two gene mutations that have been reported to disrupt spermiogenesis. The study of the oligotriche gene mutation (*olt*) was stimulated by a preliminary report that suggested oligotriche mutant mice had defective spermiogenesis (Moutier, 1976). Our studies of the quaking gene (*qk*) mutation are included since this mutation has been documented to disrupt

mouse spermiogenesis (Bennett et al, 1971), but its action on testicular steroidogenesis has not been examined. The actions of the quaking gene mutation may be expressed differently than in the original report by Bennett and colleagues (1971), since it currently is maintained in a different mouse strain.

The studies provide data that suggest spermatogenesis before spermiogenesis is not impaired by either gene mutation. However, both mutations interfered with sperm tail formation by different mechanisms. The deleterious effects on spermiogenesis did not stem from a defect in testicular steroidogenesis, since testosterone secretion by both oligotriche and quaking mouse testes was normal.

Materials and Methods

Animals

Oligotriche mice (*olt/olt*) and their normal siblings were obtained from breeding pairs generously provided by R. Moutier (Centre de Sélection et d'Élevage d'Animaux de Laboratoire, Centre National de la Recherche Scientifique, Orléans Cedex, France). The oligotriche gene mutation was maintained in C3H/HeOrl mice. Mice homozygous for the oligotriche mutation can be identified visually due to the thinning of the ventral coat. Quaking mice (*qk/qk*) and their normal siblings were purchased from The Jackson Laboratory (Bar Harbor, ME). The quaking gene mutation was maintained in

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C57BL/6J X C3 (N6F1) mice. Mice homozygous for the quaking gene mutation can be identified visually by their tremors. The gene symbol used for normal siblings was $?/+$ since they were either homozygous for the wild-type gene ($+/+$) or unaffected heterozygotes. Male siblings were housed together in an environmentally controlled mouse room (23°C; 12 hours light:12 hours dark). Feed and water were available at all times. Mice were 13 to 14 weeks old when sacrificed by cervical dislocation.

Analysis of Testicular Steroidogenesis

Steroidogenesis was analyzed by determining testosterone secretion by mouse testes perfused *in vitro* (Chubb and Desjardins, 1983). In brief, capsular arteries of isolated testes were cannulated and perfused with a defined medium consisting of 0.2% glucose, 3% bovine serum albumin, and 25% bovine erythrocytes in Krebs-Ringer-bicarbonate buffer. A maximally stimulating concentration of luteinizing hormone (100 ng/ml of NIADDK-oLH-24) was added to the medium to assess the maximum potential of the testes to secrete testosterone. Testes were perfused for 4 hours at a rate of 2 ml/hour and the venous effluent supernatant was stored at -20°C until analysis. Testosterone was quantified with a double-antibody radioimmunoassay specific for testosterone (Chubb and Desjardins, 1983).

Analysis of Spermatogenesis

Spermatogenesis was assessed by two analytical methods. In the first method, homogenization-resistant spermatids were enumerated in testis and epididymis homogenates using phase-contrast microscopy (Desjardins and Lopez, 1983). Steps 12 through 16 mouse spermatids are homogenization-resistant (Meistrich et al, 1977). This method yields a general overview of testicular spermatogenesis. In the second method, randomly selected testes were perfusion-fixed with 2% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4), dehydrated with graded ethanol washes, and embedded in methacrylate (JB-4 Embedding Kit; Polysciences, Warrington, PA). Two-micron-thick sections were stained with Sidman's acid fuchsin-toluidine blue (Dougherty, 1981) and observed using a $\times 100$ oil immersion objective. Germ cell nuclei and Sertoli cell nucleoli were counted in round cross-sections of tubules in stages I and VII of spermatogenesis as defined by Oakberg (1956). A total of 25 tubules at each stage of spermatogenesis was studied in testes from three mice for each mutant and control category. Three tubules per testis have been reported to be sufficient for germ cell counts (Amann, 1986). Totals of 15,268 and 14,106 cells were identified for the oligotriche and quaking studies, respectively.

Sertoli and germ cells were identified using descriptions provided by Bellvé and coworkers (1977). Spermatids at different steps of spermiogenesis were distinguished by nuclear condensation and cellular association. Crude cell counts were corrected for differences in nuclear or nucleolar size with Abercrombie's formula (Berndtson, 1977). Germ cell counts were standardized for differences in tubule size by expressing them as per 10 Sertoli cells, since Sertoli cells cease to proliferate in mice 12 days postpartum (Kluin et al, 1984).

Electron microscopic observations were completed for testes from two oligotriche mice and two normal siblings. Testes were perfusion-fixed with 2% glutaraldehyde in 0.1 mol/L cacodylate

buffer (pH 7.4), postfixed with unbuffered 1% osmium tetroxide, dehydrated with graded ethanol washes, and embedded in Epon-Araldite.

Statistical Procedures

The significance of the difference between means was determined by student's *t* test. All results are expressed as mean \pm SE.

Results

Oligotriche Mice

Oligotriche mice exhibited statistically normal body weight (Fig 1). Testicular steroidogenesis in oligotriche mouse testes was normal, as estimated by testosterone secretion by *in vitro* perfused testes and the maintenance of androgen-dependent seminal vesicles (Fig 1). In addition, Leydig cells appeared histologically normal (Fig 2).

The oligotriche gene mutation did affect spermatogenesis, as suggested by the significant decreases in oligotriche mouse testis weight and homogenization-resistant testicular spermatids (Fig 1). The decrease in homogenization-resistant spermatozoa in oligotriche mouse epididymides was also significant: $0.6 \pm 3 \times 10^4/\text{mg}$ (*olt/olt*) compared to $513 \pm 6 \times 10^4/\text{mg}$ ($?/+$); $n = 8$; $P < 0.001$). However, there were no significant differences in germ cell numbers up to step 7 spermatids at stage VII of spermatogenesis (Table 1). Table 1 does not include step 16 spermatids because Abercrombie's formula cannot be used with spermatids containing nonspherical nuclei. However, uncorrected spermatid counts were used to determine that

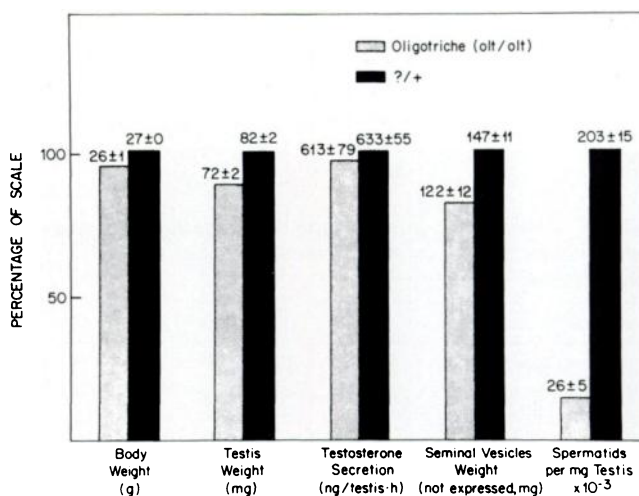


FIG. 1. Summary of data for oligotriche and normal sibling ($?/+$) mice. Data for mutant mice are displayed as percentages of normal mouse values. Absolute values are presented above the columns ($n = 6-9$). The only significant differences between mutant and normal mouse values were for testis weight ($P < 0.005$) and spermatid number ($P < 0.001$).

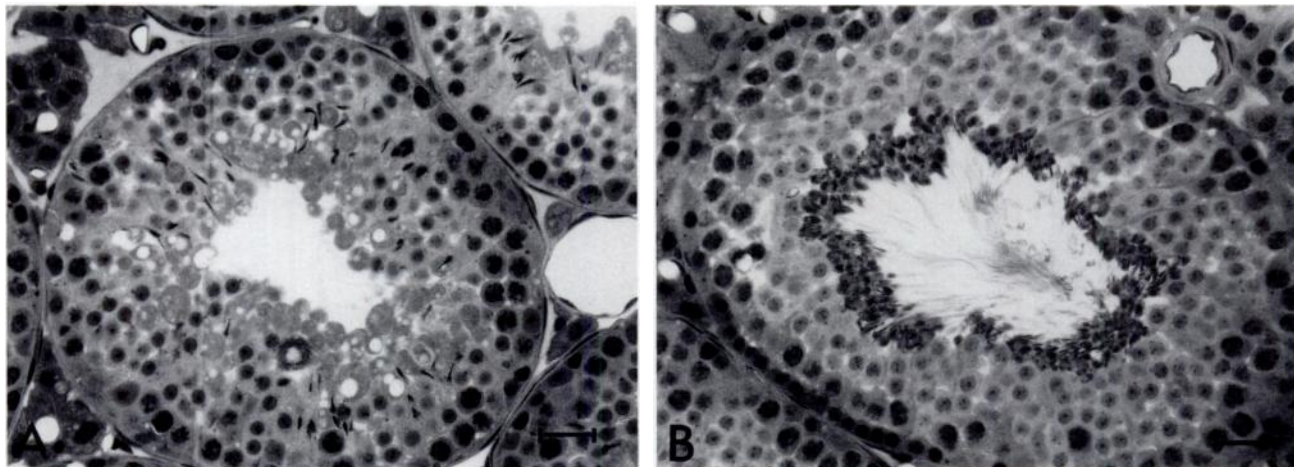


FIG. 2. Photomicrographs of cross-sections of seminiferous tubules from (A) oligotriche (*olt/olt*) and (B) normal sibling (*?/+*) mice. The absence of sperm tails in the lumen is a prominent characteristic of oligotriche mouse testes. Bar = 30 μ m.

spermatogenesis in oligotriche mice was characterized by a significant decrease in the number of spermatids after step 13 of spermiogenesis (Fig 3).

The oligotriche gene mutation prevented sperm tail development. Sperm tails were never observed in tubule lumens of mutant mouse testes, although condensed heads of elongated spermatids were evident (Fig 2). Electron micrographs demonstrate that mature spermatids in oligotriche mice did not have any tail structures, although manchettes and condensed nuclei were prominent (Fig 4). A careful search of the sections did not reveal any sperm tails, although centrioles were observed.

Quaking Mice

Mice homozygous for the quaking gene mutation were characterized by 20% to 24% decreases in body, testis, and seminal vesicle weights (Fig 5). The decreased organ weights were allometric because, when expressed per gram

Table 1. Germ cell counts at Stage VII of spermatogenesis in seminiferous tubules of oligotriche and normal sibling mouse testes*

Germ cell class	<i>olt/olt</i>	<i>?/+</i>
Spermatogonium A	2.2 \pm 0.2 [†]	1.8 \pm 0.2
Preleptotene spermatocyte	33.8 \pm 1.9	31.6 \pm 2.0
Pachytene spermatocyte	36.4 \pm 2.1	35.2 \pm 2.4
Step 7 spermatid	87.0 \pm 5.5	87.6 \pm 5.6

olt/olt = oligotriche mouse testes; *?/+* = normal sibling mouse testes.

* Cell counts were corrected with Abercrombie's formula and expressed as per 10 Sertoli cells (Sertoli cell numbers per tubule were 1.8 \pm 0.1 [*olt/olt*] and 2.5 \pm 0.2 [*?/+*]). Step 16 spermatid counts could not be corrected because of the nonspherical shape of their nuclei and were not included in this table.

[†] Mean \pm SE of counts in 25 tubules from 3 mice of each genotype.

of body weight, there were no significant differences between mutant and normal mice.

Testosterone secretion per testis was decreased in quaking mice (Fig 5). However, when expressed as per milligram of testis, mutant and normal mouse testes secreted similar amounts of testosterone (6.2 \pm 0.5 ng/mg testis \times hour [*qk/qk*] and 6.3 \pm 0.7 ng/mg testis \times hour [*?/+*]; n = 6 to 8; NS). The latter results correlate with the normal appearance of Leydig cells in mutant mouse testes (Fig 6).

The decreased number of homogenization-resistant spermatids per milligram of quaking mouse testis (Fig 5) was

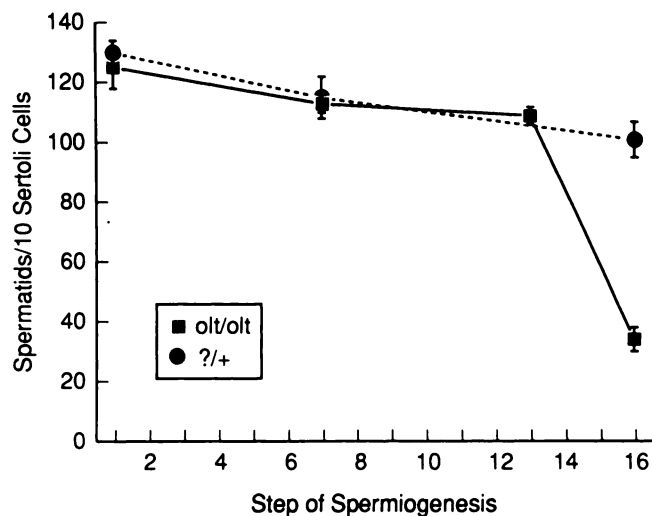


FIG. 3. Spermatid numbers at different steps of spermiogenesis in oligotriche (*olt/olt*) and normal (*?/+*) mice. Crude cell counts were used in the construction of this graph due to the nonspherical nuclei of steps 8 through 16 spermatids. Spermatids at each step of spermiogenesis were counted in 25 tubules from 3 mice of each genotype. The difference between mutant and normal mouse step 16 spermatids was significant ($P < 0.001$).

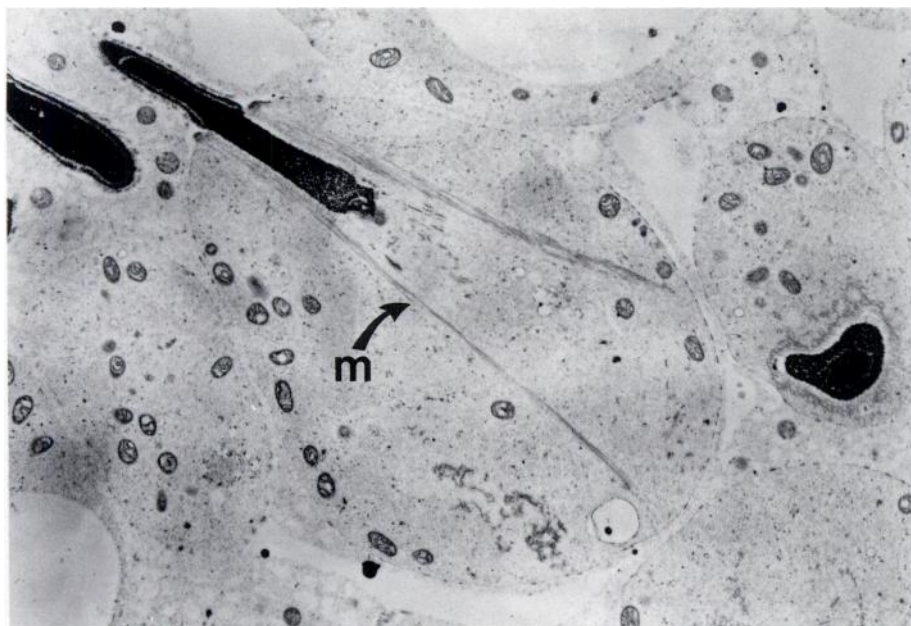


FIG. 4. An electron micrograph of a mature spermatid in oligotriche mouse testes. The condensation of the nucleus and acrosome formation appear to occur normally, although the tail is missing completely. The manchette (m) is apparent. Original magnification $\times 5,000$.

accentuated by the decreased number of homogenization-resistant spermatozoa in the quaking mouse epididymis: $4 \pm 7 \times 10^4/\text{mg}$ (*qk/qk*) and $640 \pm 3 \times 10^4/\text{mg}$ (*?/+*); $n = 7$ to 8 ; $P < 0.001$). The decrease in homogenization-resistant testicular spermatids was confirmed by counts of spermatids at specific steps of spermiogenesis (Fig 7). Although spermatid numbers were decreased in quaking mice, a normal complement of prespermatid germ cells was present in mutant mouse tubules (Table 2).

In addition to decreased spermatid numbers, quaking mouse testes had clear tubule lumens (Fig 6), which is

indicative of abnormal sperm tail development. Evidence supporting this assumption resulted from the absent or abnormal tails of spermatozoa extruded from cauda epididymides of the mutant mice.

Discussion

Studies presented in this report provide new evidence that the oligotriche gene is required for normal spermiogenesis. If the mouse is homozygous for the oligotriche gene mutation, spermatocytogenesis and meiosis are normal but spermiogenesis is disrupted. Spermatid numbers drop precipitously after step 13 of spermiogenesis, and sperm tail formation is not initiated. None of the other gene mutations known to interfere with mouse spermiogenesis causes a complete absence of the sperm tail (see Chubb, 1992). For example, quaking mouse spermatids develop a tail that starts to become disorganized after step 9 (Bennett et al, 1971).

The actions of the oligotriche and quaking gene mutations on reproduction are expressed only in one aspect of gametogenesis: spermiogenesis. This conclusion is supported by data presented in this report. Also, female mice homozygous for either gene mutation are fertile (Moutier, 1976; Green, 1989), supporting the specificity of the gene mutation action for spermiogenesis.

Although the oligotriche gene mutation resembles the quaking gene mutation in the fact that they both disrupt sperm tail formation, their mechanisms of action must differ. For example, the quaking gene mutation decreased spermatid numbers starting with step 1 of spermiogenesis. Bennett and coworkers (1971) reported a similar reduction in steps 1 through 8 spermatids in quaking mouse testes but

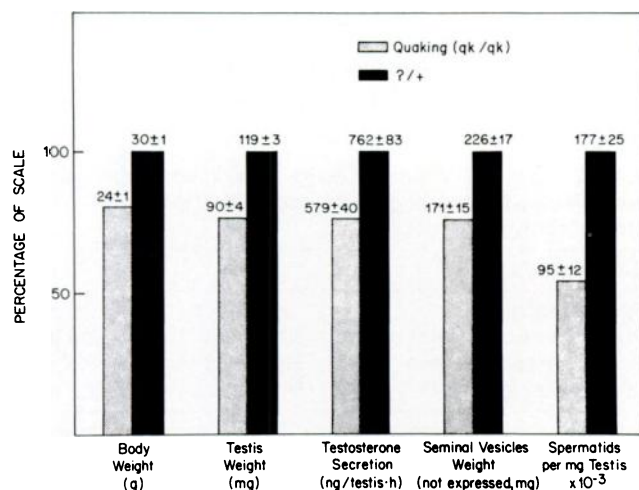


FIG. 5. Data summary for quaking and normal sibling (*?/+*) mice. Data for mutant mice are displayed as percentages of normal mouse values. Absolute values are presented above the columns ($n = 6-8$). All parameters for mutant and normal mice were significantly different ($P < 0.001$).

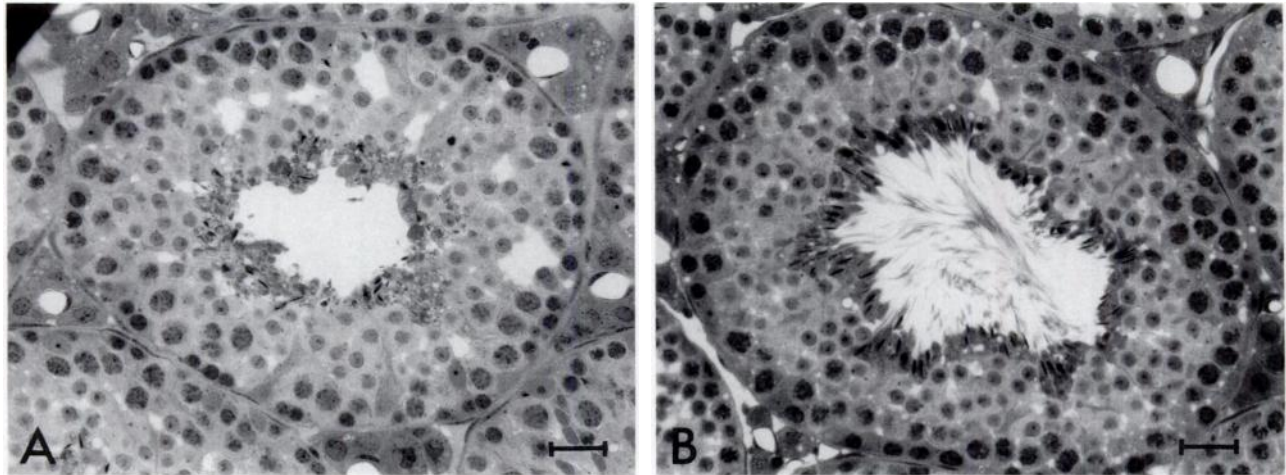


FIG. 6. Photomicrographs of cross-sections of seminiferous tubules from (A) quaking (qk/qk) and (B) normal sibling ($?/+$) mice. The absence of sperm tails in the lumen is a prominent characteristic of quaking mouse testes. Bar = 30 μ m.

did not enumerate steps 9 through 16 spermatids. Spermatid numbers in oligotriche mice were not altered until after step 13. Also, sperm tail development was not initiated in oligotriche mice, whereas quaking mouse spermiogenesis has been demonstrated to have normal sperm tail development in the early steps (Bennett et al, 1971). The steroidogenic potential of oligotriche and quaking mouse testes was not impaired, as evidenced by biochemical, physiologic, and microanatomic evidence of normal Leydig cell function. This information suggests that a decrease in steroidogenesis was not a factor in the defective spermiogenesis.

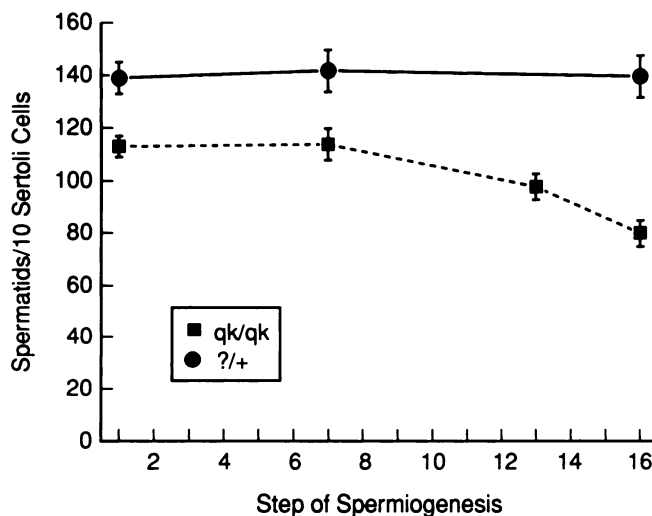


FIG. 7. Spermatid numbers at different steps of spermiogenesis in quaking (qk/qk) and normal ($?/+$) mice. Crude cell counts were used in the construction of this graph due to the nonspherical nuclei of steps 8 through 16 spermatids. Spermatids at each step of spermiogenesis were counted in 25 tubules from 3 mice of each genotype. The number of spermatids in quaking mouse testes was significantly decreased starting at step 1 ($P < 0.005$).

Although the normal mice in our study were either heterozygous or homozygous for the wild-type gene, the low variance in germ cell count suggests that mice heterozygous for either gene mutation have normal spermatogenesis. This conclusion agrees with previous reports that indicated male mice heterozygous for the oligotriche or quaking gene mutations are unaffected (Moutier, 1976; Green, 1989). These results would not be expected if spermatids carrying the mutated gene in heterozygotes were affected. Spermatids bearing the mutated genes could receive the products of normal oligotriche and quaking genes from unaffected spermatids in heterozygotes. Spermatids have the potential to share gene products via cytoplasmic bridges (Braun et al, 1989). Alternatively, the gene mutations could be altering the function of Sertoli cells, which would be detrimental in homozygotes only.

Both oligotriche and quaking gene mutations have pleiotropic actions. For example, oligotriche mice have thinning

Table 2. Germ cell counts at Stage VII of spermatogenesis in seminiferous tubules of quaking and normal sibling mouse testes*

Germ cell class	qk/qk	$?/+$
Spermatogonium A	2.2 \pm 0.3 \dagger	2.0 \pm 0.2
Preleptotene spermatocyte	30.0 \pm 2.4	32.8 \pm 2.3
Pachytene spermatocyte	32.9 \pm 1.8	35.5 \pm 2.2
Step 7 spermatid	84.1 \pm 4.7 \ddagger	107.0 \pm 5.8

qk/qk = quaking mouse testes; $?/+$ = normal sibling mouse testes.

* Cell counts were corrected with Abercrombie's formula and expressed as per 10 Sertoli cells (Sertoli cell numbers per tubule were 1.7 \pm 0.2 [qk/qk] and 1.9 \pm 0.2 [$?/+$]). Step 16 spermatid counts could not be corrected because of the nonspherical shape of their nuclei and were not included in this table.

\dagger Mean \pm SE of counts in 25 tubules from 3 mice of each genotype.

\ddagger Significantly different from control value ($P < 0.005$).

of the ventral coat (Moutier, 1976), and quaking mice have myelination deficiencies (Green, 1989). These actions in other cell types could be helpful in determining common pathways affected by the gene mutations.

The oligotriche and quaking gene mutations have not been studied at the molecular level. The type of mutation (deletion, point, etc.) and number of gene loci encompassed are unknown. Insight into the importance of this information was provided by Bode (1984). He induced a mutation (qk^k) that was allelic to the quaking gene but resulted in defective myelination without a concomitant effect on male reproduction. This evidence suggests that the spontaneous quaking gene mutation (qk) may encompass two genes, one affecting spermiogenesis and the other involved with myelination.

In summary, the current studies provide new information that elucidates the effects of oligotriche and quaking gene mutations on spermiogenesis. The total absence of sperm tails in oligotriche mice supports the conclusion that the oligotriche gene mutation may allow valuable insights about the factors regulating spermiogenesis.

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