The LGI1 gene involved in lateral temporal lobe epilepsy belongs to a new subfamily of leucine-rich repeat proteins

Wenli Gu[a], Andrea Wevers[b], Hannsjörg Schröder[b], Karl-Heinz Grzeschik[c], Christian Derst[d], Eylert Brodtkorb[e], Rob de Vos[f], Ortrud K. Steinlein[a,*]

[a] *Institute of Human Genetics, University Hospital Bonn, Wilhelmstrasse 31, D-53111 Bonn, Germany*
[b] *Institute for Anatomy II, Neuroanatomy, University of Cologne, Joseph-Stelzmann Strasse 9, 50931 Cologne, Germany*
[c] *Department of Human Genetics, University of Marburg, Bahnhofstrasse 7, 35037 Marburg, Germany*
[d] *Institute of Physiology II, University of Freiburg, Herman Herder Strasse 7, 79104 Freiburg, Germany*
[e] *Department of Neurology, Trondheim University Hospital, N-7006 Trondheim, Norway*
[f] *Laboratorium Pathologie Oost Nederland, Burgemeester Edo Bergsmalaan 1, 7512 AD Enschede, The Netherlands*

Received 14 March 2002; revised 10 April 2002; accepted 12 April 2002

First published online 25 April 2002

Edited by Guido Tettamanti

Abstract Recently mutations in the LGI1 (leucine-rich, glioma-inactivated 1) gene have been found in human temporal lobe epilepsy. We have now identified three formerly unknown LGI-like genes. Hydropathy plots and pattern analysis showed that LGI genes encode proteins with large extra- and intracellular domains connected by a single transmembrane region. Sequence analysis demonstrated that LGI1, LGI2, LGI3, and LGI4 form a distinct subfamily when compared to other leucine-rich repeat-containing proteins. In silico mapping and radiation hybrid experiments assigned LGI2, LGI3, and LGI4 to different chromosomal regions (4p15.2, 8p21.3, 19q13.11), some of which have been implicated in epileptogenesis and/or tumorigenesis.

Key words: Epilepsy; Glioma; Chromosomal localization; Expression profile; Gene family

1. Introduction

Mutations in the LGI1 gene (leucine-rich, glioma-inactivated 1) were recently found in families with autosomal dominant lateral temporal lobe epilepsy (ADLTE) [1], a rare idiopathic partial epilepsy syndrome. LGI1 encodes a putative membrane-anchored protein of unknown function, which is characterized by a leucine-rich repeat (LRR) domain flanked by conserved cysteine clusters. LGI1 has been cloned from the t(10:19) balanced translocation breakpoint in a glioblastoma cell line, and was therefore named according to its possible function as a tumor-suppressor gene. The possible role in tumorigenesis was further supported by the observation of LGI1 down-regulation in malignant gliomas [2]. Thus it came as a surprise that LGI1 is associated with epilepsy in several families not known for an unusual accumulation of brain tumors or other malignancies [1]. So far all genes known to be mutated in human idiopathic epilepsies were encoding ion channels [3]. Other genes, like the gene encoding the anticonvulsant peptide dynorphin, are associated with an increased risk for convulsions, but cannot cause epilepsy by themselves [4]. LGI1 presented the first evidence that genes not encoding ion channels can be mutated in these common neurological disorders. Genes that are functionally or structurally related to LGI1 now have to be regarded as candidates for different epilepsies. We therefore used a combined in silico- and bench-cloning approach to search for sequences with high similarities to LGI1 and identified three formerly unknown genes named LGI2, LGI3, and LGI4. Comparison of predicted protein sequences and structures showed that the four LGI genes belong to a formerly unknown subfamily within the superfamily of LRR proteins. We describe the cDNA and deduced amino acid sequences, as well as differences in mRNA expression patterns for the human (*h*) LGI1–LGI4 genes. Furthermore, we examine the phylogenetic origin and present chromosomal assignments for *h*LGI and murine LGI genes.

2. Materials and methods

2.1. Database search and sequence computation

To identify *h*LGI sequences a translated basic local alignment search tool nucleotide (tBLASTn) search with the known *h*LGI1 peptide sequence (NM005097) was carried out using the NCBI human expressed sequence tag (EST) database (http://www.ncbi.nlm.nih.gov). The genomic sequences containing cDNA fragments were retrieved by basic local alignment tool (BLAT) search from the Human Genome Project Working Draft database (HGWD) (http://www.genome.ucsc.edu) (*h*LGI2, *h*LGI4) and by BLASTN search from human genomic sequence scaffolds and unordered pieces in the Celera Genomics database (http://www.celera.com) (*h*LGI3). The *h*LGI sequences were used to retrieve mouse LGI (*m*LGI) sequences from both databases. EST contig building and sequence alignment was carried out using the Heidelberg Unix Sequence Analysis Resources (HUSAR) DNA analysis package (http://genius.embnet.dkfz-heidelberg.de).

*Corresponding author. Fax: (49)-228-287 2380.
E-mail addresses: wevers.anatomie@uni-koeln.de (A. Wevers), grzeschi@mailer.uni-marburg.de (K.-H. Grzeschik), derst2@mailer.uni-marburg.de (C. Derst), eylert.brodtkorb@medisin.ntnu.no (E. Brodtkorb), rdevos@laborpath.nl (R. de Vos), ortrud.steinlein@ukb.uni-bonn.de (O.K. Steinlein).

Abbreviations: ADLTE, autosomal dominant lateral temporal lobe epilepsy; BLAT, basic local alignment tool; EST, expressed sequence tag; HUSAR, Heidelberg Unix Sequence Analysis Resources; LGI, leucine-rich glioma-inactivated; LRR, leucine-rich repeat; tBLASTn, translated basic local alignment search tool nucleotide

PII: S0014-5793(02)02713-8

2.2. RT-PCR, genomic PCR and cDNA amplification

To clone the complete reading frames of *h*LGI genes, RT-PCR was performed using the Titan RT-PCR kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's manual. For verification of the intron–exon boundaries PCR reactions were performed with genomic control DNA as template, which was prepared from the blood of a healthy German individual. The PCR profile consisted of an initial 3 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, with a final incubation of 10 min at 72°C. Amplified products were sequenced using an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The distribution of *h*LGI mRNA expression was analyzed using the Multiple Tissue cDNA Panel (Clontech, Palo Alto, CA, USA). The amplification protocol included an initial denaturation step (94°C, 3 min), followed by 35 cycles of amplification (94°C, 30 s; 55°C, 30 s; 72°C, 30 s) and a final extension step (72°C, 5 min).

2.3. Radiation hybrid mapping

For chromosomal assignment of *h*LGI2, *h*LGI3, and *h*LGI4 gene specific amplification from the GenBridge 4 radiation hybrid panel was performed. Primer pairs are given in the legend to Fig. 1.

2.4. Immunohistochemical visualization of LGI1 in the human cortex

Brain specimens were obtained upon autopsy from control individuals who were unknown to be affected by neurological diseases during life. Furthermore, the brains of all individuals studied were free of apparent neuropathological changes, like amyloid plaques, neurofibrillary tangles, vascular lesions, or obvious structural abnormalities. Immunohistological staining was performed on 10 μm cryostat sections from the frontal cortex of a 38-year-old male and a 44-year-old female as well as on 7 μm paraffin sections from the temporal cortex (five individuals, age range 10–59 years, three males, two females). Cryostat sections were incubated either with a commercially available goat antiserum (10 μg/ml) raised against a peptide mapping at the amino-terminus of LGI1 (N-18, sc-9581, Santa Cruz, CA, USA) or with a rabbit antiserum specific for LGI1 (gift of J. Cowell) diluted 1:200, whereas paraffin sections were incubated with the rabbit antiserum diluted 1:50. Incubations with the primary antibodies were carried out overnight at 4°C. Visualization of antibody binding was achieved by a biotinylated secondary antibody and a streptavidin–peroxidase complex (ABC technique) using the chromogen diaminobenzidine according to standard protocols. In case of paraffin sections pretreatment with 0.05% trypsin (pH 7.8, 20 min, 37°C) was mandatory prior to the application of rabbit antiserum. For control purposes, the working dilution of sc-9581P antiserum was preincubated with its peptide (50 ng and 100 ng, sc-9581, Santa Cruz) prior to application.

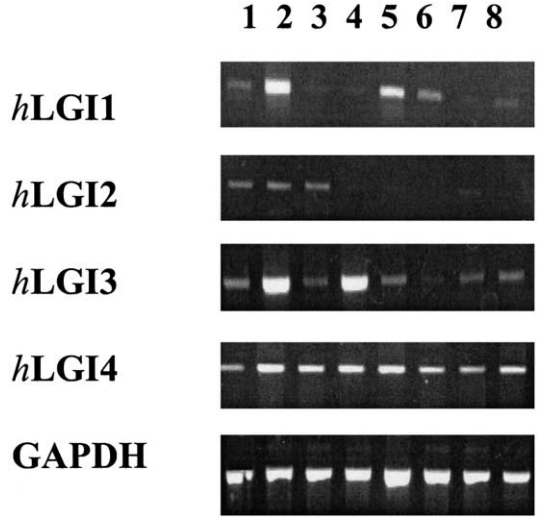

Fig. 1. *h*LGI1–*h*LGI4 mRNA expression patterns. Semi-quantitative PCR was carried out using the following primer pairs: *h*LGI1, 5′-ATTTTCCTTGAATGGGACCATGTGG-3′, 5′-TGAGGCGTTCTGAGTGTCTGAGGT-3′; *h*LGI2, 5′-TTTGTCATCGCAGACAGCTC-3′, 5′-AAGCGGGTAAGGGAAAGGTA-3′; *h*LGI3, 5′-CTGTTTGGCGGCTCTTACAT-3′, 5′-GAGCTGCTGGACACAATCAG-3′; *h*LGI4, 5′-GAAGGGCAACCCTGCTTC-3′, 5′-ATGTAGCGTGTGAGGCACAG-3′. GADPH primers were used to allow for normalization of RNA concentration. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, muscle; lane 7, kidney; lane 8, pancreas. The *h*LGI2, *h*LGI3, and *h*LGI4 primers given here were also used for radiation hybrid mapping.

3. Results

3.1. Cloning of LGI2, LGI3, and LGI4

The tBLASTn search identified different human EST fragments, which encoded peptides with high similarity to parts of LGI1. RT-PCR was performed to verify the in silico-cloned sequences and to fill in the gaps. The cDNA sequences of three novel genes, containing the intact open reading frames, could thus be identified. The genes were named LGI2, LGI3, and LGI4, their sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/GenBank) (accession numbers: AF467954–AF467956). The sequences of the four *m*LGI genes were determined in silico.

3.2. Sequence comparison

*h*LGI2, *h*LGI3 and *h*LGI4 encode predicted proteins of 545, 549, and 538 amino acid residues, respectively. Fig. 2 shows the alignment of the four proteins. Hydropathy calculation (MacMolly Tetra Lite, Soft Gene, Berlin, Germany) indicated a signal peptide at the N-terminus and a putative transmembrane region at a position conserved among all LGI peptides (Fig. 2). Human and murine LGI peptides have identical overall structures, with four complete and one partial LRR in the putative extracellular part. Cysteine-rich regions flank the LRRs on both the N-terminal and the C-terminal side, the N-terminal region showing the conserved cysteine motif CX_3CXCX_6C [5,6]. Such cysteine-consensus flanks are characteristic for most extracellular LRR proteins. With the exception of the third LRR of LGI4, all LGI LRRs have the conserved phenylalanine at position 20 and thus belong to the so-called 'F-20' family of LRR, which also includes proteins like slit, biglycan and osteomodulin [6]. The predicted intracellular parts of the four LGI peptides were highly similar to each other, no known sequence motifs could be identified for either LGI peptide. For each of the LGI peptides the intracellular part contained several different putative phosphorylation site consensus sequences, some of them were conserved within the subfamily (Fig. 2). The high degree of similarity among the LGI peptides is not only present in the LRR repeats, but was found through the entire length of the peptides. The extracellular parts of the peptides share 61–72% similarity with each other, the intracellular parts 62–72%. The transmembrane domain represents the most conserved region of the peptides, with 75–99% similarity among the different LGI members. The overall similarity between the *m*LGI and *h*LGI genes was 97% for LGI1, 98% for LGI2, 97% for LGI3, and 93% for LGI4.

3.3. Gene structure

The genomic organizations of the new *h*LGI and murine LGI genes were deduced from the alignment of their cDNA sequences with the corresponding genomic fragments. The positions of intron–exon boundaries in *h*LGI1 and *h*LGI4

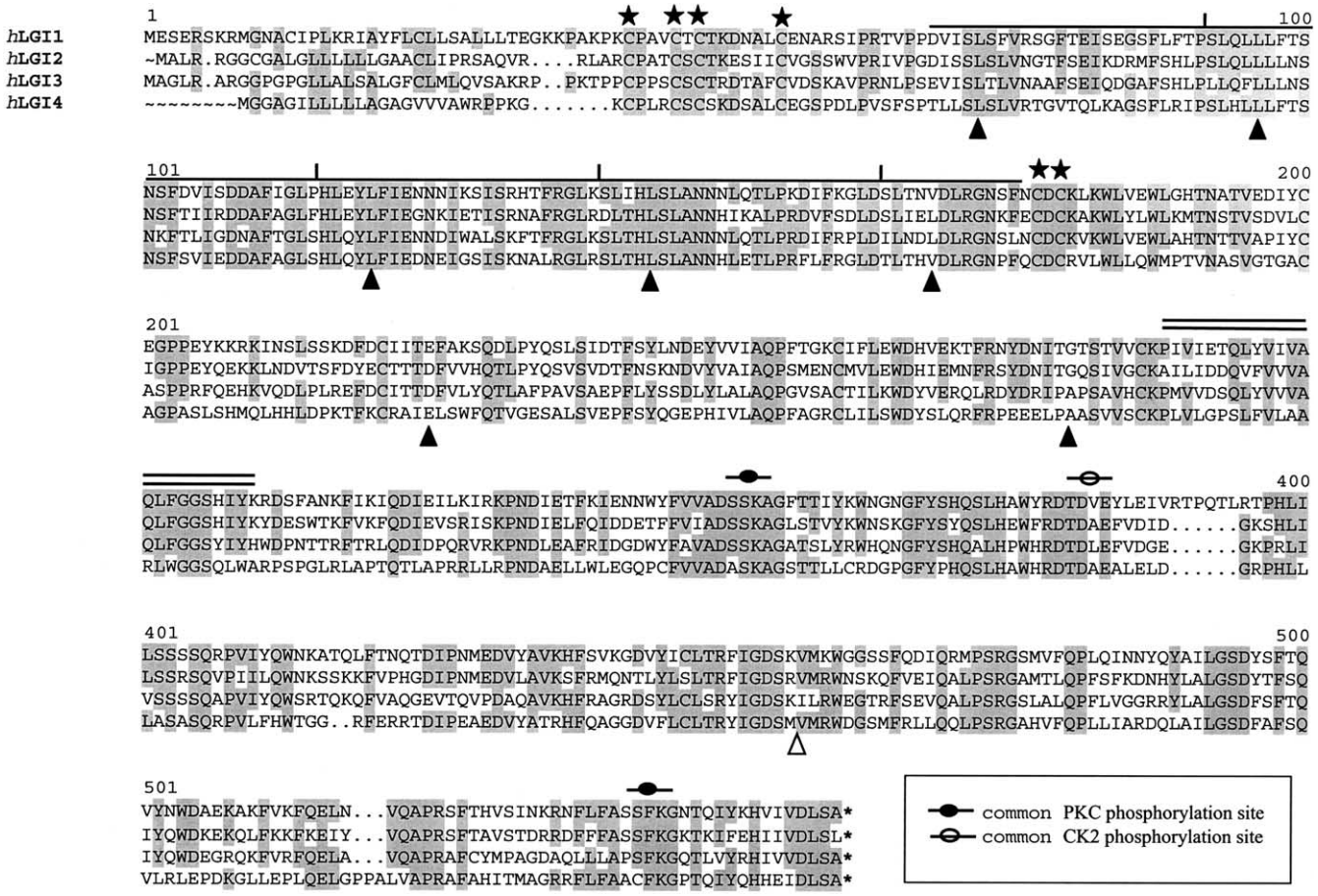

Fig. 2. Alignment of deduced amino acid sequences of *h*LGI1, *h*LGI2, *h*LGI3 and *h*LGI4. Shaded boxes indicate the shared amino acid sequence identity between at least three genes. Stars indicate conserved cysteine residues, the LRR-containing regions are marked by a single line (LRRs are separated by vertical bars), and a double line indicates the regions predicted to contain the transmembrane domain. Dots indicate amino acids not found in one or more LGI genes. The positions of introns in the genomic sequence are marked by inverted triangles (filled triangles, introns shared by all four genes; open triangle, intron only found in LGI4). The numbers refer to amino acid residues in the LGI1 protein sequence. An asterisk indicates stop codons. Potential glycosylation sites found in at least three LGI genes are marked by half moons (filled half moon, casein kinase II phosphorylation site; open half moon, protein kinase C phosphorylation site).

were experimentally verified by PCR amplification of genomic DNA. As shown in Fig. 2, seven introns are inserted at strictly conserved positions in all four LGI genes. The same intron positions were found in the respective murine genes (data not shown). The first five introns are inserted between the second and third nucleotides of the codons for leucine or valine found in the fifth amino acid position of each LRR repeat. Except for these seven introns that are conserved between all four genes, LGI4 has an extra intron within the C-terminal part, located between codons M433 and V434.

3.4. Genomic localizations of LGI genes

By running a BLAT search of cDNA sequences against the December 22, 2001 draft assembly of the HGWD database, cytogenetic positions were derived for *h*LGI1 (10q23.33), *h*LGI2 (4p15.2), and *h*LGI4 (19q13.11). Radiation hybrid mapping results were compatible with these predictions (Table 1), and helped to assign the *h*LGI3 sequence, which was not found in the HGWD database (8p21.3). The murine LGI genes were located on mouse chromosomes 19, 5, 14 and 7, respectively, in subregions synthenic to the localization of the corresponding human genes.

3.5. mRNA expression patterns

The distribution patterns of *h*LGI mRNA expression in different tissues were studied by semi-quantitative PCR with cDNA from eight different tissues as templates (Fig. 1). Both *h*LGI1 and *h*LGI4 are expressed in all tissues studied, and their expression was apparently highest in brain. *h*LGI2 mRNA could only be detected in brain, heart, and placenta. PCR from these cDNA samples gave rather weak bands. *h*LGI3 was present in all tissues, but the signals from brain and lung were apparently stronger than those from other tissues (Fig. 1). However, in semi-quantitative PCR the differences in signal intensity might not correlate directly to the amount of mRNA present in the respective tissue.

3.6. LGI1 protein expression in human brain

Immunohistological experiments demonstrated that the *h*LGI1 mRNA is translated into protein. LGI1-immunoreactive neurons were found in all layers of the frontal (Fig. 3) and temporal (Fig. 4) cortex with strongest labeling intensities in layers II/III. Most of the labeled neurons displayed a pyramidal shape. The immunoprecipitate was located within the perikaryon sparing the nucleus and sometimes extending into

Table 1
Radiation hybrid mapping of LGI genes

Gene symbol	Primer pair[a] (product size)	Data vector[b]	Cytogenetic map localization	Genetic map interval GeneMap 99	Whitehead framework physical map location	Whitehead map flanking markers
*h*LGI2	LGI2for/rev (299 bp)	0100000111001000110000110100100010000100001011100011000010001100011000000000001101100010000010	4p15.2	chromosome 4	6.3 cR from D4S1021	D4S863
				36.5 cM	–	SGC30299
				–	2.3 cR from WI-6539	–
				38.3 cM		AFMB355YA5 SGC34745
*h*LGI3	LGI3for/rev (292 bp)	0000000001100000001010000000110000000110110111000100011001000100001111001001000000201010100	8p21.3	chromosome 8	4.7 cR from WI-4688	WI-17471
				42.2 cM	–	WI-4688
				–	2.8 cR from WI-6536	–
				44.9 cM		WI-6304 WI-6536
*h*LGI4	LGI4for/rev (312 bp)	0001000101001000000000111101110100000010110011101010000010000110010011110110010111000010000111	19q13.11	chromosome 19	9.7 cR from CHLC.GATA10A05.799	SGC33351
				58.1 cM	–	WI-16546
				–	7.3 cR from D19S224	–
				58.7 cM		WI-15149 D19S224

By physical mapping using the GenBridge 4 radiation hybrid mapping panel, LGI genes were mapped with respect to Whitehead framework markers (http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl). Using markers flanking the genes on the Whitehead map, the position in an interval of the GeneMap 99 (http://www.ncbi.nlm.nih.gov/genemap/) and in a cytogenetic interval (http://genome.cse.ucsc.edu/goldenPath/hgTracks.html) were derived. In addition to the physical distance to the flanking Whitehead framework markers, for each of the genes the closest flanking markers within the Whitehead RH map, the genetic map intervals according to GeneMap 99, and the cytogenetic position are listed.

[a]Primer pairs for *h*LGI2, *h*LGI3, *h*LGI4: see legend to Fig. 3.

[b]0, negative; 1, positive; 2, uncertain data.

the apical (Figs. 3D and 4) and, in rare cases, also into the basal dendrites (Fig. 4A). The distribution pattern of immunoreactive cells was similar in all cortices investigated, however, the labeling intensity showed slight variations. Control sections preincubated with the sc-9581 peptide did not show any immunoreactive cells (Fig. 3B).

4. Discussion

Combining bioinformatics with confirmatory cloning and sequencing, we found three novel genes, LGI2, LGI3, and LGI4, homologous to LGI1. All four genes encode proteins consisting of an extracellular domain containing an LRR region flanked by conserved cysteine clusters, a putative transmembrane region, and an intracellular domain without obvious structural elements. Three different conserved patterns of flanking cysteine clusters are found in most extracellular LRR proteins. The conserved spacing of the N-terminal cysteine cluster, CX_3CXCX_6C, is present in all four LGI proteins as well as in class I small leucine-rich proteoglycans (SLRP). Except for the conserved cysteine cluster, the LGI proteins showed no significant similarity to class I SLRPs like decorin. The phylogenetic analysis (Fig. 5) showed that the four *h*LGI peptides and their murine counterparts are closely related to each other, while proteins showing different types of conserved cysteine clusters [5] were placed more distantly on the evolutionary scale. The branching pattern of the dendrogram presented here (Fig. 5) strongly suggests that the four LGI proteins belong to a formerly unknown subfamily of LRR proteins.

Northern blot experiments detected *h*LGI1 expression only in brain and skeletal muscle [2]. Using the more sensitive PCR method we were able to show that *h*LGI1 mRNA can be found in several other tissues. The observed expression patterns of *h*LGI1 and *h*LGI4 mRNA are largely identical. *h*LGI2 expression was only found in three of the tissues investigated here, while for *h*LGI3 at least faint bands were detected in all other tissues. None of the tissues showed expression of only one LGI gene, and some tissues like heart, brain and placenta even expressed all four different mRNAs. A survey of LRR proteins suggests that the major function of the LRRs may be to provide a structural framework for the formation of protein–protein interactions [7]. Thus, provided that the mRNAs detected here are indeed translated into protein, it will be interesting to see if the observed co-expression reflects related functions and if the LGI proteins are able to associate with each other.

LGI1 is so far the only LGI gene found to be associated with a human disorder. Previous publications have already shown that LGI1 mRNA can be detected in different parts of the brain, but the presence of the LGI1 protein in the human brain has not been confirmed yet. We now demonstrated that *h*LGI1 protein could indeed be found in the human temporal lobe, the part of the brain which probably plays the most critical role in the generation of seizures in ADLTE patients. The *h*LGI1 protein was mainly found in

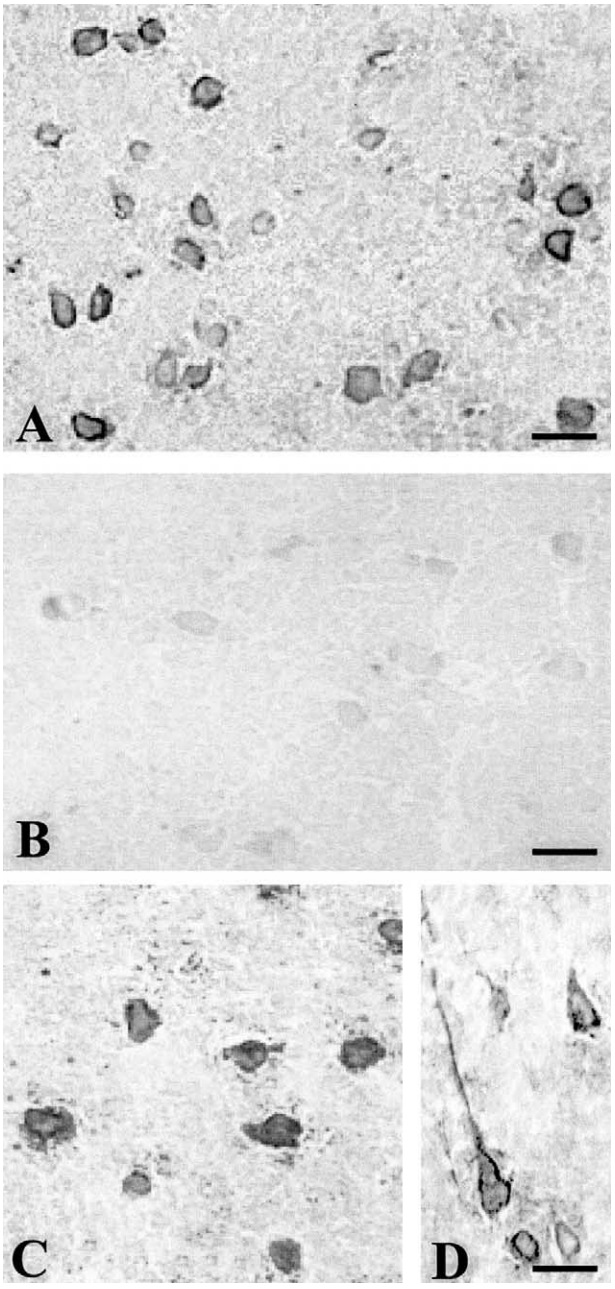

Fig. 3. *h*LGI1 immunohistochemistry in human frontal cortex. In cryostat sections, the staining and distribution pattern of *h*LGI1-immunoreactive cells obtained after incubation with sc-9581 goat antiserum (A) and the rabbit antiserum (C,D), respectively, was similar as shown here for layers II/III of the frontal cortex. The immunoprecipitate was located in the perikaryon sparing the nucleus (A,C,D), sometimes extending into the apical dendrites (D). Note that there was no signal in sections treated with pre-adsorbed sc-9581 goat antiserum (B). Bars: 70 μm.

pyramidal neurons located in lamina II–III, but contrary to its name, no apparent *h*LGI1 expression was seen in glial cells. The immunohistological approach used here would also be useful to study the precise anatomical distribution of LGI1 protein and its role in epileptogenesis.

Besides the involvement in epileptogenesis LGI1 is suspected to act as a tumor-suppressor gene. It was therefore interesting to see if the homologous genes *h*LGI2–*h*LGI4 are located in candidate regions for epilepsy genes or in regions showing loss of heterozygosity. Allelic losses of the short arm of chromosome 4 are frequently found in several tumor types, and the region 4p15.1–3 containing *h*LGI2 is suspected to contain one or more tumor-suppressor genes [8–10]. Fine mapping studies are not available yet, so it remains unclear if *h*LGI2 is indeed located within the region of common deletion. Various types of seizures and EEG abnormalities frequently occur in patients with the 4p-syndrome (also named Wolf–Hirschhorn syndrome), but *h*LGI2 is located outside the critical region that is associated with most of the pattern of the syndrome [11]. The chromosomal region containing *h*LGI3, 8p21.3, was found to be associated with different tumor types [12], but is not known as a candidate region for unknown epilepsy genes, yet. Loss of heterozygosity of chromosome 19q is a frequent event in different tumor types, but at least in gliomas *h*LGI4 seems to be located outside the region of common deletion [13–15]. *h*LGI4 can be considered as a candidate gene for benign familial infantile convulsions, a rare type of idiopathic epilepsy for which a gene had been previously mapped to 19q13 [16].

So far the functional properties of LGI proteins remain unknown. Since all other genes known to cause idiopathic human epilepsies are encoding ion channels [3] the possibility exists that LGI1 interacts with an unknown channel protein. Thus it would be interesting to evaluate the possibility of LGI

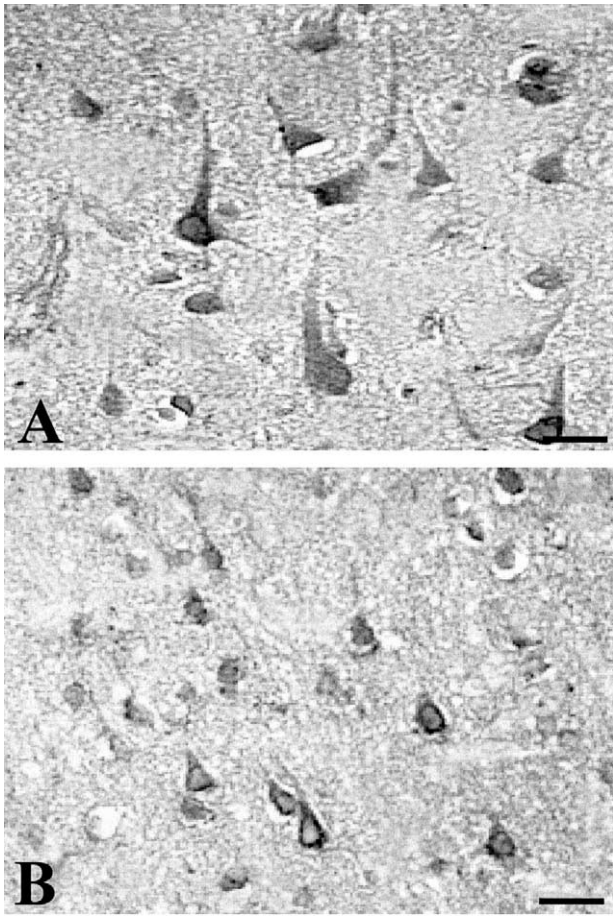

Fig. 4. LGI1 immunohistochemistry in human temporal cortex. Localization of *h*LGI1-immunoreactive neurons in layers II/III of the temporal cortex of a 27-year-old male (A) and of an 11-year-old female (B) neuropathological healthy individual. In particular the pyramidal-shaped neurons were intensely labeled. Bars: 70 μm.

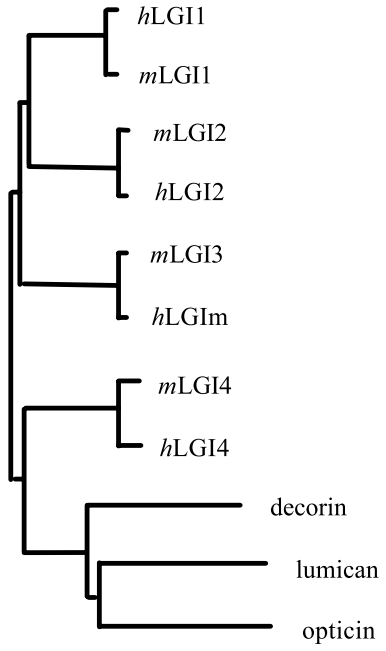

Fig. 5. Dendrogram showing the predicted relationships within the subfamily of LGI genes and between LGI genes and other LRR genes. Representative genes were chosen from the LRR gene classes I (decorin), II (lumican) and III (opticin) [5]. Horizontal distances of bars are proportional to the evolutionary distances and based upon protein sequences. The dendrogram was generated using the program Clustree (HUSAR DNA analysis package).

proteins acting as accessory subunits for ion channels. Elucidation of the biochemical and physiological features of the different members of this new subfamily of LRR proteins should lead to further understanding of the biological functions of LGI proteins and their role in epileptogenesis. Furthermore, it should help to clarify the question if members of this new subfamily of LRR genes are indeed involved in tumorigenesis [2,17]. The latter could have serious implications for families with LGI-associated epilepsies.

Acknowledgements: We would like to thank J. Cowell for providing the LGI1 rabbit antiserum used in this study. The excellent technical assistance of Sigrun Kuhlage and Hartmut Engel, and the photographical artwork of Inge Koch are gratefully appreciated. Part of this study was supported by Grants from the Deutsche Forschungsgemeinschaft to O.K.S. (SFB-TR3/A5).

References

[1] Kalachikov, S., Evgrafov, O., Ross, B., Winawer, M., Barker-Cummings, C., Boneschi, F.M., Choi, C., Morozov, P., Das, K., Teplitskaya, E., Yu, A., Cayanis, E., Penchaszadeh, G., Kottmann, A.H., Pedley, T.A., Hauser, W.A., Ottman, R. and Gilliam, T.C. (2002) Nat. Genet., advance online publication 002;DOI:10.1038/ng832.
[2] Chernova, O.B., Somerville, R.P. and Cowell, J.K. (1998) Oncogene 17, 2873–2881.
[3] Steinlein, O.K. (2001) Am. J. Med. Genet. 106, 139–145.
[4] Stogmann, E., Zimprich, A., Baumgartner, C., Aull-Watschinger, S., Hollt, V. and Zimprich, F. (2002) Ann. Neurol. 51, 260–263.
[5] Reardon, A.J., Le Goff, M., Briggs, M.D., McLeod, D., Sheehan, J.K., Thornton, D.J. and Bishop, P.N. (2000) J. Biol. Chem. 275, 2123–2129.
[6] Buchanan, S.G. and Gay, N.J. (1996) Prog. Biophys. Mol. Biol. 65, 1–44.
[7] Kobe, B. and Kajava, A.V. (2001) Curr. Opin. Struct. Biol. 11, 725–732.
[8] Shivapurkar, N., Virmani, A.K., Wistuba, I.I., Milchgrub, S., Mackay, B., Minna, J.D. and Gazdar, A.F. (1999) Clin. Cancer Res. 5, 17–23.
[9] Richard, F., Pacyna-Gengelbach, M., Schluns, K., Fleige, B., Winzer, K.J., Szymas, J., Dietel, M., Petersen, I. and Schwendel, A. (2000) Int. J. Cancer 89, 305–310.
[10] Shivapurkar, N., Maitra, A., Milchgrub, S. and Gazdar, A.F. (2001) Hum. Pathol. 32, 169–177.
[11] Wright, T.J., Ricke, D.O., Denison, K., Abmayr, S., Cotter, P.D., Hirschhorn, K., Keinanen, M., McDonald-McGinn, D., Somer, M., Spinner, N., Yang-Feng, T., Zackai, E. and Altherr, M.R. (1997) Hum. Mol. Genet. 6, 317–324.
[12] Martinez-Climent, J.A., Vizcarra, E., Sanchez, D., Blesa, D., Marugan, I., Benet, I., Sole, F., Rubio-Moscardo, F., Terol, M.J., Climent, J., Sarsotti, E., Tormo, M., Andreu, E., Salido, M., Ruiz, M.A., Prosper, F., Siebert, R., Dyer, M.J. and Garcia-Conde, J. (2001) Blood 98, 3479–3482.
[13] Smith, J.S., Tachibana, I., Lee, H.K., Qian, J., Pohl, U., Mohrenweiser, H.W., Borell, T.J., Hosek, S.M., Soderberg, C.L., von Deimling, A., Perry, A., Scheithauer, B.W., Louis, D.N. and Jenkins, R.B. (2000) Genes Chromosomes Cancer 29, 16–25.
[14] Mora, J., Cheung, N.K., Chen, L., Qin, J. and Gerald, W. (2001) Clin. Cancer Res. 7, 1358–1361.
[15] Shao, J.Y., Huang, X.M., Yu, X.J., Huang, L.X., Wu, Q.L., Xia, J.C., Wang, H.Y., Feng, Q.S., Ren, Z.F., Ernberg, I., Hu, L.F. and Zeng, Y.X. (2001) Anticancer Res. 21, 3021–3029.
[16] Guipponi, M., Rivier, F., Vigevano, F., Beck, C., Crespel, A., Echenne, B., Lucchini, P., Sebastianelli, R., Baldy-Moulinier, M. and Malafosse, A. (1997) Hum. Mol. Genet. 6, 473–477.
[17] Cowell, J.K. (2002) Nat. Med. 8, 219–220.