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## Mutations in the Human Laminin $\beta$ 2 (LAMB2) Gene and the Associated Phenotypic Spectrum

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## Mutations in the Human Laminin $\beta 2$ (*LAMB2*) Gene and the Associated Phenotypic Spectrum

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## ABSTRACT

Mutations of *LAMB2* typically cause autosomal recessive Pierson syndrome, a disorder characterized by congenital nephrotic syndrome, ocular and neurologic abnormalities, but may occasionally be associated with milder or oligosymptomatic disease variants. *LAMB2* encodes the basement membrane protein laminin  $\beta 2$  which is incorporated in specific heterotrimeric laminin isoforms and has an expression pattern corresponding to the pattern of organ manifestations in Pierson syndrome. Herein we review all previously reported and several novel *LAMB2* mutations in relation to the associated phenotype in patients from 39 unrelated families. The majority of disease-causing *LAMB2* mutations are truncating, consistent with the hypothesis that loss of laminin  $\beta 2$  function is the molecular basis of Pierson syndrome. While truncating mutations are distributed across the entire gene, missense mutations are clearly clustered in the N-terminal LN domain, which is important for intermolecular interactions. There is an association of missense mutations and small in frame deletions with a higher mean age at onset of renal disease and with absence of neurologic abnormalities, thus suggesting that at least some of these may represent hypomorphic alleles. Nevertheless, genotype alone does not appear to explain the full range of clinical variability, and therefore hitherto unidentified modifiers are likely to exist.

**Key words:** *LAMB2*, Pierson syndrome, nephrotic syndrome, autosomal recessive, podocyte, laminin, ocular malformation



## BACKGROUND

Mutations of *LAMB2* (MIM# 150325), the gene encoding laminin  $\beta$ 2, were first detected in patients who suffered from congenital nephrotic syndrome (NS) histologically presenting as diffuse mesangial sclerosis, in combination with a complex ocular maldevelopment the most impressive clinical sign of which is extreme and fixed narrowing of the pupils (microcoria) (Zenker et al., 2004a). This unusual association was first described by Pierson et al. in 1963 (Pierson et al., 1963), and therefore the term Pierson syndrome was coined for this disorder (MIM# 609049) (Zenker et al., 2004b). Microcoria-congenital nephrosis syndrome is a synonym. Patients with Pierson syndrome are also at risk for severe neurodevelopmental deficits including congenital muscular weakness / myasthenia and developmental retardation (Maselli et al., 2009; Wuhl et al., 2007). The clinical manifestations in Pierson syndrome correspond well to the defects observed in mice deficient of laminin  $\beta$ 2, who display severe glomerular kidney disease, ocular and neurologic abnormalities (Miner et al., 2006; Noakes et al., 1995a; Noakes et al., 1995b).

Laminins represent a group of cross-shaped heterotrimeric proteins each consisting of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits joined together through a coiled coil. Laminins are indispensable basement membranes constituents with important roles in cell adhesion, proliferation, differentiation and migration (Miner and Yurchenco, 2004; Ryan et al., 1996; Tunggal et al., 2000). Laminin-521 (consisting of  $\alpha$ 5,  $\beta$ 2, and  $\gamma$ 1 subunits; formerly called laminin-11) is the most common laminin isoform that contains a  $\beta$ 2-chain (Miner and Patton, 1999). This isoform is specifically expressed at distinct sites such as the glomerular basement membrane, various ocular structures, and the neuromuscular system,

1  
2  
3 consistent with the pattern of organ involvement in Pierson syndrome (Zenker et al.,  
4  
5 2004a).  
6

7  
8 The human *LAMB2* gene maps to chromosome band 3p21 and is composed of 32  
9  
10 densely packed exons spanning about 12 kb of genomic DNA (Fig. 1a). The gene  
11  
12 encodes a protein of 1,798 amino acids, containing the typical laminin domains: the N-  
13  
14 terminal globular laminin domain (LN) making interactions with neighboring laminins,  
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16 multiple EGF-like repeats (LE) with an interjacent second globular domain (LF) whose  
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18 function is currently unknown, and a coiled coil domain (LCC) (Fig. 1b). The most N-  
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20 terminal 32 amino acids represent a cleavable signal peptide.  
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25 Although Pierson syndrome was not recognized as a separate entity before 2004,  
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27 several reports on single cases and a few patient series have appeared in the years  
28  
29 since then (Bredrup et al., 2008; Choi et al., 2008; Hasselbacher et al., 2006; Kagan et  
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31 al., 2008; Maselli et al., 2009; Matejas et al., 2006; VanDeVoorde et al., 2006; Wuhl et  
32  
33 al., 2007), indicating that this disease had likely been overlooked before. The current  
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35 literature also includes some descriptions of milder variants of the disease as well as  
36  
37 two observations of apparent isolated infantile NS caused by homozygous or compound  
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39 heterozygous mutations of *LAMB2* (Choi et al., 2008; Hasselbacher et al., 2006; Kagan  
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41 et al., 2008; Matejas et al., 2006). Based on the finding of missense mutations or small  
42  
43 in frame deletions at least on one allele in these cases in contrast to the predominance  
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45 of biallelic truncating mutations in the classic Pierson syndrome, it has been proposed  
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47 that the level of residual laminin  $\beta 2$  function/expression is the main modifier of the  
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49 phenotype (Hasselbacher et al., 2006; Kagan et al., 2008). Herein we review a total of  
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49 mutations of *LAMB2* including 12 novel ones, we summarize clinical findings and discuss genotype phenotype correlations.

For Peer Review

## VARIANTS IN THE *LAMB2* GENE

All *LAMB2* sequence changes published thus far, together with the novel variants characterized in our laboratories since 2004, are divided into three subgroups and listed in Tables 1, 3, and 4. The genotype and phenotype features of patients with *LAMB2* mutations are listed in Table 2.

### Mutations

Sequence changes regarded as disease-causing mutations are presented in Table 1. They comprise missense, nonsense, and splice site mutations, as well as small deletions and insertions, found either as homozygous or compound heterozygous sequence changes in patients affected by typical Pierson syndrome or its milder variants (Choi et al., 2008; Hinkes et al., 2007; Kagan et al., 2008; Maselli et al., 2009; Matejas et al., 2006; VanDeVoorde et al., 2006; Wuhl et al., 2007; Zenker et al., 2004a, 2005), as well as in two previously published siblings with isolated NS (Hasselbacher et al., 2006). The 12 novel mutations were identified by automated sequencing of PCR products of genomic DNA as described previously (Zenker et al., 2004a). All coding exons and flanking intronic regions were analyzed in each patient. Sequence changes were classified as causative mutations, if they produce a premature translational stop codon, if they affect the conserved nucleotides at the splice acceptor and donor sites, respectively, or if they delete or substitute a conserved amino acid and were observed together with a mutation on the second allele.

The majority of mutations (35 out of 49) are predicted to lead to a premature translational stop codon. These mutations include 14 nonsense, 19 frameshift, and two

1  
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3 splice site mutations whose consequences on splicing could be confirmed on the mRNA  
4 level. The precise effect of four additional splice site mutations (c.1036+6\_9delTGAG;  
5 c.3798-2A>C; c.3982+1G>T; c.4573+1G>A) has not been determined experimentally,  
6 because no appropriate material from the respective patients was available. However,  
7 in all of them the most likely consequence predicted by *in silico* analysis is either exon  
8 skipping or intron inclusion, shifting the reading frame. This is also true for the mutation  
9 c.1036+6\_9delTGAG, the only identified splice site mutation that does not affect the  
10 invariant nucleotides +1 or +2 of the splice donor, but which is nevertheless predicted to  
11 lead to loss of splice donor function. Only eight missense changes and two in frame  
12 deletions leading to the loss of a single amino acid have been identified as likely  
13 causative mutations, to date. All of them affect highly conserved amino acid residues of  
14 the laminin  $\beta$ 2 protein (Supp. Figure S1) and were found either in the homozygous state  
15 or in compound heterozygosity with another *bona fide* mutation on the second allele  
16 (Table 2). All missense mutations except for two (p.L139P, p.S80R) have been reported  
17 previously (Choi et al., 2008; Hasselbacher et al., 2006; Kagan et al., 2008; Matejas et  
18 al., 2006; Zenker et al., 2004a). None of these changes was found in over 200 controls.  
19 Five mutations were recurrent (Table 2). Four of them (c.1405+1G>A, c.1477delT,  
20 c.3174\_3175delTG, and c.4504delA) were found in two unrelated families each, while  
21 the mutation p.R246W was independently observed in 5 unrelated families. The  
22 remaining changes are “private mutations” observed only in single families.

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25 Mutations creating premature stop codons are almost evenly distributed along the  
26 *LAMB2* gene (Fig. 1a). They may either lead to nonsense mediated mRNA decay or  
27 result in truncated proteins. Notably, the mutation c.5258dupA, which is predicted to  
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3 produce the premature translational stop located most closely to the 3' end of the  
4 mRNA and to delete only 39 amino acids from the protein, has been demonstrated to  
5 result in complete lack of protein expression (Zenker et al., 2004a). This may be due to  
6 the fact that the C-terminus of laminin  $\beta$ 2 is important for the proper assembly of the  
7 laminin chains. Mutant laminin  $\beta$ 2 chains that cannot be stably assembled into a trimeric  
8 laminin complex are probably degraded (Utani et al., 1994). Consequently, all truncating  
9 mutations known to date likely represent functional null alleles.

10  
11 In contrast, missense mutations and small in frame deletions obviously cluster in the LN  
12 domain of laminin  $\beta$ 2 (Fig. 1b). This protein domain is critical for interacting with the LN  
13 domains of  $\alpha$  and  $\gamma$  chains of neighboring laminins to form the monolayer network which  
14 represents a scaffold for basement membrane assembly (Cognato et al., 1999;  
15 Yurchenco and Cheng, 1993). This suggests that changes of highly conserved amino  
16 acid residues in that domain might perturb laminin polymerization. However, it has also  
17 been shown that the mutation p.R246W leads to significant reduction in protein  
18 expression (Zenker et al., 2004a). This may be due to disturbances at various stages of  
19 protein processing. Consistently, studies on a mouse model expressing the laminin  $\beta$ 2  
20 mutant R246Q suggested that the impact of this mutant on glomerular function stems in  
21 part from impaired laminin secretion (Cheng et al., 2008). The missense mutation  
22 p.C321R affects one of the invariant cysteines in the first EGF-like domain, LEa1 (Fig.  
23 1c). As these cysteine residues form disulfide bonds that stabilize the structure of the  
24 EGF-like domains, substitution by other amino acids probably result in alteration or  
25 destabilization of protein structure. The consequences of the missense change  
26 p.L1393F affecting the LCC domain remain elusive (Hasselbacher et al., 2006).

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3 In our own study population, the causative sequence changes were identified on both  
4 alleles in 29 out of 30 unrelated patients with typical Pierson syndrome (as defined by  
5 the presence of congenital microcoria plus NS). In a single patient (case 3.1, Table 2)  
6 only one allele, a nonsense mutation (p.Q125X) was identified, while the second allele  
7 has remained undetected despite sequencing of all introns and the presumed promoter  
8 region as well as screening for larger genomic deletions (using long range PCR  
9 covering the entire gene). RT-PCR on mRNA from kidney tissue of this patient,  
10 however, showed severely reduced *LAMB2* mRNA expression from the allele that was  
11 not affected by the nonsense mutation (data not shown). These findings strongly  
12 support the existence of a mutation on the second allele, which escaped the employed  
13 screening methods (maybe an inversion or translocation affecting the *LAMB2* locus).  
14 Together, these results provide clear evidence that Pierson syndrome is not  
15 heterogeneous and that *LAMB2* mutation detection rate reaches 98-100% in typical  
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### 39 **Polymorphisms**

40 Table 3 lists sequence variants that likely do not lead to development of Pierson  
41 syndrome or NS. These changes were quoted as probable neutral polymorphisms  
42 because they have been found either in homozygous state in healthy controls, in  
43 compound heterozygosity together with a clearly disease causing mutation in healthy  
44 Pierson syndrome carriers, or together with two *bona fide* mutations in patients.  
45 Altogether, 26 polymorphisms have been detected by sequencing of more than 200  
46 individuals of various ethnic backgrounds (Table 3). Eight of them are known  
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3 polymorphisms listed in the dbSNP database, while 18 variants are novel. 15 of the  
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5 latter are either intronic with no obvious effect on splicing or do not cause changes on  
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7 the protein level and, thus, are not supposed to affect the protein structure or function. A  
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9 heterozygous variant in exon 24, predicting a substitution of glycine by arginine  
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11 (p.G1243R) was identified in a patient with Pierson syndrome together with the mutation  
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13 p.G1693VfsX20 on the same allele. Moreover, interspecies alignment revealed only  
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15 poor conservation of the glycine at position 1243, together suggesting that this change  
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17 likely represents a neutral polymorphism. Similarly, the sequence variant c.4140C>A in  
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19 exon 26 leading to an exchange of asparagine by lysine (p.N1380K) was detected in a  
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21 patient together with the mutation p.L1393F on the same allele. Based on evolutionary  
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23 conservation and on the fact that p.N1380K but not p.L1393F was also found in one of  
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25 96 controls, the former was regarded as a probable polymorphism and the latter as the  
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27 disease-causing mutation (Hasselbacher et al., 2006). The c.5293G>A variant  
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29 (predicting the change p.A1765T) was found repeatedly in both patients and healthy  
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31 controls, and it was found in one family to be located on the same allele as a truncating  
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33 mutation (p.Q1006NfsX144) (Matejas et al., 2006). For evolutionary conservation of  
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35 these missense variants see Supp. Figure S2.  
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### 46 **Sequence Variants of *LAMB2* with Unknown Phenotype Effect**

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48 For nine missense variations in the *LAMB2* gene and three variations located 5' to the  
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50 start codon in the potential promoter region, the pathogenetic significance could not  
51  
52 definitively be determined. These changes are listed in Table 4. All but two of them have  
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54 been found as heterozygous changes in patients with NS who lacked other typical  
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3 features of Pierson syndrome. None of these patients was found to have a disease-  
4 causing change on the second allele. Nevertheless, we cannot exclude the possibility  
5 that the *LAMB2* alteration might have contributed to the renal disorder in these patients.  
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7  
8 The amino acids affected by those changes show various levels of evolutionary  
9 conservation (Supp. Figure S2). Three variations are located in the putative *LAMB2*  
10 promoter region. Two of them were heterozygous (c.-1925G>C and c.-  
11 408\_404delTAGTT) while the c.-165C>A substitution was found in a homozygous state  
12 in a patient with isolated NS. Their significance cannot be determined clearly, since the  
13 *LAMB2* promoter is poorly characterized, so far. The heterozygous variant p.P37A  
14 affects the relatively conserved signal peptide cleavage site and is predicted to possibly  
15 favour aberrant cleavage 4 amino acids more downstream (SignalP 3.0 Server at  
16 <http://www.cbs.dtu.dk/services/SignalP/>), thus leading to a protein that is slightly  
17 shortened at its N-terminus. A heterozygous substitution p.H882Y was found in a  
18 healthy carrier for Pierson syndrome, who was heterozygous for the mutation p.C374X,  
19 but whether the variation p.H882Y was on the same allele could not be determined,  
20 because the affected children were not available for genetic testing (Zenker et al.,  
21 2005). Since His-882 is relatively conserved (Supp. Figure S2) and we cannot  
22 absolutely exclude the possibility of two clinically significant sequence changes on the  
23 same allele, we conservatively classified p.H882Y in this category.  
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### 51 **Haplotype Analysis of Recurrent *LAMB2* Mutations**

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53 In patients harbouring the five recurrent *LAMB2* mutations we determined haplotypes by  
54 genotyping 13 microsatellite markers within a range of 15 Mb flanking the *LAMB2* gene  
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3 as well as 5 to 6 common intragenic single nucleotide polymorphisms in the index  
4 patient and his/her parents, as far as parental DNAs were available (Supp. Figure S3).  
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6 The mutation p.R246W, which has been observed in 5 unrelated families, was found on  
7  
8 the same haplotype in three families of Portuguese origin (two from Portugal and one  
9  
10 from Brazil), thus suggesting a founder effect in this population. However, the same  
11  
12 mutation was also found in one family of Asian origin on a different haplotype and in a  
13  
14 patient of African origin. Shared haplotypes were also found in two families with Slavic  
15  
16 background (originating from Poland and the Czech Republic), who carried the mutation  
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18 c.4504delA, two families of German/French ancestry (mutation: c.1477delT), and two  
19  
20 families of Middle European origin (mutation: p.C1058X), respectively. In all cases the  
21  
22 shared haplotypes encompassed about 5 Mb. However, the precise extent could not be  
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24 determined because it is impossible to distinguish identity by descent from identity by  
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26 state for individual markers.  
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## 36 **GENOTYPE-PHENOTYPE CORRELATIONS**

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38 All individuals carrying homozygous or compound heterozygous *LAMB2* mutations were  
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40 affected by NS in the first decade, the vast majority in the first year of life. All but two  
41  
42 patients (affected siblings from family 13) had ocular anomalies (Table 2). Although an  
43  
44 ascertainment bias cannot be excluded, the current data suggest that kidney  
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46 involvement is an invariant manifestation of genetic defects of the *LAMB2* gene.  
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48 However, some patients may be recognized because of their eye findings, and the renal  
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50 symptoms only arrive thereafter (Bredrup et al., 2008). As mentioned above, there is  
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52 strong evidence that truncating mutations, even those creating truncations of less than  
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3 50 amino acids from the C-terminal part of the protein, represent functional null alleles.  
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5 Comparison of the group of patients with biallelic truncating mutations versus those with  
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7 non-truncating mutations on at least one allele reveals a somewhat earlier onset of  
8  
9 nephrotic symptoms and delayed occurrence of end stage renal disease (ESRD) at  
10  
11 least in some of them (Fig. 2). Patients with two putative functional null alleles typically  
12  
13 develop ESRD within the first year of life. ESRD may already be present at birth and  
14  
15 mask nephrotic symptoms (hypoproteinemia, edema) through the limitation of renal  
16  
17 protein waste in the presence of oliguria. However, in two patients with biallelic  
18  
19 truncating mutations and presumed complete loss of laminin  $\beta$ 2 production, ESRD was  
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21 delayed until childhood age (patients 24.1 and 29.1) (Choi et al., 2008 and unpublished  
22  
23 observation). Regarding ocular manifestations, all patients harboring biallelic nonsense  
24  
25 or frameshift mutations exhibited congenital microcoria in association with variable other  
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27 eye abnormalities as reviewed previously (Bredrup et al., 2008), except for one  
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29 unpublished case (patient 29.1). With this single exception, patients who lacked this  
30  
31 typical ocular sign of Pierson syndrome, including the two reported families with initial  
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33 presentation of isolated NS (Choi et al., 2008; Hasselbacher et al., 2006), had at least  
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35 one non-truncating allele or a splice site mutation whose effect on protein expression  
36  
37 could not be definitely determined. This may suggest that only little residual function of  
38  
39 laminin  $\beta$ 2 is required for an apparently normal development and maintenance of the iris  
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41 muscles. Notably, those patients who presented initially with only minor or without  
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43 ocular changes appear to remain at high risk of developing serious ocular  
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45 complications, e.g. retinal detachment, in later infancy or childhood (Bredrup et al.,  
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47 2008; Choi et al., 2008; Matejas et al., 2006). Generally, isolated NS is rarely caused by  
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3 *LAMB2* mutations (Hinkes et al., 2007). In addition to what was published before (Choi  
4 et al., 2008; Hasselbacher et al., 2006; Hinkes et al., 2007), we have meanwhile studied  
5 more than 70 further cases with isolated NS without detecting another *LAMB2* mutation-  
6 positive individual in this cohort. The same is true for nonspecific syndromic NS cases  
7 lacking ocular abnormalities of the Pierson syndrome spectrum. Significant  
8 neurodevelopmental abnormalities (muscular weakness / myasthenia, global delay,  
9 presumed retinal blindness) have been described repeatedly in patients with presumed  
10 complete deficiency of laminin  $\beta$ 2 (Maselli et al., 2009; Wuhl et al., 2007) . However, the  
11 nature of these deficits is not well characterized, and only few patients with the classic  
12 Pierson syndrome phenotype survived beyond the age of 2. One long-term survivor has  
13 recently been described with a neurologic picture resembling congenital myasthenia  
14 (Maselli et al., 2009). In contrast, we are aware of several patients (case 21.1, 24.1,  
15 29.1, 31.1, 32.1, and 35.1; Table 2) with either truncating or splice site mutations on  
16 each allele, who had a normal neurologic and cognitive development up to the age of 4-  
17 21 years (Bredrup et al., 2008; Choi et al., 2008; Wuhl et al., 2007). In one of them,  
18 possible residual function of one allele carrying a *de novo* splice site mutation (c.3798-  
19 2A>C) was discussed but could not be demonstrated (Wuhl et al., 2007). Most patients  
20 who showed a favourable neurodevelopmental outcome despite the presence of  
21 biallelic mutations predicting probable complete loss of function also had delayed onset  
22 of ESRD. This observation would be compatible with the existence of genetic modifiers  
23 that may to some extent compensate for the laminin  $\beta$ 2 defect, thereby rescuing the  
24 neurologic deficits and ameliorating the renal phenotype. Admittedly, considering  
25 truncating mutations as functional null alleles may be an oversimplification and does not  
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3 account for possible rescue mechanisms on the transcriptional level (Kellermayer,  
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6 2006).

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8 Taken together, the current data provide evidence of significant genotype phenotype  
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10 correlations. On the other hand, it has become obvious that the genotype alone does  
11  
12 not explain all the clinical variability among *LAMB2*-associated disorders. The  
13  
14 neurological manifestations of Pierson syndrome remain the most enigmatic part of the  
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16 disease spectrum and require further elucidation.  
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## 19 20 21 22 **CLINICAL AND DIAGNOSTIC RELEVANCE** 23

24  
25 The diagnosis of Pierson syndrome is based on the recognition of the typical  
26  
27 association of glomerular kidney disease and ocular abnormalities. In the typical cases  
28  
29 with microcoria and early onset NS the diagnosis is obvious. Molecular testing of  
30  
31 *LAMB2* will very likely confirm the diagnosis in such patients. Mutational screening by  
32  
33 sequencing of all coding exons and flanking intronic regions should particularly be  
34  
35 attempted, if the parents wish to have prenatal testing in a further pregnancy. Although  
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37 affected fetuses may also present with kidney abnormalities on prenatal ultrasound  
38  
39 (Mark et al., 2006), only molecular genetic testing allows an early and reliable prenatal  
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41 diagnosis. Molecular analysis of the *LAMB2* gene may also be indicated to identify a  
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43 familial mutation in order to offer subsequent carrier testing in healthy family members,  
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45 although the risk of having a child with Pierson syndrome in relatives of a patient is very  
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47 small (excluding consanguinity of the partners), given a presumably low carrier  
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49 frequency in the general population. In cases with congenital or infantile NS and less  
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51 specific ocular symptoms, molecular analysis of the *LAMB2* gene provides a clue in the  
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3 differential diagnosis. Among patients with isolated NS *LAMB2* mutations are apparently  
4 rare (Hinkes et al., 2007 and unpublished results). Analysis of this gene may however  
5 still be justified in patients with congenital or infantile nephrosis, mesangial sclerosis on  
6 biopsy, and negative results upon testing for mutations in the genes *NPHS1*, *NPHS2*,  
7 *WT1*, and *PLCE1*. Early development of ESRD (from birth or in the first 3 months of  
8 life), which is unusual in other types of congenital NS, may be a further indication for  
9 testing for *LAMB2* mutations. Considering the fact that microcoria may be the first  
10 presenting symptom of Pierson syndrome, *LAMB2* testing may be of important  
11 diagnostic and prognostic value in any child with congenital microcoria.  
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24 Considering the clinical variability of *LAMB2*-associated disorders, predictions on the  
25 phenotypic expression on the basis of the genotype should be made with caution.  
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27 Unfortunately, this is particularly true with respect to the possibility of relevant  
28 neurologic involvement which is an important determinant of long-term prognosis.  
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30 Within the same family, however, our current experience suggests a rather high  
31 consistency of the phenotype.  
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39 Patients with Pierson syndrome are mainly taken care of by pediatric nephrologists and  
40 ophthalmologists. There is so far no specific treatment available. Nephrectomy for the  
41 treatment of severe renal protein waste may be considered similar to the management  
42 of patients with Finnish type nephrosis (Holmberg et al., 1995), but it should be taken  
43 into account that ESRD, which usually occurs much earlier in patients with Pierson  
44 syndrome compared to Finnish type nephrosis, will spontaneously limit protein loss.  
45  
46 Kidney transplantation is currently the only long-term renal treatment option. There is so  
47 far no evidence of recurrence of mesangial sclerosis in the transplant, but the number of  
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3 successfully transplanted patients who could be monitored for a longer time is very  
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5 small. Of note, patients with a proven laminin  $\beta 2$  defect should receive careful  
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7 ophthalmological follow-up, as they are obviously at high risk of retinal detachment even  
8  
9 in the absence of significant congenital ocular anomalies. High grade myopia seems to  
10  
11 be another common feature developing in infancy and early childhood (Bredrup et al.,  
12  
13 2008).  
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16  
17 An important differential diagnosis to Pierson syndrome is the nephrosis-microcephaly  
18  
19 syndrome (Galloway-Mowat syndrome; GMS; MIM# 251300). Microcephaly which is a  
20  
21 key finding in GMS has also been described in some patients with Pierson syndrome  
22  
23 (Wuhl et al., 2007). However, in the latter microcephaly is usually not congenital, but  
24  
25 may develop during the first year of life. Moreover, other features, such as structural  
26  
27 brain anomalies, epilepsy, and hiatus hernia usually allow distinguishing GMS from  
28  
29 Pierson syndrome clinically. Notably, however, there have been a few reports on  
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31 patients with GMS and ocular changes resembling the manifestations in Pierson  
32  
33 syndrome (Mildenberger et al., 1998; Shapiro et al., 1976). Despite the obvious clinical  
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35 overlap, we have found no evidence that GMS and Pierson syndrome are allelic  
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37 disorders (Dietrich et al., 2008).  
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44 There is so far no evidence for isolated ocular anomalies or ocular plus neurologic  
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46 abnormalities without kidney involvement being caused by mutations in *LAMB2*.  
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48 Isolated microcoria was been reported as an autosomal dominant trait (MIM# 156600).  
49  
50 A provisional locus has been assigned to 13q31-q32 (Rouillac et al., 1998), thus ruling  
51  
52 out *LAMB2* as the causative gene for families linked to this locus. However, genetic  
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54 heterogeneity is not excluded and studies of a possible significance of *LAMB2*  
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3 mutations in sporadic cases with isolated microcoria or related iris symptoms as well as  
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6 in families unlinked to 13q31-q32 are warranted.  
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For Peer Review



## FUTURE PROSPECTS

Future research will be directed towards the identification of possible modifiers of the phenotype caused by laminin  $\beta 2$  defects. Considering that a lack of expression on the  $\beta 2$ -chain probably leads to the expression of aberrant laminin isoforms in the basement membrane rather than the complete absence of laminin (Noakes et al., 1995b), it is tempting to speculate that laminin  $\beta 1$  (or other laminin  $\beta$  isoforms) might to some extent be able to compensate for the lack of  $\beta 2$ . Treatment prospects might arise from the knowledge on modifying factors. As a significant proportion of *LAMB2* mutations are nonsense mutations, aminoglycosides which are known to be able to induce translational stop codon readthrough (Allamand et al., 2008; Linde et al., 2007), and which reach high concentrations particularly in the kidney, may be evaluated as a therapeutic target in the future.

A locus-specific mutation database is available at: <http://www.med.uni-magdeburg.de/LAMB2mutdb>.

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## REFERENCES

- Allamand V, Bidou L, Arakawa M, Floquet C, Shiozuka M, Paturneau-Jouas M, Gartioux C, Butler-Browne GS, Mouly V, Rousset JP, Matsuda R, Ikeda D, Guicheney P. 2008. Drug-induced readthrough of premature stop codons leads to the stabilization of laminin alpha2 chain mRNA in CMD myotubes. *J Gene Med* 10:217-224.
- Bredrup C, Matejas V, Barrow M, Blahova K, Bockenbauer D, Fowler DJ, Gregson RM, Maruniak-Chudek I, Medeira A, Mendonca EL, Kagan M, Koenig J, Krastel H, Kroes HY, Saggari A, Sawyer T, Schittkowski M, Swietliński J, Thompson D, VanDeVoorde RG, Wittebol-Post D, Woodruff G, Zurowska A, Hennekam RC, Zenker M, Russell-Eggitt I. 2008. Ophthalmological aspects of Pierson syndrome. *Am J Ophthalmol* 146:602-611.
- Chen YM, Kikkawa Y, Miner JH. 2008. Proteinuria in mice expressing a mutant laminin 2 chain (R246Q) in podocytes stems in part from impaired laminin secretion. *J Am Soc Nephrol* 19:102A.
- Choi HJ, Lee BH, Kang JH, Jeong HJ, Moon KC, Ha IS, Yu YS, Matejas V, Zenker M, Choi Y, Cheong HI. 2008. Variable phenotype of Pierson syndrome. *Pediatr Nephrol* 23:995-1000.
- Colognato H, Winkelmann DA, Yurchenco PD. 1999. Laminin polymerization induces a receptor-cytoskeleton network. *J Cell Biol* 145:619-631.
- Dietrich A, Matejas V, Bitzan M, Hashmi S, Kiraly-Borri C, Lin SP, Mildenerberger E, Hoppe B, Palm L, Shiihara T, Steiss JO, Tsai JD, Vester U, Weber S, Wühl E, Zepf K, Zenker M. 2008. Analysis of genes encoding laminin beta2 and related

1  
2  
3 proteins in patients with Galloway-Mowat syndrome. *Pediatr Nephrol* 23:1779-  
4  
5 1786.  
6

7  
8 Hasselbacher K, Wiggins RC, Matejas V, Hinkes BG, Mucha B, Hoskins BE, Ozaltin F,  
9  
10 Nurnberg G, Becker C, Hangan D, Pohl M, Kuwertz-Bröking E, Griebel M,  
11  
12 Schumacher V, Royer-Pokora B, Bakkaloglu A, Nürnberg P, Zenker M,  
13  
14 Hildebrandt F. 2006. Recessive missense mutations in LAMB2 expand the  
15  
16 clinical spectrum of LAMB2-associated disorders. *Kidney Int* 70:1008-1012.  
17

18  
19  
20 Hinkes BG, Mucha B, Vlangos CN, Gbadegesin R, Liu J, Hasselbacher K, Hangan D,  
21  
22 Ozaltin F, Zenker M, Hildebrandt F. 2007. Nephrotic syndrome in the first year of  
23  
24 life: two thirds of cases are caused by mutations in 4 genes (NPHS1, NPHS2,  
25  
26 WT1, and LAMB2). *Pediatrics* 119:e907-919.  
27

28  
29  
30 Holmberg C, Antikainen M, Ronnholm K, Ala Houhala M, Jalanko H. 1995.  
31  
32 Management of congenital nephrotic syndrome of the Finnish type. *Pediatr*  
33  
34 *Nephrol* 9:87-93.  
35

36  
37 Kagan M, Cohen AH, Matejas V, Vlangos C, Zenker M. 2008. A milder variant of  
38  
39 Pierson syndrome. *Pediatr Nephrol* 23:323-327.  
40

41  
42 Kellermayer R. 2006. Translational readthrough induction of pathogenic nonsense  
43  
44 mutations. *Eur J Med Genet* 49:445-450.  
45

46  
47 Linde L, Boelz S, Nissim-Rafinia M, Oren YS, Wilschanski M, Yaacov Y, Virgilis D, Neu-  
48  
49 Yilik G, Kulozik AE, Kerem E, Kerem B. 2007. Nonsense-mediated mRNA decay  
50  
51 affects nonsense transcript levels and governs response of cystic fibrosis  
52  
53 patients to gentamicin. *J Clin Invest* 117:683-692.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Mark K, Reis A, Zenker M. 2006. Prenatal findings in four consecutive pregnancies with  
4 fetal Pierson syndrome, a newly defined congenital nephrosis syndrome. *Prenat*  
5  
6 *Diagn* 26:262-266.  
7  
8  
9  
10 Maselli RA, Ng JJ, Anderson JA, Cagney O, Arredondo J, Williams C, Wessel HB,  
11  
12 Abdel-Hamid H, Wollmann RL. 2009. Mutations in LAMB2 causing a severe form  
13  
14 of synaptic congenital myasthenic syndrome. *J Med Genet* 46:203-208.  
15  
16  
17 Matejas V, Al-Gazali L, Amirlak I, Zenker M. 2006. A syndrome comprising childhood-  
18  
19 onset glomerular kidney disease and ocular abnormalities with progressive loss  
20  
21 of vision is caused by mutated LAMB2. *Nephrol Dial Transplant* 21:3283-3286.  
22  
23  
24 Mildenberger E, Lennert T, Kunze J, Jandeck C, Waldherr R, Versmold H. 1998. Diffuse  
25  
26 mesangial sclerosis: association with unreported congenital anomalies and  
27  
28 placental enlargement. *Acta Paediatr* 87:1301-1303.  
29  
30  
31 Miner JH, Go G, Cunningham J, Patton BL, Jarad G. 2006. Transgenic isolation of  
32  
33 skeletal muscle and kidney defects in laminin beta2 mutant mice: implications for  
34  
35 Pierson syndrome. *Development* 133:967-975.  
36  
37  
38 Miner JH, Patton BL. 1999. Laminin-11. *Int J Biochem Cell Biol* 31:811-816.  
39  
40  
41 Miner JH, Yurchenco PD. 2004. Laminin functions in tissue morphogenesis. *Annu Rev*  
42  
43 *Cell Dev Biol* 20:255-284.  
44  
45  
46 Noakes PG, Gautam M, Mudd J, Sanes JR, Merlie JP. 1995a. Aberrant differentiation of  
47  
48 neuromuscular junctions in mice lacking s-laminin/laminin beta 2. *Nature*  
49  
50 374:258-262.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Noakes PG, Miner JH, Gautam M, Cunningham JM, Sanes JR, Merlie JP. 1995b. The  
4  
5 renal glomerulus of mice lacking  $\alpha$ -laminin/laminin beta 2: nephrosis despite  
6  
7 molecular compensation by laminin beta 1. *Nat Genet* 10:400-406.  
8  
9  
10 Pierson M, Cordier J, Hervouuet F, Rauber G. 1963. [an Unusual Congenital and  
11  
12 Familial Congenital Malformative Combination Involving the Eye and Kidney]. *J*  
13  
14 *Genet Hum* 12:184-213.  
15  
16  
17 Rouillac C, Roche O, Marchant D, Bachner L, Kobetz A, Toulemont PJ, Orssaud C,  
18  
19 Urvoy M, Odent S, Le Marec B, Abitbol M, Dufier JL. 1998. Mapping of a  
20  
21 congenital microcoria locus to 13q31-q32. *Am J Hum Genet* 62:1117-1122.  
22  
23  
24 Ryan MC, Christiano AM, Engvall E, Wewer UM, Miner JH, Sanes JR, Burgeson RE.  
25  
26  
27 1996. The functions of laminins: lessons from in vivo studies. *Matrix Biol* 15:369-  
28  
29 381.  
30  
31  
32 Shapiro LR, Duncan PA, Farnsworth PB, Lefkowitz M. 1976. Congenital microcephaly,  
33  
34 hiatus hernia and nephrotic syndrome: an autosomal recessive syndrome. *Birth*  
35  
36 *Defects Orig Artic Ser* 12:275-278.  
37  
38  
39 Tunggal P, Smyth N, Paulsson M, Ott MC. 2000. Laminins: structure and genetic  
40  
41 regulation. *Microsc Res Tech* 51:214-227.  
42  
43  
44 Utani A, Nomizu M, Timpl R, Roller PP, Yamada Y. 1994. Laminin chain assembly.  
45  
46 Specific sequences at the C terminus of the long arm are required for the  
47  
48 formation of specific double- and triple-stranded coiled-coil structures. *J Biol*  
49  
50 *Chem* 269:19167-19175.  
51  
52  
53 VanDeVoorde R, Witte D, Kogan J, Goebel J. 2006. Pierson syndrome: a novel cause  
54  
55 of congenital nephrotic syndrome. *Pediatrics* 118:e501-505.  
56  
57  
58  
59  
60

- 1  
2  
3 Wuhl E, Kogan J, Zurowska A, Matejas V, Vandevoorde RG, Aigner T, Wendler O,  
4  
5 Lesniewska I, Bouvier R, Reis A, Weis J, Cochat P, Zenker M. 2007.  
6  
7 Neurodevelopmental deficits in Pierson (microcoria-congenital nephrosis)  
8  
9 syndrome. *Am J Med Genet A* 143:311-319.  
10  
11  
12 Yurchenco PD, Cheng YS. 1993. Self-assembly and calcium-binding sites in laminin. A  
13  
14 three-arm interaction model. *J Biol Chem* 268:17286-17299.  
15  
16  
17 Zenker M, Aigner T, Wendler O, Tralau T, Muntefering H, Fenski R, Pitz S, Schumacher  
18  
19 V, Royer-Pokora B, Wuhl E, Cochat P, Bouvier R, Kraus C, Mark K, Madlon H,  
20  
21 Dötsch J, Rascher W, Maruniak-Chudek I, Lennert T, Neumann LM, Reis A.  
22  
23 2004a. Human laminin  $\beta$ 2 deficiency causes congenital nephrosis with mesangial  
24  
25 sclerosis and distinct eye abnormalities. *Hum Mol Genet* 13:2625-2632.  
26  
27  
28  
29 Zenker M, Pierson M, Jonveaux P, Reis A. 2005. Demonstration of two novel LAMB2  
30  
31 mutations in the original Pierson syndrome family reported 42 years ago. *Am J*  
32  
33 *Med Genet A* 138:73-74.  
34  
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36  
37 Zenker M, Tralau T, Lennert T, Pitz S, Mark K, Madlon H, Dotsch J, Reis A, Muntefering  
38  
39 H, Neumann LM. 2004b. Congenital nephrosis, mesangial sclerosis, and distinct  
40  
41 eye abnormalities with microcoria: an autosomal recessive syndrome. *Am J Med*  
42  
43 *Genet* 130A:138-145.  
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## LEGENDS TO FIGURES

**Figure 1.** *LAMB2* gene, laminin  $\beta 2$  protein domains, and distribution of known mutations. **a**, Genomic representation of the human *LAMB2* gene. Vertical bars represent exons. Untranslated regions are shown in reduced height. Coloring corresponds to functional protein domains with grey color in parts of exon 1 and 2 encoding the signal peptide. Numerals within the boxes indicate exon numbers. Location of truncating mutations (nonsense, frameshift) and splice site mutations are indicated by arrows on top of the cartoon in black and blue, respectively. Positions of missense mutations and small in frame deletions are indicated by arrows at the bottom of the cartoon and show obvious clustering in exons 2 to 7. **b**, In its biologically active form laminin  $\beta 2$  is part of a heterotrimeric complex shown here exemplarily as laminin-521. Laminin  $\alpha 5$  and  $\gamma 1$  chains are depicted in light and dark grey, respectively. Coloring of the human laminin  $\beta 2$  chain corresponds to functional protein domains: LN, laminin N-terminal globular domain; LEa/b, laminin EGF-like modules; LF, domain IV, globular domain; LCC, laminin coiled coil domain; L $\beta$ , Laminin  $\beta$  loop; LG, C-terminal globular modules (belonging to  $\alpha$  chain). Positions of non-truncating mutations are indicated by asterisks. Most of them affect the LN domain (yellow asterisks), while one is located in the first EGF-like module (pink) and another one in the LCC domain (orange). **c**, The first EGF-like module (LEa1) is shown in detail with letters corresponding to the one letter amino acid code. The

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3 highly conserved cysteine residues are highlighted and connecting lines  
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5 indicate disulfide bonds. The mutated Cys-321 is shown in pink.  
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10 **Figure 2.** Age of onset of gross proteinuria / nephrosis (**a**) and age of onset of end  
11 stage renal disease (ESRD) (**b**) are plotted on the Y axis in two genotypic  
12 classes: patients with either nonsense or frameshift mutations on both  
13 alleles (“truncating”) and patients with a missense mutation or small in frame  
14 deletion on at least one allele (“missense”). Each bullet represents one  
15 individual patient. Grey filled circles indicate the age when the respective  
16 patient developed NS and ESRD, respectively, while open circles represent  
17 patients who have not developed this feature (nephrosis or ESRD) at the  
18 given age of their last follow-up examination. Black bullets in (**b**) represent  
19 the age of death of those patients who died without having developed  
20 ESRD. Boxes indicate the range between upper and lower quartiles. Black  
21 horizontal line represents median. Whiskers reach from maximum to  
22 minimum excluding outliers. Arrowheads represent observations out of the  
23 range of the diagram (age in months given in parentheses).  
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**Table 1. Mutations in the *LAMB2* gene causing Pierson syndrome and milder disease variants\***

Exon/Intron	DNA Variant <sup>a</sup>	Predicted and/or	Family-ID	Previous Publications
Exon 2	c.235_237delGTC	p.V79del	1	Matejas et al. [2006]
Exon 2	<b>c.240T&gt;G</b>	<b>p.S80R</b>	2	This report
Exon 3	c.373C>T	p.Q125X	3	Bredrup et al. [2008]
Exon 4	<b>c.416T&gt;C</b>	<b>p.L139P</b>	4	This report
Exon 4	c.447_449delTAT	p.I149del	5	Bredrup et al. [2008]
Exon 5	c.499G>T	p.D167Y	6	Kagan et al. [2008]
Exon 5	c.536C>T	p.S179F	7	Choi et al. [2008]
Exon 7	c.736C>T	p.R246W	8, 9, 10, 11,12	Zenker et al. [2004]
Exon 7	c.737G>A	p.R246Q	13	Hasselbacher et al. [2006]
Exon 7	<b>c.825T&gt;A</b>	<b>p.Y275X</b>	14	This report
Exon 8	c.961T>C	p.C321R	15	Hasselbacher et al. [2006]
Intron 8	<b>c.1036+6_9delTGAG</b>	<b>No mRNA studies</b>	16	This report
Exon 9	c.1122T>A	p.C374X	17	Zenker et al. [2005]
Exon 10	c.1241_1242dupCC	p.M415PfsX83	18	Wühl et al. [2007]
Exon 10	c.1252C>T	p.Q418X	18	Wühl et al. [2007]
Intron 10	c.1405+1G>A	p.S409X	19, 20	Bredrup et al. [2008]
Exon 11	c.1477delT	p.C493AfsX4	21, 22	Wühl et al. [2007]
Exon 11	c.1478delG	p.C493SfsX4	23	Maselli et al. [2009]
Exon 11	c.1503_1504delAT	p.C502X	24	Choi et al. [2008]
Exon 13	c.1723C>T	p.R575X	9	Bredrup et al. [2008]
Exon 14	c.1875_1879delGCGCT	p.L627AfsX5	25	Bredrup et al. [2008]
Exon 16	c.2067C>G	p.Y689X	17	Zenker et al. [2005]
Exon 17	c.2283_2286delCTCT	p.S762RfsX29	7	Choi et al. [2008]
Exon 18	c.2422delG	p.V808WfsX343	26	Bredrup et al. [2008]
Exon 19	c.2602C>T	p.Q868X	27	Bredrup et al. [2008]
Exon 21	c.3015delG	p.Q1006NfsX145	28	Zenker et al. [2004]
Exon 21	<b>c.3015dupG</b>	<b>p.Q1006AfsX49</b>	29	This report
Exon 21	c.3094C>T	p.R1032X	30	Bredrup et al. [2008]
Exon 22	c.3174_3175delTG	p.C1058X	27, 31	Bredrup et al. [2008]
Intron 22	c.3327+2T>C	p.R1037LfsX18	21	Wühl et al. [2007]
Exon 24	<b>c.3440dupC</b>	<b>p.R1148SfsX27</b>	29	This report
Exon 24	c.3780_3781delGG	p.E1260DfsX8	19	Bredrup et al. [2008]
Intron 24	c.3798-2A>C	No mRNA studies	31	Bredrup et al. [2008]
Exon 25	c.3902delA	p.E1301GfsX58	22	Wühl et al. [2007]
Intron 25	<b>c.3982+1G&gt;T</b>	<b>No mRNA studies</b>	32	This report
Exon 26	c.4177C>T	p.L1393F	15	Hasselbacher et al. [2006]
Exon 27	c.4267delT	p.C1423VfsX29	24	Choi et al. [2008]
Exon 27	c.4504delA	p.R1502GfsX18	33, 25	Zenker et al. [2004]
Exon 27	c.4519C>T	p.Q1507X	33	Zenker et al. [2004]
Exon 27	<b>c.4534delC</b>	<b>p.L1512FfsX8</b>	34	This report
Intron 27	<b>c.4573+1G&gt;A</b>	<b>No mRNA studies</b>	35	This report
Exon 28	c.4684C>T	p.R1562X	36	Zenker et al. [2004]
Exon 28	<b>c.4780dupA</b>	<b>p.R1594KfsX5</b>	37	This report
Exon 29	c.4804delC	p.Q1602RfsX52	23	Maselli et al. [2009]
Exon 29	<b>c.4907_4908delAG</b>	<b>p.E1636AfsX22</b>	38	This report
Exon 30	c.5078delG	p.G1693VfsX21	5	Bredrup et al. [2008]
Exon 31	c.5182C>T	p.Q1728X	1	Matejas et al. [2006]
Exon 31	<b>c.5197C&gt;T</b>	<b>p.Q1733X</b>	10	This report
Exon 31	c.5258dupA	p.E1754GfsX7	39	Zenker et al. [2004]

\*The numbering for the nucleotide changes are based on cDNA sequence in accordance with the GenBank entries NM\_002292.3, NP\_002283.3, and NT\_022517.18 (Genome Reference Consortium Human Build 37). Novel mutations are printed in bold.

<sup>a</sup>For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence

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2 **Table 2. Patients with *LAMB2* mutations\*<sup>a</sup>: genotype and phenotypic features**  
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Family #	Patient	Genotype <sup>a</sup>	Total number of affected individuals	Age of death (†) / last follow-up	Age at diagnosis of nephrotic proteinuria	Onset of ESRD / Age at initiation of RRT	Kidney biopsy result	Microcoria	Other major ocular abnormalities	Microcephaly	Neurodevelopmental deficits	Reference
14	1.1	[p.V79del]+[p.Q1728X]	7	20 y	NA	4 y	NA	-	NA	-	-	Matejas et al.
15	1.2			8 y (†)	NA	5 y	DMS	-	C, R, VI	-	-	
16	1.3			NA	4.5 y	16 y	FSGS	-	Mi, C, R, VI	-	-	
18	2.1	[p.S80R]+ [p.S80R]	1	12 y	6.5	-	atypical DMS	-	My, R	-	-	This report
20	3.1	[p.Q125X] + ?	1	2 m (†)	1 w	2 w	DMS, MGN	+	L	+	H	Bredrup et al.[2008]
22	4.1	[p.L139P] + [p.L139P]	3	12 m (†)	3 m	-	DMS?	+	L, VI	-	H, M, C	This report
23	4.2			1.5 w (†)	< 1 w	-	NA	-	VI	-	NA	
24	4.3			5 m (†)	< 1 w	-	NA	-	L, VI	-	H, M	
26	5.1	[p.I149del]+[p.G1693VfsX21]	1	2m (†)	1 w	< 1m	NA	+	VI	-	H	Bredrup et al.[2008]
29	6.1	[p.D167Y]+ [p.D167Y]	2	3.7 y	1 m	19 m	MCD	-	My, R, VI	-	-	Kagan et al. [2008]
31	7.1	[p.S179F]+[p.S762RfsX29]	1	1.5 y	2 w	1.5 y	FSGS	-	R, VI	-	-	Choi et al. [2008]
33	8.1	[p.R246W]+[p.R246W]	2	8 m (†)	5 m	-	DMS	+	L	-	H, M	Zenker et al. [2004] <sup>2</sup>
35	8.2			8 m (†)	1 w	-	DMS	+	L	-	H, M	
37	9.1	[p.R246W]+[p.R575X]	2	16 m	1 w	5 m	NA	+	L, VI	+	H, M, C	Bredrup et al.[2008]
39	9.2			1 y (†)	< 1 m	-	DMS	+	N, My	-	H, M, C	
40	10.1	[p.R246W]+[p.Q1733X]	1	2 m	1 m	-	NA	+	NA	-	NA	This report
41	11.1	[p.R246W]+[p.R246W]	1	19 m (†)	2 w	13 m	FSGS	+	L	-	M	Bredrup et al.[2008]
43	12.1	[p.R246W]+[p.R246W]	5	3 m (†)	< 1 m	NA	NA	NA	N	-	NA	This report

1														
2	13	13.1	[p.R246Q]+[p.R246Q]	2	5 y (†)	1 m	< 12m	FSGS	-	-	-	-		Hasselbacher et al. [2006]
3														
4		13.2			3 y	1 w	< 12 m	NA	-	-	-	-		
5	14	14.1	[p.Y275X]+[p.Y275X]	2	1 m	< 1 w	< 1 w	DMS	+	VI	-	NA		This report
6														
7	15	15.1	[p.C321R]+[p.L1393F]	2	7 y	3 m	5.7 y	MCD	-	N, My, R, VI	-	-		Hasselbacher et al. [2006]
8														
9		15.2			3.5 y	1 w	8 m	O	(+)	R, VI	-	-		
10	16	16.1	[c.1036+6_9del]+[c.1036+6_9del]	1	2 m	7 w	7 w	NA	+	Mi, R	-	M, S		This report
11														
12	17	17.1	[p.C374X]+[p.Y689X]	2	2 w (†)	1 w	1 w	DMS	+	L	-	H		Zenker et al. [2005]
13														
14		17.2			1.5 w (†)	1 w	1 w	DMS	+	L	-	NA		
15	18	18.1	[p.M415PfsX83]+[p.Q418X]	1	15 m (†)	< 1mo	3 m	NA	+	Mi, L, VI	+	H, M, C		Wühl et al. [2007]
16														
17														
18	19	19.1	[c.1405+1G>A]+[p.E1260DfsX8]	2	6 w (†)	< 1w	1 m	NA	+	L, VI	NA	NA		Bredrup et al.[2008]
19														
20	20	20.1	[c.1405+1G>A]+[c.1405+1G>A]	1	16 m (†)	1 w	3 w	DMS	+	N, R, VI	-	H, M, C		Bredrup et al.[2008]
21														
22	21	21.1	[p.C493AfsX4]+[c.3327+2T>C]	1	8.3 y	1.5 m	2.9 y	DMS	+	My, L, VI	-	-		Wühl et al. [2007]
23														
24	22	22.1	[p.C493AfsX4]+[p.E1301GfsX58]	1	5 y	< 1m	< 1m	DMS	+	VI	-	H, M, C		Wühl et al. [2007]
25														
26	23	23.1	[p.C493SfsX4]+[p.Q1602RfsX52]	1	20 y	1 w	12 m	DMS	+	My	-	H, M		Maselli et al. [2009]
27														
28	24	24.1	[p.C502X]+[p.C1423VfsX29]	1	7.5 y	8 m	-	MCD	+	N, My, VI	-	-		Choi et al. [2008]
29														
30	25	25.1	[p.L627AfsX5]+[p.R1502GfsX18]	2	4 w (†)	1 w	2 w	DMS	+	L, Mi, VI	-	H		Bredrup et al.[2008]
31														
32	26	26.1	[p.V808WfsX343]+[p.V808WfsX343]	2	8 m (†)	1 w	2 m	DMS	+	N	+	H, M, S		Bredrup et al.[2008]
33														
34	27	27.1	[p.Q868X]+[p.C1058X]	1	4.5 m (†)	2 w	4 m	atypical DMS	+	N, VI	-	H		Bredrup et al.[2008]
35														
36	28	28.1	[p.Q1006NfsX145]+[p.Q1006NfsX145]	8	2-8 w	< 1m	< 1m	DMS	+	NA	-	NA		Zenker et al. [2004] <sup>1</sup>
37														
38	29	29.1	[p.Q1006AfsX49]+[p.R1148SfsX27]	1	18 y	3 m	3 y	DMS	-	VI (glaucoma)	-	-		This report
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3	30	30.1	[p.R1032X]+[p.R1032X]	1	4 m (†)	1 w	1 m	DMS	+	L	+	NA		Bredrup et al.[2008]	
4															
5	31	31.1	[p.C1058X]+[c.3798-2A>C]	1	10 y	2 m	3 m	NA	+	N, My, VI	-	-		Bredrup et al.[2008]	
6															
7	32	32.1	[c.3982+1G>T]+[c.3982+1G>T]	2	5 y	< 3 y	-	DMS	+	L, N, Mi, VI	-	-		This report	
8															
9		32.2			6 m (†)	< 6 m	6 m	NA	NA	NA	NA	NA			
10	33	33.1	[p.R1502GfsX18]+[p.Q1507X]	1	2 m (†)	1w	2 m	DMS	+	N, VI, L	-	NA		Zenker et al. [2004] <sup>2</sup>	
11															
12	34	34.1	[p.L1512FfsX8]+[p.L1512FfsX8]	1	1m	< 1w	2 w	DMS	+	-	-	-		This report	
13	35	35.1	[c.4573+1G>A]+[c.4573+1G>A]	3	5 y	< 1 w	-	NA	-	N, My, R	-	-		This report	
14		35.2			21 y	3 y	21 y	MGN	-	N, My, R	-	-			
15		35.3			15 y	5 y	9 y	FSGS	-	N, My, R	-	-			
16															
17	36	36.1	[p.R1562X]+[p.R1562X]	3	1 m (†)	< 1m	< 1m	DMS	+	L		H		Zenker et al. [2004] <sup>1</sup>	
18															
19	37	37.1	[p.R1594KfsX5]+[p.R1594KfsX5]	1	1m (†)	< 1w	< 1m	NA	+	L	-	C		This report	
20	38	38.1	[p.E1636AfsX22]+[p.E1636AfsX22]	1	6 w (†)	1 w	< 1 m	DMS	+	L	-	H, M		This report	
21															
22	39	39.1	[p.E1754GfsX7]+[p.E1754GfsX7]	1	19 m (†)	< 1m	< 1m	DMS	+	L, N, VI	+	H, M		Zenker et al. [2004] <sup>2</sup>	

23 The numbering for the nucleotide changes are based on cDNA sequence in accordance with the GenBank entries NM\_002292.3, NP\_002283.3, and NT\_022517.18  
24 (GRCh37).

25 For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence

26 y, year(s); m, month(s); w, week(s); NA, not done / not available; MCD, minimal changes; DMS, diffuse mesangial sclerosis; FSGS, focal and  
27 segmental glomerulosclerosis; MGN, membranous glomerulonephritis; O, other; +, present; -, not present / none; Mi, microphthalmia; My, high  
28 myopia (> 5 diopters); N, nystagmus; L, abnormal lens (either lenticonus or cataract); R, retinal detachment; VI, severe visual impairment of any  
29 cause and despite correcting glasses; H, significant hypotonia / muscular weakness / myasthenia; M, significant motor delay; C, suspected or  
30 proven cognitive deficits, speech delay; S, seizures; MR, mental retardation

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**Table 3. Single nucleotide polymorphisms in the *LAMB2* gene**

Exon/Intron	DNA variant <sup>a</sup>	Predicted and/or demonstrated effect on protein	Status	Protein domain
Exon 3	c.306C>T	p.=	novel	LN
Intron 3	c.250-97A>G	-	novel	-
Exon 8	c.1014C>T	p.=	novel	LEa1
Exon 14	c.1764C>T	p.=	rs33942096	LF
Intron 14	c.1890+25G>A	-	rs9865051	-
Exon 16	c.2034T>C	p.=	novel	LF
Exon 17	c.2307C>T	p.=	novel	LF
Intron 18	c.2489-62C>T	-	novel	-
Exon 19	c.2673C>T	p.=	novel	LEb3
Exon 20	c.2740G>A	p.G914R	rs35713889	LEb3
Exon 20	c.2754G>T	p.=	novel	LEb3
Exon 21	c.2959G>A	p.E987K	rs34759087	LEb5
Intron 21	c.3110-15T>C	-	novel	-
Intron 22	c.3327+28T>C	-	novel	-
Intron 22	c.3328-36T>G	-	novel	-
Exon 23	c.3387A>G	p.=	rs34290943	LEb7
Exon 24	c.3645G>A	p.=	rs13082063	LCC
Exon 24	c.3727G>C	p.G1243R	novel	LCC
Exon 25	c.3858G>T	p.=	rs34967349	LCC
Exon 26	c.4140C>A	p.N1380K	novel	LCC
Intron 26	c.4224+19G>A	-	novel	-
Intron 27	c.4573+26A>G	-	novel	-
Intron 29	c.4923+17A>G	-	novel	-
Intron 29	c.4923+49G>A	-	novel	-
Intron 29	c.4924-35G>A	-	rs72936885	-
Exon 32	c.5293G>A	p.A1765T	novel	LCC

\*The numbering for the nucleotide changes are based on cDNA sequence in accordance with the GenBank entries NM\_002292.3, NP\_002283.3, and NT\_022517.18 (GRCh37).

<sup>a</sup>For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence

**Table 4. *LAMB2* sequence variants with unknown phenotypic effects**

Exon/Intron	DNA variant <sup>a</sup>	Predicted and/or demonstrated effect on protein	Status	Protein Domain
5'UTR	c.-1925G>C	-	novel	-
5'UTR	c.-404_408delTAGTT	-	novel	-
5'UTR	c.-165C>A	-	novel	-
Exon 2	c.109C>G	p.P37A	novel	Signal peptide cleavage site
Exon 9	c.1193C>T	p.T398I	novel	LEa2
Exon 10	c.1403G>T	p.R468L	novel	LEa3
Exon 13	c.1724G>A	p.R575Q	novel	LF
Exon 16	c.2099G>A	p.G700E	novel	LF
Exon 19	c.2644C>T	p.H882Y	novel	LEb3
Exon 22	c.3155_3157delCTC	p.P1053del	novel	LEb6
Exon 26	c.4118A>G	p.D1373G	novel	LCC
Exon 27	c.4370G>A	p.R1457Q	novel	LCC

\*The numbering for the nucleotide changes are based on cDNA sequence in accordance with the GenBank entries NM\_002292.3, NP\_002283.3, and NT\_022517.18 (GRCh37).

<sup>a</sup>For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence

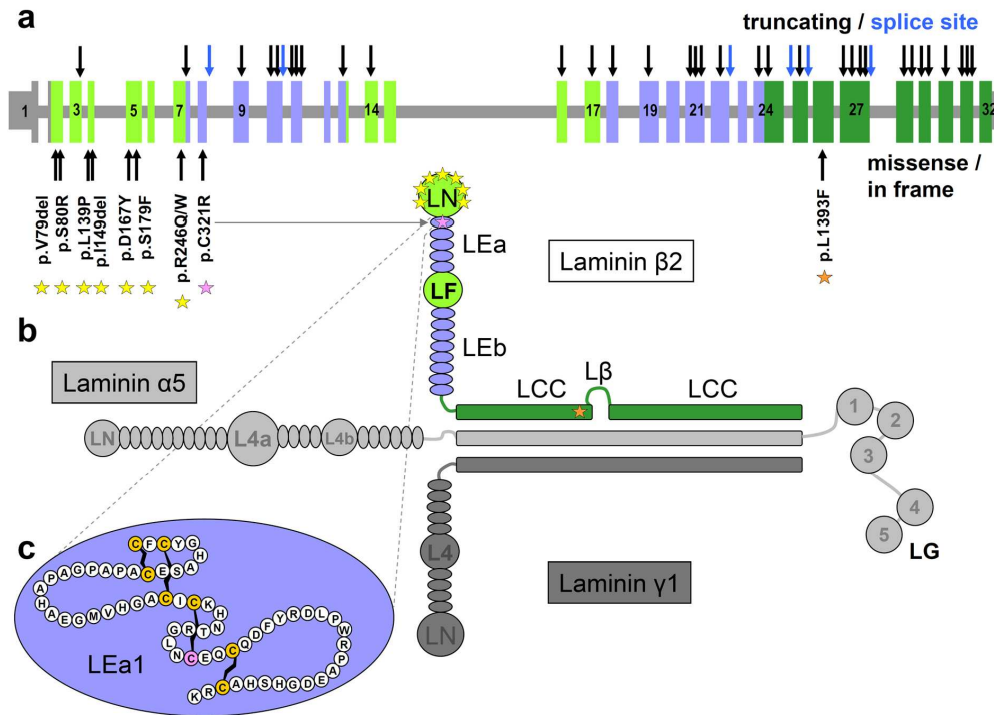


Figure 1 RGB colors, 300 dpi  
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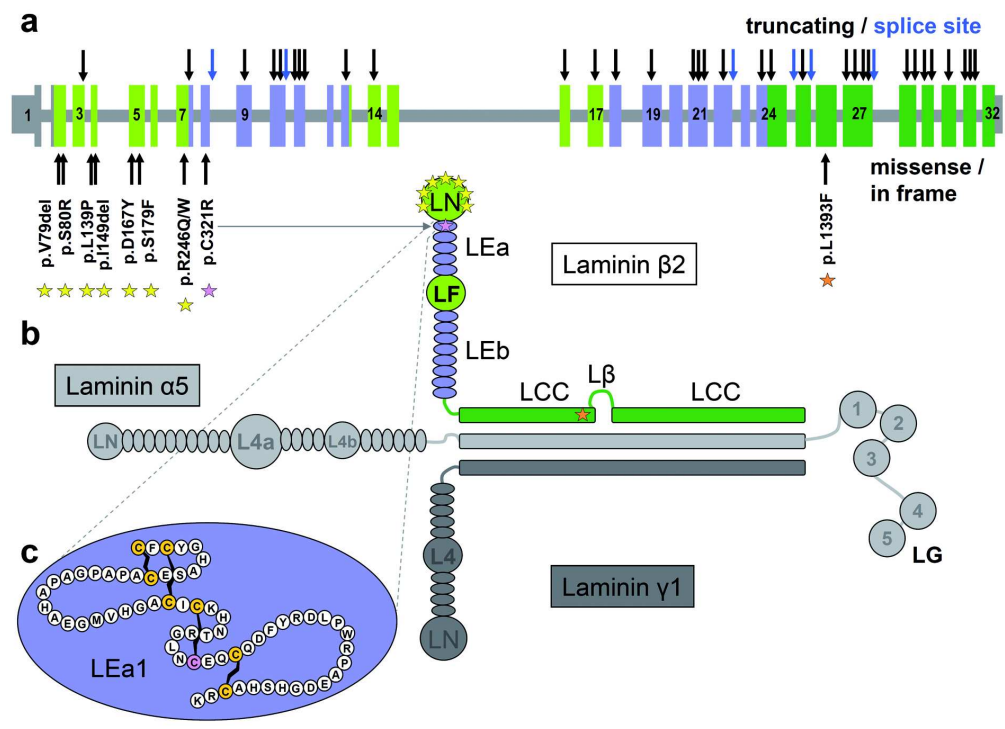
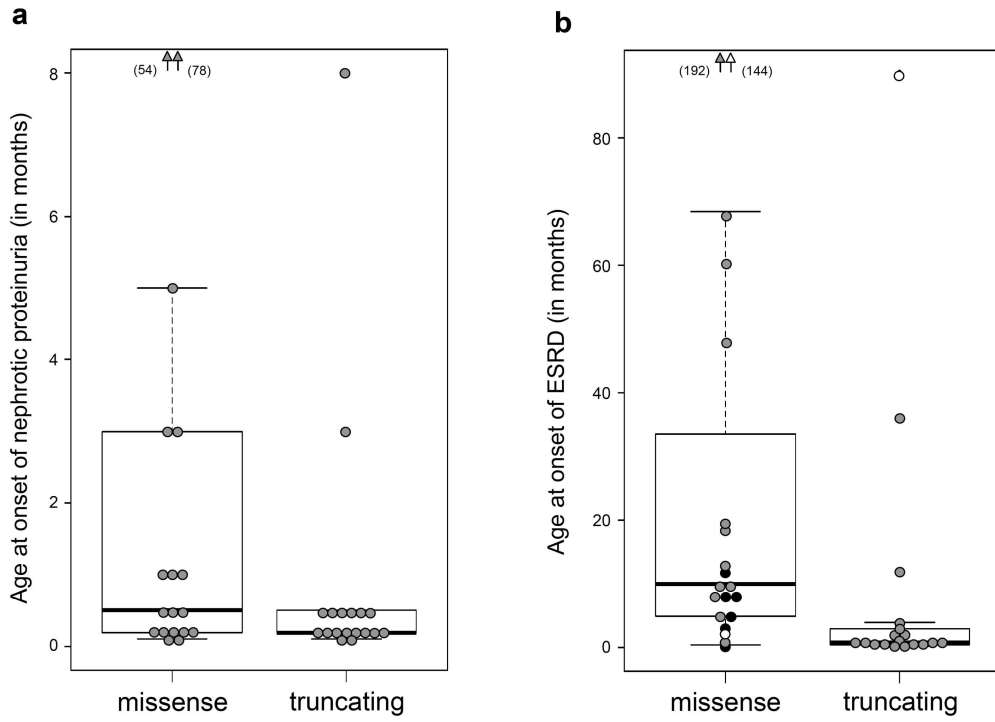


Figure 1, CMYK colors, 300 dpi  
160x119mm (300 x 300 DPI)

review

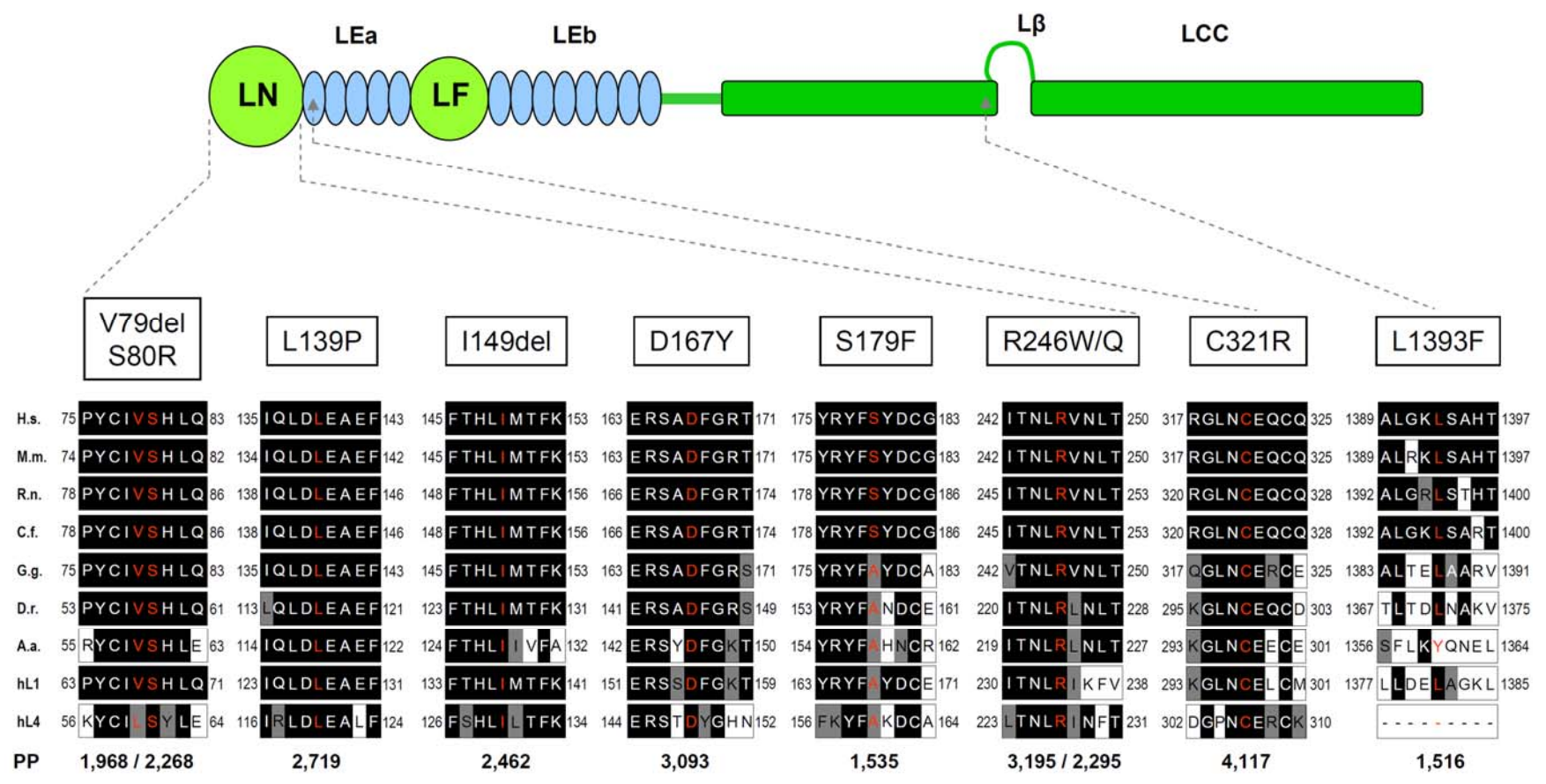


120x86mm (600 x 600 DPI)

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**Supp. Figure S1. LAMB2 missense mutations.** Localization of missense mutations and small deletions are shown in a schematic of the laminin β2 protein (corresponding to Fig. 1), and evolutionary conservation is demonstrated in partial amino acid sequence alignments created according to outputs of BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and the Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd>). Conserved amino acids are shown with black background and similar amino acids with a grey background. H.s., Homo sapiens; M.m., Mus musculus; R.n., Rattus norvegicus; C.f., Canis familiaris; G.g., Gallus gallus; D.r., Danio rerio; A.a., Aedes aegypti; hL1, human Laminin β1; hL4, human Laminin β4. PP, PSIC score differences calculated by PolyPhen (prediction of functional effect of human nsSNPs available at <http://genetics.bwh.harvard.edu/pph/>). Scores above 1.5 suggest that the change is probably damaging.

**Supp. Figure S2. Evolutionary conservation of amino acid residues affected by changes classified either as polymorphisms or variants with unknown phenotypic effect (Tables 3 and 4).**

**P37A**

Homo sapiens	21	GLLLSVLAATLAQAPA <b>P</b> DVP-GCSRGSYCYPATGDL	54
Macaca mulatta	20	ELLSPVLAATLAQAPAPDVP-GCSRGSYCYPATGDL	53
Rattus norvegicus	24	GLLLSVLAATLAQVP <b>S</b> LDVP-GCSRGSYCYPATGDL	57
Canis familiaris	24	GLLLSVLATALAQALAPDMP-GCSRGSYCYPATGDL	57
Gallus gallus	20	VPLPSGPGGTAWAASSPDSPOGCAAGSCYPATGDL	54
Danio rerio	1	MVRKVLSAVSAQE--PDAAHGCTHGSCYPATGDL	38
Aedes aegypti	1	MLNTNRRPPTTNKQPIHRVHPCEQSSCYPATGNL	34
human LAMB1	8	AFSFLALCRARVRAQEPEFSYGC <b>A</b> EGSCYPATGDL	42
human LAMB4	6	TLFLHLGLWLSYSKAQD-----DCNRGACHPTTGDL	35

**T398I**

Homo sapiens	383	GRHCELCRPFFFYRDP <b>T</b> KDLRDPVAVCRSCDCD	413
Macaca mulatta	382	GRHCELCRPFFFYRDP <b>T</b> KDLRDPVAVCRSCDCD	412
Rattus norvegicus	386	GRHCELCRPFFFYRDP <b>T</b> KDMRDPAA <b>C</b> RP <b>C</b> DCD	416
Canis familiaris	386	GRHCELCRPFFFYRDP <b>S</b> KDLRDPAMCRSCDCD	416
Gallus gallus	383	GRHCHLCKPFYYKDP <b>S</b> KDLRDP <b>T</b> VCRA <b>C</b> NCY	413
Danio rerio	361	GS <b>N</b> CE <b>S</b> CKPFYYQDP <b>T</b> RD <b>I</b> RD <b>P</b> GV <b>C</b> V <b>A</b> CD <b>C</b> D	391
Aedes aegypti	359	GYHCEECSPFFYRDP <b>L</b> ED <b>I</b> QSPYVCK <b>P</b> CD <b>C</b> D	389
human LAMB1	371	GR <b>N</b> CE <b>Q</b> CKPFYY <b>Q</b> HPERD <b>I</b> RD <b>P</b> N <b>F</b> CER <b>C</b> T <b>C</b> D	401
human LAMB4	368	G <b>Q</b> H <b>C</b> DR <b>C</b> RP <b>L</b> FYRDP <b>L</b> K <b>T</b> I <b>S</b> DPY <b>A</b> C <b>I</b> P <b>C</b> E <b>C</b> D	398

**R468L**

Homo sapiens	452	CRDGF <b>F</b> GLS <b>I</b> SDRLG <b>C</b> R <b>C</b> Q <b>C</b> NARGTVPG <b>S</b> T	482
Macaca mulatta	451	CRDGF <b>F</b> GLS <b>I</b> SD <b>P</b> LGRR <b>C</b> Q <b>C</b> NARGTVPG <b>S</b> T	481
Rattus norvegicus	455	CRDGF <b>F</b> GLS <b>A</b> SN <b>P</b> RG <b>C</b> Q <b>C</b> NS <b>R</b> GTVP <b>G</b> GT	485
Canis familiaris	455	CRDGF <b>F</b> GLS <b>A</b> SD <b>P</b> AGRR <b>C</b> Q <b>C</b> DARGTV <b>P</b> G <b>T</b> T	485
Gallus gallus	452	CKAG <b>F</b> FGLS <b>A</b> AN <b>P</b> Q <b>G</b> Q <b>R</b> CR <b>C</b> DP <b>R</b> GT <b>V</b> AD <b>G</b> S	482
Danio rerio	430	CK <b>P</b> G <b>F</b> FGLS <b>A</b> SD <b>P</b> RG <b>C</b> Q <b>P</b> CK <b>C</b> DP <b>R</b> GT <b>V</b> S <b>G</b> SS	460
Aedes aegypti	428	CK <b>E</b> G <b>F</b> W <b>N</b> F <b>D</b> EN <b>N</b> P <b>D</b> GC <b>Q</b> T <b>C</b> SN <b>I</b> L <b>G</b> T <b>V</b> D <b>N</b> AG	458
human LAMB1	440	CK <b>E</b> G <b>F</b> Y <b>D</b> LS <b>E</b> DP <b>F</b> G <b>C</b> K <b>S</b> C <b>A</b> C <b>N</b> PL <b>G</b> T <b>I</b> P <b>G</b> GN	470
human LAMB4	437	CK <b>P</b> N <b>H</b> Y <b>G</b> LS <b>A</b> T <b>D</b> PL <b>G</b> Q <b>P</b> C <b>D</b> C <b>N</b> PL <b>G</b> SL <b>P</b> -FL	466

**R575Q**

Homo sapiens	560	FR <b>F</b> PLDHL <b>I</b> WE <b>A</b> ED <b>T</b> -----R <b>G</b> Q <b>V</b> LD <b>V</b> VER <b>L</b> V	586
Macaca mulatta	559	FR <b>F</b> PLDHL <b>I</b> WE <b>A</b> ED <b>T</b> -----R <b>G</b> Q <b>V</b> LD <b>V</b> VER <b>L</b> V	585

Rattus norvegicus	563	FRPFLDHLTWEAEGA-----HGQVLEVVERLV	589
Canis familiaris	563	FRPFLDHLTWEAEDT-----RGQVLDVVERLV	589
Gallus gallus	560	YRINLDHYTYEAEDA-----RLHMGSVVEREP	586
Danio rerio	538	FFMALDHYIYEAEETAK-----LGQVRDFFEREY	565
Aedes aegypti	535	FIPTL-HQVLEAEFPGTVN-----CTHLSQNC SAVIREH	567
human LAMB1	548	YFATLDHYLYEAEAN-----LPGVSIIVERQY	575
human LAMB4	544	FFAPLNIFYLYEAEAEATTLQGLAPLGSETFGQSPAVHVVLGEP	585

**G700E**

Homo sapiens	684	EPGISYKHLHLKLVRTGSAQPETPYSGPGLLID	716
Macaca mulatta	683	EPAISYKHLHLKLVRTGGSAQPETPYSGPGLLID	715
Rattus norvegicus	687	EPGLSYKLLKLTGTGGRAHPETPYSGSGLLID	719
Canis familiaris	687	EPGISYKLLKLVRTGGSAQTEAPYSGPSLLID	719
Gallus gallus	684	ERGVSYTIRLELGCATGQQDP-TAS----VLID	711
Danio rerio	663	ESGVSYKLRVELIRYADRNSIITSTNA-FVLVD	694
Aedes aegypti	666	ENGKTYKFIVTFQR----HDPYRDNGAAQILID	694
human LAMB1	673	EKGINYYTVRLELPQYTSSDSDVES---PYTLID	702
human LAMB4	679	EPDVQYSIDVYFSQPLQGESHAHS----HVLVD	707

**H882Y**

Homo sapiens	870	GFPSRCPCVCNGHADECNHTTGACLGC	896
Macaca mulatta	869	GFPSRCPCVCNGHADECNTHTTGACLGC	895
Rattus norvegicus	873	GFPNCRPCVCNGRADECDAHTGACLGC	899
Canis familiaris	873	GFPSQPCPCVCNGHADECNTHTGACVGC	899
Gallus gallus	865	GFPTCRPCQCNHGADEC DPQTGSCLRC	891
Danio rerio	848	GFPNCRPCQCNHGADEC HQRTGACLNC	874
Aedes aegypti	848	NFPNCQMCFCNHTPTCNSKTGEC SQC	874
human LAMB1	860	GFPSQPCPCQCNHADD CDPVTGEC LNC	886
human LAMB4	856	GFPSCHPCPCNRFAELCDPETGSCFNC	882

**P1053del**

Homo sapiens	1038	CTCNLLGTNPQQCPS- <b>F</b> DQCHCDPSSGQCPCLPNV	1071
Macaca mulatta	1037	CTCNLLGTNPQQCPS-PDQCHCDRSSGQCPCLPNV	1070
Rattus norvegicus	1041	CTCNLLGTD PQR CPS-TDLCHCDPSTGQCPCLPNV	1074
Canis familiaris	1041	CTCNLLGTD PQQ CPS-IDRCNCDPSSGQCPCLPNV	1074
Gallus gallus	1033	CSCNTLGTDPNTCG--PQQCQCDQRS GQCHCLPHV	1065
Danio rerio	1016	CTCNFLGTERSQCLS-RDDCVCQRATGQCQCLPNV	1049
Aedes aegypti	1016	CDCNVLGTN-----QTVQHCDRFTGQCPCLANV	1043
human LAMB1	1028	CVCNYLGTVQEHCN--GSDCQCDKATGQCLCLPNV	1060
human LAMB4	1022	CSCHASGVSPMECPGGGACLCDPVTGACPCLPNV	1056

**G1243R**

Homo sapiens	1128	ESSFVHMQEKLGIVQ <b>G</b> IVGARNTSAAST	1255
Macaca mulatta	1127	ESSFVHMQEKLGIVQ <b>G</b> IVGARNTSAAST	1254
Rattus norvegicus	1131	ESSFLN <b>L</b> QKLG <b>M</b> VQ <b>A</b> IV <b>A</b> ARNTSAAST	1258
Canis familiaris	1131	ESSFW <b>R</b> I <b>Q</b> EK <b>L</b> G <b>T</b> V <b>Q</b> GIVGARNTSAAST	1258
Gallus gallus	1122	EGTFRR <b>L</b> EE <b>S</b> L <b>A</b> T <b>I</b> R <b>D</b> A <b>V</b> A <b>A</b> R <b>N</b> A <b>T</b> A <b>A</b> T <b>A</b>	1249
Danio rerio	1206	ERR <b>F</b> KE <b>L</b> ED <b>M</b> LA <b>Q</b> ARD <b>I</b> V <b>N</b> ARNATAEAV	1233
Aedes aegypti	1200	KKE <b>F</b> D <b>S</b> M <b>G</b> K <b>I</b> D <b>I</b> I <b>K</b> G <b>L</b> L <b>T</b> N <b>T</b> I <b>S</b> D <b>R</b> E-I	1226
human LAMB1	1217	RE <b>T</b> V <b>D</b> S <b>V</b> ER <b>K</b> V <b>S</b> E <b>I</b> K <b>D</b> I <b>L</b> A <b>Q</b> S-P <b>A</b> E <b>E</b> P <b>L</b>	1243
human LAMB4	1214	E <b>A</b> D <b>F</b> K <b>D</b> L <b>R</b> G <b>N</b> V <b>S</b> E <b>I</b> E <b>R</b> I <b>L</b> K <b>H</b> P <b>V</b> F <b>P</b> S <b>G</b> K <b>F</b>	1241

**D1373G**

Homo sapiens	1356	SPVSN <b>S</b> A <b>S</b> A <b>R</b> H <b>R</b> T <b>E</b> A <b>L</b> M <b>D</b> A <b>Q</b> K <b>E</b> D <b>F</b> N <b>S</b> K <b>H</b> M <b>A</b> N <b>Q</b> R <b>A</b> L	1390
Macaca mulatta	1355	SPVSN <b>S</b> A <b>S</b> A <b>R</b> H <b>R</b> T <b>E</b> A <b>L</b> M <b>D</b> A <b>Q</b> N <b>E</b> D <b>F</b> N <b>S</b> K <b>H</b> M <b>A</b> N <b>Q</b> R <b>A</b> L	1389
Rattus norvegicus	1359	SPVSN <b>S</b> A <b>D</b> T <b>R</b> R <b>R</b> A <b>E</b> V <b>L</b> M <b>G</b> A <b>Q</b> R <b>E</b> N <b>F</b> N <b>R</b> O <b>H</b> L <b>A</b> N <b>Q</b> O <b>A</b> L	1393
Canis familiaris	1359	SPVSN <b>S</b> A <b>D</b> T <b>R</b> H <b>R</b> T <b>E</b> V <b>L</b> M <b>S</b> A <b>Q</b> R <b>E</b> D <b>F</b> N <b>R</b> K <b>H</b> K <b>A</b> N <b>Q</b> O <b>A</b> L	1393
Gallus gallus	1350	SPV <b>S</b> A <b>S</b> A <b>T</b> R <b>H</b> H <b>T</b> E <b>Q</b> L <b>L</b> A <b>S</b> R <b>R</b> D <b>A</b> F <b>N</b> R <b>O</b> N <b>A</b> A <b>S</b> R <b>R</b> A <b>L</b>	1384
Danio rerio	1334	ST <b>V</b> S <b>Q</b> S <b>A</b> D <b>T</b> R <b>K</b> K <b>T</b> E <b>R</b> L <b>I</b> G <b>Q</b> K <b>R</b> D <b>D</b> F <b>N</b> R <b>K</b> N <b>A</b> A <b>N</b> K <b>R</b> T <b>L</b>	1368
Aedes aegypti	1323	EL <b>N</b> D <b>N</b> A <b>E</b> R <b>Q</b> C <b>K</b> R <b>T</b> E <b>I</b> L <b>L</b> K <b>K</b> Q <b>Q</b> D <b>H</b> F <b>D</b> H <b>L</b> H <b>D</b> T <b>N</b> E <b>A</b> S <b>F</b>	1357
human LAMB1	1344	ST <b>V</b> E <b>Q</b> S <b>A</b> L <b>M</b> R <b>D</b> R <b>V</b> E <b>D</b> V <b>M</b> M <b>E</b> R <b>E</b> S <b>Q</b> F <b>K</b> E <b>K</b> Q <b>E</b> E <b>Q</b> A <b>R</b> L <b>L</b>	1378
human LAMB4	1330	ST <b>I</b> N <b>T</b> S <b>A</b> N <b>T</b> R <b>N</b> D <b>L</b> L <b>T</b> I <b>L</b> D <b>T</b> L <b>T</b> S <b>K</b> G <b>N</b> -----LS	1356

**N1380K**

Homo sapiens	1365	R <b>H</b> R <b>T</b> E <b>A</b> L <b>M</b> D <b>A</b> Q <b>K</b> E <b>D</b> F <b>N</b> S <b>K</b> H <b>M</b> A <b>N</b> Q <b>R</b> A <b>L</b> G <b>K</b> L <b>S</b> A	1395
Macaca mulatta	1364	R <b>H</b> R <b>T</b> E <b>A</b> L <b>M</b> D <b>A</b> Q <b>N</b> E <b>D</b> F <b>N</b> S <b>K</b> H <b>M</b> A <b>N</b> Q <b>R</b> A <b>L</b> R <b>K</b> L <b>S</b> A	1394
Rattus norvegicus	1368	R <b>R</b> R <b>A</b> E <b>V</b> L <b>M</b> G <b>A</b> Q <b>R</b> E <b>N</b> F <b>N</b> R <b>O</b> H <b>L</b> A <b>N</b> Q <b>O</b> A <b>L</b> G <b>R</b> L <b>S</b> T	1398
Canis familiaris	1368	R <b>H</b> R <b>T</b> E <b>V</b> L <b>M</b> S <b>A</b> Q <b>R</b> E <b>D</b> F <b>N</b> R <b>K</b> H <b>K</b> A <b>N</b> Q <b>O</b> A <b>L</b> G <b>K</b> L <b>S</b> A	1398
Gallus gallus	1359	R <b>H</b> H <b>T</b> E <b>Q</b> L <b>L</b> A <b>S</b> R <b>R</b> D <b>A</b> F <b>N</b> R <b>O</b> N <b>A</b> A <b>S</b> R <b>R</b> A <b>L</b> T <b>E</b> L <b>A</b> A	1389
Danio rerio	1343	R <b>K</b> K <b>T</b> E <b>R</b> L <b>I</b> G <b>Q</b> K <b>R</b> D <b>D</b> F <b>N</b> R <b>K</b> N <b>A</b> A <b>N</b> K <b>R</b> T <b>L</b> T <b>D</b> L <b>N</b> A	1373
Aedes aegypti	1332	C <b>K</b> R <b>T</b> E <b>I</b> L <b>L</b> K <b>K</b> Q <b>Q</b> D <b>H</b> F <b>D</b> H <b>L</b> H <b>D</b> T <b>N</b> E <b>A</b> S <b>F</b> L <b>K</b> Y <b>Q</b> N	1362
human LAMB1	1353	R <b>D</b> R <b>V</b> E <b>D</b> V <b>M</b> M <b>E</b> R <b>E</b> S <b>Q</b> F <b>K</b> E <b>K</b> Q <b>E</b> E <b>Q</b> A <b>R</b> L <b>L</b> D <b>E</b> L <b>A</b> G	1383
human LAMB4	1339	R <b>N</b> D <b>L</b> L <b>T</b> I <b>L</b> D <b>T</b> L <b>T</b> S <b>K</b> G <b>N</b> -----LS <b>L</b> E <b>R</b> L <b>K</b>	1361

**R1457Q**

Homo sapiens	1438	G <b>G</b> L <b>S</b> C <b>N</b> -G <b>A</b> A <b>T</b> A <b>D</b> L <b>A</b> L <b>G</b> R <b>A</b> R <b>H</b> T <b>Q</b> A <b>E</b> L <b>Q</b> R <b>A</b> L <b>A</b> E <b>G</b> G <b>S</b> I <b>L</b> S	1475
Macaca mulatta	1437	G <b>G</b> L <b>N</b> C <b>N</b> -G <b>A</b> V <b>A</b> T <b>A</b> D <b>L</b> A <b>L</b> G <b>R</b> A <b>R</b> H <b>T</b> Q <b>A</b> E <b>L</b> Q <b>R</b> A <b>L</b> A <b>E</b> G <b>G</b> S <b>I</b> L <b>S</b>	1474
Rattus norvegicus	1441	G <b>G</b> L <b>G</b> C <b>S</b> -G <b>A</b> A <b>T</b> A <b>D</b> L <b>A</b> L <b>G</b> R <b>A</b> R <b>H</b> T <b>Q</b> A <b>E</b> L <b>Q</b> R <b>A</b> L <b>V</b> E <b>G</b> G <b>G</b> I <b>L</b> S	1478
Canis familiaris	1441	G <b>G</b> L <b>G</b> C <b>N</b> -G <b>A</b> V <b>A</b> M <b>A</b> D <b>L</b> A <b>L</b> G <b>R</b> A <b>R</b> H <b>T</b> Q <b>A</b> E <b>L</b> Q <b>R</b> A <b>L</b> A <b>E</b> G <b>G</b> G <b>I</b> L <b>S</b>	1478
Gallus gallus	1432	G <b>G</b> L <b>S</b> C <b>S</b> -G <b>A</b> V <b>S</b> T <b>A</b> D <b>S</b> A <b>L</b> D <b>R</b> A <b>R</b> H <b>A</b> Q <b>E</b> E <b>L</b> R <b>R</b> A <b>G</b> E <b>V</b> A <b>Q</b> -L <b>S</b>	1468

Danio rerio	1416	GGLN <b>CN</b> -GAVAVADTALDRSKHAEKELDKAMGVVEELFK	1453
Aedes aegypti	1400	GGLTCDK <b>GAL</b> TRSEKALQYAKKTEQTIKEKEELADDILR	1438
human LAMB1	1426	GGPGCG-GLVTVAHNAWQKAMDLDQDVLSALAEVEQLSK	1463
human LAMB4	1404	RGPGCH-GSLTLSTNALQKAQEAQSIIRNLDKQVRGLKN	1441

**A1765T**

Homo sapiens	1748	KLQRLQ <b>EE</b> LEGT <b>YE</b> ENER <b>A</b> LESKAAQLD <b>GLE</b>	1777
Macaca mulatta	1747	KLQRLQ <b>EE</b> LEGT <b>YE</b> ENER <b>A</b> LESKAAQLD <b>GLE</b>	1776
Rattus norvegicus	1751	KLQRLQ <b>EE</b> LEGT <b>YE</b> ENER <b>E</b> LEV <b>K</b> AAQLD <b>GLE</b>	1780
Canis familiaris	1751	KLQRLQ <b>EE</b> LEGT <b>YE</b> ENER <b>A</b> LE <b>G</b> KAAQLD <b>GLE</b>	1780
Gallus gallus	1742	KLQKLRA <b>LE</b> EAYERN <b>ER</b> VLDAK <b>V</b> AQLD <b>GLE</b>	1771
Danio rerio	1726	KLQRLA <b>E</b> LEKDY <b>EE</b> NQKVLE <b>G</b> KARQLD <b>GLE</b>	1755
Aedes aegypti	1710	QLNKLHDLYKTY <b>EQ</b> NQNELGS <b>LE</b> TNIQ <b>GLT</b>	1739
human LAMB1	1736	KLQLLKD <b>LE</b> RKYEDNQR <b>Y</b> LEDKAQ <b>EL</b> AR <b>LE</b>	1765
human LAMB4	1710	KIRRI <b>TD</b> LERKIQDLNLSRQAKAD <b>QL</b> R <b>ILE</b>	1739

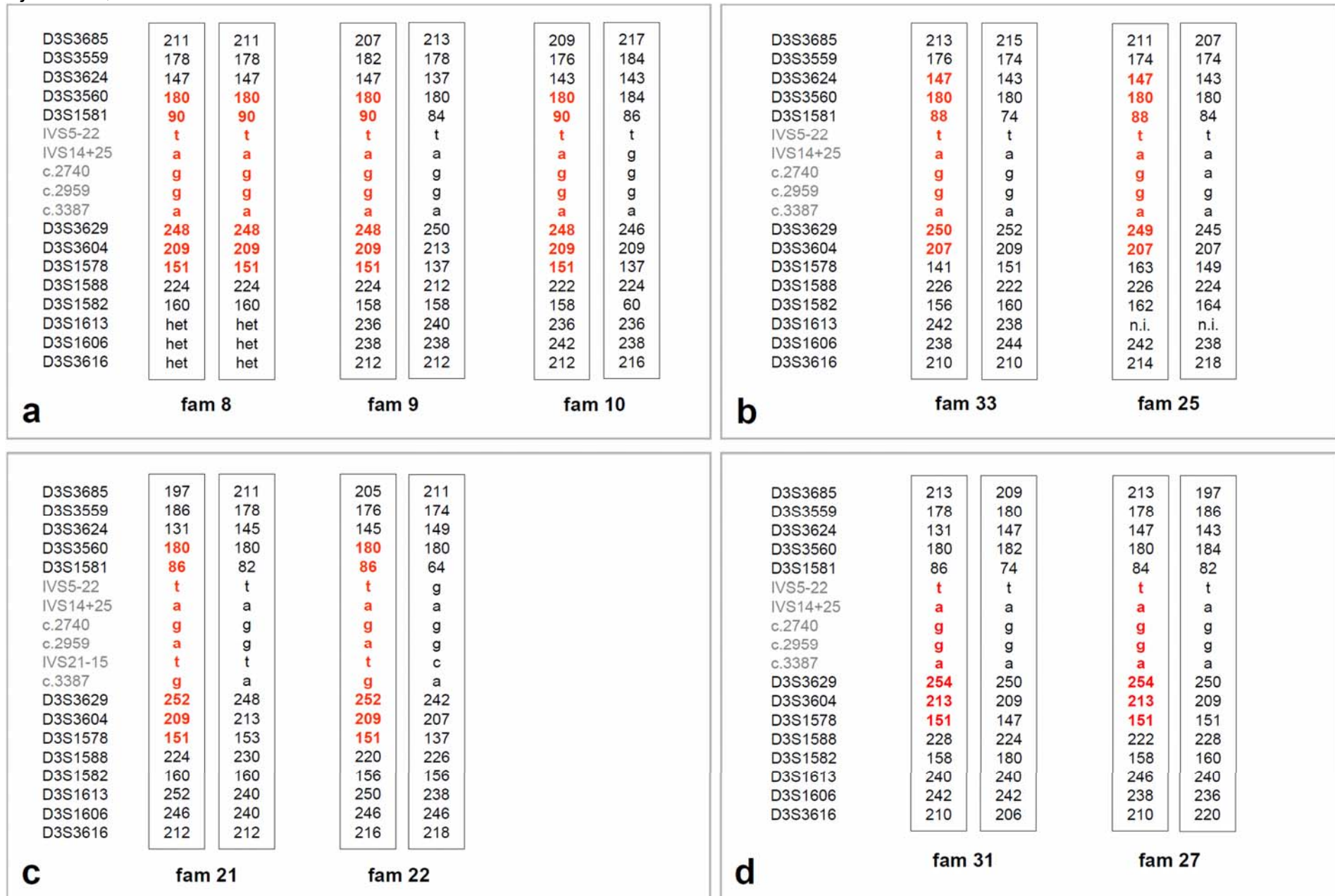
Alignment of human laminin  $\beta$ 2 and laminin  $\beta$ 2 orthologues from various species, as well as the human  $\beta$ -laminin paralogues showing the degree of conservation of the respective amino acid residues affected by variants. Conserved amino acids are shown with black background and similar amino acids with a grey background. Alignments were created according to outputs of BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and the Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd>).

**Reference sequence information:**

Homo sapiens:	NP_002283.3	laminin, beta 2 precursor
Macaca mulatta:	XP_001109982.1	lamb2, isoform 1 / lamc1
Rattus norvegicus:	NP_037106.1	laminin, beta 2 GENE ID: 25473=P15800
Canis familiaris:	XP_533831.2	similar to Laminin beta-2 chain precursor
Gallus gallus:	NP_989497.1	laminin, beta 2
Danio rerio:	XP_689856.2	similar to LOC494988
Aedes aegypti:	Q17EW2	laminin, beta-2 chain
Human LAMB1:	NP_002282.2	
Human LAMB4:	NP_031382.2	

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**Supp. Figure S3.** Haplotype analysis in families harbouring recurrent mutations. Haplotype analysis, including microsatellites flanking the LAMB2 locus (black) and intragenic SNPs (grey), in families with the recurrent LAMB2 mutations p.R246W, p.C1058X,



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c.1477delT and c.4504delA. Red lettering marks the shared haplotype containing the respective mutation. Haplotype of parents and siblings are not shown. a, Haplotypes of three patients of Portuguese origin. The alleles highlighted in red represent the shared haplotype of at least 5,5 Mb (D3S3560 to D3S1578) harboring the missense mutation p.R246W. het, heterozygous, phase indefinable in absence of parental samples. b, Haplotypes of two affected with Slavic background. Alleles highlighted in red indicate the shared haplotype of at least 5,4 Mb (D3S3624 to D3S3604) containing the mutation c.4504delA. n.i., not informative. c, Haplotypes of two affected with German-French origin, respectively. Shared haplotypes of at least 5,5 Mb (D3S3560 to D3S1578) bearing the mutation c.1477delT are coloured in red. d, Haplotypes of two patients with mixed European background. Alleles highlighted in red demonstrate the shared haplotype of at least 5,1 Mb (LAMB2 gene to D3S1578) containing the nonsense mutation p.C1058X.

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