Loss of p53 in quaking viable mice leads to Purkinje cell defects and reduced survival

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The qkv mutation is a one megabase deletion resulting in abnormal expression of the qkl gene. qkv mice exhibit hypomyelination of the central nervous system and display rapid tremors and seizures as adults. The qkI locus on 6q26-27 has also been implicated as a candidate tumor suppressor gene as the qkI locus maps to a region of genetic instability in Glioblastoma Multiforme (GBM), an aggressive brain tumor of astrocytic lineage. As GBM frequently harbors mutations affecting p53, we crossbred qkv and p53 mutant mice to examine whether qkv mice on a p53−/− background have an increased incidence of GBM. qkv−/−; p53−/− mice had a reduced survival rate compared to p53+/− littermates, and the cause of death of the majority of the mice remains unknown. In addition, immunohistochemistry revealed Purkinje cell degeneration in the cerebellum. These results suggest that p53 and qkl are genetically linked for neuronal maintenance and survival.

The study of neurological diseases has been greatly advanced with the use of mouse models. In particular, spontaneously occurring mutant mice have played an important role in identifying key components required for proper myelination. One such mouse, the autosomal recessive qkv mutant, was first described in 1964 by Sidman and colleagues. The qkv−/− mouse exhibited severe hypomyelination of the central and peripheral nervous system and severe hindlimb shaking. The mutation responsible for the qkv−/− phenotype was later identified as a one megabase deletion that results in loss of parkin (park2) and parkin co-regulated (pacrg) function, whereas qkl expression becomes abnormally expressed. In qkv−/− mutants, qkI-6/7 expression is preferentially lost in myelin producing cells of the central and peripheral nervous systems, while for the most part expression remains unaffected in all other cell types. The qkl gene encodes for the alternatively spliced KH domain RNA binding proteins QKI-5, -6, and -7. QKI has been shown to regulate important mRNA targets involved in myelination and oligodendrocyte differentiation. QKI is implicated in myelinogenesis by stabilizing myelin basic protein (MBP) mRNA, regulating the alternative splicing pattern of myelin associated glycoprotein (MAG), and oligodendrocyte differentiation and Schwann cell differentiation. The overexpression of QKI-6 in qkv−/− mutants has been shown to rescue the myelination defect.

In addition to myelination defects, qkv−/− mice also exhibit ciliopathies including male sterility and mild hydrocephalus. Cilia lining the ventricles of the brain are responsible for proper circulation of cerebral spinal fluid (CSF). Ciliogenesis is normal in qkv−/− mice, however the cilia are functionally impaired leading to decreased CSF flow and hydrocephalus. Hydrocephalus in qkv−/− mice becomes fatal on a patched1 heterozygous background. The long arm of chromosome 6 is known to harbor a common fragile site that is frequently lost or mutated in a variety of cancers. The breakpoint includes several genes, one of which has been identified as the qkl gene. Chromosomal aberrations at 6q26–27 are common in Glioblastoma Multiforme (GBM). In addition to mapping to a region of genomic instability, expression of qkl mRNA transcripts have been shown to be altered specifically in human gliomas compared to other brain cancers. Thus, it has been proposed that QKI may function as a tumor suppressor and play a role in GBM progression. Evidence for a suppressor role was shown for the C. elegans GLD-1, a QKI homolog. The GLD-1 C. elegans homolog of QKI is known to associate with the p53 cep-1 mRNA, influencing its activity. In addition, QKI was discovered to be down-regulated in colorectal cancers. QKI over-expression in the colon epithelium resulted in increased levels of p27kip1 as well as an increase in membrane bound β-catenin, an indicator of gastric cell differentiation. These observations along with our data
that QKI-6/-7 cause cell cycle arrest, a property often observed for tumor suppressors, suggest that the absence of QKI proteins may promote tumorigenesis.

Loss of heterozygosity and mutations of the p53 gene is commonly associated with a variety of cancers in multiple organ sites. Individuals with the Li Fraumeni syndrome, in part caused by mutations in the p53 gene, suffer from early onset of many different types of cancer. Mutations abrogating p53 function and allelic loss of chromosome 17p were among the first genetic lesions identified in GBM. p53 losses are present in all grades of astrocytoma at an average of 30%, suggesting that the inactivation of p53 is an early event in gliomagenesis. p53-/- and p53-/- mice have been found to develop a broad spectrum of tumors, including lymphomas, osteosarcomas, and fibrosarcomas. These results suggest that the absence of QKI proteins may promote tumorigenesis.

Here we report that qkv mice on a p53-/- background had a reduced survival rate compared to qkv-/-; p53-/- and p53-/- controls, and the cause of death of the majority of the mice remains unknown. qkv-/-; p53-/- mice also displayed neurological defects including Purkinje neuron degeneration.

**Results**

The qkv-/- mice contain a recessive autosomal mutation of ~1.1 Mb on chromosome 17 affecting the expression of quaking (qkl), parkincoregulated gene (paercg) and parkin (park2). The mutation results in aberrant expression of alternatively spliced qkl transcripts, leading to the dysmyelination defects observed in the qkv mice. Deletion of the paercg gene in qk mice causes ciliopathies including hydrocephalus and male sterility. To examine whether the p53 pathway genetically interacts with the paercg-parkin-qkl locus in the regulation of the phenotypes of the qkv mice, we crossed the qkv mice with p53 null mice. We initially assessed the myelin sheath of resulting qkv-/-; p53-/- progeny. Post-natal day 30 (P30) coronal sections of the corpus callosum were stained with an antibody specific to myelin basic protein (MBP). As expected, qkv-/- mice showed reduced thickness of the corpus callosum (98.8 ± 16.4 μm) compared to wild-type (wt) (172.7 ± 7.03 μm) and qkv+/-; p53-/- (191.8 ± 5.99 μm) littersmates (Figure 1). However, qkv+/-; p53-/- mice did not show an increased severity of hypomyelination compared to qkv-/- mice (86.9 ± 8.89 μm, Figure 1), and corpus callosum thickness was not found to be statistically different between the mice (p = 0.287). Although corpus callosum thickness was reduced in mice with the qkl mutation, sparse high intensity MBP staining was observed along the myelin tracts. These MBP-positive “blebs” have previously been observed in spinal cord white matter of qkv-/- mice, corresponding to redundant loops of uncompacted myelin. These results suggest that p53 does not cooperate with qkl in the regulation of myelin formation in vivo. The loss of both wt p53 alleles did not affect the onset of hindlimb shaking in mice pups, since both qkv-/-; p53-/- mice, qkv-/-; p53-/- mice and qkv-/- mice displayed tremors by P14 (data not shown). Common husbandry procedures and animal handling are sources of acute stress and elicit tonic clonic seizures in qkl mice. The onset of stress induced tonic-clonic seizures in qkv-/-; p53-/- mice was significantly earlier than that of qkv-/- mice. qkv-/- mice began demonstrating stress-induced seizures at 12 weeks of age, whereas qkv-/-; p53-/- mice and qkv-/-; p53-/- mice were observed to have seizures and ataxic movements as early as 4 weeks of age (data not shown). As expected, mice heterozygous for the qkl mutation did not demonstrate tonic-clonic seizures or hindlimb shaking.

We assessed the cellular morphology of Purkinje cells by immunostaining P30 coronal brain sections with an antibody against Calbindin, a Purkinje neuron-specific marker. Immunostaining of the brains of qkv-/-; p53-/- mice revealed Purkinje cell body loss as well as loss of Calbindin-positive Purkinje cell dendritic arbors, whereas control mice demonstrated normal Purkinje cell appearance.

**Figure 1** | qkv-associated hypomyelination is not exacerbated on a p53-/- background. Brains were frozen over acetone-dry ice and cryostat sectioned at a thickness of 10 μm. Coronal sections of age and sex-matched mouse cortices were stained with anti-MBP antibody. Scale bar represents 100 μm.

**Figure 2** | qkv-/-; p53-/- mice display Purkinje cell defects. (A) qkv-/-; p53-/- mice display Purkinje cell body and dendritic arbor loss at P30 (arrows), whereas control littersmates exhibit normal cerebellar architecture. Sections were stained with anti-Calbindin antibody. Scale bar represents 50 μm. (B) qkv-/-; p53-/- show normal Purkinje cell morphology of the cerebellum at P14. Coronal sections of the cerebellum were stained with anti-Calbindin antibody. Scale bar represents 50 μm.
In order to quantitatively assess differences in Purkinje cell numbers, we analyzed the Purkinje cell linear density of P30 mice. *qkv*/*v*; *p53*/*v* mice were found to have a significantly lower Purkinje cell density (16.36 ± 1.42 cells/mm) compared with *wt* (38.77 ± 2.52 cells/mm; *p* < 0.001), *qkv*/*v*; *p53*/*v* (42.45 ± 6.5 cells/mm; *p* = 0.006), and *qkv*/*v* controls (40.16 ± 7.9 cells/mm; *p* = 0.01). Misplaced Purkinje neurons have occasionally been observed in *qkv*/*v* mice. To determine if the Purkinje defects observed were due to impaired migration or other developmental defects, immunostaining was performed on brains of P14 mice. Purkinje cell morphology was normal in *qkv*/*v*; *p53*/*v* P14 mice, suggesting that Purkinje cell loss was not due to failure in neuronal migration at earlier stages of development (Figure 2B). These results suggest that the Purkinje cell defects observed in *qkv*/*v*; *p53*/*v* mice were not due to impaired development, but cell body and dendrite degeneration and defects in neuronal maintenance and survival. Indeed, *qkI* has been shown to play a role in apoptosis in 3T3 mouse fibroblasts, HeLa cells, and primary rat oligodendrocytes.

*p53*/*v* mice develop primarily sarcomas, with an incidence of 28% over 17 months. *p53*/*v* mice have a much accelerated rate of tumorigenesis, with the majority of mice succumbing to lymphomas by six months of age. *qkv* has been implicated as a candidate tumor suppressor gene as the *qkv* locus maps to a region of genetic instability in GBM, therefore we examined whether *qkv*/*v*; *p53*/*v* mice would display a higher incidence of brain tumors and thus may die earlier than mice deficient for *p53* alone. Kaplan-Meier curve analysis was performed in order to compare mouse survival rates (Figure 3A). In agreement with previous observations, none of the *qkv*/*v* mice cohort succumbed to tumor formation at the end of the 261 day observation period. Overall, *qkv*/*v*; *p53*/*v* mice were found to have a reduced survival time compared to the other groups, with a median survival time (MST) of 119 days. *qkv*/*v*; *p53*/*v* mice showed a significantly reduced survival time compared to *qkv*/*v*; *p53*/*v* mice (MST = 142 days; *p* = 0.0135) as well as *p53*/*v* mice (MST = 172 days; *p* = 0.0443) according to the log rank/Mantel Cox test. There was no significant difference between the survival rates of *qkv*/*v*; *p53*/*v* and *p53*/*v* mice according to the Mantel Cox test (*p* = 0.9175).

Previous studies have shown that the majority of *p53*/*v* mice develop tumors of the lymphatic system and soft tissue sarcomas, with only one instance of a brain tumor reported in a *p53*/*v* mouse. Similarly, the *p53*/*v* and *qkv*/*v*; *p53*/*v* mice we generated developed primarily sarcomas and lymphomas, with no occurrence

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![Figure 3](image-url)
of any brain malignancies (Table 1). In contrast, none of the qkv/v; p53−/− mice demonstrated any occurrence of sarcomas, lymphomas, or tumors previously described in p53−/− mice (Table 1). Several qkv/v; p53−/− and qkv/v; p53−/− mice displayed neurological symptoms characteristic of hydrocephalus. Brains examined from these mice showed enlarged lateral ventricles with an accumulation of CSF, similar to the hydrocephalic phenotype observed in qkv/v; pchtl1/v−/− mice5. The brain of one qkv/v; p53−/− mouse showed increased vascularization of the cerebellum and abnormal gross cerebellar architecture. Further histological analysis of cerebellar sections revealed granule cell layer invasion of the surrounding parenchyma, consistent with medulloblastoma (Figure 3B). Cytological analysis confirmed the presence of medulloblastoma, as tumor cell nuclei were densely packed and polygonal in shape. Tumor cells were also found to express markers of astrocytic (GFAP), neuronal (NFH) and vascular (CD31) lineage (Fig. 3B). Cells expressing markers of multiple lineages indicate cells of a stem cell precursor nature, consistent with primitive neuroectodermal tumors such as medulloblastoma. Medulloblastoma has not previously been observed to occur spontaneously in either p53−/− or qkv/v mice3,4,5. One study identified only one instance of a brain malignancy out of a cohort of p53−/− mice (n = 40), whereas another study did not observe any tumors of the nervous system in a cohort of p53−/− mice (n = 35) or p53−/− (n = 96) mice5,12,13. The one p53−/− brain tumor identified was an ependymoma, a tumor originating from the walls of the ventricular system5,13. p53 mutations contribute to approximately 10% of human medulloblastoma cases44; in addition, many mouse models of medulloblastoma have been developed by the combination of a specific tumor suppressor mutation and the loss of p53. Although qkv has been mapped to the common fragile site 6q25-26 in GBM, loss of 6q25-26 in medulloblastoma is rare44, suggesting that somatic qkl mutations in clinical medulloblastoma do not confer any specific tumorigenic advantage. QKI has been shown to be expressed in post-natal neural progenitor cells of the SVZ46, suggesting that QKI may also be expressed in other populations of multi-potent precursor cells including the rostral rhombic lip progenitor cells that give rise to granule neuronal precursor cells. It has not been determined if the qk mutation affects QKI expression in neural progenitor cells including granule neuronal precursor cells, therefore it remains a possibility that the loss of qkl-6/7 in the absence of p53 could provide sufficient oncogenic stress to induce transformation.

**Discussion**

Our study indicates that qkv/v; p53−/− mice do not have decreased corpus callosum thickness or MBP-staining compared to qkv/v littermate controls, suggesting that the qk-associated myelination defect is not further impaired by the loss of p53. These findings indicate that qkl does not cooperate with p53 in vivo to regulate the oligodendrocyte differentiation pathway. qkv/v; p53−/− mice displayed Purkinje-cell defects in the cerebellum characterized by dendritic arborization defects and cell body loss, suggesting that qkl is required for neuronal cell maintenance in the absence of p53. However, the exact mechanism by which this phenotype occurs has yet to be elucidated. Cerebellar defects have previously been documented in mice homozygous for the qk mutation. Three month old qkv/v mice displayed axonal swellings in both the Purkinje and granular cell layer, indicative of axonal injury47. Axonal swelling is characteristic of inflammatory lesions in Multiple Sclerosis and experimental autoimmune encephalitis, suggesting that axonal pathology is secondary to myelination defects48–49. While our work showed that the qk dysmyelinating phenotype was not exacerbated on a p53−/− background, Purkinje cell degeneration was only observed in qkv/v; p53−/− mice and not qk−/− controls, suggesting that the neuronal pathology was not due to myelination defects. Interestingly, qkv/v; p53−/− mice displayed stress-induced tonic-clonic seizures and ataxic movements at about one month of age that was not observed in their qk−/− counterparts. Disruption in normal cerebellar architecture is one cause of ataxia50, thus Purkinje cell degeneration may be related to the ataxic-like phenotype observed in qkv/v; p53−/− mice. Indeed, 29 QKI-interacting partners were identified in a protein interaction network generated for human inherited cerebellar ataxias4,5.

qkv/v; p53−/− mice failed to develop tumors characteristic of p53−/− mice, but demonstrated a reduced survival rate compared to both qkv/v; p53−/− and p53−/− mice. One case of medulloblastoma was documented in a cohort of qkv/v; p53−/− mice (n = 11), while 2 other mice demonstrated signs of hydrocephalus. Recently, we have shown that qk−/− mice on a pchtl1−/− background have a reduced survival rate due to the development of fatal hydrocephalus5. A mild hydrocephalic phenotype occurs in qk−/− mice due to the loss of PACRG expression in the ciliated ependymal cells lining the ventricular walls, leading to cilia dysfunction48. Similarly, qkv/v; pchtl1−/− mice showed abnormal cilia function, leading to accumulation of CSF in the ventricles and hydrocephalus.

The majority of p53−/− and qkv/v; p53−/− mice in our study succumbed to thymic lymphoma, demonstrating clear symptoms including difficulty breathing due to tumor-induced compression of the lungs, enlarged ribcages, and lethargy due to tumor burden. Necropsy of these mice revealed highly vascularized, enlarged thymic epithelia, enlarged thymic morphologies. In addition, necropsy of qkv/v; p53−/− mice demonstrated signs of hydrocephalus, including ataxic-like phenotype observed in qkv/v; p53−/− mice. The qk−/− mutation results in brainstem defects in the absence of p53, but demonstrated a reduced survival rate compared to both qkv/v; p53−/− and p53−/− mice. One case of medulloblastoma was documented in a cohort of qkv/v; p53−/− mice (n = 11), while 2 other mice demonstrated signs of hydrocephalus. Recently, we have shown that qk−/− mice on a pchtl1−/− background have a reduced survival rate due to the development of fatal hydrocephalus. A mild hydrocephalic phenotype occurs in qk−/− mice due to the loss of PACRG expression in the ciliated ependymal cells lining the ventricular walls, leading to cilia dysfunction. Similarly, qkv/v; pchtl1−/− mice showed abnormal cilia function, leading to accumulation of CSF in the ventricles and hydrocephalus.

### Table 1 | Tumors in qkv/v; p53−/− mice

| Genotype  | Total no. of mice | Brain tumors | Hydrocephalus | Subcutaneous tumors | Thymic lymphoma | Intraperitoneal tumors | Other |
|-----------|------------------|--------------|---------------|---------------------|-----------------|------------------------|-------|
| qkv/v; p53−/− | 11 | 1 (11%) | 2 (18%) | 0 | 0 | 0 | 0 |
| qkv/v; p53−/− | 33 | 0 | 0 | 5 (15%) | 0 | 0 | 0 |
| qkv/v; p53−/− | 12 | 0 | 0 | 2 (15%) | 8 (61%) | 3 (23%) | 0 |
| p53−/− | 9 | 0 | 0 | 0 | 5 (55%) | 3 (33%) | 1 (11%) |

a. Tumors were classified according to tumor location.
function. Our data suggests that highlighting the pivotal role of RNA binding proteins in cellular and have been implicated in multiple diverse biological pathways, highlighting the pivotal role of RNA binding proteins in cellular function. Our data suggests that qkl interacts with p53 in vivo to regulate neuronal maintenance and survival.

Methods
Antibodies. Monoclonal MBP and NFH antibodies were obtained from Sternberger Monoclonals (Baltimore, MD), Monoclonal Calbindin-D-28K and GFAP antibodies were purchased from Sigma (St. Louis, MO). Anti-CD31 was purchased from Millipore (Billerica, MA). Antibodies against QKI-5, -6, and -7 were generated as described previously23.

Animals. Mice were monitored daily and were handled and sacrificed in accordance with a protocol approved by the Animal Care Committee at McGill University. The mice were housed in ventilated cages with a 12/12 hour light/dark cycle. p53+/– mice (lab stock # 002101) and qkl-/- mice (lab stock # 005006) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice colonies were maintained on a C57BL/6 background. Mice homozygous for the p53 mutation were crossed to heterozygous qkl+/– mice and subsequent trans-heterozygous crosses were used to generate qkl–/– males. These males were subsequently crossed to trans-heterozygous females to generate double mutant mice. Mice were screened for the p53 wild-type allele via genomic PCR using oligonucleotides (5′-ATA GGT CGG GGT TTC AT-3′ and 5′-GCC GAG TAT CTG GAC GAC AG-3′). The mutant p53 allele was identified using the following oligonucleotides: (5′-CTT GGG TGG AGA GGC TAT G-3′ and 5′-GGT CGC CAT GGT CTC GAG-3′). The qkl mutation was amplified using primers directed against the breakpoint (5′-TCT AAA GAG CAT TTT CGA AGT-3′ and 5′-TTG CTA ACT GAA TAT TAC T-3′).

Immunohistochemistry. Mice were anesthetized with isoflurane and perfused with ice-cold phosphate buffered saline followed by 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose overnight and embedded in OCT compound (Tissue-Tek, Markham, ON) over dry ice in acetone. Tissues were cryostat sectioned at a thickness of 10 µm and collected on +/+ slides (Fisher, Ottawa, ON). Tissue sections were blocked in 10% goat serum in Tris-buffered saline + 0.3% Triton X-100 for 1 hr followed by incubation with primary antibodies overnight at room temperature. Slides were incubated with Alexa-flour 488 or 546 Immunoglobulin G (Invitrogen, Carlsbad, CA) at a dilution of 1:400 for 4 hr. Corpus callosum thickness was visualized by MBP staining and quantified for 4 different areas using Axiovision software (Zeiss). P14 and P30 mice cerebellar coronal sections were evaluated at bregma ~5.8 mm, and representative images were taken of the simple lobule adjacent to the primary fissure. Purkinje cell linear density was quantified for 3 different areas using Axiovision software. Purkinje cell linear density was quantified as Calbindin-positive cell bodies/mm Purkinje cell layer. Statistical significance was calculated according to a two-sample two-tailed paired student t-test.

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Author contributions
The project and all experiments were designed by C.G. and S.R. All experiments and statistical analysis were performed by C.G. The manuscript was written by C.G. and S.R.

Additional information
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