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A Fluorescence *in Situ* Hybridization Map of 6q Deletions in Acute Lymphocytic Leukemia: Identification and Analysis of a Candidate Tumor Suppressor Gene

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ABSTRACT

With the objective of identifying candidate tumor suppressor genes, we used fluorescence *in situ* hybridization to map leukemia-related deletions of the long arm of chromosome 6 (6q). Twenty of 24 deletions overlapped to define a 4.8-Mb region of minimal deletion between markers D6S1510 and D6S1692 within chromosome 6 band q16. Using reverse transcription-PCR, we found evidence of expression in hematopoietic cells for 3 of 15 genes in the region (*GRIK2*, *C6orf111*, and *CCNC*). Comparison between our own and published deletion data singled out *GRIK2* as the gene most frequently affected by deletions of 6q in acute lymphocytic leukemia (ALL). Sequence analysis of *GRIK2* in 14 ALL cases carrying heterozygous 6q deletions revealed a constitutional and paternally inherited C to G substitution in exon 6 encoding for an amino acid change in one patient. The substitution was absent among 232 normal alleles tested, leaving open the possibility that heterozygous carriers of such mutations may be susceptible to ALL. Although low in all normal hematopoietic tissues, quantitative reverse transcription-PCR showed higher baseline *GRIK2* expression in thymus and T cells than other lineages. Among T-cell ALL patients, 6q deletion was associated with a statistically significant reduction in *GRIK2* expression ($P = 0.0001$). By contrast, elevated *GRIK2* expression was measured in the myelomonocytic line THP-1 and in one patient with common ALL. Finally, we detected significant levels of *GRIK2* expression in prostate, kidney, trachea, and lung, raising the possibility that this gene may be protective against multiple tumor types.

INTRODUCTION

Biallelic inactivation of genes, through a variety of mechanisms, has been shown to contribute to the development of both familial and sporadic cancers. The position of these tumor suppressor genes, which function either to retard cell cycle progression, promote apoptosis, or maintain DNA integrity, are flagged by cytogenetic deletions. It is therefore expected that other sites commonly deleted in related malignancies will be found to harbor similar tumor-protective genes.

Microscopically visible deletions of the long arm of chromosome 6 (6q) accompany the malignant transformation of cells from a number of tissues, including breast, prostate, liver, skin, and the central nervous, urogenital, and hematopoietic systems (1–6). Similarly, molecular techniques demonstrate that loss of constitutional heterozygosity of markers on 6q characterize a spectrum of different tumors (7–12). Among the hematological malignancies, cytogenetic abnormalities involving loss of 6q have been reported in 14–31% of non-Hodgkin's lymphoma (13), 4–13% of pediatric and adult B- and T-lineage acute lymphocytic leukemia (ALL; Refs. 14–16), in 4.5% of B-cell chronic lymphocytic leukemia (17), and in 43.5% of natural killer cell lymphoma/leukemia (18). Unbalanced chromosomal rearrangements of 6q occur in myeloid disease but are rare (6).

Cytogenetic analysis has been used to correlate deletion of specific chromosomal bands on 6q with clinical subtypes of hematological malignancy. Analysis of 126 cases of non-Hodgkin's lymphoma with 6q abnormalities revealed preferential loss of three distinct regions at bands 6q25-q27, 6q25-q21, and 6q25-q23 in intermediate, high and low grade lymphoma, respectively (19). In ALL, 6q deletions have been associated with a T-cell immunophenotype and either an intermediate or poor outcome (20–24). In one study, all deletions were thought to include band q21 (15).

More precisely defined region(s) of minimal deletion (RMD) on 6q have been established in various lymphoid malignancies using either fluorescence *in situ* hybridization (FISH) or microsatellite analysis. Using FISH with yeast artificial chromosomes, we previously defined two distinct ALL-specific RMD, within 6q16-q21, one proximal to marker D6S447 and the second distal to yeast artificial chromosome 860f10 (telomeric to D6S447 and equivalent to marker CHLC.GGAT16C02; Refs. 25–27). These data have been corroborated by other investigators who identified RMD that coincide with either the proximal or both these regions (28–32). However, despite accumulating evidence that deletion of chromosomal bands 6q16-q21 is a critical event in ALL, no tumor suppressor gene contributing to the pathogenesis of lymphoid disease has yet been identified in this region.

In this present study, we have constructed a detailed map of cytogenetically visible deletions of 6q using FISH with P1-derived artificial chromosome and bacterial artificial chromosome clones on a series of new ALL cases. A minimal region of overlap of 4.8Mb, positioned within chromosomal band 6q16 and coincident with the more proximal of our previously characterized RMD, was identified. Using a combination of gene scanning, the selection of genes specifically expressed in hematopoietic tissues and, comparing our own with published data, a candidate tumor suppressor gene, the *glutamate receptor ionotropic kainate 2* (*GRIK2* or *GluR-6*), was identified. This gene was additionally analyzed for inactivating mutations in cases of ALL carrying heterozygous chromosome 6q deletions and for levels of expression in a range of normal and malignant hematopoietic cells.

MATERIALS AND METHODS

FISH Analysis

Patient Samples and Cell Lines. Fixed cell suspensions prepared for routine cytogenetic analysis were available from diagnostic or relapse bone marrow samples of patients with acute leukemia. The corresponding G-banded karyotypes were described according to the International System of Human Cytogenetic Nomenclature (33). Additional ALL samples were provided by member laboratories of the United Kingdom Cancer Cytogenetics Group on request through the Leukemia Research Fund United Kingdom Cancer Cytogenetics Group Karyotype Database in Acute Leukemia (34). Slides were made according to standard techniques and stored at −20°C for future analysis by FISH. In total, 24 ALL and 7 acute myeloid leukemia patients with chromosomal abnormalities involving 6q were analyzed, of which, 2 have been previously reported (cases 2 and 22 in this study corresponding to cases 21 and 15 in the earlier study; Ref. 27). Three cell lines derived from patients with T-

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ALL and carrying deletions of 6q (PEER, MOLT 4, and RPMI 8402) were obtained from the German National Tissue Culture collection. Cell lines were grown and cytogenetic preparations made using standard procedures.

Chromosome 6 Clones and FISH Procedure. P1-derived artificial chromosome (from library RPCI-1) and bacterial artificial chromosome (from library RPCI-11) clones positioned on chromosome 6 by Human Genome Mapping Project (35) were obtained on request through the United Kingdom Human Genome Mapping Project Resource Centre, Hinxton (Cambridge, United Kingdom).[1] DNA prepared from the clones using a standard SDS lysis technique was labeled by incorporation of Spectrum Green or Spectrum Red-conjugated dUTP (Vysis, Richmond, United Kingdom) using a nick translation kit (Vysis). Hybridization and washing procedures were performed as described previously (36). Visualization of FISH signals was performed on an Axioplan fluorescence microscope (Karl Zeiss, Göttingen, Germany) equipped with appropriate filters (Chroma Technology, Battleboro, VT) and MacProbe software (Applied Imaging International, Newcastle Upon Tyne, United Kingdom). For analysis of leukemic samples, probes were hybridized in pairs labeled with Spectrum Red and Spectrum Green. Probes labeled in Spectrum Red were either from the 6q centromeric region (RP1-71H19) or the 6q subtelomeric region (RP1-167A14 or RP1-57M24). Twenty-one Spectrum Green-labeled probes positioned on Sanger Institute P1-derived artificial chromosome/bacterial artificial chromosome 6 contigs[2] between the centromeric and subtelomeric clones were used for the initial analysis (Fig. 2). Analysis was restricted to metaphase cells and signals defined by the appearance of pairs of closely aligned red or green spots, one on each of the two sister chromatids of a chromosome. A metaphase cell was scored only if two clearly positive red and/or green signals were visible (or three or four signals in the case of clones trisomic/tetrasomic for chromosome 6). A cell was scored as deleted for a probe if either a red or green signal only was seen on a chromosome, and the missing signal was not present on any other chromosome (Fig. 1). Clonal deletion was defined as the loss of signal in a minimum of five metaphase cells and in no $<$20% of the total cells observed. The appearance of red and green signals separated on two different chromosomes was interpreted as translocation of 6q with a breakpoint between the two probes. By performing sequential FISH experiments with probes from the primary panel, the boundaries of deletion or the translocation breakpoints were defined in each case. Additional probes were obtained to refine the breakpoints of critical deletions (Fig. 3).

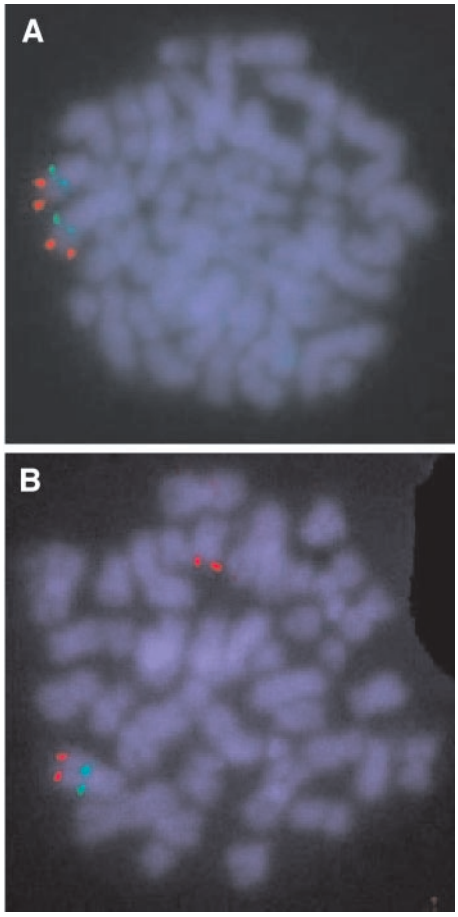

Fig. 1. Example of fluorescence *in situ* hybridization probes hybridized to metaphase cells from an acute lymphocytic leukemia patient (no. 2) with del (6q). *A*, pairs of red and green signals are present on both the deleted 6q and the normal chromosome 6 (probe retained). *B*, *red* and *green signals* mark the normal chromosome 6, but *red signals* only are seen on the deleted 6q (probe deleted).

Analysis of Gene Expression within the FISH RMD

The following forward (F) and reverse (R) primers (Sigma-Genosys, Cambridge, United Kingdom) were designed to amplify coding sequences from 15 genes positioned within the RMD: *PU3F2*, F 5′-AGCGCAGAGCCTGGTGCAG-3′ and R 5′-CAGCTCGTCTCCGCGGC-3′; *FBXL4*, F 5′-TCAGAGCCAGGACTATGTGGAACTTACTT-3′ and R 5′-CCACTTACGAAGGGTTCGAGCG-3′; *Novel (1)*, F 5′-CGGGAGACAGATCATGATCGATACAG-3′ and R 5′-GGTCGGAGTACTTAAGACTCAGTGCAGAC-3′; *Q9B552*, F 5′-CATCGCTTTCCCTTTGCAGGAT-3′ and R 5′-GAATAAATTCCTACCGACTGAATGGCATAGTC-3′; *COQ3*, F 5′-GAACCAGCTCAGTGGGACTCTACAGA-3′ and R 5′-CCGGGACCGAGTGTTTACCA-3′; *C6orf111*, F 5′-GCTTGGATTGCCCAAAGAGAAGCT-3′ and R 5′-GACTGTTGCTGTCTTCAGAAGGAGGA-3′; *Novel (2)*, F 5′-CCGCTGCTCAGGGGATTG-3′ and R 5′-GTGACATCAAGGACACGAGTTTGAATACC-3′; *Q9BRU1*, F 5′-GCTGAGAATCTGTGGTCAGTTTGCTC-3′ and R 5′-CTCAAGGTCTTGTCTCGGGGTAACATA-3′; *CCNC*, F 5′-GCCACTGCTACGGTATATTTCAAGAGATTC-3′ and R 5′-CAGAAGTAGCAGCAGCAATCAATCTTG-3′; *PRDM13*, F 5′-CCGAGCATTGCGAGACGTC-3′ and R 5′-GGACGCAAAGGTGACGCAC-3′; *GPR145*, F 5′-CAGTCATCCTCCCTTCCATGATTG-3′ and R 5′-ACCAAATCAGCCACAGCCAGG-3′; *SIM1*, F 5′-GATCATGTACATCTCAGAGACAGCCTCAG-3′ and R 5′-GCTCGC GAGGAAGAAGGACTCC-3′; *043738*, F 5′-CTGAGCAGGCAGAGAGATGCAGA-3′ and R 5′-AATATAGGTTCTGAATCTACTCTAGCCTGTCGACC-3′; *DJ467N11.1*, F 5′-TTTGGGCCTGACATGGAAGAAGATA-3 and R 5′-TCCTCTTTTCCATCCAGCCTCCTCTTC-3′; and *GRIK2*, F 5′-GGCGCACCGTTAAACTCCTGCTCTG-3′ and R 5′-GTAGCAATGTTCTGTTTCTGTTAATTGTGTTC-3′.

The primers were initially used to assess expression of the candidate tumor suppressor genes in normal brain and bone marrow RNA by conventional reverse transcription-PCR (RT-PCR) using a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Twenty-μl reactions containing 5 μl of cDNA, 0.2 μl of TaqDNA polymerase (Perkin-Elmer), 2 μl of 10$\times$ buffer, 2 μl of 2 mM deoxynucleoside triphosphates, and 200 ng of each primer were denatured at 94°C for 10 min then subjected to 35 cycles at 94°C denaturation for 1 min, 65°C annealing for 1 min, and a 72°C extension for 2 min followed by a final extension step of 72°C for 10 min. Reaction products were assessed by ethidium bromide staining of a 1.5% agarose gel using conventional electrophoresis.

Mutation Analysis of *GRIK2* in ALL Patients and Cell Lines

Patients, Cell Lines, and Normal Controls. DNA from 14 cases of ALL, with evidence for heterozygous deletion of the *GRIK2* region, was obtained as described previously (37). Seven patients with loss of heterozygosity for at least one 6q microsatellite marker within *GRIK2* (cases A–G; Ref. 38) were referred to the department through Medical Research Council childhood and adult ALL treatment trials UKALLX, UKALLXI, and UKALLXII. An additional 5 patients that had been referred to this department for routine cytogenetic investigation (cased I–M), and the cell lines PEER and RPMI 8402 (cases N and O) were chosen because they carried deletions of *GRIK2* mapped by FISH (as part of this or a previous study; Ref. 27). A panel of 96 Caucasian human random control DNA samples (HRC-1) were obtained from the European collection of cell cultures (Sigma-Aldrich, Gillingham, Dorset, United Kingdom). Twenty additional normal control DNA samples were prepared from the peripheral blood of consenting volunteers from this department and from the parents of 1 patient. All DNA samples were prepared using the Purescript DNA isolation kit (Gentra Systems/Flowgen, Ashby de la Zouch, Leicestershire, United Kingdom).

[1] Internet address: http://www.hgmp.mrc.ac.uk.
[2] Internet address: http://www.sanger.ac.uk/HGP/Chr6/.

Sequence Analysis. Exons 1–15 and 17 of *GRIK2* were positioned on sequenced fragments AP002531, AP002530, and AP002529 by performing a homology search with the cDNA sequence (accession no. NM_021956) using the BLAST facility of the National Center for Biotechnology Information.[3] Although not present in the publicly available cDNA sequence, exon 16 had been recently characterized by electronic-PCR and RT-PCR (39). The following forward (F) and reverse (R) primers from the genomic regions flanking each exon were then designed and synthesized (Sigma-Genosys): *exon 1*, F 5′-CCCAGGAGCCGAACGCTAGATC-3′ and R 5′-CATCCGGCTGCCTGGGAG-3′; *exon 2*, F 5′-CAGAAAACTTCTGATATTTTCTTTATCTTGTGA-3′ and R 5′-GAATACCTAGAAATTTGAATTCCAGGAAAC-3′; *exon 3*, F 5′-CTGGCACCTCTCCTCTCATTATTGACA-3′ and R 5′-CAATAAGAACCTGGAATGGGCTTCAG-3′; *exon 4*, F 5′-ATGAGTGTTTTCTGATTCTTTGCC-3′ and R 5′-AGAAGATCATATTGTAAGTGCAAGTTTATGAA-3′; *exon 5*, F 5′-CTTTATCACATCCTACTATATTTTGTTCTCACTTG-3′ and R 5′-CTACAAAGGAATCAGTCATAATACTTCTTACTAAA-3′; *exon 6*, F 5′-CATGCCTGTGAAGATACTCTGTCC-3′ and R 5′-CTGAAGTTTATATGTATGCATATAAACCCAGAA-3′; *exon 7*, F 5′-CTCTTCCTCCTTGCAAACCATCTACCA-3′ and R 5′-AGGACACTTGCTAGAAAAACCACAATAATCTCAC-3′; *exon 8*, F 5′-TGAAAAGTAATGAATATAAGTTTCCTACTTTTGTG-3′ and R 5′-TCAATGAAATGTGACTACAACAGAGAATATTT-3′; *exon 9*, F 5′-CTCTCTACCACGTGCCTGATG-3′ and R 5′-GCACTTCTGTGTTCATCTACATTTAACTAAG-3′; *exon 10*, F 5′-GATGATGAGTTTCATGATTAACTGTTACCTC-3′ and R 5′-AAAAGAGTAAACCTTGTAGCAACATAACAAACTA-3′; *exon 11*, F 5′-TGATTACTGATTTTTTCTTGTGACTGAAAAT-3′ and R 5′-ATTAGTTAGATAAAGGAGGTAACAATTGCCAA-3′; *exon 12*, F 5′-AATGCTGGATAGAATTTCTTCCCACTGC-3′ and R 5′-CAGCAAAGAGTGGGACATGGTGC-3′; *exon 13*, F 5′-TTGCTGATCAAATTCTCTATATTCGTTTCACCT-3′ and R 5′-GAGAGAAGTTTGCGCTCTATCATTTTAATTTGTAA-3′; *exon 14*, F 5′-TGTGTCTAAGCATTATAGGAGCACATGGAAACTA-3′ and R 5′-CCTGTCTTACAGCAACTCAGATTAATGAACA-3′; *exon 15*, F 5′-CTGCCCTCCTCTCATCTTGCT-3′ and R 5′-ATAGTTCTATGCATTATCATTAGTTGGTAACATAA-3′; *exon 16*, F 5′-CCAGATCTACATTGTTTCAAGAATTAGAGAT-3′ and R 5′-GGTCAGTTCCTTAGGATGAAAACAAC-3′; and *exon 17*, F 5′-TAATATTGATCTTGGACAGTTACAGTTTATGTATC-3′ and R 5′-GAAACATTCTGGCTAAATTGTTTGG-3′.

Primer pairs were designed to amplify the entire exon and splice recognition sites and to have a T_m of either 70°C or 65°C. Genomic DNA from the 14 leukemic samples was amplified using the paired primers. PCR reactions contained: 200 ng of DNA; 200 ng of each primer, 2 units of Taq polymerase; and 4 μl of 10× Taq buffer containing $MgCl_2$ at 1.5 mM final working concentration (Promega, Southampton, United Kingdom) in a total volume of 40 μl. For primer pairs with a T_m of 70°C, the reactions were denatured at 94°C for 10 min, then amplified for 35 cycles at 94°C for 1 min, 65°C for 1 min, and 72°C for 7 min, followed by a final extension of 72°C for 7 min. For primer pairs with a T_m of 65°C, the annealing temperature was reduced to 60°C. PCR products were purified using a GFX DNA purification kit (Amersham Biosciences, Chalfont, Buckinghamshire, United Kingdom), and the eluted products diluted with 100 μl of PCR grade water before direct sequence reactions were prepared. Forward and reverse sequence reactions were performed using the BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer/Applied Biosystems, Beaconsfield, Bucks, United Kingdom) in an Applied Biosystems Gene Amp PCR System 9700. All sequencing reactions were analyzed in an automated sequencing system (ABI 377; Applied Biosystems) using apparatus and protocols supplied by the manufacturer.

Forward and reverse sequences for each exon from each patient were then compared with the wild-type sequence using the Megalign software (DNASTAR, Inc., Madison, WI). Any discrepancy between the sequence obtained and wild-type sequence was verified by repeating the sequencing reactions. For sequences that differed consistently from the wild type, PCR reactions were repeated to rule out PCR errors.

Rapid Screening for a bp Substitution by Restriction Enzyme Digestion. PCR products containing exon 6 of *GRIK2* were prepared as described for sequence analysis using normal control DNA samples. The GFX-purified products were digested overnight with Taq I at 65°C (New England Biolabs, Hitchin, Herts, United Kingdom) and then analyzed by gel electrophoresis on a 1.5% agarose gel.

Quantitative Analysis of Expression of *GRIK2*

Preparation of RNA and cDNA. Cell lines and patient samples were obtained as described above for FISH and mutation analysis. RNA was prepared from peripheral blood or whole bone marrow from ALL patients at presentation, EBV-transformed B lymphocytes, T-cell lines (JURKAT, PEER and MOLT-4), B-cell lines (REH, U-266, and RAJI), myeloid cell lines (K-562, THP-1, and HL-60), and isolated granulocytes, T cells, B cells, natural killer cells, and monocytes. Karyotypes were available for all leukemic samples and cases with del(6q) selected only if a minimum of 80% of G-banded metaphases were reported to carry the deletion.

Granulocytes were obtained from peripheral blood of a normal donor after mononuclear cells were removed over a Lymphoprep (Nycomed Pharma AS, Oslo, Norway) gradient. Other lineages were isolated from the remaining lymphocytes using anti CD14 (monocytes), CD19 (B cells) and CD56 (natural killer cells) phycoerythrin-conjugated antibodies and anti- phycoerythrin microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). T cells were obtained by negative depletion of other lineages. RNA was prepared using the Purescript RNA isolation kit (Gentra Systems/Flowgen).

cDNA was prepared from the different hematopoietic RNAs and from commercially available RNA from whole brain, lung, kidney, prostate, and trachea (Ambion Europe Ltd., Huntingdon, United Kingdom). For each preparation, 2 μg of RNA were reverse transcribed and cDNA quality assessed in PCR reactions with *G6PD* primers as described previously (40).

Quantitative Real-Time RT-PCR Analysis. Relative expression levels of *GRIK2* were determined in a range of different hematopoietic and other tissues using the *GRIK2* and *G6PD* primers in real-time RT-PCR reactions. All experiments were performed using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) following the manufacturer's instructions. Ten-μl reactions were set up in glass capillary tubes, each consisting of 5.2 μl of cDNA, 100 ng each of the forward and reverse primers, 0.8 μl of 25 mM $MgCl_2$, 0.5 μl (1 μg/ml) of BSA, 0.5 μl of 1 unit/μl Uracil DNA Glycosylase (Roche Molecular Biochemicals), and 1 μl of LightCycler DNA Mastermix containing SYBR Green 1, deoxynucleoside triphosphates (with dUTP in place of dTTP), PCR buffer, and TaqDNA polymerase (Roche Molecular Biochemicals). Reactions were sealed and allowed to stand for 10 min at room temperature before denaturing at 96°C for 10 min. Amplification was carried out over 45 cycles of 95°C for 15 s, 65°C for 3 s, and 72°C for 10 s with fluorescence measurements taken at the end of the elongation phase. A melt curve was generated after amplification by raising the temperature from 75°C to 95°C at a rate of 0.1°C/second.

Log dilution curves for quantification of *GRIK2* and *G6PD* expression were prepared from serial dilutions ($1–10^{-6}$) of brain cDNA using the *GRIK2* primers and from JURKAT cDNA using the *G6PD* primers for amplification. Reactions were performed in triplicate and mean crossing points plotted against the log concentration of standards to generate a standard curve. Triplicate PCR reactions were also performed using the *GRIK2* and *G6PD* primers and cDNA samples prepared from the cell lines, presentation ALL samples, isolated hematopoietic cells, and tissues. Brain or JURKAT standard dilutions and non–template controls were included in each experiment and levels of expression of *GRIK2* and *G6PD* relative to the standards calculated for each sample by plotting mean crossing points on the standard curves. *G6PD* expression was assumed to be constant across tissue and cell types, and a final level of expression (measured as percentage of *GRIK2* relative to that in brain tissue) was calculated according to the following formula: expression of *GRIK2* relative to brain $= a \times c/b \times 100$, where a = expression of *GRIK2* in tissue relative to expression in brain standard, b = expression of *G6PD* in tissue relative to expression in JURKAT standard, and c = expression *G6PD* in brain relative to expression in JURKAT standard. To avoid the possibility of spurious loss of *GRIK2* transcript detection, as a result of poor cDNA quality, only samples with expression levels of *G6PD* of 10% of the JURKAT standard or greater were analyzed.

Comparison was made between levels of expression of *GRIK2* in T–ALL and B–ALL samples and between cases with and without a 6q deletion overall and among T–ALL cases alone. Paired distributions were tested for compara-

[3] Internet address: http://www.ncbi.nlm.nih.gov.

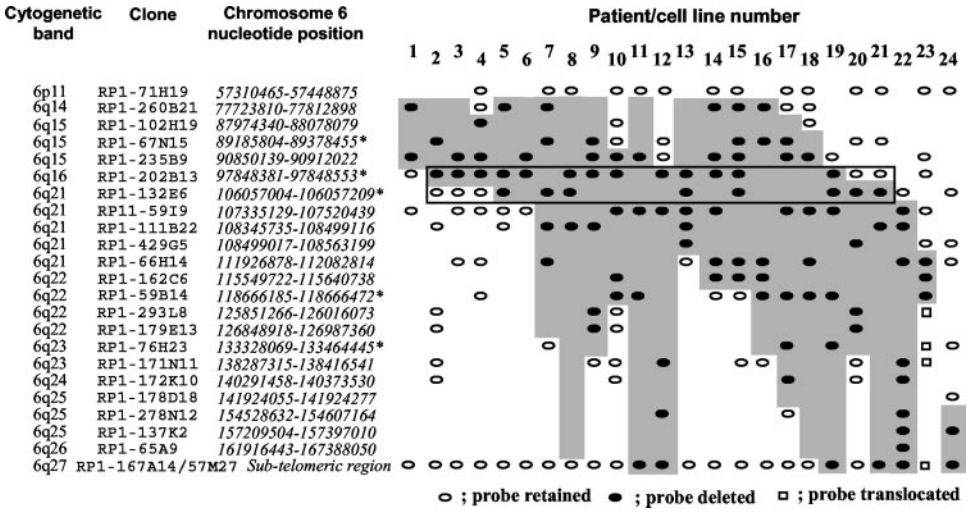

Fig. 2. Primary fluorescence *in situ* hybridization analysis of cases of acute leukemia with deletions of 6q. Chromosome 6 nucleotide positions and chromosomal band assignments are as annotated in the ENSEMBL human genome browser. *, clone not fully sequenced; position refers to a corresponding marker or was estimated from the position of overlapping fully sequenced clones. The maximum extent of each deletion is indicated by *grey infill* and a putative common region of overlap between RP1-202B13 and RP1-132E6, enclosed within the *rectangle*.

bility of variance by F-test. Differences between means of data sets with comparable variance (F-test, $P > 0.05$) were tested by conventional nonpaired student *t* test. Welch's modified student *t* test was used to determine the significance of differences between the means of distributions with significantly different variance as determined by F-test ($P < 0.05$).

RESULTS

FISH Analysis. A deletion of 6q was identified by one or more probes in 24 of 34 patients or cell lines with G-banded evidence of 6q abnormalities. For each case, the position of deleted probes is presented in Fig. 2, whereas clinical details are provided in Table 1. In all except 4 of the 24 cases, deletions included the region between probe RP1-202B13 (6q16) and the adjacent probe RP1-132E6 (6q21). Three cases (2, 3, and 4) were deleted for RP1-202B13 but not RP1-132E6, and 2 cases (20 and 21) retained RP1-202B13 but showed loss of RP1-132E6. FISH analysis with six additional clones demonstrated that five of the deletions overlapped to define a 4.8-Mb RMD between RP1-48F9 and RP1-299C21 (Fig. 3). The new RMD was positioned within band q16, falling within the more proximal of those previously defined (27), and it was deleted in 20 (83%) of the cases included in this study.

Table 1 Patients' and cell lines' clinical details.

	Age at diagnosis (in yrs)	Sex	Disease subtype[a]
1	12	F	Common ALL
2	13	F	Pre-B ALL
3	13	M	T-Cell ALL
4	10	M	T-Cell ALL
5[b]	16	F	T-Cell ALL
6	8	M	T-Cell ALL
7	15	M	T-Cell ALL
8	15	M	T-Cell ALL
9	13	M	T-Cell ALL
10	4	M	Common ALL
11	3	M	Unusual ALL
12	4	M	Common ALL
13[b]	19	M	T-Cell ALL
14	16	M	T-Cell ALL
15	8	M	T-Cell ALL
16[b]	5	F	T-Cell ALL
17	4	F	Pre-B ALL
18	11	M	Common ALL
19	10	M	Common ALL
20		F	ALL
21		M	AML
22	10	M	Null ALL/AML
23	8	F	Common ALL
24		M	AML

[a] ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia.
[b] Cell line derived from a leukemic sample.

Gene Content of the RMD. The region between RP1-48F9 and RP1-299C21 has been fully sequenced (35), and gene scanning using the ENSEMBL human genome browser[4] revealed the presence of 15 genes. Of these, 6 had been previously characterized, 7 corresponded to full-length or near full-length cDNA or protein sequences, and 2 were predicted on the basis of open reading frame analysis and similarity to expressed sequence tags (ESTs) or known protein sequences. Three criteria were applied to prioritize investigation of genes from within the RMD. First, known functional characteristics and expression patterns were considered; second, we evaluated gene expression patterns in hematopoietic tissues by RT-PCR; and third, we compared the position of our RMD with those published by other groups.

Known Functional Characteristics and Expression Patterns. Known or predicted function and published expression profiles of genes from within the RMD are summarized in Table 2. Several were newly identified and thus poorly characterized. These included two putative ubiquitin processing enzymes (*Novel 2* and *FBXL4*), an oxidoreductase (*Novel 1*), a RNA helicase (043738), a methyltransferase (COQ3), and 5 genes of essentially unknown function (*Q9B552*, *C6orf111*, *Q9BRU1*, *PRDM13*, and *DJ467N11.1*). Expression of *Q9BRU1* was found in a chronic myeloid leukemia bone marrow sample but evidence for transcription in normal hematopoietic tissue had not been reported for any of these genes.

The 5 remaining genes included two functionally related transcription factors, *BRN-2* (*POU3F2*) and *SIM-1*, both of which play a role in embryonic neuronal development. *SIM-1* is essential for early stages of development but is also required to maintain expression of *BRN-2* that in turn directs terminal differentiation (41). *BRN-2* is additionally required for melanocyte development and *SIM-1* for differentiation of neuronal cells that regulate aspects of behavior such as appetite. A second gene in the region, the G protein-coupled receptor (*MCH2*), is activated by melanin concentrating hormone and also involved in appetite regulation (42). Lastly, *CCNC* and *GRIK2* have reported functions consistent with a tumor suppressor role. *GRIK2* encodes a transmembrane receptor subunit involved in the transduction of proapoptotic signals (43), and CCNC has been implicated in the negative regulation of cell growth (44). *CCNC* is known to be widely expressed and to have functional activity in lymphocytes

[4] Internet address: http://www.ensembl.org.

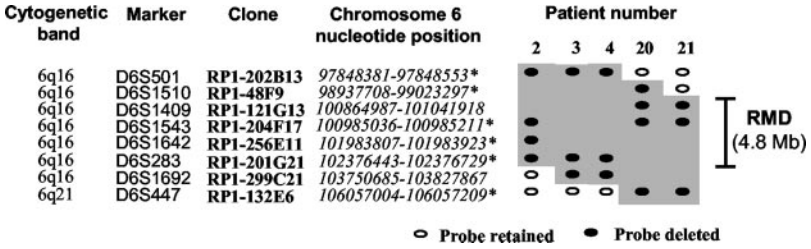

Fig. 3. Secondary fluorescence *in situ* hybridization analysis of the region between P1-derived artificial chromosomes RP1-202B13 and RP1-132E6 in 5 patients with leukemia-related deletions of 6q. Chromosome 6 nucleotide positions and chromosomal band assignments are as annotated in the ENSEMBL human genome browser. *, clone not fully sequenced; position refers to a corresponding marker or was estimated from the position of overlapping fully sequenced clones. A region of minimal deletion (RMD) was defined between P1-derived artificial chromosomes RP1-48F9 (containing marker D6S1510) and RP1-229C21 (containing marker D6S1692).

Table 2 *Gene content of the 6q regions of minimal deletion*

Gene[a]	Function/predicted function[b]	Tissues expression[c]
POU3F2	Also known as brain-specific homeobox/POU domain protein 2 (BRN-2) or nerve system-specific octamer-binding transcription Facter *N*-OCT 3; member of 8 member POU domain protein family	Foetal brain/melanocytes
FBXL4	F box protein, putative ubiquitin protein ligase	Heart, kidney, liver, lung, pancreas, and placenta
Novel (1)	Short chain dehydrogenase/reductase	Unknown
Q9B552	Unknown function	Adenocarcinomas of kidney and ovary
COQ3	Hexaprenyldihydroxybenzoate methyltrannsferase, involved in ubiquinone/coenzyme Q biosynthesis	Unknown
C6orf111	Unknown function	Embryonic kidney
Novel (2)	Ubiquitin COOH-terminal hydrolase 16	Unknown
Q9BRU1	Unknown function	CML[d] bone marrow
CCNC	Cellular cyclin, binds to and activates cyclin-dependent kinase 8 that phosphorylates a subunit of RNA polymerase II; negative regulator of cell growth	Widely expressed
PRDM13	PR-domain zinc finger protein 13, unknown function	Unknown
GPR145	Melanin concentrating hormone receptor 2 (MCH2/MCHR2), G protein-coupled receptor: thought to be involved in the regulation of food intake and energy balance	Predominantly brain
SIM1	Homologue of (Drosophila) single-minded 1, transcription factor involved in embryogenesis	Foetal kidney, CNS, mesoderm, and endoderm
043738	RNA helicase (fragment), member of U5 small ribonuclear protein family	Melanoma
DJ467N11.1	Unknown function	Unknown
GRIK2	Glutamate receptor subunit, member of ionotropic glutamate receptor family; prominent role in neurotransmission and neuronal development/apoptosis	CNS, heart

[a] Genes are listed in order according to position in a centromeric to a telomeric orientation.
[b] Gene names and function are as annotated in the ENSEMBL human genome browser.
[c] Tissue expression patterns are taken from the National Center for Biotechnology Information Reference Sequence database, Human Genome Organisation Gene Cards, or the literature.
[d] CML, chronic myelogenous leukemia; CNS, central nervous system.

(45), whereas *GRIK2* expression has been reported only in the central nervous system and heart.

Expression in Hematopoietic Tissue as Assessed by RT-PCR. We performed RT-PCR using paired primers specific for exon sequences from each of the genes within the RMD and cDNA from whole bone marrow or brain. A visible product of the expected size was obtained with *CCNC*-, *C6orf111*-, and *GRIK2*-specific primers using cDNA from bone marrow samples and with *CCNC*, *C6orf111*, *GRIK2*, and *MCH2* primers using brain cDNA. Amplification from the brain sample with the *GRIK2* primers produced a markedly stronger signal than from bone marrow (by ethidium bromide staining of electrophoresed PCR products; Fig. 4), whereas levels of expression of *CCNC* and *C6orf111* were comparable in the two tissues.

Relative Position of Published Regions of Minimal Deletion. Genetic markers, defining the proximal and distal boundaries of eight regions of minimal deletion, were identified from seven published studies; all included cases of ALL with 6q deletions mapped by FISH or loss of heterozygosity (Table 3). Base pair positions of each marker were obtained from the ENSEMBL human genome browser version 14. The position of the new FISH RMD was then mapped relative to each of those published and found to overlap with six of them (Fig. 5). More detailed analysis of published data revealed that all but one of the six regions included exons of a single gene, *GRIK2*. Furthermore, individual patients from one study showed a pattern of loss of heterozygosity consistent with deletions confined to within this gene (Fig. 6). Because *GRIK2* was also 1 of the 3 genes within our RMD for which we could detect expression in bone marrow (albeit at low level), the gene was selected for additional analysis. A homology search using the BLAST facility of National Center for Biotechnology Information of genomic sequences containing the *GRIK2* exons (accession nos. AP002528 to AP002531) also identified 18 ESTs (accession nos. AI151464, AI458823, AI871030, AA416738D, AW172953, BE004183, D44785, D60804, D60803, H09902, H09809, AI904894, AA984511, AW162049, AA985125, AA911579, AF017693, and AA479978). On the basis of sequence homology to common repeats, lack of evidence for splicing, and lack of cross-species conservation, it seemed unlikely that any of the ESTs represented functional genes and none were additionally analyzed.

Mutation Analysis of *GRIK2* Exons. To investigate the possibility that mutations within the coding region of *GRIK2* underlie selection of 6q deletions in ALL, we sequenced each exon in 14 patients displaying heterozygous loss of FISH probes or microsatellite markers

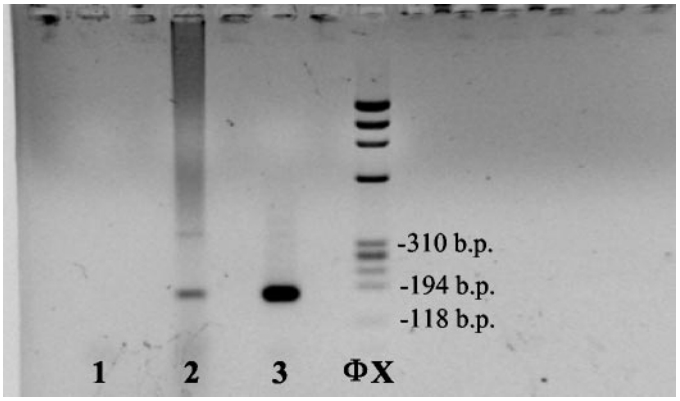

Fig. 4. Reverse transcription-PCR analysis of *GRIK2* using H_2O (*Lane 1*), bone marrow cDNA (*Lane 2*), brain cDNA (*Lane 3*). A product of the expected 173-bp size was amplified from bone marrow and brain samples.

Table 3 *The relative position of 6q regions of minimal deletion taken from studies that include cases of ALL*[a]

Reference no.	Type of analysis used[b]	Patients included in study[c]	Proximal boundary of RMD[d]		Distal boundary of RMD[d]	
			Marker	Nucleotide position	Marker	Nucleotide position
32	LOH	T-ALL	D6S1652	85900740	D6S1644	89676208
30	LOH	ALL^{ped}	D6S275	93013258	D6S283	102228253
30	LOH	ALL^{ped}	D6S283	102228003	D6S302	111834590
31	LOH	ALL^{ped}	D6S1709	102226866	D6S434	102304176
28	FISH^{int}	ALL, CLL, NHL	D6S1565	99627218	D6S434	102304176
29	LOH	ALL^{ped}	D6S301	103530714	D6S1594	108344938
26	FISH^{met}	NHL, ALL	D6S447	105869805	D6S246	108300123
38	LOH	ALL	D6S1709	102226866	D6S278	108163643

[a] ALL, acute lymphocytic leukemia; RMD, region(s) of minimal deletion; LOH, loss of heterozygosity; FISH, fluorescence *in situ* hybridization.
[b] FISH analysis of interphase cells (FISH^{int}) or metaphase cells (FISH^{met}); LOH by PCR of microsatellites (LOH).
[c] Disease classification: mixed adult and childhood B- and T-cell ALL (ALL), pediatric B- and T-cell ALL (ALL^{ped}), adult T-cell ALL (T-ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma (NHL).
[d] Markers are the retained proximal and distal markers flanking the published RMD and nucleotide position refers to the chromosome 6 bp positions of the markers as annotated in the ENSEMBL database. For proximal RMD boundaries, the distal bp position of markers is used and, for distal boundaries, the proximal bp position.

in the region. In all cases, the expected wild-type sequences were obtained, except for exon 6 in 1 patient (A) and exon 15 in patients B, E, H, and I. The bp change seen in exon 15 was identified as a silent G to A transition at position 114 in all 4 patients and was found to correspond to a polymorphism identified by the single nucleotide polymorphism consortium[5] (DbSNP: 2227283). By contrast, no known single nucleotide polymorphism existed at the site of the C to G transversion (bp 113) observed in exon 6 in patient A (Fig. 7, *A* and *B*). Moreover, the substitution resulted in an amino acid change from serine (TCG) to tryptophan (TGG) at position N297C of the GRIK2 protein. To determine whether the C to G bp change arose through somatic mutation, DNA from the patient's remission marrow sample was amplified with the exon 6 primers and sequenced. Examination of the sequence trace (Fig. 7*C*) revealed the coexistence of both C and G residues at the site of transversion, demonstrating that it was of germ line origin.

The C to G bp change found in patient A also resulted in the abolition of a Taq I restriction enzyme site and therefore could be screened for by digestion of exon 6 PCR-amplified DNA. We performed Taq I digests of *GRIK2* exon 6 amplified from 116 random control DNA samples and observed only digestion products associated with two known polymorphisms. Taken together with the 14 ALL samples, only one among 246 tested alleles therefore carried the C to G transversion. We also tested DNA samples from the patient's parents and found a similar pattern in the paternal DNA (confirmed by sequencing analysis). The paternal side of the family was confirmed to be of Caucasian origin, although additional medical history was unavailable.

Quantitative Analysis of *GRIK2* Expression in Hematopoietic and Other Tissues. Using real-time quantitative RT-PCR expression of *GRIK2* in normal hematopoietic cells was either low or undetectable but with the highest levels in thymus and purified T cells (0.4 and 0.33%, compared with brain; Tables 4 and 5). Furthermore, *GRIK2* expression was higher in T-cell leukemia (compared with B-cell malignancies) and in the T-cell line JURKAT (compared with the myeloid cell lines K-562 and HL-60 and B-cell lines RAJI and U-266). In cell lines and patients without deletions of 6q, *GRIK2* expression was detectable in 9 of 10 T-ALLs but in only 6 of 12 B-cell ALLs. Excluding patient 23, which showed evidence for aberrant up-regulation, the median expression of *GRIK-2* was lower in B-ALL (0.067) compared with T-ALL (0.1423), although overall the difference between these two groups was not statistically significant.

A much reduced or totally absent level of *GRIK2* expression was detected in 5 cases of T-ALL and in 2 cases of B-ALL with 6q deletions. In patients with T–lineage ALL, 6q deletion was associated with a statistically significant reduction in *GRIK*2 expression (from a mean value of 0.1423 to 0.00097). Because in more than half of B-cell leukemias, *GRIK2* expression was undetectable, the effect of del(6q) in the 2 cases investigated was not meaningful.

Exceptionally high levels of *GRIK2* expression (8.55 and 9.14% of the level detected in brain) were found in 1 case of common ALL (patient 23) and in the myelomonocytic cell line THP-1. This finding is of particular relevance because *GRIK2* expression could not be detected in isolated normal monocytes or B cells (Table 4). Immunophenotypic and karyotypic analysis confirmed the THP-1 cells to be myelomonocytic and carrying the expected published chromosomal abnormalities (German tissue culture collection animal and human cell line database) with no 6q abnormality. Expression levels of *GRIK2* were also confirmed to be high on a second cDNA sample made from freshly prepared THP-1 RNA and from the patient 23 sample. Expression was comparatively abundant in whole prostate (9.6%), kidney (6.0%), and trachea (8.2%) tissue and intermediate in lung (2.6%).

DISCUSSION

The ionotropic glutamate receptors function as membrane bound ligand-gated ion channels and are classified according to their pharmacological agonists *N*-methyl-D-aspartate, alpha-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA), or kainate (46). Binding of homomeric or heteromeric glutamate receptors by their ligands causes Ca^{2+} or Na^{2+} efflux and can also transduce signals through cytoplasmic proteins. In brain, glutamate is the predominant neurotransmitter but has other functions such as the regulation of growth, migration, and survival of developing neurons (47–49). Recently, it has become evident that different tissues throughout the body possess glutamate receptors (reviewed in Ref. 50).

We identified a kainite receptor (*GRIK2*) as 1 of 3 genes within a RMD-derived from FISH analysis of 24 leukemic samples. Of the 2 remaining genes, 1 (*CCNC*) had known functions compatible with a tumor suppressor role but no mutations of *CCNC* had been found by others (51) in 8 patients with lymphoid malignancies and del(6q). Furthermore, a comparison of published deletion data clearly singled out *GRIK2* as worthy of further study. We observed a novel, germline, and paternally transmitted C to G transversion in exon 6 of *GRIK2* in 1 of 14 ALL samples with heterozygous 6q deletion. The mutation resulted in the substitution of serine for tryptophan in the extracellular domain of the GRIK2 protein. Currently, there are only two annotated polymorphisms of *GRIK2* that result in changes at the protein level (National Center for Biotechnology Information single

[5] Internet address: http://snp.cshl.org.

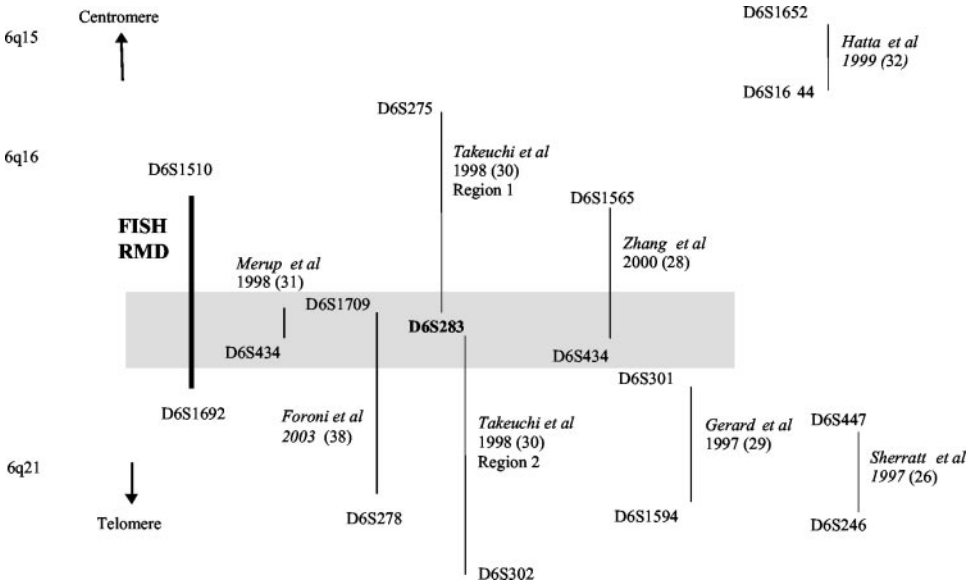

Fig. 5. The relative position of published regions of minimal deletion (RMD) derived from fluorescence *in situ* hybridization (FISH) or loss of heterozygosity analysis of lymphoid malignancies in relation to the new FISH RMD. The chromosome 6 nucleotide position of the boundaries of each RMD, method of analysis, and disease subtypes of patients included in each study are presented in Table 3. Six of nine RMD overlapped to give a consensus region centered on marker D6S283 (highlighted by the *grey rectangle*).

nucleotide polymorphism database[6] numbers rs3213608 and rs2235076) but neither falls within the extracellular domain. Moreover, the substitution is centered within a region (N19-407C) that has been strictly conserved between mouse and man and therefore, it is likely to affect receptor function. The patient's father to date remains unaffected by leukemia. However, as with those underlying familial retinoblastoma, this mutation might act in a semipenetrant fashion with malignancy developing only if a critical second hit (deletion of the second allele of *GRIK2* as detected cytogenetically in our patient) takes place. Because the patient carrying the abnormal *GRIK2* sequence was 4 years old at the time of diagnosis, it is also possible that, as is the case with familial retinoblastoma, the second hit has pathological consequences only if acquired before a certain age or perhaps *in utero*. Loss of the *GRIK2* region appears to be associated with childhood rather than adult ALL as seen in our study and those of others (28, 30, 31, 38), supporting a model in which the gene is tumor protective in the immature hematopoietic system. Several cytogenetic rearrangements have strong association with childhood leukemia. Fusion transcripts from at least two of these (*ETV6-AML1* and *AML1-ETO*) are detectable in the blood of neonates at a frequency that is 100-fold greater than that of the corresponding leukemia (52), suggesting that a somatically acquired translocation is leukemogenic only if a critical second hit occurs relatively early in hematopoietic development.

We demonstrated that somatic mutations within the coding region of *GRIK2* are not commonly associated with cytogenetic deletion in ALL, although we did not rule out the possibility that inactivation of the retained copy of the gene occurred through epigenetic silencing, post-transcriptional mechanisms, or mutation within upstream regulatory sequences. We also cannot exclude that haploinsufficiency of *GRIK2* contributes to ALL. However, although investigated specifically for neurological dysfunction, no leukemia was observed in mice carrying a homozygous *GRIK2* deletion (53) at least up to 6 months of age.

Expression of *GRIK2* was low in normal hemopoietic cells compared with levels in brain but was most prominent in those of T-lineage, and it was consistently detected among T-cell leukemias, except in the presence of 6q deletion. These observations support the possibility that in T-ALL at least, haploinsufficiency might reduce *GRIK2* expression below a critical tumor protective threshold level.

[6] Internet address: http://www.ncbi.nlm.nih.gov/SNP/.

A number of recent studies have demonstrated that various neurotransmitters contribute to signaling in T cells (54). Glutamate was reported to bind specific sites on T-lymphocyte plasma membranes and impair the activity of macrophages and lymphocytes *in vitro* (55–57). In carcinoma patients, elevated plasma glutamate levels were linked to a reduced lymphocyte response to mitogens and a high death rate (58, 59). Specific mediators of glutamate signaling were not identified in these studies, but involvement of the AMPA-specific ionotropic glutamate receptor GluR-3, in integrin-mediated adhesion and chemotactic migration of human T cells, was reported recently (60). Our study places additional emphasis on the potential signifi-

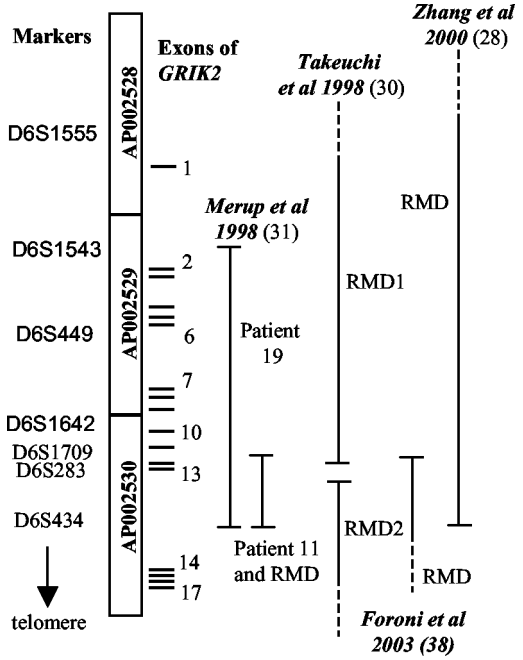

Fig. 6. The positional relationship between the new fluorescence *in situ* hybridization (FISH) region of minimal deletion (RMD), exons of *GRIK2*, and published RMD derived from loss of heterozygosity or FISH analysis of cases of acute lymphocytic leukemia. Markers used to define the RMD and exons of *GRIK2* were positioned on sequenced fragments AP002528, AP002529, and AP002530 by paired blast search. In total, six RMD encompassed exons of *GRIK2*, including two that were derived from a single study (RMD1 and RMD2; Ref. 30). Individual patients from one study displayed a pattern of loss of heterozygosity consistent with the presence of deletions confined to within the coding region of *GRIK2* (patients 11 and 19; Ref. 31).

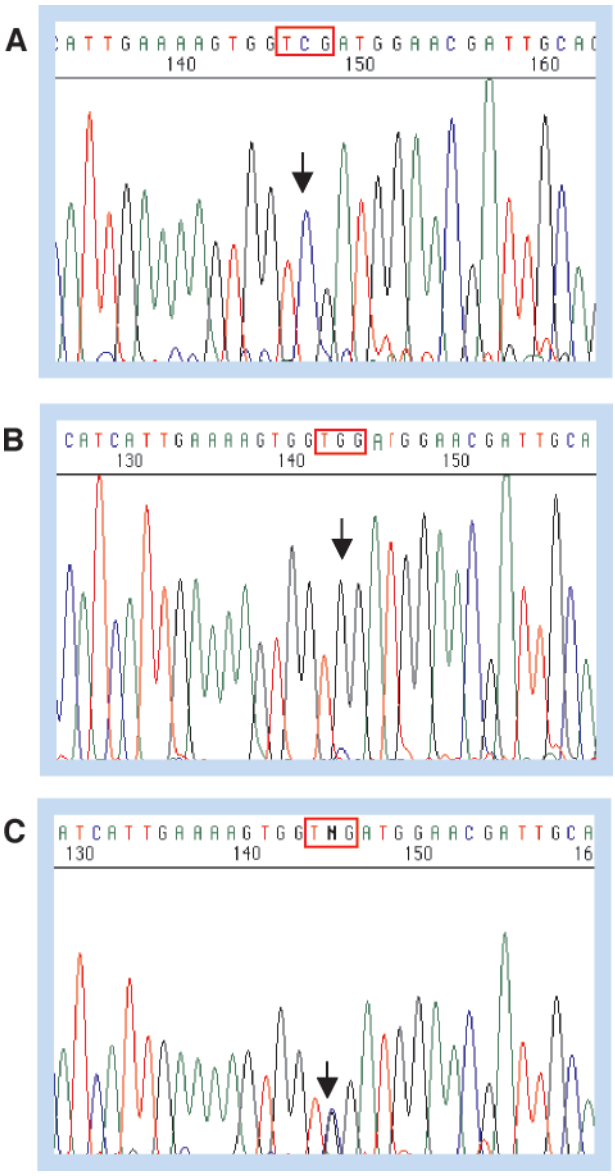

Fig. 7. Partial sequences of *GRIK2* exon 6, the *arrow* marks position 113 in each case. *A*, normal sequence with C at position 113 as part of triplet TCG encoding serine. *B*, presentation sample from patient A with G at position 113 as part of triplet TGG encoding tryptophan. *C*, remission sample showing coexistence of C and G at position 113.

Table 4 *Relative expression of GRIK2 in whole tissues and isolated hematopoietic cells*

	Origin of c-DNA	Level of GluR-6 expression relative to brain (%)
Whole tissue	Brain	100
	Thymus	0.4 ± 0.36
	Prostate	9.6 ± 1.7
	Kidney	6.0 ± 1.3
	Bone marrow	0.00
	Lung	2.6 ± 0.94
	Trachea	8.2 ± 1.0
Isolated hematopoietic cells	Granulocytes	0.0047
	Monocytes	0.00
	T cells	0.33 ± 0.18
	B cells	0.00
	Natural killer cells	0.00

cance of glutamate signaling in T cells and is the first to demonstrate that a kainate-specific glutamate receptor is expressed in the hematopoietic system.

Although non-T-cell leukemias showed less consistent evidence for *GRIK2* expression, unexpectedly high levels of transcription (~9% of the level in brain) were found in 1 patient with common ALL and in the cell line THP-1. The reasons for selective up-regulation in these two different types of cells remain unclear but demonstrate that relatively high levels of *GRIK2* transcription can occur in hemopoietic cells and be associated with a malignant phenotype. Ectopic expression of the metabotropic glutamate receptor 1 has recently been reported in melanomas (61), and it is possible that similar aberrant gene deregulation drives expression of *GRIK2* in THP-1 and patient 23. Alternatively, these clones may have arisen through transformation of normal hematopoietic cells with endogenous high levels of *GRIK2*.

Reflecting the diverse consequences of glutamate signaling, both up- and down-regulation of glutamate receptor expression have been reported in brain tumors when compared with their normal cellular counterparts (62, 63). Tumor-related loss of glutamate receptor function is likely to be driven by evasion of apoptosis, induced by glutamate through Ca^{2+} mobilization, or activation of signaling cascades via cytoplasmic proteins (43). Other functions such as trophic effects of glutamate signaling are likely to play a part in the tumor related up-regulation of glutamate receptors. A tumor-promoting role for glutamate is not restricted to cells of the central nervous system because *N*-methyl-D-aspartate and AMPA receptor antagonists have been reported to inhibit growth and invasion of several non-neuronal malignancies and their use as anticancer agents has been proposed (64, 65). The exceptionally high levels of *GRIK2* expression observed in 2 of 34 (5.8%) leukemic samples analyzed suggest that glutamate signaling might positively regulate clonal expansion of some leukemic cell types. THP-1 cells are known to excrete high levels of glutamate (66), so glutamate receptors potentially form part of an

Table 5 *Relative expression of GRIK2 in presentation ALL[a] samples and leukemic cell lines with and without deletion of 6q*

Cell line or patient no.	Immunophenotype	% of cells with del(6q)	Level of GRIK-2 expression relative to brain (%)
JURKAT	T	0	0.17 ± 0.03
K-562	Myeloid	0	0.010 ± 0.00013
RAJI	B	0	0.014 ± 0.011
THP-1	Myelomoncytic	0	9.14 ± 0.55
U-266	B	0	0.0062 ± 0.00233
REH	Myeloid	0	0.00
HL60	Myeloid	0	0.00
PEER	T	100	0.00071 ± 0.00051
MOLT-4	T	100	0.00
Patient 1	T	0	0.26 ± 0.091
Patient 2	T	0	0.58 ± 0.30
Patient 3	T	0	0.018 ± 0.0067
Patient 4	T	0	0.061 ± 0.0022
Patient 5	T	0	0.278 ± 0.021
Patient 6	T	0	0.00
Patient 7	T	0	0.017 ± 0.0032
Patient 8	T	0	0.0138 ± 0.0082
Patient 9	T	0	0.025 ± 0.0023
Patient 10	T	98	0.00414 ± 0.0002
Patient 11	T	82	0.00
Patient 12	T	80	0.00
Patient 13	B	0	0.00
Patient 14	B	0	0.00
Patient 15	B	0	0.00
Patient 16	B	0	0.027 ± 0.0043
Patient 17	B	0	0.010 ± 0.0030
Patient 18	Pre B	0	0.40 ± 0.12
Patient 19	Pre B	0	0.00
Patient 20	Common	0	0.00
Patient 21	Common	0	0.00
Patient 22	Common	0	0.00
Patient 23	Common	0	8.55 ± 0.78
Patient 24	Common	100	0.00
Patient 25	Common	100	0.00

[a]ALL, acute lymphocytic leukemia; T, T-cell ALL; B, B-cell All; Pre B, Pre B-cell ALL; Common, common B-cell ALL.

autostimulatory loop in this line. It will therefore be of interest to additionally characterize the role of GRIK2 and other glutamate receptors in THP-1 and in hematological malignancies in general.

Expression of *GRIK2* has only been previously reported in the central nervous system and in the rat heart (67). In addition to hematopoietic cells, we detected variable levels in thymus, prostate, kidney, lung, and trachea, suggesting that it functions in a wide range of tissues. Because deletions of chromosome 6 are also common to diverse tumor types, our data raise the possibility that *GRIK2* acts as a multilineage tumor suppressor gene.

The present and published data support the possibility that complete or partial loss of *GRIK2* function contributes to some of the lymphoid leukemic proliferations. Of other genes within our RMD, we also observed expression of CCNC and C6orf111, making both of these candidate ALL tumor suppressors, which should not be excluded from future analysis. We found no evidence for expression of the remaining genes recognized by ENSEMBL and positioned within our RMD. However, numerous ESTs that did not correspond to computer predicted open reading frame or known cDNA or protein sequences could be mapped to the region. Both the large number and the lack of support for any functional role of these ESTs made them unsuitable for additional investigation, although we recognize that some of these noncoding RNA molecules may still play regulatory roles in the expression of other genes. Finally, heterogeneity in the position of RMD identified in different studies may reflect the existence of more than 1 ALL-related tumor suppressor gene on 6q and genes positioned proximal and distal to the one we have investigated cannot be excluded as potential candidates.

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