Brief Report

The Human Homolog of the QKI Gene Affected in the Severe Dysmyelination "Quaking" Mouse Phenotype: Downregulated in Multiple Brain Regions in Schizophrenia

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Objective: The authors sought to understand the origins of oligodendrocyte/myelin gene expression abnormalities in the brains of persons with schizophrenia.

Method: Twelve cortical regions (Brodmann's areas 8, 10, 44, 46, 23/31, 24/32, 20, 21, 22, 36/28, 7, and 17) and three noncortical regions (caudate, hippocampus, and putamen) of 16 elderly schizophrenia patients and 14 matched comparison subjects were examined using 450 separate microarrays. The mRNA levels of QKI and its isoforms were then measured in a larger cohort by using quantitative real-time polymerase chain reaction (qPCR) in the cingulate cortex of schizophrenia subjects and matched comparison subjects.

Results: Expression of QKI mRNA was decreased in seven cortical regions and the hippocampus in the schizophrenia subjects. QKI gene expression deficits detected by microarray were validated by qPCR in the cingulate cortex, where the expression of isoforms QKI-5, QKI-6, and QKI-7 were profoundly perturbed in schizophrenia.

Conclusions: Since QKI plays a fundamental role in oligodendrocyte differentiation and in myelination, its underexpression may be pivotal to, and upstream of, other myelin-associated gene expression abnormalities in schizophrenia. Given the role of QKI in determination of oligodendrocyte fate, these results not only confirm oligodendrocyte-related gene expression abnormalities in schizophrenia but suggest that the physiology of glial progenitor cells may be altered in schizophrenia.

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Recent microarray studies, supported by several other independent lines of evidence (1, 2), have identified white matter abnormalities and decreased mRNA expression of numerous oligodendrocyte and myelin-associated genes in multiple brain regions as characteristics of the pathophysiology of schizophrenia (3). The neurobiological origins of these abnormalities, however, remain unclear.

Qk^{V} is an autosomal recessive mutation in mice that leads to severe dysmyelination of the CNS due to defects in oligodendrocyte maturation and RNA metabolism of myelin components (4, 5). The *qk* gene produces several RNA binding proteins by alternative splicing. The three major isoforms, QKI-5, QKI-6, and QKI-7, are mostly expressed in the oligodendrocytes (6, 7).

QKI-5 regulates alternative splicing of myelin-associated glycoprotein (5) and nuclear retention of myelin basic protein mRNAs (8). QKI-6 and QKI-7 promote the differentiation of oligodendrocytes (4). These data demonstrate that the different isoforms of QKI regulate RNA metabolism of several myelin structural genes as well as differentiation of progenitor cells into oligodendrocytes. The regulation of aspects of both oligodendrocyte genesis and myelination

TABLE 1. Sequences of TaqMan Probes and Primers[a]

Assay	Accession Number(s)	Applied Biosystems Assay ID	TaqMan Probe Context or Sequence	Primers
QKI	NM_006775; NM_206853; NM_206854; NM_206855	Hs00287641_m1	ATTGGTACCTGCAGCAGAAGGAGAA	
QKI-5	NM_006775	Hs00916681_m1	GTGACCGCAGACCGAGCCGCCACCG	
QKI-6[b]	NM_206853	Assay-by-Design	TTTCGTTGGGAAAGCCA	Forward: TTGAGTATCCTATTGA ACCTAGTGGTGTA; Reverse: GAGGGTTCAGTTAAGACCGTTCT
QKI-7	NM_206854	Hs00920546_m1	GTATTAGAGTGGATTGAAATGCCAG	
18S rRNA			TaqMan ribosomal RNA control reagents	

[a] For pre-designed assays, the manufacturer does not provide the primer and probe sequences.
[b] The primer/probe sequences for QKI-6 (designed from NM_206853) are also present in the QKI-7 isoform (NM_206854). Therefore, theoretically, this primer/probe pair could amplify sequences from QKI-7 mRNA as well as from the QKI-6 mRNA. However, the expected QKI-7 amplicon would have to be 1,358 bp long. Under the TaqMan assay conditions employed, a DNA sequence of 1,358 bp length cannot be amplified. In fact, the DNA sequence amplified under the current TaqMan assay conditions is 89 bp long corresponding to the length expected for QKI-6 (NM_206853).

TABLE 2. Characteristics of the Sample Groups Used in Microarray and qPCR Studies

Study Type and Characteristic	Comparison Subjects		Schizophrenia Subjects	
	N	**%**	**N**	**%**
Microarray analyses				
Number of subjects[a]	18	100.0	21	100.0
Gender				
Male	8	44.4	14	66.6
Female	10	55.6	7	33.3
	Mean	**SE**	**Mean**	**SE**
Age (years)	80.7	3.02	74.7	2.37
Brain pH	6.52	0.06	6.50	0.06
Postmortem interval (min)	505	100	707	72
	N	**%**	**N**	**%**
qPCR studies				
Number of subjects	22	100.0	30	100.0
Gender[b]				
Male	6	27.3	19	63.3
Female	16	72.7	11	36.7
	Mean	**SE**	**Mean**	**SE**
Age (years)	82.3	2.4	75.9*	1.8
Brain pH	6.46	0.06	6.52	0.05
Postmortem interval (min)	447.1	87.5	830.7*	85.2

[a] The number of subjects included in analyses varied from brain region to brain region (comparison subjects: median=13, range=8–18; schizophrenia subjects: median=13, range=9–21).
[b] Male-female ratio significantly differed between groups ($p<0.05$).
*$p<0.05$.

by QKI suggests that abnormalities of QKI expression may trigger the downregulation of some of the other oligodendrocyte- and myelin-associated genes that have been observed in schizophrenia.

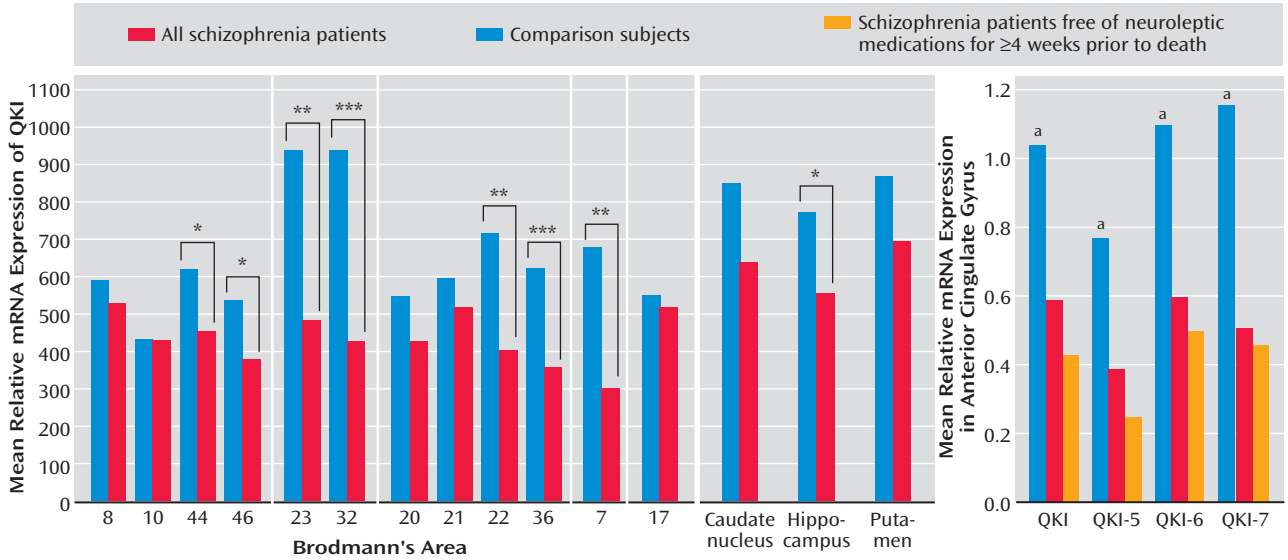
Here we examine using microarrays the gene expression profile of QKI in multiple brain regions of schizophrenia and comparison subjects postmortem. The QKI findings were then expanded and replicated in the anterior cingulate cortex of a larger independent cohort by quantitative real-time polymerase chain reaction (qPCR).

Method

Human brain samples (Brodmann's areas 8, 10, 44, 46, 24/32, 23/31, 7, 20, 21, 22, 36/28, and 17 as well as the hippocampus, caudate nucleus, and putamen) were obtained from the Brain Bank of the Department of Psychiatry of the Mount Sinai School of Medicine/Bronx VA Medical Center and prepared for microarray (HG-U133 A&B Human genome, Affymetrix GeneChip®, Santa Clara, Calif.) and qPCR analysis as described (3, 9). The mRNA levels of QKI (all isoforms) and its three main isoforms (QKI-5, QKI-6, and QKI-7) were measured by qPCR using QKI TaqMan MGB probe and primer sets (Applied Biosystems, Foster City, Calif.). Sequences of primers and probes as well as of the endogenous reference 18S rRNA (9) are given in Table 1.

All subjects (Table 2) died of natural causes with no history of licit or illicit drug abuse or neurological disease. Patients were diagnosed antemortem according to DSM-IV criteria as previously described (9). Comparison subjects (nursing home residents) evidenced no neurological or neuropsychiatric diseases (9). Diagnostic and postmortem consent procedures were approved by the Mount Sinai, Bronx VA, and Pilgrim Psychiatric Center institutional review boards.

FIGURE 1. Microarray-Based Gene Expression Profile of Human QKI in Multiple Brain Regions and Relative mRNA Expression of QKI and Its Isoforms in the Anterior Cingulate Gyrus of Patients With Schizophrenia and Comparison Subjects Measured by qPCR



[a] Significantly higher expression relative to all schizophrenia subjects (all F values >9.00, all p<0.004) and those free of neuroleptic medications for 4 weeks or more before death (all F values >11.00, all p<0.002).
*p<0.05. **p<0.01. ***p<0.001.

For microarray analyses, filtering and transformation were performed using GX™ Explorer version 2.0 (Gene Logic Inc., Gaithersburg, Md.) tools (expression, comparative, and contrast analyses) following normalization with Affymetrix MAS version 5.0 (3). The qPCR results were analyzed by analyses of covariance (ANCOVA) with sex, age, brain pH, or postmortem interval as covariates when significantly different between the diagnostic groups. The expression of the QK transcripts studied did not correlate significantly with sex, age, tissue pH, or postmortem interval.

Results

Analysis of variance for the expression of QKI derived from the microarray study revealed a significant main effect of diagnosis (F=49.5, df=1, 361, p=0.000001) and brain region (F=3.9, df=14, 361, p=0.000003) but no significant diagnosis-by-region interaction. We used t scores (3) as a standardized measure of gene expression change in schizophrenia across all of the analyzed brain regions. QKI mRNA expression was significantly decreased in seven cortical regions and in the hippocampus of subjects with schizophrenia (Figure 1).

Of the four different probe sets that were used for qPCR in the cingulate cortex, one was a pan-QKI probe that did not distinguish between isoforms, whereas the others were specific to the QKI-5, QKI-6, and QKI-7 isoforms. ANCOVA showed that the mRNA expression of QKI, QKI-5, QKI-6, and QKI-7 was reduced in schizophrenia subjects relative to comparison subjects (Figure 1).

Eleven of the schizophrenia subjects in the qPCR study had been free of neuroleptic medications for 4 weeks or more prior to death (range=4 weeks to 7 years). The mRNA expressions of QKI, QKI-5, QK-6, and QKI-7 were significantly reduced in this group relative to the comparison subjects, suggesting that the observed QKI expression changes were independent of the acute effects of antipsychotic medications (Figure 1).

Discussion

These results provide evidence for a profound schizophrenia-associated disruption in the expression of a gene (*QKI*) known to play a pivotal role in oligodendroglial cell fate, expression levels and splicing of several myelin-associated genes, and abnormal myelination in mice with mutations of this gene. These deficits are evident in multiple brain regions of known significance to schizophrenia and appear to generalize to three of the major isoforms of QKI.

The qk^v mutation in mice is characterized by severe CNS dysmyelination, reduced number of myelin lamellae, lack of myelin sheath compaction, abnormalities in the structure of nodal regions (10), and alteration of dopamine system parameters, including increased dopamine metabolism and increased dopamine D_2 receptor binding (11). Many of these alterations have been observed in studies of schizophrenia (2). In addition, QKI is a pivotal determinant of glial cell fate in progenitor cells (4) and regulation of alternative splicing and stability of mRNAs for several myelin structural components (4, 5) that have been implicated in schizophrenia. Myelin-associated glycoprotein is among the genes whose expression levels and isoforms are governed by QKI, and reductions in the expression of myelin-associated glycoprotein and its isoforms are among the most consistently reported myelin-associated

deficits in schizophrenia (2, 12). Taken together, these findings suggest that QKI may be upstream of—and central to—the downregulation of at least some of the many abnormally expressed myelin-associated genes in schizophrenia. It should be kept in mind, however, that the possibility of QKI gene expression having been influenced by factors such as long-term neuroleptic exposure, cigarette smoking, and postmortem artifacts cannot be completely ruled out.

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While this report was in press, two papers by Aberg and colleagues have since been published reporting decreased QKI gene expression in schizophrenia: "Human QKI, a new candidate gene for schizophrenia involved in myelination" (Am J Med Genet B Neuropsychiatr Genet 2006; 141:84-90) and "Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia" (Proc Natl Acad Sci USA 2006; 103:7482-7487).

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