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Tissue-specific expression of the human laminin α5-chain, and mapping of the gene to human chromosome 20q13.2-13.3 and to distal mouse chromosome 2 near the locus for the ragged (*Ra*) mutation

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Abstract To investigate the function of the laminin α5-chain, previously identified in mice, cDNA clones encoding the 953-amino-acid carboxy terminal G-domain of the human laminin α5-chain were characterized. Northern blot analysis showed that the laminin α5-chain is expressed in human placenta, heart, lung, skeletal muscle, kidney, and pancreas. The human laminin α5-chain gene (*LAMA5*) was assigned to chromosome 20q13.2-q13.3 by in situ hybridization, and the mouse gene (*Lama5*) was mapped by linkage analysis to a syntonic region of distal chromosome 2, close to the locus for the ragged (*Ra*) mutation.

Key words: Basement membrane; cDNA cloning; Gene mapping; Laminin

1. Introduction

The laminins are the principal non-collagenous components of basement membranes, and they also influence cell behavior by interacting with a number of cell-surface receptors [1–4]. Each laminin molecule is a composed of three non-identical subunits, called the α-, β-, and γ-chains. In mammals 10 different laminin chains have been identified, which can assemble into laminin heterotrimers of diverse structure and function [3,4]. The five known laminin α-chains have diverse N-terminal structures, but they all possess an ∼100 kDa globular domain, the G-domain, at their C-termini. The G-domain is made up of five homologous repeats (G1–G5) of ∼180 amino acids and is part of the major cell-binding site located in the long arm of the laminin heterotrimer. The biomedical importance of the laminin α-chains is emphasized by the discovery of disease-causing mutations in the genes for at least two α-chains: deficiencies in the α2-chain lead to muscular dystrophy in humans and mice [5–7], and mutations in the α3-chain are found in some cases of the lethal blistering human skin disorder Herlitz junctional epidermolysis bullosa [8].

The most recently described laminin α-chain, α5, was initially cloned in mice and found to be more homologous to a *Drosophila* laminin α-chain than to the other four mammalian α-chains [9]. Expression of the laminin α5-chain mRNA in several developing and adult mouse tissues, including the heart and kidney, suggests that it may be a major constituent of basement membranes in these tissues [9,10]. Whether the α5-chain is a subunit of biologically active laminin isoforms isolated from human placenta [11] and bovine kidney [12] remains to be determined. To aid in elucidating the function of the laminin α5-chain, we have isolated cDNA clones encoding the G-domain of the human laminin α5-chain and determined the tissue-specific expression and chromosomal localization of the human gene. Mapping of the mouse gene to distal chromosome 2 by linkage analysis indicates that it may be a possible candidate gene for the ragged (*Ra*) mutation.

2. Materials and methods

2.1. Isolation and sequencing of cDNA clones

Potential human laminin α5-chain expressed sequence tags were identified by searching the Human cDNA Database of The Institute for Genome Research (available via the World Wide Web at URL http://www.tigr.org) against the C-terminus of the translated mouse laminin α5-chain cDNA sequence (GenBank U37501 [9]). The 0.6 kb insert of one cDNA clone (IMAGE Consortium clone no. 155198/GenBank R70310; obtained from the American Type Culture Collection) was used to screen a human placenta cDNA library in λgt11 (Clontech H1008b) by plaque hybridization [13], resulting in the isolation of a clone with a 1.6 kb insert, clone 1047. The remainder of the G-domain cDNA was obtained by PCR amplification from a human placenta cDNA library in pACT2 (Clontech HL4025AH), using *Pfu* polymerase (Stratagene) and a sense primer corresponding to nt 7959–7978 of the mouse cDNA sequence (5′-GGTGCCATGAAGTTCAATG) and an antisense primer derived from a sequence at the 5′ end of clone 1047 (5′-GTTGTAATGGTGCCAGACTC). The 1.6 kb PCR product was cloned into the pCR-Script SK vector (Stratagene) to give plasmid 1079. An additional cDNA clone with a 2.9 kb insert (1140) was isolated by screening a human placenta 5′ STRETCH PLUS library in λgt11 (Clontech HL5014b) with a restriction fragment of 1079. The inserts of the cDNA clones in λgt11 were subcloned into pBluescript SK+ (Stratagene) for further analysis. The cDNA was sequenced on both strands using Sequenase enzyme and reagents (Amersham), and analysis of the sequence was carried out using the software programs of the Wisconsin Package, Version 8.1, of the Genetics Computer Group.

2.2. Northern blot hybridization

A human multiple tissue Northern blot, containing ∼2 μg per lane of poly A-enriched RNA from eight human tissues, was purchased from Clontech (7760-1). Total RNA was extracted from cultured human cell lines using the Trizol reagent (Life Technologies), fractionated by formaldehyde–agarose gel electrophoresis, and blotted onto nylon membranes using standard protocols [13]. The insert of clone 1047 was labeled with [^{32}P]dCTP by the random primer method using a kit from Amersham. Hybridization was performed for 1 h at 68°C in 10 ml per blot of QuikHyb solution (Stratagene), and the blots were washed to a final stringency of 0.1×SSC at 50°C and exposed to Kodak X-Omat AR film at −70°C with intensifying screens.

2.3. Mapping of the human gene by in situ hybridization

Clone 1047 in pBluescript was labeled with tritium by nick-translation and used as a probe for in situ hybridization to chromosome

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A

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   1 tcaggggtgcagctgcgcaccccacgggatcttgccgaccttgctgcctacactgccctcaagttctacctgcagggcccagagcctgagcctgggcagggtaccgaggatcgctttgtg
   1 S  G  V  Q  L  R  T  P  R  D  L  A  D  L  A  A  Y  T  A  L  K  F  Y  L  Q  G  P  E  P  E  P  G  Q  G  T  E  D  R  F  V
     ↪ G1
 121 atgtacatgggcagccgccaggccactggggactacatgggtgtgtctctgcgtgacaagaaggtgcactgggtgtatcagctgggtgaggcgggccctgcagtcctaagcatcgatgag
  41 M  Y  M  G  S  R  Q  A  T  G  D  Y  M  G  V  S  L  R  D  K  K  V  H  W  V  Y  Q  L  G  E  A  G  P  A  V  L  S  I  D  E

 241 gacattggggagcagttcgcagctgtcagcctggacaggactctccagtttggccacatgtccgtcacagtggagagacagatgatccaggaaaccaagggtgacacggtggccccctggg
  81 D  I  G  E  Q  F  A  A  V  S  L  D  R  T  L  Q  F  G  H  M  S  V  T  V  E  R  Q  M  I  Q  E  T  K  G  D  T  V  A  P  G

 361 gcagaggggctgctcaacctgcggccagacgacttcgtcttctacgtcgggggtaccccagtaccttcacgccccctccccctgcttcgcttccccggctaccggggctgcatcgagatg
 121 A  E  G  L  L  N  L  R  P  D  D  F  V  F  Y  V  G  G  Y  P  S  T  F  T  P  P  P  L  L  R  F  P  G  Y  R  G  C  I  E  M

 481 gacacgctgaatgaggaggtggtcagcctctacaacttcgagaggaccttccagctggacacggctgtggacaggccttgtgcccgctccaagtcgaccgggggacccgtggctcacggac
 161 D  T  L  N  E  E  V  V  S  L  Y  N  F  E  R  T  F  Q  L  D  T  A  V  D  R  P  C  A  R  S  K  S  T  G  D  P  W  L  T  D
                                                                  ↪ G2
 601 ggctcctacctggacggcaccggcttcgcccgcatcagcttcgacagtcagatcagcaccaccaagcgcttcgagcaggagctgcggctcgtgtcctacagcggggtgctcttcttcctg
 201 G  S  Y  L  D  G  T  G  F  A  R  I  S  F  D  S  Q  I  S  T  T  K  R  F  E  Q  E  L  R  L  V  S  Y  S  G  V  L  F  F  L

 721 aagcagcagagccagttcctgtgcttggccgtgcaagaaggcagcctcgtgctgttgtatgactttggggctggcctgaaaaaggccgtcccactgcagccccaccgccccgacctcg
 241 K  Q  Q  S  Q  F  L  C  L  A  V  Q  E  G  S  L  V  L  L  Y  D  F  G  A  G  L  K  K  A  V  P  L  Q  P  P  P  P  L  T  S

 841 gccagcaaggcgatccaggtgttcctgctgggggcagccgcaagcgtgtgctggtgcgtgtggagcgggccacggtgtacagcgtggagcaggacaatgatctggagctggccgacgcc
 281 A  S  K  A  I  Q  V  F  L  L  G  G  S  R  K  R  V  L  V  R  V  E  R  A  T  V  Y  S  V  E  Q  D  N  D  L  E  L  A  D  A

 961 tactacctgggggcgtgccgcccgaccagctgccccgagcctgcgatggctcttccccaccggaggctcagtccgtggctgcgtcaaaggcatcaaggccctgggcaagtatgtggac
 321 Y  Y  L  G  G  V  P  P  D  Q  L  P  P  S  L  R  W  L  F  P  T  G  G  S  V  R  G  C  V  K  G  I  K  A  L  G  K  Y  V  D

1081 ctcaagcggctgaacacgacaggcgtgagcgccggctgcaccgccgacctgctggtggggcgcgccatgactttccatggccacggcttccttcgcctggcgctctcgaacgtggcaccg
 361 L  K  R  L  N  T  T  G  V  S  A  G  C  T  A  D  L  L  V  G  R  A  M  T  F  H  G  H  G  F  L  R  L  A  L  S  N  V  A  P
                                       ↪ G3
1201 ctcactggcaacgtctactccggcttcggcttccacagcgcccaggacagtgccctgctctactaccgggcgtccccggatgggctatgccaggtgtccctgcagcagggccgtgtgagc
 401 L  T  G  N  V  Y  S  G  F  G  F  H  S  A  Q  D  S  A  L  L  Y  Y  R  A  S  P  D  G  L  C  Q  V  S  L  Q  Q  G  R  V  S

1321 ctacagctcctgaggactgaagtgaaaactcaagcgggcttcgccgatggtgccccccattacgtcgccttctacagcaatgccacgggagtctggctgtatgtcgatgaccagctccag
 441 L  Q  L  L  R  T  E  V  K  T  Q  A  G  F  A  D  G  A  P  H  Y  V  A  F  Y  S  N  A  T  G  V  W  L  Y  V  D  D  Q  L  Q

1441 cagatgaagccccaccgggaccacccccgagctccagccgcagcctgaggggccccgaggctcctcctgggaggcctgcctgagtctggcaccatttacaacttcagtggctgcatc
 481 Q  M  K  P  H  R  G  P  P  P  E  L  Q  P  Q  P  E  G  P  P  R  L  L  L  G  G  L  P  E  S  G  T  I  Y  N  F  S  G  C  I

1561 agcaacgtcttcgtgcagcggctcctgggcccacagcgcgtatttgatctgcagcagaacctgggcagcgtcaatgtgagcacgggctgtgcacccgccctgcaagcccagaccccgggc
 521 S  N  V  F  V  Q  R  L  L  G  P  Q  R  V  F  D  L  Q  Q  N  L  G  S  V  N  V  S  T  G  C  A  P  A  L  Q  A  Q  T  P  G
                                                                                          ↪ spacer
1681 ctggggcctagaggactgcaggccaccgcccggaaggcctcccgccgcagccgtcagcccgcccggcatcctgcctgcatgctgccccacacctcaggaccacccgagactcctaccag
 561 L  G  P  R  G  L  Q  A  T  A  R  K  A  S  R  R  S  R  Q  P  A  R  H  P  A  C  M  L  P  P  H  L  R  T  T  R  D  S  Y  Q
                                                                  ↪ G4
1801 tttgggggttccctgtccagtcacctggagtttgtgggcatcctggcccgacataggaactggcccagtctctccatgcacgtcctcccgcgaagctcccgaggcctcctcctcttcact
 601 F  G  G  S  L  S  S  H  L  E  F  V  G  I  L  A  R  H  R  N  W  P  S  L  S  M  H  V  L  P  R  S  S  R  G  L  L  L  F  T

1921 gcccgtctgaggcccggcagcccctccctggcgctcttcctgagcaatggccacttcgttgcacagatggaaggcctcgggactcggctccgcgcccagagccgccagcgctcccggcct
 641 A  R  L  R  P  G  S  P  S  L  A  L  F  L  S  N  G  H  F  V  A  Q  M  E  G  L  G  T  R  L  R  A  Q  S  R  Q  R  S  R  P

2041 ggccgctggcacaaggtctccgtgcgctgggagaagaaccggatcctgctggtgacggacggggcccgggcctggagccaggaggggccgcaccggcagcaccaggggggcagagcacccc
 681 G  R  W  H  K  V  S  V  R  W  E  K  N  R  I  L  L  V  T  D  G  A  R  A  W  S  Q  E  G  P  H  R  Q  H  Q  G  A  E  H  P

2161 cagccccacaccctctttgtgggcggcctcccggccagcagccacagctccaaacttccggtgaccgtcgggttcagcggctgtgtgaagagactgaggctgcacgggaggccccctgggg
 721 Q  P  H  T  L  F  V  G  G  L  P  A  S  S  H  S  S  K  L  P  V  T  V  G  F  S  G  C  V  K  R  L  R  L  H  G  R  P  L  G

2281 gcccccacacggatggcaggggtcacaccctgcatcttgggccccctggaggcgggcctgttcttcccaggcagcgggggagttatcactttagacctcccaggagctacactgcctgat
 761 A  P  T  R  M  A  G  V  T  P  C  I  L  G  P  L  E  A  G  L  F  F  P  G  S  G  G  V  I  T  L  D  L  P  G  A  T  L  P  D
                                   ↪ G5
2401 gtgggcctggaactggaggtgcggcccctggcagtcaccggactgatcttccacttgggccaggcccggacgcccccctacttgcagttgcaggtgaccgagaagcaagtcctgctgcgg
 801 V  G  L  E  L  E  V  R  P  L  A  V  T  G  L  I  F  H  L  G  Q  A  R  T  P  P  Y  L  Q  L  Q  V  T  E  K  Q  V  L  L  R

2521 gcggatgacggagcaggggagttctccacgtcagtgacccgcccctcagtgctgtgtgatggccagtggcaccggctagcggtgatgaaaagcgggaatgtgctccggctggaggtggac
 841 A  D  D  G  A  G  E  F  S  T  S  V  T  R  P  S  V  L  C  D  G  Q  W  H  R  L  A  V  M  K  S  G  N  V  L  R  L  E  V  D

2641 gcgcagagcaaccacaccgtgggccccttgctggcggctgcagctggtgccccagcccctctgtacctcgggggcctgcctgagcccatggccgtgcagccctggccccccgcctactgc
 881 A  Q  S  N  H  T  V  G  P  L  L  A  A  A  A  G  A  P  A  P  L  Y  L  G  G  L  P  E  P  M  A  V  Q  P  W  P  P  A  Y  C

2761 ggctgcatgaggaggctggcggtgaaccggtccccgtcgccatgactcgctctgtggaggtccacggggcagtgggggccagtggctgcccagccgcctaggacacagccaaccccggc
 921 G  C  M  R  R  L  A  V  N  R  S  P  V  A  M  T  R  S  V  E  V  H  G  A  V  G  A  S  G  C  P  A  A  *

2881 ccctggtcaggcccctgcagctgcctcacaccgccccttgtgctcgcctcataggtgtctatttggactctaagctctacgggtgacagatcttgtttctgaagatggtttaagttatag

3001 cttcttaaacgaaagaataaaatactgcaaaatgtttttatatttggccctccacccatttttaattgtgagagatttgtcaccaatcatcactggttcctccttaaaaattaaaaagt
                                  ↑
3121 aacttctgtgtaaaaaaaaaa
```

B

Human Laminin α5 Chain Sequence *versus*

	G1	G2	G3	G4	G5	G_{TOTAL}
mouse α5	82	83	75	61	61	72
human α1	21	27	22	19	27	23
human α2	18	26	19	25	23	21
human α3	44	27	34	31	37	34
human α4	38	28	25	25	36	30

Fig. 1 (*figure caption on page 298*).

spreads obtained from human lymphocytes. Preparation of chromosomes, hybridization, autoradiography, and chromosome banding were performed as previously described [14,15]. For fluorescence in situ hybridization, the same probe was labeled with biotin–16-dUTP (Boehringer-Mannheim) and hybridized to metaphase spreads according to the method of Pinkel et al. [16]. The hybridized probe was

Fig. 1. (A) Composite sequence of the human laminin α5-chain cDNA clones encoding the C-terminal G-domain. The nucleotide sequence is on the top line, and the deduced amino acid sequence, in the one letter code, is on the bottom line. An asterisk indicates the TAG stop codon. The two polyadenylation signals found in the cDNA clones are underlined, and the poly A addition site in clone 1047 is marked by an up arrow. Cysteine residues are circled. The boundaries between the subdomains of the G-domain (the G1–G5 repeats and the spacer region between the G3 and G4 repeats) were assigned according to the criteria of Garrison et al. [27] and Richards et al.[28], and a right arrow is placed beneath the first residue of each domain. (B) Comparison of the human laminin α5-chain G-domain sequence to that of the mouse laminin α5-chain (residues 2662–3610 of GenBank U37501 [9]) and to the G domains of the other four human α-chains: α1 (residues 3126–3075 of SWISS-PROT P25391 [29]), α2 (residues 2154–3110 of GenBank Z26653 [30]), α3 (residues 777–1713 of GenBank L34155 [31]), and α4 (residues 835–1816 of GenBank X91171 [28]). The sequences were aligned and the percent identity calculated with the GAP program using the default parameters.
←

detected by means of fluorescein isothiocyanate-conjugated avidin (Vector Laboratories), and chromosomes were counterstained and R-banded with propidium iodide as described in Lemieux et al. [17].

2.4. Linkage mapping of the mouse gene

To screen for informative DNA polymorphisms, we amplified and sequenced a PCR product from both C57BL/6J and *Mus spretus* (SPRET/Ei) genomic DNAs (obtained from The Jackson Laboratory Mouse DNA Resource), using primers derived from the mouse cDNA sequence. The C57BL/6J allele contained a *Stu*I site (AGGCCT, located in a 111-bp intron between nt 9267 and 9268 of the cDNA sequence) that was absent in the *M. spretus* allele (AGGCTT). The 94 progeny of the Jackson Laboratory (C57BL/6JEi×SPRET/Ei)-F1×SPRET/Ei backcross panel (designated BSS) [18] were genotyped for the polymorphic *Stu*I site. Aliquots of genomic DNA (100 ng/50 μl reaction) were amplified with the primers 5′-dTTTGGCTTTCGTGGCACCCAGG (nt 9214–9235) and 5′-dATAGAAGGCAACATAGTGAGGAGC (nt 9361–9384), using AmpliTaq DNA polymerase and reagents (Perkin-Elmer). After an initial denaturation at 94°C for 2 min, 35 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 60 s were run. The purified PCR products were digested with *Stu*I and separated on 5% polyacrylamide/0.5×TBE gels to score for presence of the C57BL/6J allele, indicated by cleavage of the 282-bp PCR product to yield bands of 98 and 184 bp. Analysis of the data and chromosomal assignment of the mouse gene were performed by The Jackson Laboratory Backcross Mapping Service.

3. Results and discussion

We have isolated and characterized overlapping cDNA clones that encode the C-terminal G-domain of the human laminin α5-chain. The composite sequence contains an open reading frame of 2859-bp encoding 953 amino acids, followed by a TAG stop codon and a 3′ untranslated region of either 179 nt (clone 1047 and clone 155198) or 269 nt (clone 1140), due to alternative use of an AATAAA polyadenylation signal at nt 3016 or an ATTAAA polyadenylation signal at nt 3111 (Fig. 1A). The amino acid sequences of the human and mouse laminin α5-chain G-domains have an overall identity of 72%, with a greater similarity in the G1 and G2 repeats (>80% identity) than in the G4 and G5 repeats (61% identity) (Fig. 1B). An extra pair of cysteine residues (amino acids 430 and 920) are present in the human sequence, and the spacer region between the G3 and G4 repeats is seven residues longer than in the mouse sequence. When compared to the sequence of the other four known laminin α-chains, the laminin α5 G-domain has the greatest similarity to that of the α3-chain and the least to that of the α2-chain (Fig. 1B). The degree of sequence identity varies among the five G-repeats, suggesting that they may perform different functions.

The distribution of laminin α5-chain mRNA in human tissues was analyzed by hybridizing the ^{32}P-labeled insert of clone 1047 to a multiple tissue Northern blot. A single laminin α5-transcript of ∼12 kb was detected in placenta and in adult heart, lung, kidney, skeletal muscle, and pancreas, with little or no expression in brain and liver (Fig. 2). The laminin α5 message was also observed on Northern blots of total RNA extracted from several human cell lines, including clone A colon carcinoma, A431 epidermoid carcinoma, and JAR choriocarcinoma cells (not shown), suggesting that human cell lines may prove useful for studying the synthesis and processing of the α5-chain.

The chromosomal localization of the human laminin α5-chain gene (*LAMA5*) was determined by in situ hybridization. Upon examination of 100 metaphase cells hybridized to a tritiated α5-chain cDNA probe, 183 silver grains were associated with chromosomes; 46 silver grains were located on human chromosome 20, with 41 of these (89%) non-randomly distributed on the q13.2-q13.3 bands of the long arm, with the maximum on the q13.3 band (Fig. 3A). When fluorescence in situ hybridization was performed using a biotinylated probe, 90% of 50 metaphase cells examined showed specific fluorescent signals on the 20q13.3 band (not shown). The mouse gene (*Lama5*) was mapped by linkage analysis using an interspecific backcross panel. Segregation of a polymorphic *Stu*I site in the backcross progeny revealed that *Lama5* was linked to markers on distal mouse chromosome 2 (Fig. 3B,C). *Lama5* was mapped 3.2±1.8 cM distal to *Gnas*, the gene encoding the α-subunit of the stimulatory G-protein, and 1.1±1.1 cM proximal to the microsatellite markers *D2Mit74*, *D2Mit266*, and *D2Mit457*. *LAMA5* and *Lama5* are members of a large conserved linkage group that includes all of human chromosome 20 and the distal region of mouse chromosome 2 [19]. Based on the position of *Lama5* relative to other genes on mouse chromosome 2, *LAMA5* is predicted to reside distal to *GNAS1* (the human homologue of *Gnas*) and *EDN3* (endothelin-3) on human chromosome 20.

The human laminin α5-chain gene does not appear to be a likely candidate gene for any genetic disorders assigned so far to the long arm of human chromosome 20 [20], but the mouse gene maps very close to the locus of semidominant ragged (*Ra*) mutation on distal chromosome 2 [21]. *Ra* heterozygotes have sparse fur due to a deficiency of certain hair follicle types, while *Ra* homozygotes lack fur, are frequently edema-

Fig. 2. Northern blot analysis of the tissue distribution of the human laminin α5-chain mRNA. The ^{32}P-labeled insert of clone 1047 was hybridized to a human multiple tissue Northern blot containing 2 μg per lane of poly A+ RNA from heart (lane 1), brain (2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and pancreas (lane 8). The signal in lane 2 may be an artifact.

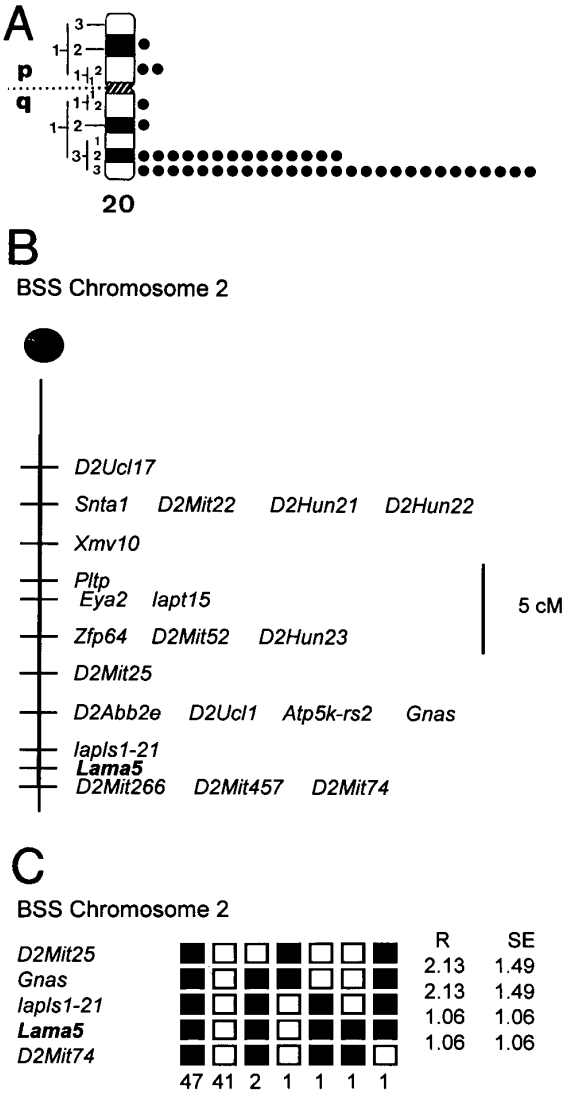

Fig. 3. Chromosomal localization of the human and mouse laminin α5-chain genes. (A) Idiogram of G-banded human chromosome 20, showing the distribution of labeled sites observed when the tritium-labeled human laminin α5-chain cDNA clone 1047 was hybridized to chromosome preparations. (B): Linkage map of the distal region of mouse chromosome 2, showing the position of *Lama5* in relation to flanking markers. (C) Haplotype analysis of the 94 Jackson Laboratory BSS backcross progeny for *Lama5* and selected flanking markers. Columns represent the genotypes of the backcross progeny, with white boxes indicating mice homozygous for the *M. spretus* allele and black boxes indicating mice heterozygous for the C57BL/6J and *M. spretus* alleles. The number of progeny with each haplotype is given below the columns. To the right, the column labeled R lists the distances between markers in cM, and the column marked SE lists the standard errors for the map distances. Complete data for *Lama5* and other markers mapped on the Jackson Laboratory BSS backcross panel are available electronically at World Wide Web URL http://www.jax.org/resources/documents/cmdata.

tous, and display a high rate of pre- and perinatal lethality due to unknown causes [22,23]. The laminin α5-chain is expressed in skin [9], consistent with a role in hair follicle development, and it is also present in the heart and kidney [9,10], two organs in which dysfunction can produce edema. The *Ra* mutation occurs in the ROP (*Ra*/+ *Os*/+ *Pt*/+) mutant strain of mice, which have nephrogenic defects resulting from the *Os* (oligosyndactyly) mutation [24]. The ROP mice were found to have enhanced levels of glomerulosclerosis and glomerular laminin protein and laminin β1-chain mRNA accumulation compared to *Os*/+ mice on a C57BL/6J genetic background [25]. Loss of one laminin chain may lead to overexpression of another, as was observed in the laminin β2-chain knock-out mice [26]. Thus, the laminin α5-chain should be evaluated as a potential candidate gene for the *Ra* mutation; alternatively, it may serve as a marker for positional cloning of the *Ra* gene.

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