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Reproductive and neurological *Quaking^{viable}* phenotypes in a severe combined immune deficient mouse background

Tammy A. Tucker¹, Jean A. Kundert², Alla A. Bondareva^{1,3}, and Edward E. Schmidt^{1,✉}

¹ *Veterinary Molecular Biology, Montana State University, Molecular Biosciences Building, 960 Technology Boulevard, Bozeman, MT, 59718, USA*

² *Animal Resource Center, Montana State University, Bozeman, MT, 59717, USA*

³ *Department of Biochemistry and Molecular Biology, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, T2N4N1, Canada*

Abstract

The *quaking^{viable}* (*qk^v*) mutation, a spontaneous deletion of a multigenic region encompassing roughly 1 Mb at 5.9 cM on the proximal end of mouse chromosome 17, causes severe trembling in all homozygous animals and infertility in all homozygous males. Physiologically, quaking mice exhibit dysmyelination and postmeiotic spermatogenic arrest. Molecular defects in *Qk^v* mice occur in the affected tissues, indicating the primary causes of these pathologies are cell autonomous. However, because both the reproductive and neurological defects are in immune-privileged sites and because some similar pathologies at both sites have been shown to be immune mediated, we tested whether the immune system participates secondarily in manifestation of *Qk^v* phenotypes. The *qk^v* mutation was bred into a severe combined immune-deficient mouse line (SCID; devoid of mature B and T cells) and penetrance of the neurological and the male sterile phenotypes was measured. Results showed that neither defect was ameliorated in the immune-deficient background. We conclude that the *Qk^v* pathologies do not likely involve a B- or T-cell-dependent response against these immune-privileged sites.

Keywords

Dysmyelination; Immune-privileged; Male sterile; Neurodegenerative; Spermatogenesis

Introduction

Quaking^{viable} (*Qk^v*) mice exhibit two severe recessive traits. First, all homozygous animals show a trembling phenotype that becomes detectable roughly 10 days after birth, increases in severity until 3 weeks of age, and persists for the life of the animal (Sidman et al. 1964). Trembling correlates with an axonal myelin deficiency in the central nervous system (CNS) (Friedrich 1974; Sidman et al. 1964). Second, adult homozygous males are nearly aspermic. Histological analyses show normal premeiotic spermatogenic cell types as well as apparently normal haploid round spermatids; however, later stages are absent (Bennett et al. 1971). The few morphologically mature spermatozoa that can be isolated from young males are nonfunctional in in vitro fertilization (Bennett et al. 1971); however, they can produce zygotes that mature into adult mice when used for fertilization via intracytoplasmic sperm injection (Yanagimachi et al. 2004).

The *qk^v* mutation is a deletion of ~1 Mb on chromosome 17 that has been shown to entirely eliminate the mouse *parkin* and *pacrg* (*parkin-coregulated*) genes (Lorenzetti et al. 2004a,b) and to disrupt regulation of the nearby *quaking* (*qk*) gene (Ebersole et al. 1996; Hardy 1998; Hardy et al. 1996; Lorenzetti et al. 2004a). Null mutations in the human *parkin* gene cause loss of dopaminergic neurons in autosomal juvenile Parkinson's disease (Kitada et al. 1998); however, simple *parkin*-null mice exhibit less severe neurological defects and are not deficient in dopaminergic neurons (Goldberg et al. 2003). No role for the loss of the *parkin* gene has yet been ascribed to the *Qk^v* mouse phenotype. Indeed, despite similarities between the trembling behavior in *Qk^v* mice and in human Parkinson's disease, each condition exhibits distinct neurophysiological characteristics (Kitada et al. 1998; Lorenzetti et al. 2004a). The trembling phenotype in *Qk^v* mice appears to result not from the deletion of *parkin* (Lorenzetti et al. 2004a,b), but rather, from misregulation of *qk* (Ebersole et al. 1996; Hardy 1998; Hardy et al. 1996; Lu et al. 2003; Wu et al. 2002). This neurological effect has been partially phenocopied in chemically mutagenized mice having *qk* disruptions (Cox et al. 1999). Conversely, transgenic studies show that spermiogenesis and male fertility can be restored to *Qk^v* mice by a *pacrg* cDNA transgene, strongly implicating *pacrg* in the spermiogenic defect (Lorenzetti et al. 2004b).

The *qk* gene encodes a family of at least five different proteins, the QKI proteins, as a result of alternative pre-mRNA splicing (Kondo et al. 1999). All QKI proteins share an identical 311-amino-acid core including an RNA-binding domain, but differ at their C-termini (8 to 30 amino acids) (Kondo et al. 1999). QKI isoforms show different cell-type-specific expression and different subcellular localization (Hardy et al. 1996; Lu et al. 2003; Wu et al. 1999). Based on domains conserved with other proteins and on functional studies, the QKI proteins have been proposed to act as RNA-binding proteins, regulators of alternative splicing, signal transducers, or inducers of apoptosis (Ebersole et al. 1996; Hardy 1998; Hardy et al. 1996; Pilotte et al. 2001; Wu et al. 1999, 2002).

The *qk^v* mutation affects regulation of splicing of the *qk* pre-mRNA, thereby preventing expression of two QKI protein isoforms (QKI-6 and QKI-7) in oligodendrocytes (Ebersole et al. 1996) and reducing expression of another isoform (QKI-5) in the brain (Wu et al. 1999). Correlative data link the phenotype in *Qk^v* mice to misexpression of myelin-associated glycoprotein (MAG) isoforms (Frail and Braun 1985), which results from incorrect splicing of the MAG pre-mRNA (Fujita et al. 1990). Cotransfection studies show that overexpression of QKI-5 can disrupt splicing of a MAG minigene pre-mRNA in cell culture (Wu et al. 2002). Thus, it is likely that splicing-dependent misregulation of relative QKI protein levels results in splicing-dependent misregulation of MAG protein isoform accumulation in *Qk^v* mice (Wu et al. 2002).

Both the postmeiotic spermatogenic compartment of the testis and the myelinated compartment of the CNS are immune-privileged tissues (Ferguson et al. 2002; Hedger 2002; Martin et al. 1992). Antigens from either of these compartments can, upon disruption of their privileged environment or status, cause either severe or chronic auto-immune reactions leading to pathologies (Mancardi et al. 2000; Tung et al. 1989; Yule and Tung 1993). In the case of *Qk^v* mice, molecular and genetic data strongly suggest that the primary defects that cause dysmyelination and that cause aspermia are manifested in the pathology-affected tissues (CNS and testis, respectively, see above). However, it is untested whether these defects are pathological on their own or whether a secondary immune reaction to the primary molecular defect is involved in the pathology. As an indication of whether the *Qk^v* phenotypes likely involved a secondary immune component, we tested whether either defect could be ameliorated in a severely immune-compromised background. We produced mice with the *qk^v* mutation in a homozygous *scid*-null background (devoid of mature B and T cells; Bosma and Carroll 1991) and showed that neither of the quaking pathologies was reduced in these mice.

Materials and methods

Animals

B6.Cg-*T^{2J}*+/+ *Qk* (*Qk^v*), C57Bl/6J, NOD.CB17-*Prkdc^{scid}*/J (SCID), and BALB/cJ mouse lines were purchased from Jackson Laboratories and were maintained in our colony. The *qk^v* mutation is on chromosome 17 and the *scid* mutation is on chromosome 16 (Blunt et al. 1996). Outbred CD1 mice were purchased from Charles River Laboratories and were maintained in our colony. For marker confirmation, we used mice bearing the *thp^{ΔN}*-targeted mutation on chromosome 17, on a C57Bl/6J background (more than eight generations backcrossed) (Hobbs et al. 2002), which are maintained in our colony. For replacement fostering, timed matings between wild-type CD1 mice were synchronized with matings involving trembling *Qk^v* females. At birth, pups were taken from the *Qk^v* dams and were mixed into the litters of newborn CD1 mice (when necessary, CD1 pups were culled to keep litter sizes ≤11 total pups), and CD1 surrogates were allowed to rear the pups to weaning.

All animals were housed under aseptic specialized-care conditions in HEPA-filtered forced air cages (Techniplast) and were provided with sterilized diet and water. Protocols used in this study were approved by the Montana State University Institutional Animal Care and Use Committee.

Genotype, marker, and statistical analyses

Genomic DNA or total RNA was harvested by standard procedures from mouse tail tips (~0.5 cm) at weaning (~3 weeks of age) for survival genotypic analyses, or from kidneys at sacrifice (Schmidt and Schibler 1995). The *scid* mutation was followed by direct sequencing (Fig. 1a) as described previously (Sealey et al. 2002). The proximal arm of chromosome 17 was followed using haplotype-specific markers in the region. The primary marker was an expressed tag for the major histocompatibility class I (MHC-I) *H2-D1* gene at 19.09 cM on chromosome 17. *H2-D1* is expressed in all nucleated cells (Margulies 1999; Pamer and Cresswell 1998), which allowed us to develop a reliable and sensitive haplotype-specific RNase protection assay for this marker using a riboprobe transcribed from a 189-bp *H2-D1^b* (haplotype b) cDNA fragment that we amplified by RT-PCR from C57Bl/6 mice. The primers for RT-PCR-based cloning were sense primer, 5'-TATGAATTCTCGAGGAGCCCC GGTACATCTC-3' and antisense primer, 5'-TATGGATCCT CAGGCTCACTCGGAACCACT-3'. This primer set amplifies a broad range of MHC-I cDNAs; *H2-D1^b* was identified from a library of clones by sequence analysis. Sequence polymorphisms between *H2-D1^b* and *H2-D1^d* provided an RNA:RNA hybrid that was haplotype-specifically cleaved by RNase, yielding haplotype-specific band sizes in RNase protection experiments (Fig. 1b), performed as described previously (Schmidt et al. 2003). This assay distinguished C57Bl/6 and either BALB/c or CB.17 *H2-D1* mRNAs (Fig. 1b and unpublished data), and could be performed either on RNA harvested from mice at sacrifice or on RNA isolated from tail snips at weaning (survival genotyping). The marker was confirmed by co-inheritance with molecular markers on either side of *qk^v*, namely D17mit164 at position 4.1 cM on chromosome 17 (Copeland et al. 1993) (Fig. 1c) and an engineered mutation in the *TATA-binding protein* gene, *thp*, at 8.25 cM on chromosome 17 (Hobbs et al. 2002) (Fig. 1d and data not shown). Genetic data were analyzed using the Student's *t* test to compare observed data to predicted Mendelian distributions of genotypes or phenotypes, or to compare control and experimental data sets, as described in table legends.

Results and discussion

Events that compromise the integrity of immune-privileged sites including the CNS and the postmeiotic testis can result in autoimmune-mediated pathologies. For example, injury to the testis, either from trauma or infectious disease, can lead to sterility via autoimmune-mediated

clearance of post-meiotic germ cells (Ferguson et al. 2002; Sakamoto et al. 1995; Suominen 1995). Also, quaking-like neurological disorders have been induced in rodents by immunizing animals with myelin basic protein (Mancardi et al. 2000; Martin et al. 1992; Swanborg 2001), by induction with the proinflammatory cytokine interferon γ (Corbin et al. 1996), or by transgenic misexpression of MHC-I antigens in oligodendrocytes (Yoshioka et al. 1991). The pathology of some neurodegenerative disorders, including Down's syndrome, Huntington's, Pick's, Parkinson's, and Alzheimer's diseases, multiple sclerosis, and the AIDS-dementia complex, are thought to involve secondary autoimmune reactions against neuronal tissues bearing a primary defect that invokes the response (Martin et al. 1992; Wersinger and Sidhu 2002). More importantly, although in many of these pathologies the primary defect is not in an immune-related process, the immune system participates in manifestation of the pathology. As a result, many of these pathologies can be abrogated with anti-inflammatory treatments or immunodepletion (Martin et al. 1992; Swanborg 2001). In the current study, we examined whether the immune system played a role in the phenotypic manifestation of Qk^v mice by testing whether the *scid* mutation could abrogate or ameliorate the Qk^v pathologies.

To produce the F1 hybrid generation, trembling (homozygous) adult B6.Cg- $T^{2J}+/+Qk$ dams (" qk^v/qk^v ," C57Bl/6J background, haplotype b) were mated with either homozygous NOD.CB17-*Prkdc*^{*scid*}/J ("*scid/scid*") sires or with wild-type BALB/cJ sires (both haplotype d). As such, the only source of chromosome 17 bearing a haplotype b MHC-I entering this experiment carried the qk^v mutation; the only source of chromosome 17 bearing a haplotype d MHC-I was wild type ("+") at the qk locus; and all F1 pups were $qk^v/+;scid/+$ for the experimental group, or $qk^v/+;+/+$ for the controls. Because the severe trembling phenotype of the qk^v/qk^v dams can interfere with their ability to nurse and care for pups, F1 litters were replacement-fostered into the litters of outbred CD1 mice that had just given birth. The agouti coat color of the F1 $qk^v/+;scid/+$ or $qk^v/+;+/+$ hybrid pups was used to distinguish them from the surrogate's biological offspring (white) at weaning. Because all parents in all the experimental and control matings were purely inbred, F1 animals retained 100% linkage between the proximal chromosome 17 markers we used and the qk^v mutation.

F1 hybrid sibs were mated and F2 pups were reared to sexual maturity (8 weeks), at which time animals were scored for trembling (Table 1). Mature trembling males were each housed with two mature wild-type females for 3 weeks and were then harvested. In the experimental double-heterozygous F1 $\times$ F1 mating, we expected 1/16th of the progeny to be homozygous mutant at both loci ($qk^v/qk^v;scid/scid$). In the experimental and the control groups, similar penetrance of the trembling phenotype was observed (Table 1). The *scid* alleles showed a Mendelian distribution, including two trembling males that were *scid/scid* (Table 1). Wild-type females housed with these trembling males did not become pregnant and testes from all trembling F2 males, including the two that were *scid/scid*, showed an absence of elongated spermatids and spermatozoa at sacrifice, indicating spermiogenic arrest had occurred (Table 1).

Because the F2 generation only provided three trembling animals on an *scid/scid* background, we further tested penetrance of the Qk^v phenotypes on an *scid/scid* background by sib matings using selected F2 or later progeny (Table 2). These crosses provided 28 trembling females, of which 19 were *scid/scid*, and 25 trembling males, of which 16 were *scid/scid*. All trembling males, including the 16 that were *scid/scid*, were infertile (Table 2). Results confirmed that the trembling phenotype and the spermiogenic arrest phenotype were manifested in severely immune-compromised animals, which indicated that mature B and T cells do not participate in the pathology underlying either phenotype.

Previous studies have shown that the molecular defects in Qk^v mice are manifested in the cells in which the pathological states underlying the phenotypes occur (Ebersole et al. 1996; Hardy

1998; Hardy et al. 1996; Lorenzetti et al. 2004a,b). However, it was untested whether a secondary autoimmune response triggered by these defects might play a role in the pathologies. Our results indicate that B and T cells do not likely participate in the Qk^v phenotypes. Thus, whereas Qk^v mice provide a useful model for understanding the developmental processes underlying myelination and spermiogenesis, as well as for studying some genetic diseases that disrupt these developmental processes, they are unlikely to provide an accurate model for studying pathologically similar immune-mediated disorders.

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Fig. 1.

Molecular marker analyses for *scid* and for the proximal end of chromosome 17. **a** Direct sequence-based analysis of *scid* locus on chromosome 16. Genomic DNA isolated from mouse tail snips at weaning were amplified by PCR and the PCR products were sequenced using the primer set and methods described in Sealey et al. (2002). Genotypes were read directly from the chromatograph scans to ensure accurate detection of heterozygotes. The arrows indicate the site of the *scid*⁻ point mutation; genotypes are indicated below. **b** Analysis of haplotype of expressed *H2-D1* alleles in F1 hybrid mice. Kidney total RNA samples (5 μg) from five experimental (samples 1–5) and three control (samples 6–8) mice were supplemented with yeast RNA to 50 μg, hybridized to 5 fmol of radiolabeled *H2-D1*^b probe, and digested with RNase A and RNase T1, as described previously (Schmidt and Schibler 1995). Samples were then resolved on denaturing polyacrylamide sequencing gels and protected fragments visualized by autoradiography. Control lanes contained either 0.05 fmol of undigested probe (lane P) or 5 fmol probe hybridized to 50 μg yeast RNA (lane Y, negative control). The positions of protected fragments from haplotype b and haplotype d mice are indicated at right, as is the position of a universal protected probe fragment that arises from hybridization to MHC-I transcripts. The determined haplotype of the MHC locus of each mouse is indicated at bottom. **c** Identification of the D17Mit164 marker at 4.1 cM on chromosome 17. PCR primers for D17Mit164 were described elsewhere (Copeland et al. 1993) and their sequences were obtained on the Jackson Laboratory genomics resources web page (<http://www.informatics.jax.org/>). **d** Identification of the *thp*^{ΔN} marker at 8.25 cM on chromosome 17 used the PCR primers and conditions described previously (Hobbs et al. 2002).

Table 1

F2 penetrance of Qk^v phenotypes is unaffected by the *scid* allele

Group ^a	Parental genotypes ^b		F1 genotype (strain)	F2 generation	
	Paternal (strain)	Maternal (strain)		All F1 progeny	n
Experimental	$q^{+/+};s^{-/-}$ (CB.17)	$q^{-/-};s^{+/+}$ (C57Bl/6)	$q^{+/-};s^{+/-}$ (hybrid) $q^{+/-};s^{+/-}$ (hybrid)		94
Control	$q^{+/+};s^{+/+}$ (BALB/c)	$q^{-/-};s^{+/+}$ (C57Bl/6)			110
					Trembling ^c (%) <i>d</i>
					17 (18.1) 18 (16.4)

^aExperimental group, homozygous NOD.CB17-Prkdc^{scid}/J founder males×homozygous trembling B6.Cg-*T2J*+/+ *Qk* founder females. Control group, wild-type BALB/cJ founder males×homozygous trembling B6.Cg-*T2J*+/+ *Qk* founder females. Because trembling dams are poor at nurturing young, experimental group F1 progeny were replacement-fostered onto CD1 surrogates at birth, as described in Materials and methods

^b q , qk^v allele; s , *scid* allele; +, wild-type version of allele; −, mutant version of allele

^cTrembling animals in the experimental group were genotyped for the *scid* allele by direct sequencing (see Materials and methods). Results showed an *scid* genotype ratio of 5:9:3 ($s^{+/-};s^{+/-};s^{-/-}$; control matings did not harbor an s^{-} allele and were not genotyped), which did not differ significantly from the predicted Mendelian ratio of 1:2:1 ($P>0.25$). Two trembling males and one trembling female were $s^{-/-}$. Trembling males were assessed for fertility and spermiogenic progression after 8 weeks of age, as described in Materials and methods. Results showed that all trembling males, including the two that were $s^{-/-}$, had arrested spermiogenesis (no elongated spermatids or spermatozoa) and were infertile

^dThe number of trembling animals was significantly less than the predicted F2 Mendelian value of 25% (experimental and control groups combined, $N=204$, 17.2% tr, $P<0.01$), suggesting that $q^{-/-}$ mice have a reduced likelihood of surviving to weaning. This was confirmed on a genotypic level using the chromosome 17 molecular markers described in Materials and methods (data not shown). More importantly, the percent trembling did not differ significantly between the experimental and control groups ($P>0.25$), indicating that the *scid* allele did not diminish phenotypic manifestation of the q^{-} allele

Table 2

Qk^v phenotypes on an SCID background

Mating set	Paternal genotype	Maternal genotype ^a	n	tr (<i>s</i> ^{-/-} ; <i>s</i> ^{+/-}) ^b	Calm	Mendelian tr:calm ^c	tr males (sterile) ^d
1	<i>q</i> ^{+/-} ; <i>s</i> ^{-/-}	<i>q</i> ^{-/-} ; <i>s</i> ^{-/-}	6	3 (3:0)	3	1:1	2 (2)
2	<i>q</i> ^{+/-} ; <i>s</i> ^{-/-}	<i>q</i> ^{+/-} ; <i>s</i> ^{-/-}	47	11 (11:0)	36	1:3 ^e	4 (4)
3	<i>q</i> ^{+/-} ; <i>s</i> ^{-/-}	<i>q</i> ^{-/-} ; <i>s</i> ^{+/-}	82	39 (21:18)	43	1:1 ^e	19 (19) ^f

tr trembling phenotype, *calm* normal nontrembling behavior; other abbreviations as in Table 1

^aProgeny from the first and third mating sets were replacement-fostered onto CD1 surrogates at birth, as described in Materials and methods

^b*scid* genotype of trembling progeny. Top two sets had only mutant *scid* alleles, as verified by direct sequencing on breeders. Third mating set had a maternal wild-type *scid* allele, giving an expected progeny ratio for *s*^{-/-}; *s*^{+/-} of 1:1

^cPredicted phenotypes based on Mendelian inheritance of a recessive trait. First and third mating sets used homozygous trembling (*q*^{-/-}) dams; second set used heterozygous nontrembling (*q*^{+/-}) dams. All sires were heterozygous nontrembling at the *qk^v* locus (*q*^{+/-}) and homozygous mutant at the *scid* locus (*s*^{-/-})

^dTrembling males were assessed for fertility and spermiogenic progression after 8 weeks of age as described in Materials and methods. Infertility and spermiogenic arrest showed 100% penetrance

^ePredicted Mendelian phenotype ratio does not differ significantly from observed tr:calm ratio, *P*>0.25

^f*scid* genotypes of trembling males in mating set 3, ten *s*^{-/-}; nine *s*^{+/-}