LRRC8 involved in B cell development belongs to a novel family of leucine-rich repeat proteins

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Abstract In a previous study, we isolated a novel gene, LRRC8 (leucine-rich repeat-containing 8), in a girl with congenital agammaglobulinemia. We have now identified four unknown LRRC8-like genes, named TA-LRRP, AD158, LRRC5, and FLJ23420. Their predicted structures are very similar to each other, and highly conserved between humans and the mouse. All five genes encode proteins consisting of 16 extracellular leucine-rich repeats (LRRs), all of which have four transmembrane regions except for FLJ23420. These genes belong to a novel family, designated the LRRC8 family, within the superfamily of LRR proteins. TA-LRRP, AD158, and LRRC5 might be implicated in proliferation and activation of lymphocytes and monocytes.

Key words: B cell development; Leucine-rich repeat; Gene family

1. Introduction

In a previous study [1], we isolated a novel gene, LRRC8 (leucine-rich repeat-containing 8) from the t(9:20)(q33.2;q12) balanced translocation breakpoint in a girl with congenital agammaglobulinemia, absence of B cells in peripheral blood, and minor facial anomalies. LRRC8 encodes a putative four-pass transmembrane protein of unknown function, which has one isolated and eight sequentially located leucine-rich repeats (LRRs), and is highly conserved between humans and the mouse; 99% of amino acid sequences are identical. Transplantation experiments with murine bone marrow cells, which were forced to express the truncated LRRC8 found in the patient, showed that expression of the truncated protein inhibited B cell development. This therefore indicated that LRRC8 was responsible for the B cell deficiency in this patient and is required for B cell development.

LRR proteins participate in many biologically important processes, such as hormone–receptor interactions, enzyme inhibition, cell adhesion, and cellular trafficking. A number of recent studies revealed the involvement of LRR proteins in early mammalian development, neural development, cell polarization, regulation of gene expression, and apoptosis signaling. In all these processes, the LRR domains probably mediate protein–protein interactions [2]. For example, CD14 (lipopolysaccharide (LPS) receptor) and Toll-like receptor 4, which are responsible for LPS signaling, have sequentially located LRRs [3]. Glycoproteins Ib, V, and IX also have LRRs, and form a complex as the receptor for von Willebrand factor [4]. Furthermore, receptors of chorionic gonadotropin, luteinizing hormone, and thyroid-stimulating hormone are LRR-containing G protein-coupled receptors [5].

The LRRC8 protein may therefore have a novel, specific ligand which is essential for B cell development. In addition, proteins that are functionally or structurally related to LRRC8 have to be regarded as candidates for critical molecules in the immune system. We therefore used bioinformatics and laboratory works to search for sequences with high similarities to LRRC8 and identified four unknown genes named TA-LRRP, AD158, LRRC5, and FLJ23420. Comparison of predicted protein sequences and structures showed that the five genes belonged to a novel family within the superfamily of LRR proteins. In this report we describe a novel five-membered family of genes in humans and the mouse, designated the LRRC8 family. We have analyzed the pattern of expression of the human LRRC8 family genes, particularly in the immune system. Furthermore, we have examined the phylogenetic origin of human and murine LRRC8 family genes.

2. Materials and methods

2.1. Database search and sequence computation

To identify sequences of the LRRC8 family genes, a protein–protein basic local alignment search tool (BLASTp) search with the LRRC8 peptide sequence (AY143166) was carried out using the NCBI protein database (http://www.ncbi.nlm.nih.gov/BLAST/). Genomic structures of the genes were retrieved by a basic local alignment tool (BLAT) search from the Human Genome Project Working Draft database (HGWD) (http://genome.ucsc.edu/). Sequences of the mouse LRRC8 family genes were also retrieved from both databases. The computation program of the given sequence alignment was MacDNASIS v. 3.2 (Hitachi Software Engineering Company). The structural elements of the LRRC8 family proteins were identified with Protein families database of alignments and HMMs (Pfam) (http://www.sanger.ac.uk/Software/Pfam/search.shtml). Prediction of transmembrane helices in proteins was performed with TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Secondary structure of LRRC8 protein was predicted with the PSIPRED protein structure

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Abbreviations: BLASTp, protein–protein basic local alignment search tool; BLAT, basic local alignment tool; HGWD, Human Genome Project Working Draft database; LPS, lipopolysaccharide; LRR, leucine-rich repeat; LRRC8, leucine-rich repeat-containing 8; Pfam, Protein families database of alignments and HMMs; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate

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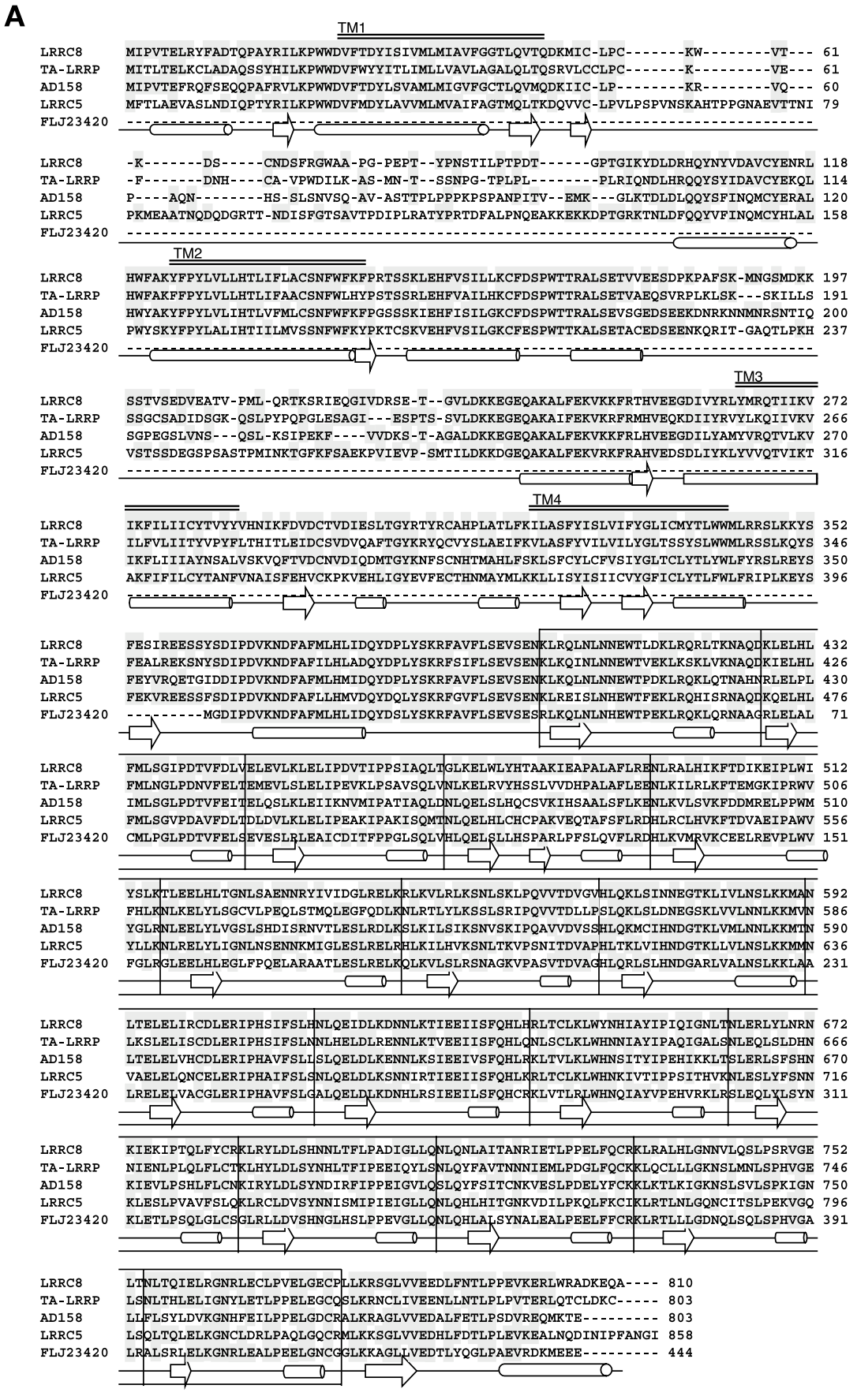
A
TM1
TM2
TM3
TM4
LRRC8 MIPVTELRYFADTQPAYRILKPWWDVFTDYISIVMLMIAVFGGTLQVTQDKMIC-LPC-------KW--------VT--- 61
TA-LRRP MITLTELKCLADAQSSYHILKPWWDVFWYYITLIMLLVAVLAGALQLTQSRVLCCLPC-------K---------VE--- 61
AD158 MIPVTEFRQFSEQQPAFRVLKPWWDVFTDYLSVAMLMIGVFGCTLQVMQDKIIC-LP--------KR--------VQ--- 60
LRRC5 MFTLAEVASLNDIQPTYRILKPWWDVFMDYLAVVMLMVAIFAGTMQLTKDQVVC-LPVLPSPVNSKAHTPPGNAEVTTNI 79
FLJ23420
LRRC8 -K-------DS-----CNDSFRGWAA-PG-PEPT--YPNSTILPTPDT------GPTGIKYDLDRHQYNYVDAVCYENRL 118
TA-LRRP -F-------DNH----CA-VPWDILK-AS-MN-T--SSNPG-TPLPL--------PLRIQNDLHRQQYSYIDAVCYEKQL 114
AD158 P----AQN--------HS-SLSNVSQ-AV-ASTTPLPPPKPSPANPITV--EMK---GLKTDLDLQQYSFINQMCYERAL 120
LRRC5 PKMEAATNQDQDGRTT-NDISFGTSAVTPDIPLRATYPRTDFALPNQEAKKEKKDPTGRKTNLDFQQYVFINQMCYHLAL 158
FLJ23420
LRRC8 HWFAKYFPYLVLLHTLIFLACSNFWFKFPRTSSKLEHFVSILLKCFDSPWTTRALSETVVEESDPKPAFSK-MNGSMDKK 197
TA-LRRP HWFAKFFPYLVLLHTLIFAACSNFWLHYPSTSSRLEHFVAILHKCFDSPWTTRALSETVAEQSVRPLKLSK---SKILLS 191
AD158 HWYAKYFPYLVLIHTLVFMLCSNFWFKFPGSSSKIEHFISILGKCFDSPWTTRALSEVSGEDSEEKDNRKNNMNRSNTIQ 200
LRRC5 PWYSKYFPYLALIHTIILMVSSNFWFKYPKTCSKVEHFVSILGKCFESPWTTKALSETACEDSEENKQRIT-GAQTLPKH 237
FLJ23420
LRRC8 SSTVSEDVEATV-PML-QRTKSRIEQGIVDRSE-T--GVLDKKEGEQAKALFEKVKKFRTHVEEGDIVYRLYMRQTIIKV 272
TA-LRRP SSGCSADIDSGK-QSLPYPQPGLESAGI---ESPTS-SVLDKKEGEQAKAIFEKVKRFRMHVEQKDIIYRVYLKQIIVKV 266
AD158 SGPEGSLVNS---QSL-KSIPEKF----VVDKS-T-AGALDKKEGEQAKALFEKVKKFRLHVEEGDILYAMYVRQTVLKV 270
LRRC5 VSTSSDEGSPSASTPMINKTGFKFSAEKPVIEVP-SMTILDKKDGEQAKALFEKVRKFRAHVEDSDLIYKLYVVQTVIKT 316
FLJ23420
LRRC8 IKFILIICYTVYYVHNIKFDVDCTVDIESLTGYRTYRCAHPLATLFKILASFYISLVIFYGLICMYTLWWMLRRSLKKYS 352
TA-LRRP ILFVLIITYVPYFLTHITLEIDCSVDVQAFTGYKRYQCVYSLAEIFKVLASFYVILVILYGLTSSYSLWWMLRSSLKQYS 346
AD158 IKFLIIIAYNSALVSKVQFTVDCNVDIQDMTGYKNFSCNHTMAHLFSKLSFCYLCFVSIYGLTCLYTLYWLFYRSLREYS 350
LRRC5 AKFIFILCYTANFVNAISFEHVCKPKVEHLIGYEVFECTHNMAYMLKKLLISYISIICVYGFICLYTLFWLFRIPLKEYS 396
FLJ23420
LRRC8 FESIREESSYSDIPDVKNDFAFMLHLIDQYDPLYSKRFAVFLSEVSENKLRQLNLNNEWTLDKLRQRLTKNAQDKLELHL 432
TA-LRRP FEALREKSNYSDIPDVKNDFAFILHLADQYDPLYSKRFSIFLSEVSENKLKQINLNNEWTVEKLKSKLVKNAQDKIELHL 426
AD158 FEYVRQETGIDDIPDVKNDFAFMLHMIDQYDPLYSKRFAVFLSEVSENKLKQLNLNNEWTPDKLRQKLQTNAHNRLELPL 430
LRRC5 FEKVREESSFSDIPDVKNDFAFLLHMVDQYDQLYSKRFGVFLSEVSENKLREISLNHEWTFEKLRQHISRNAQDKQELHL 476
FLJ23420 ---------MGDIPDVKNDFAFMLHLIDQYDSLYSKRFAVFLSEVSESRLKQLNLNHEWTPEKLRQKLQRNAAGRLELAL 71
LRRC8 FMLSGIPDTVFDLVELEVLKLELIPDVTIPPSIAQLTGLKELWLYHTAAKIEAPALAFLRENLRALHIKFTDIKEIPLWI 512
TA-LRRP FMLNGLPDNVFELTEMEVLSLELIPEVKLPSAVSQLVNLKELRVYHSSLVVDHPALAFLEENLKILRLKFTEMGKIPRWV 506
AD158 IMLSGLPDTVFEITELQSLKLEIIKNVMIPATIAQLDNLQELSLHQCSVKIHSAALSFLKENLKVLSVKFDDMRELPPWM 510
LRRC5 FMLSGVPDAVFDLTDLDVLKLELIPEAKIPAKISQMTNLQELHLCHCPAKVEQTAFSFLRDHLRCLHVKFTDVAEIPAWV 556
FLJ23420 CMLPGLPDTVFELSEVESLRLEAICDITFPPGLSQLVHLQELSLLHSPARLPFSLQVFLRDHLKVMRVKCEELREVPLWV 151
LRRC8 YSLKTLEELHLTGNLSAENNRYIVIDGLRELKRLKVLRLKSNLSKLPQVVTDVGVHLQKLSINNEGTKLIVLNSLKKMAN 592
TA-LRRP FHLKNLKELYLSGCVLPEQLSTMQLEGFQDLKNLRTLYLKSSLSRIPQVVTDLLPSLQKLSLDNEGSKLVVLNNLKKMVN 586
AD158 YGLRNLEELYLVGSLSHDISRNVTLESLRDLKSLKILSIKSNVSKIPQAVVDVSSHLQKMCIHNDGTKLVMLNNLKKMTN 590
LRRC5 YLLKNLRELYLIGNLNSENNKMIGLESLRELRHLKILHVKSNLTKVPSNITDVAPHLTKLVIHNDGTKLLVLNSLKKMMN 636
FLJ23420 FGLRGLEELHLEGLFPQELARAATLESLRELKQLKVLSLRSNAGKVPASVTDVAGHLQRLSLHNDGARLVALNSLKKLAA 231
LRRC8 LTELELIRCDLERIPHSIFSLHNLQEIDLKDNNLKTIEEIISFQHLHRLTCLKLWYNHIAYIPIQIGNLTNLERLYLNRN 672
TA-LRRP LKSLELISCDLERIPHSIFSLNNLHELDLRENNLKTVEEIISFQHLQNLSCLKLWHNNIAYIPAQIGALSNLEQLSLDHN 666
AD158 LTELELVHCDLERIPHAVFSLLSLQELDLKENNLKSIEEIVSFQHLRKLTVLKLWHNSITYIPEHIKKLTSLERLSFSHN 670
LRRC5 VAELELQNCELERIPHAIFSLSNLQELDLKSNNIRTIEEIISFQHLKRLTCLKLWHNKIVTIPPSITHVKNLESLYFSNN 716
FLJ23420 LRELELVACGLERIPHAVFSLGALQELDLKDNHLRSIEEILSFQHCRKLVTLRLWHNQIAYVPEHVRKLRSLEQLYLSYN 311
LRRC8 KIEKIPTQLFYCRKLRYLDLSHNNLTFLPADIGLLQNLQNLAITANRIETLPPELFQCRKLRALHLGNNVLQSLPSRVGE 752
TA-LRRP NIENLPLQLFLCTKLHYLDLSYNHLTFIPEEIQYLSNLQYFAVTNNNIEMLPDGLFQCKKLQCLLLGKNSLMNLSPHVGE 746
AD158 KIEVLPSHLFLCNKIRYLDLSYNDIRFIPPEIGVLQSLQYFSITCNKVESLPDELYFCKKLKTLKIGKNSLSVLSPKIGN 750
LRRC5 KLESLPVAVFSLQKLRCLDVSYNNISMIPIEIGLLQNLQHLHITGNKVDILPKQLFKCIKLRTLNLGQNCITSLPEKVGQ 796
FLJ23420 KLETLPSQLGLCSGLRLLDVSHNGLHSLPPEVGLLQNLQHLALSYNALEALPEELFFCRKLRTLLLGDNQLSQLSPHVGA 391
LRRC8 LTNLTQIELRGNRLECLPVELGECPLLKRSGLVVEEDLFNTLPPEVKERLWRADKEQA---- 810
TA-LRRP LSNLTHLELIGNYLETLPPELEGCQSLKRNCLIVEENLLNTLPLPVTERLQTCLDKC----- 803
AD158 LLFLSYLDVKGNHFEILPPELGDCRALKRAGLVVEDALFETLPSDVREQMKTE--------- 803
LRRC5 LSQLTQLELKGNCLDRLPAQLGQCRMLKKSGLVVEDHLFDTLPLEVKEALNQDINIPFANGI 858
FLJ23420 LRALSRLELKGNRLEALPEELGNCGGLKKAGLLVEDTLYQGLPAEVRDKMEEE--------- 444

Fig. 1.

prediction server (http://bioinf.cs.ucl.ac.uk/psipred/). Phylogenetic tree analysis of amino acid sequences from human and mouse LRRC8 family was performed with TreeTop of GeneBee services (http://www.genebee.msu.su/services/phtree_reduced.html).

2.2. Isolation and stimulation of PBMCs and monocytes

Peripheral blood mononuclear cells (PBMCs) were purified from normal, healthy volunteers. Heparinized blood was obtained, and PBMCs were isolated by Ficoll-Paque centrifugation using standard procedures. The PBMCs were washed and resuspended in RPMI 1640 (Nikken seibutsuigaku-kenkyusho, Kyoto, Japan)/10% fetal calf serum (FCS) (JRH Biosciences, Australia) with 100 U/ml of penicillin and 100 μg/ml of streptomycin. Monocytes were further enriched as previously detailed [6]. PBMCs were suspended at 10×10^7/ml in RPMI 1640/10% FCS and cultured at 37°C for 1 h on a plastic Petri dish coated overnight with cold 100% FCS. The cells were washed twice to remove non-adherent cells, and subsequently the adherent monocytes were obtained by culture with cold phosphate-buffered saline/0.2% EDTA-Na/5% FCS at 4°C for 20 min. Enriched monocytes were washed and suspended in RPMI 1640/10% FCS. 10 μg/ml of phytohemagglutinin (PHA; Sigma, St. Louis, MO, USA), 10 ng/ml of phorbol 12-myristate 13-acetate (PMA; Sigma), and 100 ng/ml LPS (*Escherichia coli* 0127: B8; Sigma) were used to stimulate PBMCs and monocytes at 37°C, 5% CO_2.

2.3. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from PBMCs and monocytes by the phenol–guanidinium method, followed by isopropanol precipitation. The RNA (1 μg) was used for cDNA synthesis with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. LRRC8 family mRNA expression patterns were analyzed using the Multiple Tissue cDNA Panel (Clontech, Palo Alto, CA, USA). Semi-quantitative PCR was carried out using the following primer pairs: LRRC8, 5′-TGCAGAAGCTGTCCATCAAC-3′, 5′-TTGAACAGGTCCTCCTCCAC-3′; TA-LRRP, 5′-GTCAACCTCAAGGAGCTTCG-3′, 5′-CAGGAGGAAGTGTTTCCAGGT-3′; AD158, 5′-CCTAGCACCTTCTCCAGTCG-3′, 5′-CCAGTCATGTCCTGAATGTCC-3′; LRRC5, 5′-AGTTCCCAGCATGACAATCC-3′, 5′-CTTAAGGTGCCGCAACTCTC-3′; FLJ23420, 5′-GTCAGCGAAAGCCGTCTAAA-3′, 5′-CAGGTGGTTGTCCTTGAGGT-3′. The conditions were an initial cycle at 95°C for 30 s, followed by adequate cycles (see figures) consisting of two steps (LRRC8, 98°C for 2 s, 65°C for 15 s; TA-LRRP, AD158, LRRC5, 98°C for 2 s, 68°C for 20 s; FLJ23420, 98°C for 2 s, 70°C for 10 s). GAPDH or β-actin primers were used to normalize the cDNA content. The cycle number in semi-quantitative PCR was optimized to analyze PCR products in the exponential phase of the PCR. These experiments were repeated three times with similar results. All the reactions were performed with TaKaRa Z-taq (Takara Shuzo, Ohtsu, Shiga, Japan).

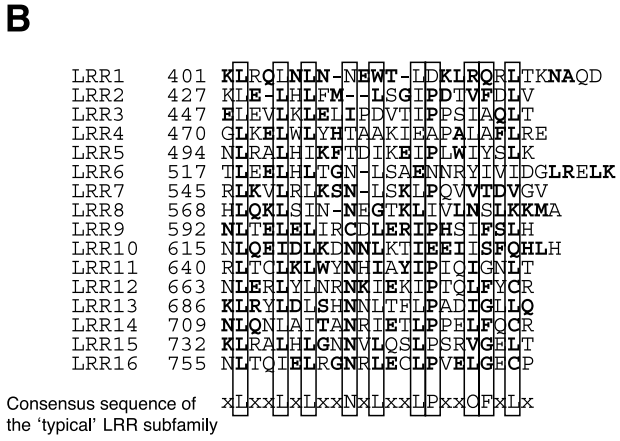

Fig. 1 (*Continued*). A: Alignment of deduced amino acid sequences of LRRC8, TA-LRRP, AD158, LRRC5, and FLJ23420. Sixteen sequentially located LRR regions are marked by boxes. Double lines indicate the regions predicted to contain the transmembrane regions. Secondary structure of LRRC8 protein is indicated by solid lines (coils), tubes (α-helices) and arrows (β-strands). Identical amino acids are highlighted in gray. B: Sequence alignment of the individual LRRs in LRRC8 protein. Bold face indicates the shared amino acids between at least four genes. Boxes denote the positions of the conserved amino acids in the consensus sequence of the 'typical' LRR subfamily. O, a non-polar residue; x, any residue.

3. Results

3.1. Identification of TA-LRRP, AD158, LRRC5, and FLJ23420

BLASTp search identified different human genes, which encoded peptides with high similarity to LRRC8. RT-PCR was performed to verify the sequences. All of the genes had already been named: TA-LRRP, AD158, LRRC5, and FLJ23420 (accession numbers: BC060782, AB081509, BC024159, and BC022216, respectively), and the *E* values of their BLASTp searches were 0.0, 0.0, 0.0, and e-130, respectively. The sequences of five murine LRRC8 family genes were determined in silico (accession numbers: mLRRC8, BC048152; mTA-LRRP, AK083378; mAD158, BC026572; mLRRC5, BC037717; mFLJ23420, XM_146206). In this report we did not experimentally verify their full predicted sequences.

3.2. Sequence comparison

Human genes for TA-LRRP, AD158, LRRC5, and FLJ23420 encoded predicted proteins of 803, 803, 858, and 444 amino acid residues, respectively. These proteins had 16 LRRs at the C-termini and four putative transmembrane regions, excluding FLJ23420. These structures were highly conserved among all LRRC8 family proteins. Pfam identified C-terminal eight to 13 sequential LRRs. However, the repeats seemed to start at LRRC8 residue 401, considering their predicted secondary structures consisting of alternately repeated β-strand and α-helix. Figs. 1A and 2 show the alignments and structures of the five predicted proteins. A high degree of similarity was found among LRRC8 family proteins not only in the LRRs but also through the entire peptides, especially in the extracellular portions. The intracellular portions, however, had no obvious structural elements and no similarity was observed between them. The overall sequence identity among the human LRRC8 family proteins was approximately 50–60%. They also had 74–99% sequence identity with their corresponding murine orthologues (Table 1). Phylogenetic tree analysis indicated that LRRC8 and TA-LRRP are more closely related to each other than to other LRRC8 family members (Fig. 3). The prevailing LRR consensus sequence of the LRRC8 family suggested that this family belongs to the 'typical' LRR subfamily [2], although the N-terminal three LRRs were non-canonical (Fig. 1B).

3.3. Gene structures and genomic localizations of LRRC8 family genes

The genomic organizations of the human and murine LRRC8 family genes were deduced from the alignment of their cDNA sequences with the corresponding genomic fragments. LRRC8 and TA-LRRP had conserved intron positions in their coding regions. The same intron positions were found in the respective human and murine orthologues of these five genes (data not shown).

By a BLAT search of cDNA sequences against the June

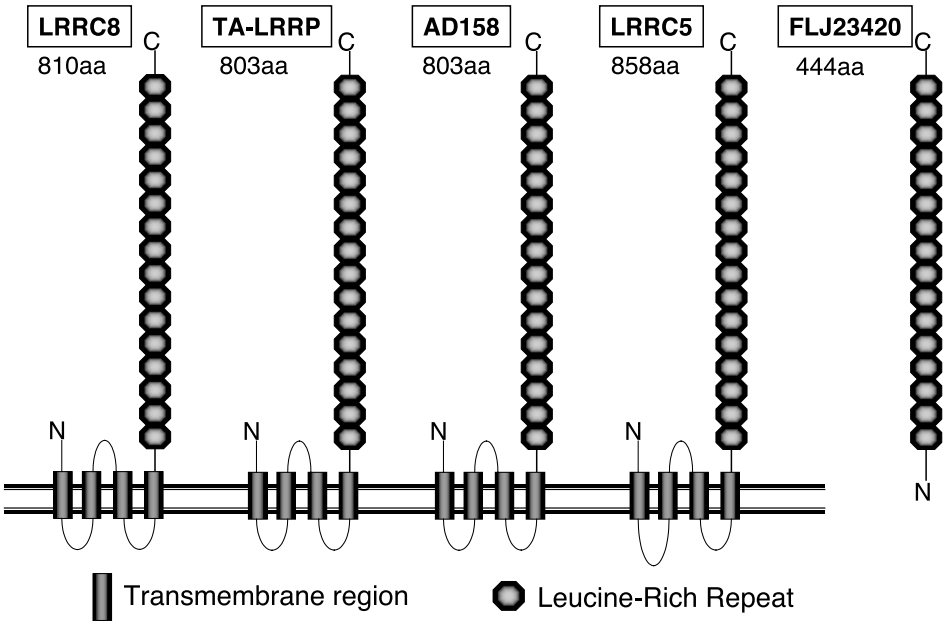

Fig. 2. Schematic structures of LRRC8 family proteins. N, N-terminal; C, C-terminal.

2002 draft assembly of the HGWD database, cytogenetic positions were derived for LRRC8 (9q34.11), AD158 (1p22.2), TA-LRRP (1p22.2), LRRC5 (1p22.2), and FLJ23420 (19p13.3). The murine genes were located on mouse chromosomes 2, 5, 5, 5, and 8, respectively. Human and mouse cytogenetic positions of TA-LRRP, AD158, and LRRC5 are so close that they appear to form a gene cluster.

3.4. mRNA expression patterns

The distribution patterns of LRRC8 family genes in different tissues were studied by semi-quantitative PCR with cDNA from eight different tissues and five subsets of lymphocytes (Fig. 4). LRRC8, TA-LRRP, AD158, and LRRC5 were expressed in almost all tissues except skeletal muscle. Their expression levels were particularly high in the brain, placenta, and lung. These four genes were expressed in T cells as well as B cells. FLJ23420 was also expressed in almost all tissues, but its signals were somewhat weak.

3.5. mRNA expressions in human PBMCs and monocytes

The time-series changes of LRRC8 family genes expression were estimated in stimulated PBMCs or monocytes (Fig. 5). FLJ23420 mRNA expression level was so weak in PBMCs that it could not be estimated. After PHA stimulation, expression levels of TA-LRRP and LRRC5 gradually increased, and AD158 mRNA expression was transiently induced; however, LRRC8 mRNA expression was eventually decreased by long-term stimulation. PMA stimulation induced transient expression of all genes. In monocytes, only LRRC8 was expressed before stimulation. After 24-h stimulation with LPS, LRRC8 mRNA expression was repressed, while LRRC5 mRNA expression was induced. Expression of all four genes was increased after 72-h stimulation with LPS, although expression levels of AD158 and TA-LRRP were somewhat weak.

4. Discussion

Using bioinformatics and laboratory works, we identified a novel five-membered human and murine gene family, designated the LRRC8 family. All five genes encode proteins consisting of 16 extracellular LRRs. All genes, except FLJ23420, encode putative four-pass transmembrane proteins, and their intracellular domains have no obvious structural elements. The structures of the five proteins were very similar to each other, and highly conserved between human and mouse, and they were ubiquitously expressed. We had reported in a previous study [1] that LRRC8 has one isolated and eight sequentially located LRRs. However, based on the conserved structures among these family proteins and their secondary structures, seven additional candidates for LRR were recognized. The major function of the LRR proteins is thought to be to provide a structural framework for the formation of

Table 1
The similarity of human and murine LPRC8 family proteins

Gene symbol	Human			Mouse		
	Amino acid residues	Cytogenetic localization	Sequence identity with LRRC8 (%)	Amino acid residues	Cytogenetic localization	Sequence identity with human orthologue (%)
LRRC8	810	9q34.11	100	810	chr.2	99
TA-LRRP	803	1p22.2	59	803	chr.5	93
AD158	803	1p22.2	59	803	chr.5	97
LRRC5	858	1p22.2	55	859	chr.5	95
FLJ23420	444	19p13.2	48	795	chr.8	74

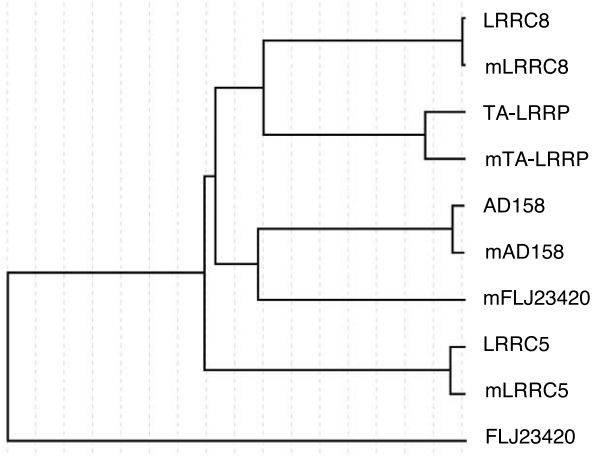

Fig. 3. Dendrogram demonstrating the evolutionary relationships between the five human and murine LRRC8 family proteins.

protein–protein interactions [2], and LRRC8 family proteins have a high degree of similarity in their extracellular regions. Among them, FLJ23420 could be distinguished from the other family proteins in terms of structure, and it might be a soluble factor. It is also possible that soluble forms of the other four proteins may exist. Thus, the characteristics of these proteins suggest that these proteins play important and fundamental roles in protein–protein interactions, though no disease is known to be caused by mutations of any of these genes except for LRRC8.

Although function has been partly delineated in LRRC8, i.e. development of pro-B cells to pre-B cells and morphogenesis, the function of the other members of the family is totally unknown. Induction profiles of these genes were therefore analyzed. PHA stimulation, which activates T cells, increased the level of TA-LRRP, AD158 (the name of AD is derived from adipocyte differentiation), and LRRC5 expression. Thus, they may be markers of activated T cells, and function within them. Peripheral resting monocytes expressed LRRC8 alone among this gene family. As they differentiated into macrophages after 24-h LPS stimulation in vitro, however, LRRC8 mRNA expression was repressed. On the other hand, the other three family genes, which were almost not expressed in resting monocytes, were noticeably induced to expression by LPS stimulation. Thus, TA-LRRP, AD158, and LRRC5 were induced by activation of monocytes as well as T cells, in contrast to LRRC8. Accordingly, these three genes seem to be functionally different from LRRC8, which may be supported by their chromosomal localization, that is, these three genes form a cluster, both in humans and in the mouse. Indeed, TA-LRRP was originally identified in inducible genes with T cell activation by microarrays, and its name was derived from T cell activation leucine repeat-rich protein. Moreover, LRRC8, TA-LRRP, AD158, and LRRC5 seemed to be regulated by protein kinase C, because PMA stimulation, which is attributed to the activation of protein kinase C, induced transient expression of all genes.

Lymphocyte development into mature and functional B or T cells is promoted by cell-to-cell contact as well as by a number of soluble factors produced in specialized microenvironments [7,8]. Moreover, antigen recognition induces changes in chemokine responsiveness in B and T cells that may help cell movements within secondary lymphoid organs [9]. Macrophages also have a broad range of surface receptors for various ligands, and they differ in appearance or function based on the environment in which they mature from monocytes [10]. Considering the structural characteristics of LRRC8 family proteins, they probably play important roles in protein–protein interactions. In addition, the time-series changes of TA-LRRP, AD158, and LRRC5 mRNA expressions in stimulated PBMCs and monocytes suggest that these proteins may function as important receptors for activated cells. These proteins may play important roles in the microenvironmental conditions of lymphocytes or monocytes, for example, migration, homing, proliferation, or differentiation, and might be involved in the interaction between immunocytes and epithelial cells. Because some adhesion molecules and growth factors produced by stromal cells are known to have roles in B cell development [11], LRRC8 might be involved in the interaction between pro-B cells and bone marrow stromal cells. Ligands of these proteins have not been discovered yet, but candidates may be extracellular proteins or secretory components of stromal cells, vascular endothelial cells, or epithelium of lymphoid organs. Elucidation of the biochemical and physiological features of this novel family of LRR proteins should lead in an innovative manner to further understanding of immune system function.

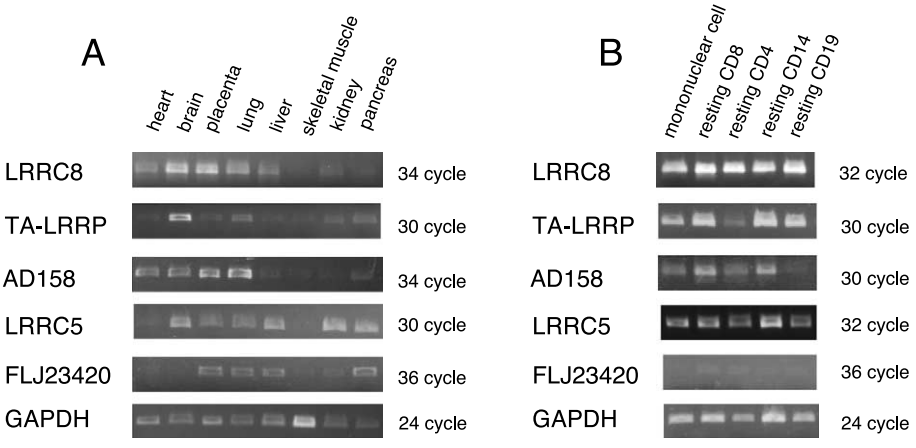

Fig. 4. Semi-quantitative analysis of LRRC8 family expression patterns by PCR: (A) in various human tissues and (B) in five subsets of lymphocytes.

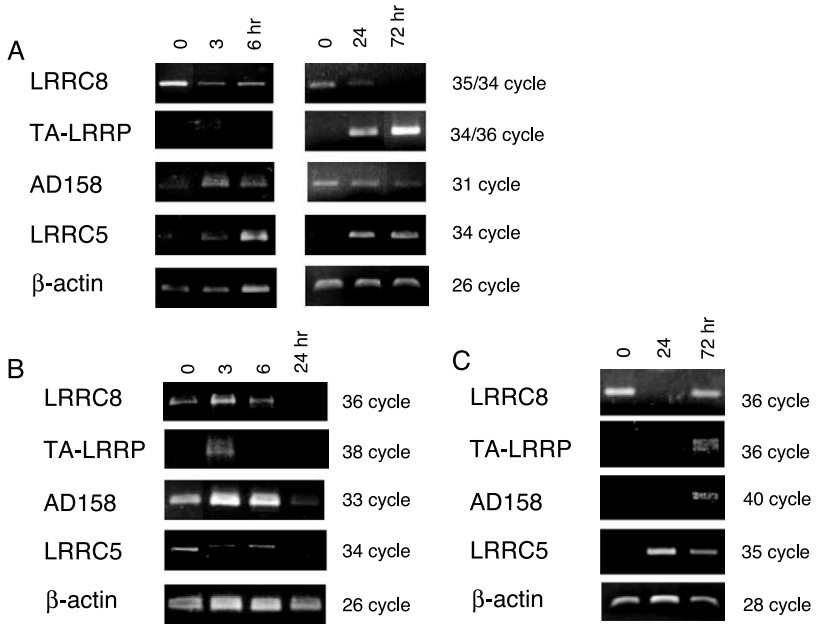

Fig. 5. Time-series changes of LRRC8 family expression were estimated by semi-quantitative PCR in PBMCs stimulated by (A) PHA (10 μg/ml), (B) PMA (10 ng/ml), and (C) in monocytes stimulated by LPS (100 ng/ml). Adequate cycles of semi-quantitative PCR are indicated on the right of each picture.

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