Developmental Expression Profile of *Quaking*, a Candidate Gene for Schizophrenia, and Its Target Genes in Human Prefrontal Cortex and Hippocampus Shows Regional Specificity

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Decreased expression of oligodendrocyte/myelin-related (OMR) genes, including *quaking* (*QKI*), is a consistent finding in gene expression studies of post-mortem brain from subjects with schizophrenia, and these changes are most prominent in the hippocampus vs. the prefrontal cortex (PFC). Although expression of *QKI* and other OMR genes has been examined in rodents, little is known about their developmental trajectory in the human brain. Therefore, we examined expression of *QKI* and several putative mRNA targets of QKI in human PFC and hippocampus at different ages. The pattern of *QKI* expression in the PFC resembled that reported in rodents, with high *QKI-5* in the fetal brain and an increase in *QKI-6* and *QKI-7* during the period of active myelination, although *QKI-5* expression did not decrease substantially during postnatal development in the PFC in humans as it does in rodent brain. Most of the putative QKI target genes also showed linear increases in expression with increasing age in the PFC. In contrast, expression of these genes showed little evidence of developmental regulation in the hippocampus. Correlations between expression levels of the nuclear vs. cytoplasmic *QKI* isoforms, and putative splicing targets of the former, also differed between tissues. Thus, we speculate that a robust increase in OMR gene expression normally occurs with age in the PFC, but not in the hippocampus, which may explain why decreases in OMR gene expression in schizophrenia are more pronounced in the latter tissue. We also suggest that OMR transcripts might be processed by different splicing proteins in different tissues. © 2007 Wiley-Liss, Inc.

Key words: myelin; human development; splicing; schizophrenia

Decreased expression of a subset of oligodendrocyte/myelin-related (OMR) genes is one of the most consistent findings among gene expression studies of post-mortem schizophrenic brain tissue (Hakak et al., 2001; Tkachev et al., 2003; Prabakaran et al., 2004; Katsel et al., 2005b; Dracheva et al., 2006; Haroutunian et al., 2007). Interestingly, although most investigators have targeted the prefrontal cortex (PFC) in these studies, the most severe changes in OMR gene expression were actually observed in the anterior cingulate cortex and hippocampus of subjects with schizophrenia (SCZ) in a comprehensive study comparing gene expression changes associated with SCZ from multiple brain regions (Katsel et al., 2005b). Notably, histological studies have shown an abnormal distribution and decreased density of oligodendrocytes in frontal regions of SCZ brains, as well as reduced cell numbers in certain cortical layers

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(Uranova et al., 2001, 2004; Flynn et al., 2003; Hof et al., 2003; Tkachev et al., 2003; Stark et al., 2004). However, reduced OMR gene expression does not appear to result only from loss of oligodendrocytes, insofar as total levels of the major myelin protein, myelin basic protein (MBP), have not been reduced in most studies (Chambers and Perrone-Bizzozero, 2004; Dracheva et al., 2006). Subsequent gene expression studies have shown that the transcript encoding quaking (*QKI*), an RNA binding protein essential for normal myelination, is also severely decreased in frontal regions (Aberg et al., 2006) and in the anterior cingulate and hippocampus of subjects with SCZ (Haroutunian et al., 2006; McCullumsmith et al., 2007). Many of the OMR genes exhibiting decreased expression in SCZ are also putative targets of the QKI protein, which influences both splicing and stabilization of mRNA transcripts. Furthermore, both linkage and association data implicate *QKI* as a potential candidate gene for SCZ. Specifically, *QKI* is strongly linked to SCZ in a large Swedish pedigree (Lindholm et al., 2001, 2004), and an independent group has demonstrated association of polymorphisms within *QKI* to SCZ in a sample of 700 cases and controls (O'Donovan, 2006). Thus, *QKI* is an excellent candidate gene for SCZ, and its decrease may be the primary upstream cause of decreases in the expression of some OMR genes in at least a subset of SCZ subjects.

Most work on the role of quaking in myelination has been conducted on the rodent ortholog qk, which is responsible for splicing of some OMR genes, including myelin-associated glycoprotein (*Mag*) and possibly proteolipid protein (*Plp*; Wu et al., 2002). Studies of splicing defects resulting from abnormal levels of the nuclear isoform qk-5 have been performed in the *quaking viable* (qk^v) mutant mouse. However, this mutant harbors an ~1-Mb homozygous deletion including the *qk* promoter, the parkin-associated coregulated gene (*Pacrg*), and part of *Parkin*. Therefore, a better model with which to study the effects of a decrease in qk isoforms on the expression of other OMR genes is the recently introduced qk^{e5} mutant, carrying a homozygous N-ethyl-N-nitrosourea (ENU)-induced point mutation somewhere in the promoter region, leading to decreased expression of all three qk isoforms.

There have not been any studies examining developmental stage-specific expression of *QKI* in humans. To understand how candidate genes are dysregulated in SCZ, it is important to establish normative gene expression data in the human brain with regional and temporal specificity. In the present study, we examined expression of the major *QKI* isoforms as well as splice forms of transcripts that are putatively bound and regulated by the QKI protein at different ages in the PFC and hippocampus.

MATERIALS AND METHODS

Human Subjects

Tissue was obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland. Subjects from the developmental cohort were 1–2 months (n = 6), 1–2 years (n = 6), 8 years (n = 3 PFC, n = 2 hippocampus), 15–16 years (n = 6), 18–22 years (n = 6), and 34–46 years (n = 6) of age for both the PFC and hippocampus. For the PFC, fetuses 16–19 weeks of gestation were also included in the analysis (n = 6). Demographic characteristics of the cohort are listed in Table I.

TABLE I. Demographic Characteristics of Human Development Cohort*

Case no.	Age	PMI	Sex	Race	Cause of Death
898	16 wks GA	1		AA	Abortion
201	18 wks GA	1	M	AA	Abortion
279	18 wks GA	1	F	AA	Abortion
980	18 wks GA	5	F	AA	Abortion
246	19 wks GA	1	M	AA	Abortion
906	19 wks GA	1	F	AA	Abortion
1157	20 days	14	F	C	Pneumonia
1537	31 days	9	F	C	Arrythmia
1404	39 days	27	M	AA	Asphyxia
1321	62 days	27	F	AA	Pneumonia
1487	64 days	24	F	AA	Premature
1210	68 days	25	M	AA	Asphyxia
1063	1 yr 123 days	21	M	AA	Dehydration
1488	1 yr 137 days	21	M	AA	Hit by car
1798	1 yr 288 days	24	F	AA	Intussusception of bowel
1864	2 yrs 178 days	8	F	C	Bronchiolitis
1275	2 yrs 57 days	21	F	AA	Myocarditis
1906	2 yrs 71 days	27	M	AA	Asthma
1706	8 yrs	20	F	AA	Cardiac transplant rejection
1674	8 yrs	36	M	C	Drowning, hypothermia
1708	8 yrs	20	F	AA	Asphyxia
1843	15 yrs	9	F	C	MVA
1297	15 yrs	16	M	AA	MVA
4591	16 yrs	14	F	C	MVA
1230	16 yrs	16	F	C	MVA
1105	16 yrs	17	M	C	MVA
1158	16 yrs	15	M	C	Cardiomegaly
1611	18 yrs	11	M	C	Hanging
1429	18 yrs	9	M	C	MVA
4727	20 yrs	5	M	C	MVA
4548	20 yrs	5	F	AA	MVA
4542	22 yrs	8	M	C	MVA
1442	22 yrs	7	M	C	MVA
1266	42 yrs	15	M	C	CVD
1410	45 yrs	16	M	C	CVD
1428	45 yrs	14	M	C	CVD
1535	34 yrs	16	M	C	Abdominal injuries
1935	43 yrs	16	M	C	Multiple Injuries
1936	46 yrs	13	M	C	CVD

*PMI, post, mortem interval; GA, gestational age; AA, African American; C, Caucasian; MVA, motor vehicle accident; CVD, cardiovascular disease.

RNA Isolation and Quantitative RT-PCR

Frozen tissue was ground over liquid nitrogen and stored at −80°C until use. Total RNA was isolated by using Trizol reagent (Invitrogen, Carlsbad, CA) and genomic DNA contamination was removed by using Turbo DNAfree reagents (Ambion, Austin, TX). RNA samples were further

purified with RNeasy MinElute columns (Qiagen, Valencia, CA). Superscript III First Strand Synthesis Supermix (Invitrogen) was used to generate first strand cDNA from 1 μg total RNA. Custom TaqMan gene expression assays were designed and synthesized by Applied Biosystems (Foster City, CA). The reactions consisted of the fluorogenic probe and primer mix together with Platinum Quantitative PCR SuperMix-UDG w/ROX (Invitrogen). When probes from the Universal Probe Library (Roche, Indianapolis, IN) were used, the final concentrations of probe and primers were 100 nM and 200 nM, respectively. The cycling conditions included a 2-min hold at 50°C, a 2-min initial denaturation at 95°C, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. Each reaction was performed in quadruplicate on an AB7900HT instrument (Applied Biosystems). The data were normalized to the geometric mean of beta-actin and GAPDH and to a calibrator that consisted of a pooled RNA sample using the $2^{-\Delta\Delta Ct}$ method that has been described previously (Livak and Schmittgen, 2001).

RT-PCR Validation of Microarray Predictions

cDNA was generated from about 2 μg of total RNA using the TaqMan Reverse Transcription Reagents kit (Applied Biosystems) with a mixture of oligo-dT and random hexamers, following the manufacturer's instructions. For PCR, about 50–100 ng cDNA was used as a template, with primer pairs designed to amplify the region containing the skipped exon. Reactions used Taq Polymerase (Promega, Madison, WI) and were run for 25–35 cycles at annealing temperatures appropriate for the primer pairs used. PCR products were run out on 2% agarose gels and stained with ethidium bromide.

Statistical Analysis

Developmental expression profiles were analyzed by one-way analysis of covariance with age group as a main factor. Sex, post-mortem interval, and race were included in the analysis as covariates, except in cases when there was an interaction effect between the main factor and a covariate. All variables were transformed by taking the square root to reduce the kurtosis and skewness of the distributions. Polynomial contrasts were performed for each dependent variable to identify linear, quadratic, or higher order trends in expression patterns across age groups. Thus, general patterns across age groups were analyzed instead of performing pairwise comparisons between specific groups of subjects. Values for statistical significance described in the text indicate that a polynomial contrast, usually linear but occasionally quadratic, was observed for a particular transcript. Pearson correlation coefficients were calculated for quaking isoforms and putative targets.

qk^{e5} Mouse Brain Tissue

Brains from homozygous qk^{e5} and wild-type mice sacrificed at 3 months of age were a kind gift from Dr. Monica Justice (Baylor College of Medicine). These mice have been described previously (Noveroske et al., 2005). Procedures involving vertebrate animals were performed according to NIH guidelines and were approved by the IACUC at the Bronx VA and Mount Sinai School of Medicine. Tissue from neocortex, hippocampus, and white matter was dissected from the right hemisphere of qk^{e5} mice and wild-type littermates (n = 3 per group). RNA was isolated using the procedures described above.

RESULTS

QKI and Target Gene Expression Across Human Development

Expression levels of the major isoforms of quaking and several of its putative splicing targets were examined in human brain at seven developmental stages in the PFC and hippocampus. Target genes included alternative splice forms of *MAG* and *PLP1* (Wu et al., 2002), *MAGI1* (Sugnet et al., 2006), and breast carcinoma amplified sequence 1 (*BCAS1*; our own preliminary data), as well as the confirmed 3′ UTR binding target cyclin-dependent kinase (CDK)-inhibitor $p27^{Kip1}$ (Larocque et al., 2005). Several of the genes examined showed evidence of developmental regulation in the PFC. In contrast, expression levels varied substantially in the hippocampus among individuals, and there were fewer significant changes.

QKI Isoforms

There were no significant differences in *QKI-5* expression across the various age groups in either region (Figs. 1A, 2A, Table II). Notably, *QKI-5* was the only isoform that was expressed at high levels in the fetal PFC. *QKI-6* expression increased in the PFC in a linear fashion with age ($P = 0.005$), with a substantial increase between 1–2 months and 1–2 years of age (Fig. 1B). In the hippocampus, *QKI-6* expression increased at 1–2 years of age, remained high in the 8-year-old and 15–16-year-old groups, and then decreased into adulthood ($P = 0.003$; Fig. 2B). The overall trend for *QKI-7a* in the PFC was a linear increase in expression with increasing age ($P < 0.001$), but there was a peak in expression at 1–2 years of age ($P = 0.003$; Fig. 1C). *QKI-7b* expression did not vary significantly with age in either region because of greater heterogeneity, although the general pattern was similar to that of *QKI-6* and *QKI-7a* (Fig. 1D). In the hippocampus, the *QKI-7a* and *QKI-7b* transcripts showed trajectories that resembled that of *QKI-6*, but there was a greater degree of variability (Fig. 2C,D).

MAGI1

MAGI1 is not specifically an OMR gene, but the protein interacts with the neuregulin receptor ERBB4, which is involved in myelination and has been implicated in SCZ (Buxbaum et al., 2007). *MAGI1* also has an exon that is differentially included in brain tissue and contains a sequence that is bioinformatically predicted to be a splicing target of the QKI protein (Sugnet et al., 2006). We measured expression of *MAGI1* with a probe crossing the brain-specific exon and either the previous or next exon ("MAGI + previous" and "MAGI + next"), as well as a probe spanning the skipped exon

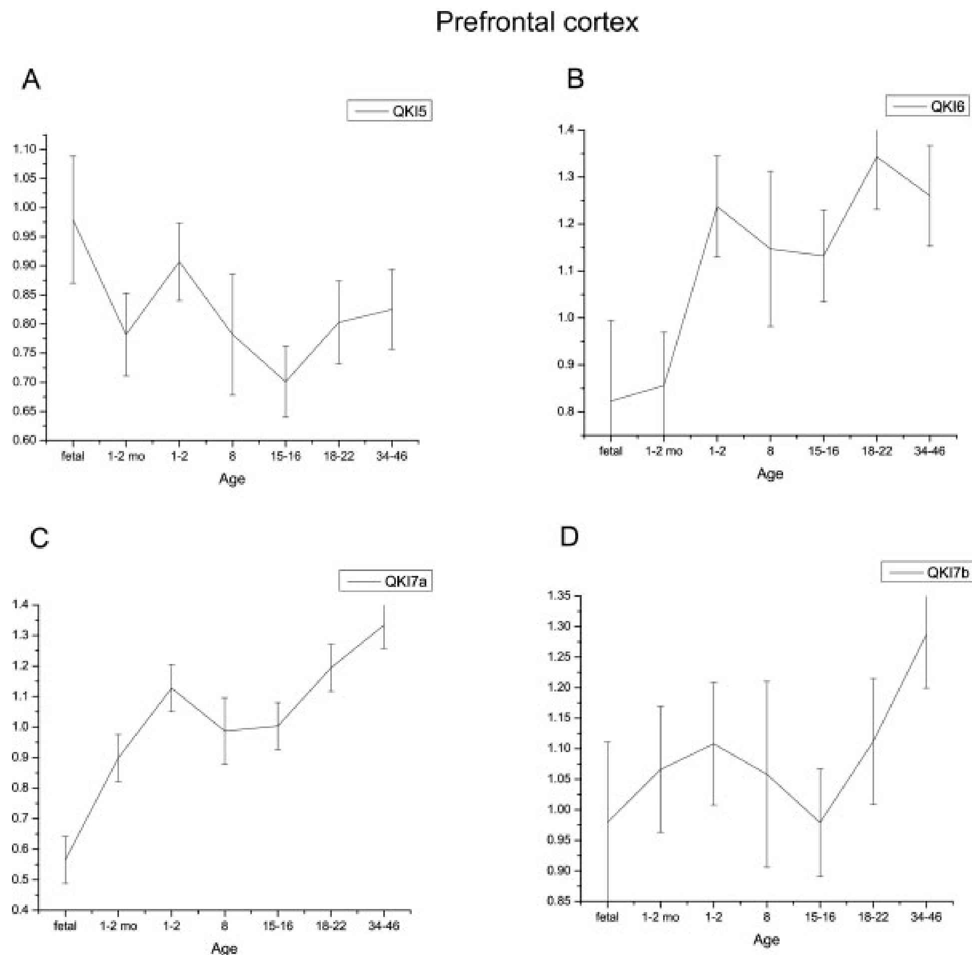

Fig. 1. Expression profile of *QKI* isoforms in the prefrontal cortex. **A:** *QKI-5* expression was high in fetal brain and did not show evidence of developmental regulation. **B:** *QKI-6* expression increased linearly with age. **C:** *QKI-7a* also showed a linear increase with age. **D:** *QKI-7b* expression did not vary significantly with age, but there was a trend toward an increase with age.

("MAGI brain skipped"). In the PFC, expression levels with all of the probes were very low in fetal brain and increased substantially at 1–2 months of age (Fig. 3A–C, Table III). Peak expression was reached at 8 years of age, after which levels dropped and then tended to level off into adulthood ($P < 0.001$). The decrease after 8 years was more pronounced for the isoform lacking the brain-specific exon. In the hippocampus, none of the isoforms showed significant developmental regulation (Fig. 4A–C). For the isoform lacking the brain-specific exon, although the ANCOVA indicated that there were no differences between the age groups because of the high heterogeneity in this tissue, there was a significant linear contrast indicating that expression was highest at 1–2 months of age and showing a gradual decrease with increasing age ($P = 0.028$). The fact that the patterns were so different between the two regions suggests that *MAGI1* may have a region-specific role in the developing brain. No studies have been reported describing developmental regulation of *MAGI1* in the rodent brain.

BCAS1

After identifying *Bcas1* as a putative splicing target of QKI in the qk^{e5} mice (see below), we examined expression of *BCAS1*, using probes targeting the equivalent of the brain-specific mouse exon 7 (Sugnet et al., 2006) as well as an exon junction in the constitutive transcript (exon 5–6), in the human samples. In the PFC, both isoforms showed a linear increase with age but a drop between 1 and 2 years of age and 8 years of age ($P < 0.001$; Fig. 3D,E). There was substantial heterogeneity in the hippocampus, and there were no significant differences across age groups for either splice variant (Fig. 4D,E).

$p27^{Kip1}$

The cell cycle molecule $p27^{Kip1}$ showed an unexpected pattern of mRNA expression in the PFC that differed from that of the other genes examined. Expression of $p27^{Kip1}$ was very high in the fetal PFC but fell significantly by 1–2 months of age (Fig. 3F). Levels

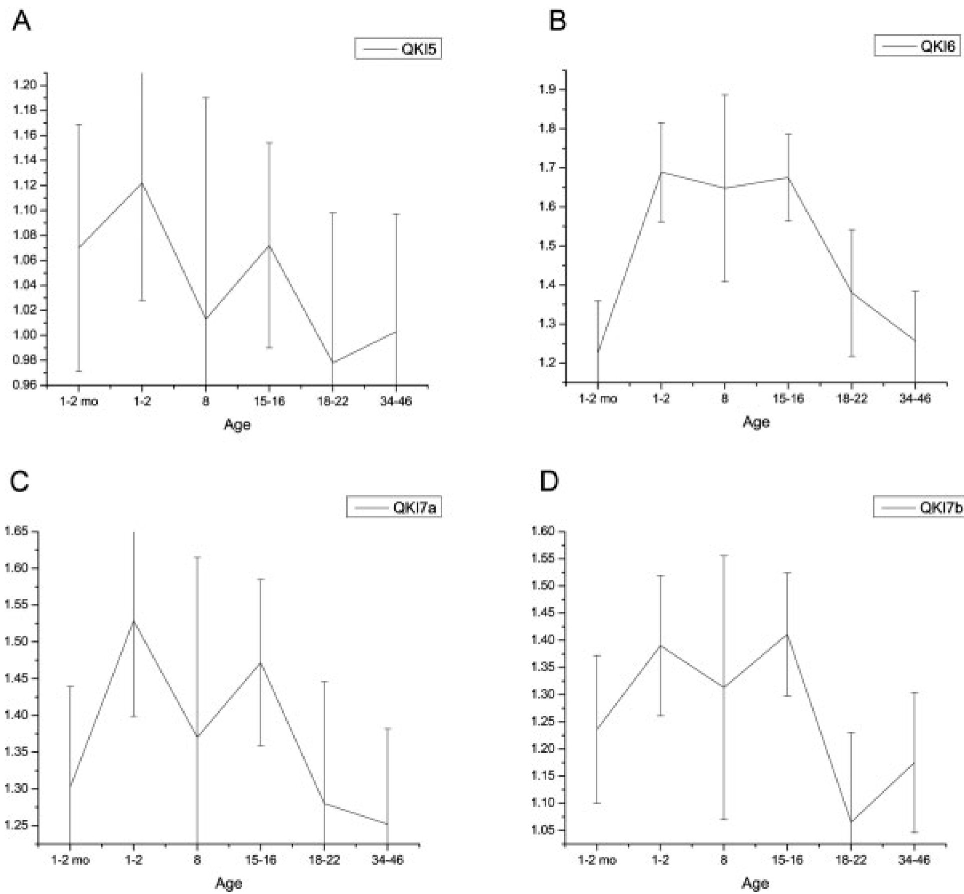

Fig. 2. Expression profile of *QKI* isoforms in the hippocampus. **A:** *QKI-5* expression was very heterogeneous and did not change with age. **B:** *QKI-6* expression reached a high level between 1–2 years and 15–16 years but decreased into adulthood. **C:** *QKI-7a* expression did not vary with age. **D:** *QKI-7b* expression did not vary with age.

increased throughout adolescence and were restored to fetal levels in the oldest subjects ($P < 0.001$). The pattern was almost completely the opposite in the hippocampus, where expression reached a peak at 1–2 months of age and decreased into adulthood (Fig. 4F). However, the differences observed in the hippocampus were not significant because of high heterogeneity within age groups. As with *MAGI1*, the strikingly different expression pattern between tissues suggests that these molecules may play different roles, possibly in different cell types, during early development in these tissues.

PLP1

Proteolipid protein (*PLP*) and *DM-20* are generated by alternative splicing of exon 3B of *PLP1* transcript in differentiating oligodendrocytes. *DM-20* is missing the 5′ end of exon 3. We examined expression of the two isoforms of *PLP1* and found that both showed a linear increase in expression with age in the PFC ($P < 0.001$; Fig. 3G,H). Expression was also developmentally regulated in the hippocampus, with a large increase between 1–2 months and 1–2 years of age, followed by a leveling off in older children and adults (Fig. 4G,H).

TABLE II. Results of ANCOVA for *QKI* Isoforms*

Isoform	Region	df	F	P
QKI-5	PFC	6,29	1.354	0.266
QKI-5	Hippo	5,23	0.262	0.929
QKI-6	*PFC*	*6,29*	*3.646*	*0.008*
QKI-6	*Hippo*	*5,23*	*3.499*	*0.017*
QKI-7a	*PFC*	*6,32*	*10.244*	*<0.001*
QKI-7a	Hippo	5,23	0.855	0.526
QKI-7b	PFC	6,31	1.337	0.271
QKI-7b	Hippo	5,23	1.011	0.434

*Transcripts that showed significant developmental regulation are indicated in italics.

MAG

In the PFC, both the *S-MAG* (the exon 12 included transcript) and the *L-MAG* (the exon 12

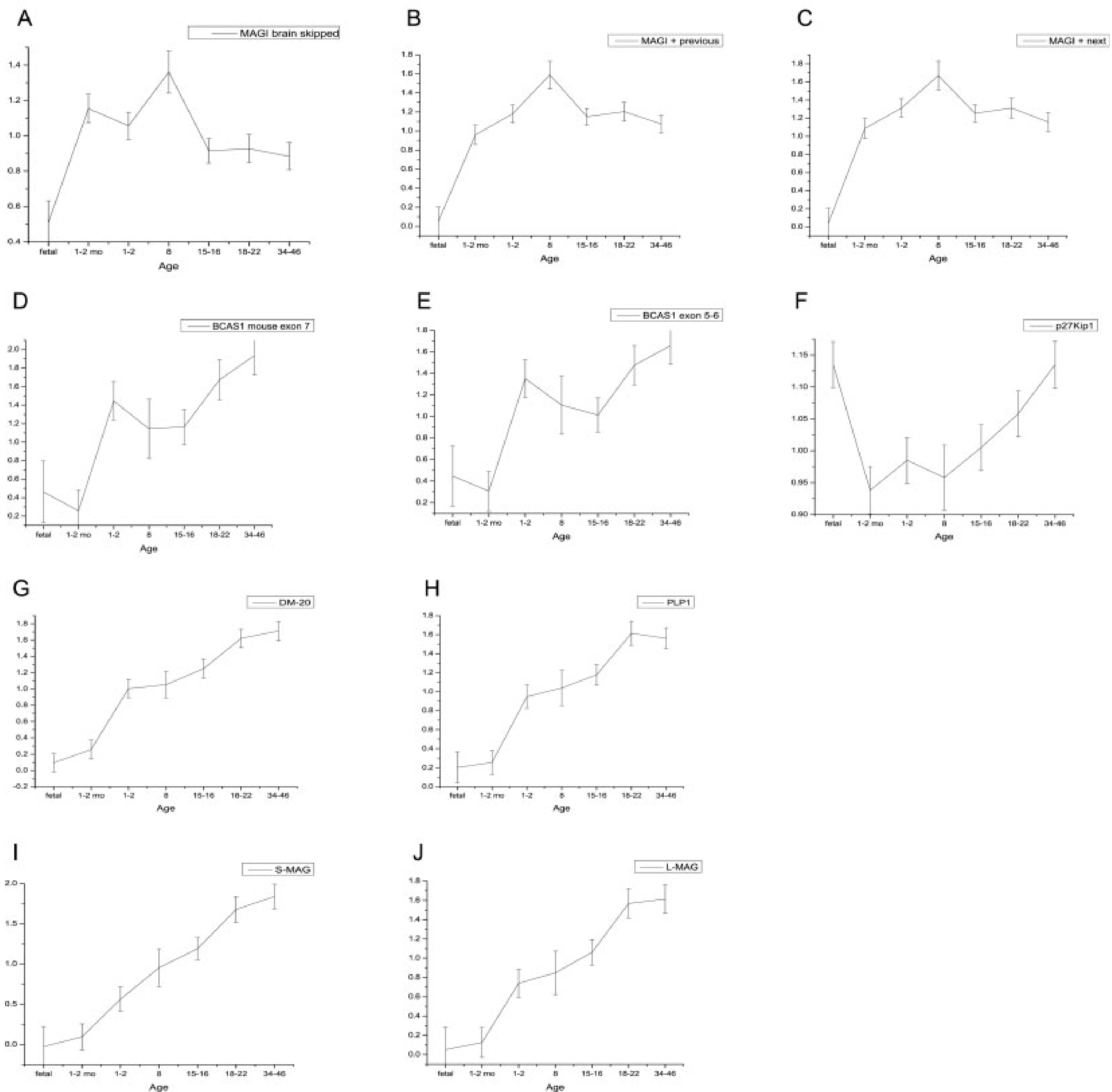

Fig. 3. Expression of putative QKI target genes in the prefrontal cortex. **A:** *MAGI1* measured with a probe spanning the brain skipped exon reached a peak at 8 years of age, then declined and leveled off into adulthood. **B,C:** *MAGI1* measured with a probe that spanned the brain exon and either the previous exon or the next exon showed a less pronounced peak at 8 years and then leveled off. **D,E:** The *BCAS1* exon 7 included transcript and the constitutive transcript measured with a probe spanning the junction of exons 5 and 6 both showed a linear increase with age. **F:** $p27^{Kip1}$ was the only QKI target gene expressed at high levels in the fetal brain, although it showed the characteristic linear increase with age postnatally. **G,H:** The *DM-20* and *PLP* isoforms of *PLP1* showed a linear increase with age. **I,J:** *S-MAG* and *L-MAG* showed a linear increase with age.

excluded transcript) splice forms were barely detectable in fetal brain and exhibited a gradual linear increase into adulthood ($P < 0.001$ for both isoforms; Fig. 3I,J). *S-MAG* also increased with age in the hippocampus during early childhood but leveled off in the adult subjects (Fig. 4I). *L-MAG* was expressed at substantial levels in the hip-

TABLE III. Results of ANCOVA for Putative QKI Targets*

Gene	Region	df	F	*P*
MAGI1 brain skipped	*PFC*	*6,29*	*4.109*	*0.004*
MAGI1 brain skipped	Hippo	5,23	1.940	0.126
MAGI1 brain + prev.	*PFC*	*6,29*	*12.600*	*<0.001*
MAGI1 brain + previous	Hippo	5,23	0.481	0.787
MAGI1 brain + next	*PFC*	*6,29*	*12.166*	*<0.001*
MAGI1 brain + next	Hippo	5,23	0.500	0.773
BCAS1 mouse exon 7	*PFC*	*6,29*	*8.725*	*<0.001*
BCAS1 mouse exon 7	Hippo	5,23	2.068	0.106
BCAS1 exon 5–6	*PFC*	*6,29*	*8.904*	*<0.001*
BCAS1 exon 5–6	Hippo	5,23	1.916	0.131
P27Kip1	*PFC*	*6,31*	*4.114*	*0.004*
P27Kip1	Hippo	5,23	1.223	0.330
DM-20	*PFC*	*6,31*	*28.304*	*<0.001*
DM-20	*Hippo*	*5,23*	*4.158*	*0.008*
PLP	*PFC*	*6,31*	*26.602*	*<0.001*
PLP	*Hippo*	*5,23*	*3.542*	*0.016*
S-MAG	*PFC*	*6,29*	*16.619*	*<0.001*
S-MAG	*Hippo*	*5,23*	*5.349*	*0.002*
L-MAG	*PFC*	*6,29*	*14.381*	*<0.001*
L-MAG	Hippo	5,23	1.474	0.237

*Transcripts that showed significant developmental regulation are indicated in italics.

pocampus beginning at 1–2 months and then remained relatively constant with high heterogeneity (Fig. 4J).

Correlations Between *QKI* and Target mRNA Expression

In general, the correlation coefficients were very similar for the different isoforms of a particular transcript. However, among the *QKI* isoforms, *QKI-6* and *QKI-7a* appeared to be virtually identical in the PFC and these transcripts were more strongly correlated with each other than with *QKI-5* and *QKI-7b* (Table IV), whereas all the *QKI* isoforms were more equally correlated in the hippocampus (Table V). In the PFC, the brain-specific exon of *MAGI1* was moderately correlated with *QKI-6* and *QKI-7a* but not at all with *QKI-5* or *QKI-7b*. In contrast, there were no meaningful correlations between any of the *MAGI1* transcripts and *QKI* in the hippocampus. In the PFC and the hippocampus, all isoforms of *QKI* were correlated with both isoforms of *BCAS1*, although the correlation was strongest for *QKI-6* and *QKI-7a* in the PFC. Both isoforms of *PLP1* and *MAG* were strongly correlated with the cytoplasmic isoforms of *QKI* but not with the nuclear isoform in the PFC. However, both nuclear and cytoplasmic isoforms of *QKI* were strongly correlated with the *PLP1* and *MAG* transcripts in the hippocampus. Finally, the 3′ UTR binding target $p27^{Kip1}$ was correlated most strongly with *QKI-7b*, followed by *QKI-5* in the PFC, whereas, again, all *QKI* isoforms were strongly correlated with $p27^{Kip1}$ in the hippocampus. In summary, unique patterns were observed among several of the *QKI* isoforms and putative target transcripts in the PFC, whereas data from the hippocampus indicated that all isoforms, with the exception of those for *MAGI1*, were correlated with all isoforms of *QKI* in this tissue.

Aberrant Myelin Gene Expression in qk^{e5} Mice

In a preliminary experiment, we hybridized brain tissue from three adult qk^{e5} mutants and three control mice to a mouse alternative splicing array in use by our collaborators (Sugnet et al., 2006). We plan to redo this experiment with larger numbers and using animals at various stages of development to assess accurately the relationship between Qk levels and splicing of OMR genes in mouse brain, so the data will not be reported here. However, we do note that the most significant differential splicing event observed between mutant and control brain tissue by the array concerned the gene *Bcas1*. Specifically, inclusion of the equivalent of the mouse brain-specific exon 7 of *Bcas1* was decreased by fourfold in the qk^{e5} mutant mice. Additionally, the exon 7-excluded constitutive transcript was detected by RT-PCR in qk^{e5} mutants but was not observed in wild-type brain (McInnes et al., 2006). RT-PCR also showed that *L-Mag* was not present at all in the qk^{e5} mutants and that *S-Mag* was decreased by twofold (data not shown). Limited access to tissue prevented us from further extensive experimentation; however, we were also able to verify decreased expression of *Qk* isoforms and the constitutive isoforms of *Mag* and *Plp1* in the qk^{e5} mutant by quantitative RT-PCR (Supplementary Table I).

DISCUSSION

QKI proteins regulate RNA splicing (Wu et al., 2002), export of target RNAs from the nucleus (Larocque et al., 2002), translation of proteins (Saccomanno et al., 1999), and RNA stability (Li et al., 2000). In rodent oligodendrocytes, QKI-5 protein is generally restricted to the nucleus, whereas QKI-6 and QKI-7 are localized to the soma and processes (Hardy et al., 1996). QKI-5 is the only isoform expressed during embryogenesis in the mouse, but all of the proteins are detectable at birth (Hardy, 1998). Expression of QKI-5 decreases substantially after P14, during the peak period of myelination in rodents (Ebersole et al., 1996; Hardy et al., 1996), accompanied by an increase in expression of the cytoplasmic isoforms QKI-6 and QKI-7. QKI-6 and QKI-7 bind to the 3′ UTR of $p27^{Kip1}$ mRNA, resulting in increased levels of the $p27^{Kip1}$ protein and cell cycle arrest, followed by differentiation of oligodendrocytes (Larocque et al., 2005). Thus, QKI-5 protein is thought to be at least partially responsible for maintaining oligodendrocytes in an undifferentiated state until QKI-6 and QKI-7 are up-regulated during the period of active myelination. QKI, presumably the nuclear isoform QKI-5, is also thought to be crucial for the developmental stage-specific splicing of several OMR genes, including *Mag* and possibly *Plp1* (Wu et al., 2002). Two other putative splicing targets include a brain-specific isoform of *Magi1* that is flanked by conserved quaking binding sequences, as well as a brain-specific exon of *Bcas1* that we observed to be differentially spliced in a preliminary experiment with qk^{e5} mutant mouse brain tissue. Therefore, we chose to investigate the developmental expres-

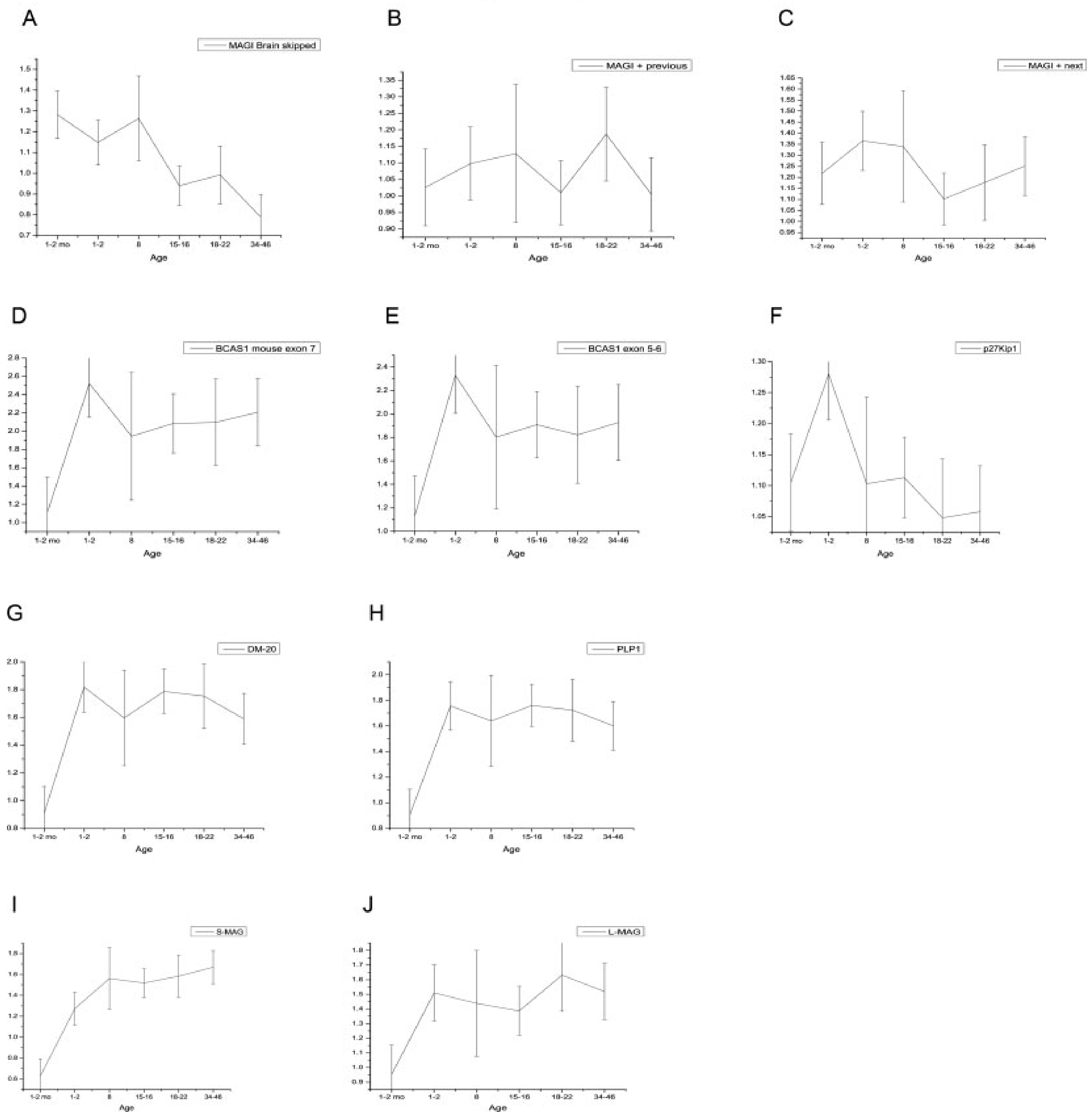

Fig. 4. Expression of QKI target genes in the hippocampus. **A:** The *MAGI1* isoform with the brain exon skipped showed a decrease in expression with increasing age. **B,C:** *MAGI1* with the brain exon included remained relatively constant across age groups with both probes. **D,E:** Both isoforms of *BCAS1* were very low at 1–2 months and increased substantially at 1–2 years, after which expression tended to level off. **F:** $p27^{Kip1}$ reached a peak at 1–2 years of age and then decreased with increasing age. **G,H:** Both isoforms of *PLP1* increased substantially at 1–2 years of age and then leveled off. **I,J:** *S-MAG* and *L-MAG* showed a gradual linear increase in expression with age that was less pronounced in the older subjects.

sion pattern of the confirmed or putative QKI targets and to determine whether they were correlated with the developmental expression profile of individual *QKI* mRNA isoforms.

The general temporal expression pattern of *QKI* mRNA isoforms observed in the human brain agrees with previous findings in the mouse brain, with the exception that *QKI-5* does not decrease dramatically

TABLE IV. Correlation Coefficients Between *QKI* Isoforms and Putative Targets in PFC (N=39)

	QKI-5	QKI-6	QKI-7a	QKI-7b
MAGI1 brain skipped	0.01	0.27	0.23	0.07
MAGI1 brain + previous	−0.16	0.45**	0.37*	−0.01
MAGI1 brain + next	−0.17	0.47**	0.39*	−0.04
BCAS1 mouse exon 7	0.44**	0.82**	0.85**	0.37*
BCAS1 exon 5-6	0.43**	0.84**	0.86**	0.37*
P27kip 1	0.47**	0.28	0.34*	0.55**
DM-20	0.16	0.76**	0.83**	0 52**
PLP	0.19	0.81**	0.85**	0.54**
S-MAG	0.06	0.60**	0.71**	0.51**
L-MAG	0.05	0.63**	0.73**	0.52**

*$P < 0.05$.
**$P < 0.01$.

TABLE V. Correlation Coefficients Between *QKI* Isoforms and Putative Targets in Hippocampus (N=32)

	QKI-5	QKI-6	QKI-7a	QKI-7b
MAGI1 brain skipped	0.24	0.15	0.16	0.28
MAGI1 brain + previous	0.13	0.10	0.01	0.11
MAGI1 brain + next	0.14	0.12	0.03	0.17
BCAS1 mouse exon 7	0.82**	0.66**	0.81**	0.43*
BCAS1 exon 5–6	0.84**	0.70**	0.85**	0.49*
$P27^{kip1}$	0.63**	0.71**	0.77*	0.71**
DM-20	0.61**	0.76**	0.84**	0 60**
PLP	0.64**	0.77**	0.86**	0.61**
S-MAG	0.43*	0.47**	0.62**	0.39**
L-MAG	0.51**	0.49**	0.66**	0.38**

*$P < 0.05$.
**$P < 0.01$.

after the peak period of myelination in the PFC of humans as it does in rodents. A possible explanation for this discrepancy is that active myelination continues in the human PFC through adulthood. As in rodents, *QKI-5* was the only isoform that was expressed at substantial levels in the human fetal brain. Another known QKI target, MBP (Ryder and Williamson, 2004), has been detected in cells as early as 5–6 weeks of gestation in human fetuses (Zecevic et al., 1998). In contrast, *QKI-6*, *QKI-7a*, and *QKI-7b* were expressed at very low levels in the fetal brain and showed a substantial increase at 1–2 years of age (for a diagram of the various *QKI* isoforms and the difference between isoforms 7a and 7b, see Kondo et al., 1999). These isoforms are known to reach a peak during the period of active myelination in the mouse brain. In the newborn human brain, there is a fivefold increase in the volume of myelinated white matter between 29 and 41 weeks postconception (Huppi et al., 1998). However, the first year after birth is the most active period of myelination (Brody et al., 1987; de Graaf-Peters and Hadders-Algra, 2006), which corresponds to the initial rise in *QKI-6* and *QKI-7* mRNA expression. After the initial peak in myelination during the first year of life, the process continues into adulthood. Thus, it is not surprising that we observed a gradual increase in *QKI-6* and *QKI-7* expression with age. The pattern was somewhat different in the hippocampus, with a decrease in expression of all the isoforms into adulthood so that adult levels were close to those observed in neonates. Therefore, we suggest that *QKI-5* expression may not fall off dramatically in humans in certain tissues characterized by ongoing myelination into adulthood, including the PFC.

The confirmed 3′ UTR QKI target, $p27^{Kip1}$, is involved in cell cycle progression but is also important for cortical development (Nguyen et al., 2006). This quaking target gene contains bioinformatically predicted binding sites for QKI proteins and is known to be an oligodendrocyte differentiation factor. Unlike the other transcripts examined in this study, QKI binds to the 3′ UTR of $p27^{Kip1}$ but does not affect its splicing, to the best of our knowledge. Although $p27^{Kip1}$ expression showed the same general trend of a linear increase with age in the postnatal brain, the high expression in the fetal brain was different from the other transcripts examined. Recent work has shown that $p27^{Kip1}$ plays a role in neuronal differentiation in the developing mouse neocortex (Itoh et al., 2007), which may explain the high expression observed in fetal brain. Notably, Qk-5 appears to be expressed in multipotential precursor cells in the ventricular zone of rodent fetal brain, and, other than *BCAS1*, $p27^{Kip1}$ was the only other gene examined whose expression was correlated with *QKI-5*, indicating a potentially unique role for these isoforms in fetal development. Correlations between *QKI* isoforms and $p27^{Kip1}$ were modest in the PFC, but all *QKI* isoforms were strongly correlated with $p27^{Kip1}$ in the hippocampus, suggesting that the QKI protein may be more important in stabilizing $p27^{Kip1}$ in the hippocampus.

Regarding the putative splicing targets of QKI, our data showed a linear increase of both isoforms of *PLP1* and *MAG* with age in the PFC and developmental regulation of *PLP1* in the hippocampus. The ratio of S-MAG and L-MAG proteins is known to be developmentally regulated, where L-MAG is the major protein in young mice and S-MAG is predominant in adults (Tropak et al., 1988). Interestingly, the pattern observed in the human brain differs from that in the rodent brain, where L-MAG is the predominant isoform in the adult central nervous system (Miescher et al., 1997). In one study, *S-MAG* and *L-MAG* mRNA expression increased as a function of age in human gray matter (Copland, 2005). In the white matter, *L-MAG* expression increased substantially in young adulthood (ages 20–24 years), whereas the major increase in *S-MAG* occurred in adolescence (ages 12–18 years). *L-MAG* expression was high throughout the remainder of life into old age, whereas *S-MAG* tended to decrease in early adulthood.

Plp1 expression has also been examined during development in rodent models. Expression of the *DM-20* transcript is detectable in the brain before the onset of myelination, whereas *Plp* is the major transcript in the adult brain (Baumann and Pham-Dinh, 2001). Surprisingly, *DM-20* is present in the mouse brain as early as

embryonic day 11, before oligodendrocytes are present (Ikenaka et al., 1992). Other work in the developing rat brain has demonstrated that Plp1 protein is produced in oligodendrocytes after they become associated with the fibers being myelinated (Hartman et al., 1982). In our human samples, we observed very low expression levels in the fetal PFC and a linear increase with age. Thus, whereas both isoforms may be present before oligodendrocytes are formed, they are detectable only at very low levels.

Strong correlations were observed between both *MAG* and *PLP1* isoforms and the cytoplasmic *QKI* isoforms but not the nuclear isoform in the PFC, indicating that the nuclear QKI-5 protein might not be directly responsible for splicing of these genes in this tissue. In fact, it has recently been shown that exon 3b of *PLP1* contains an exonic splicing enhancer bound by the splicing factor ASF/SF2. Additionally, the *MAG* alternative splicing element defined in the Artzt laboratory also lacks apparent QKI binding sites but does contain a purine-rich stretch of sequence reminiscent of ASF/SF2 binding sites. Because ASF/SF2 has conserved quaking response elements in its 3′ UTR similar to those of the known QKI target MBP (Ryder and Williamson, 2004), it may be that QKI-mediated stabilization of ASF/SF2 is important for splicing in the PFC. However, *QKI-5* is correlated with both *PLP1* and *MAG* isoforms in the hippocampus, so it is possible that unique splicing factors are responsible for similar splicing events but in different tissues. It is also possible that levels of *MAG* and *PLP1* transcripts are regulated by cytoplasmic QKI isoforms in a fashion similar to *MBP*, although they do not appear to contain canonical quaking response elements in their 3′ UTRs. Moreover, our collaborators have shown that the constitutive transcripts for *Plp1* and *Mag* are both decreased in the qk^{e5} mutant brain tissue (Supplementary Table I). Notably, this finding is different from *Mag* splicing defects observed in the qk^{v} mutant. Previously, researchers observed absent expression of *L-Mag* mRNA in developing qk^{v} mutants but overexpression of *S-Mag* such that total levels of *Mag* were not altered but the developmental ratio of *L-Mag* to *S-Mag* was inverted (Fujita et al., 1988). Expression of *L-Mag* was also absent in our adult qk^{e5} mutants; however, expression of *S-Mag* was decreased such that total levels of *Mag* were lower in the mutant vs. wild-type mouse.

Our novel putative splicing target *BCAS1* was the only gene to display a similar pattern of correlations in the hippocampus and the PFC, where all *QKI* isoforms were associated with both the brain-specific and constitutive *BCAS1*. Not much is known about this gene, but its expression does not appear to be specific to oligodendrocytes. In fact, the rat *Bcas1* ortholog has been localized to the postsynaptic density and is enriched in synaptosomes and present in the dendrites of hippocampal neurons (Ishimoto et al., 2002). Therefore, it is plausible that there may be some expression of QKI in a subset of neurons in humans responsible for brain-specific splicing of *BCAS1*. The brain-specific exon of *BCAS1* does not appear to be flanked by a canonical QKI binding sequence, although it is correlated with the *QKI-5* isoform. On the other hand, the brain-specific exon of *MAGI1* does contain bioinformatically predicted QKI binding sequences (Sugnet et al., 2006), yet the brain-specific isoform was correlated only with the cytoplasmic isoforms of *QKI* in the PFC, and there were no correlations between any of the *QKI* isoforms and the *MAGI1* isoforms in the hippocampus. Still, we might have missed important changes in gene expression specific to a particular minority cell type or a developmental window that we failed to capture in these tissues.

An important interpretation of our observation that the peak expression for most of the OMR genes examined differed between the PFC and the hippocampus is that these discrepancies may reflect differences in the rates of myelination of these structures. Furthermore, differences in the correlation of nuclear and cytoplasmic isoforms with some target transcripts in the PFC vs. the hippocampus may indicate that different proteins are involved in splicing and processing of the same targets in these regions. The general trend in the PFC was an increase with increasing age for *QKI-6* and *QKI-7* and most of the other genes examined. The increased gene expression likely corresponds to development of frontal white matter. Notably, there was a high degree of individual heterogeneity in the hippocampus that was not observed in the PFC. As a consequence, very few of the transcripts examined exhibited significant developmental regulation in the hippocampus. One possible explanation for the discrepancy is that the PFC samples likely included more white matter, allowing for a more reliable measurement of OMR genes. The variability may reflect differences in the number of axonal fibers and other white matter tracts present in the individual hippocampal dissections. In addition, earlier work has shown that the temporal course of myelination of the human cortex varies by region. Primary motor and sensory regions are myelinated in the first decade of life, whereas association cortex shows increased myelination in the second decade. Certain cortical regions including the cingulate gyrus remain poorly myelinated throughout life (Benes, 1989). Based on our results, it is possible that many OMR genes may be developmentally regulated in the PFC but not in the hippocampus, suggesting the hypothesis that vulnerability to certain neurodevelopmental disorders involving myelination may show regional specificity. Additionally, genetic defects in QKI that affect the QKI-5 isoform might be expected to have far reaching effects on the developing brain, although there is debate about whether SCZ is or is not a neurodevelopmental disorder.

Interestingly, most of the changes in myelin gene expression in SCZ have been observed in the hippocampus rather than the dorsolateral PFC (Katsel et al., 2005a). Thus, we speculate that because OMR gene expression increases into adulthood in the PFC, but not in the hippocampus, the former region may be more resilient to whatever mechanism is primarily causing a

reduction of OMR gene expression in SCZ. Alternatively, differences in the mechanisms of RNA splicing and processing in these regions might account for the differential regional severity of gene expression deficits in SCZ. For instance, all isoforms of *QKI* were correlated with the expression of all the genes tested, with the exception of *MAGI1* in the hippocampus, whereas only certain isoforms were correlated with the expression of other OMR genes in the PFC. In addition to the hippocampus, the anterior cingulate cortex is the other brain region exhibiting severe reduction of OMR genes in older subjects with SCZ. Therefore, our hypotheses could be partially verified by profiling OMR gene expression throughout the life span in the anterior cingulate cortex and showing that these genes do not increase with age as in the PFC. Additionally, as more QKI targets are verified, it will be interesting to observe whether correlations with *QKI* isoforms and these targets truly differ between brain regions.

One caveat for our study is that it is not possible to determine whether the increases in OMR gene expression observed with age are the result of increased expression per oligodendrocyte or increased production of oligodendrocytes. However, it is clear that there are regional differences in the trajectory of OMR gene expression, which may explain the increased vulnerability of the hippocampus to decreases in expression of these genes in SCZ.

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