European Journal of Human Genetics (2001) 9, 922–930
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ARTICLE

NEDD4L on human chromosome 18q21 has multiple forms of transcripts and is a homologue of the mouse *Nedd4-2* gene.

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The validation of full-length cDNA represents a crucial step in gene identification and subsequent functional analysis. In searching for candidate genes for bipolar disorder on chromosome 18q21, a novel gene homologous to *NEDD4* (Neural precursor cells expressed developmentally down-regulated) was identified using exon trapping and cDNA cloning. This novel gene is termed *NEDD4L* (Human Gene Nomenclature Committee symbol). Typical *NEDD4* orthologues that contain a C2 (Ca^{2+}/lipid-binding) and a HECT (Homologous to the E6-AP Carboxyl Terminus) ubiquitin-protein ligase domain, and multiple WW domains have been shown to regulate the epithelial sodium channel (*ENaC*). In mice, *Nedd4* has two distinct isoforms termed *Nedd4-1* that belongs to the typical *NEDD4* class, and *Nedd4-2* that is homologous to *Nedd4-1* but lacks the C2 domain. *NEDD4L* contains the WW and HECT domains seen in the *NEDD4* gene family, but lacks the C2 domain in the N-terminus. BLAST database search showed that the deduced polypeptide of *NEDD4L* has 97 and 62% sequence identity to mouse *Nedd4-2* and human *NEDD4*, respectively. Multiple forms of transcripts of *NEDD4L* have been isolated, which differ in transcription start and termination sites together with the presence or absence of an alternative spliced exon. Northern blot analysis showed a 3.4 kb mRNA species was specifically expressed in heart and skeletal muscle, while a 3.2 kb band and/or an additional 3.6 kb band is seen in other tissues tested. Striking homology of *NEDD4L* to mouse *Nedd4-2* suggests it is the human homologue of mouse *Nedd4-2*. Its position in a region of linkage for autosomal dominant orthostatic hypotensive disorder and its potential role in regulating *ENaC* make *NEDD4L* a candidate gene for this disorder.
European Journal of Human Genetics (2001) **9**, 922–930.

Keywords: NEDD4; NEDD4L; chromosome 18; exon mapping; cDNA cloning

Introduction

NEDD4 was first identified as one of the developmentally down-regulated genes expressed in mouse neural precursor cells using a subtraction cloning approach.[1] Subsequently, *NEDD4* orthologues have been identified in human, mouse, rat, Xenopus and Yeast.[2–7] The sequence structure of the *NEDD4* gene family is highly conserved in different species, and is characterised by the presence of a Ca^{2+}/lipid-binding domain (the C2 domain), multiple protein-protein interaction modules of 35–40 amino acid residues (the WW

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Received 10 August 2001; revised 11 October 2001; accepted 21 October 2001

domains), and a HECT (homologous to E6-AP carboxy terminus) ubiquitin-protein ligase domain at the C-terminal (reviewed by Harvey and Kumar).[8] Functional studies showed that *NEDD4* gene products have ubiquitin-protein ligase activity.[9–11] The WW domains of *NEDD4* gene family have been demonstrated to interact with the epithelial sodium channel (*ENaC*) through the proline-rich PY motifs (eg xPPxY) within the C-termini of *ENaC* subunits, and thereby to down regulate the intracellular Na^+ concentration.[5,12–15] The C2 domain of *NEDD4* has been shown to mediate Ca^{2+}-dependent plasma membrane localisation of the protein through phospholipid binding.[16] The WW domains of *NEDD4* have also been shown to bind to phosphoserine and phosphothreonine residues.[17] Thus, *NEDD4* family genes may not only interact with proteins containing PY motifs, but also with serine- and threonine-phosphorylated proteins, and therefore regulate a much larger repertoire of protein substrates.

In mice, *Nedd4* has two distinct isoforms: *Nedd4-1* that belongs to the typical *NEDD4* class; and *Nedd4-2* that is homologous to *Nedd4-1* but lacks the C2 domain. It has recently been reported that it is *Nedd4-2*, but not *Nedd4-1* interacts with and regulates the *ENaC* channel activity when co-expressed in Xenopus Oocytes.[3]

We cloned the full-length cDNA of the human homologue of mouse *Nedd4-2* during the course of performing exon trapping[18] and cDNA cloning to identify candidate genes for screen for genetic variations in bipolar disorder on chromosome 18q21.[19,20] Among the exons identified,[21] four were identical to portions of three GenBank published partial cDNA sequences (AB007899, BC000621, AL137469), and to a number of ESTs (for example: R19489, T74302, R07229 and W87678). The longest is AB007899 (KIAA0439) that has 4879 base pairs and contains an ORF of 2985 bp with undetermined translation initial site. Sequence analysis showed that KIAA0439 is strongly homologous to mouse *Nedd4-2*. However, it is not clear whether KIAA0439 contains the C2 domain since its full-length cDNA sequence is not completely elucidated, even though there is evidence showing that the WW domain region of KIAA0439 plays a dominant negative role in *ENaC* regulation.[22] It is highly possible that there are two distinct isoforms of human *NEDD4* genes that have sequence homology, but differ in the presence or lacking of the C2 domain.

To obtain the full-length cDNA of this novel human *NEDD4* like gene (termed *NEDD4L*), we sequenced a corresponding EST clone (W87678), and used 5′ and 3′ end RACE (rapid amplification of cDNA ends) to determine the full-length cDNA sequence. We submitted the full-length cDNA sequence of *NEDD4L* (AF210730) to GenBank in 1999. However, we observed that *NEDD4L* has multiple transcripts variably expressed in the tissues tested by Northern blot and RT–PCR analyses. Here we report full-length cDNA cloning, genomic structure and expression analysis of *NEDD4L*, and mutation screening in the coding region of *NEDD4L* in bipolar probands.

Materials and methods

Exon trapping and identification of *NEDD4L*

Exon trapping[18] was carried out as described by Chen *et al*[21] to identify the genes on human chromosome 18 (HC18) using cosmids isolated from an HC18-specific library LL18NC02. Sequence similarity database searches were performed using BLAST algorithms.[23] Among the trapped exons, four showed sequence homology to *NEDD4* gene orthologues across species. These exons are also identical to a number of human ESTs and to regions of a GenBank published partial cDNA sequence KIAA0439 (accession no. AB007899). KIAA0439 is a 4879 base pair sequence without the 5′UTR and translation start site determined. To clone the full-length cDNA of this novel gene (named *NEDD4L*), a corresponding EST clone (accession no.W87678) was ordered from Research Genetics and its 900 bp insert was sequenced. The full-length cDNA sequence of this novel *NEDD4L* gene was obtained using RACE. Primer NEDD4L.R1 (AGGAAG-TAGTTCTGTGAATCCCTC) used in RACE was designed according to the most 5′ end sequence of the EST clone, and 5′ end RACE was carried out on double-stranded cDNA derived from foetal brain and placenta polyA$^+$ RNA (Clontech) using the Marathon cDNA amplification kit (Clontech cat no. K18021-1). The RACE product was subcloned into vector pBluescript SK$^+$, and two clones were sequenced on an ABI3700 automated sequencer at the Johns Hopkins Genetics CORE facility.

Expression analysis, alternative splicing detection, and 5′ and 3′ UTR determination

The insert of EST clone (GenBank no. W87678) was used as a probe to hybridise commercial Northern blots (Clontech cat nos. 7760-1, 7756-1). The probe was labelled with ^{32}P and hybridised to the blots according to the manufacturer's protocol (PT1200-1). Post-hybridisation wash was carried out as follows: 30 min wash twice at room temperature in $2 \times$ SSC with 0.1% SDS, 30 min wash twice at 65°C in $0.1 \times$ SSC with 0.1% SDS. The filters were then exposed to an X-ray film to obtain autoradiograph.

During sequencing of the 5′ end RACE product of *NEDD4L*, an alternative splicing event was observed. To further analyse this alternative splicing event, RT–PCR was used to amplify cDNA templates derived from polyA$^+$ RNA from human foetal brain, adult brain, heart, kidney, placenta, testis (Clontech), and total RNA isolated from lymphoblast cell lines and blood. The primers used in RT–PCR were NEDD4L.F2 (5′ AGACTCTCTCGGTCTGGCTC), and NEDD4L.R2 (5′ TGTTATGATTGACATAGTATGTGC). Beta-actin cDNA was simultaneously amplified as a means of control to compare the intensity of amplification of *NEDD4L* in RT–PCR analysis.

Using Northern blot analysis we did not detect an mRNA species of 4.8 kb presumably corresponding to KIAA0439, instead a 3.4 kb band in heart and muscle, and a 3.2 kb band and/or an additional 3.6 kb band in other tissues tested were

observed. We performed a closer 5′ and 3′ RACE on double strand cDNA templates derived from polyA+ RNA from human adult brain, heart, and placenta using primers NEDD4L.R3 (5′TCATTCCATTTTGGGTTCAG), and NEDD4L.F3 (5′ TCGCCTTGACTTACCTCCAT) from exon 3 and the last exon of *NEDD4La*, respectively. The RACE products were directly sequenced.

Chromosome localisation and genomic structure of *NEDD4L*

The mapping of *NEDD4L* to chromosome 18 was carried out in two ways. First, PCR amplification of the GeneBridge4 mapping panel[24] was performed using primers E2E11F 5′ GTTCCAATGGTCCTCAGCTG and E2E11R 5′ CATGTGT-GAGCTCTGGGCAG derived from trapped exon E2E11. Second, BLAST[23] GenBank database searches at the National Center for Biological Information (NCBI) was performed to identify any genomic sequences containing the *NEDD4L* gene. The identified genomic sequence contig NT_0019090.3 was used to map the exon-intron structure of the novel *NEDD4L* gene.

Mutation screen in selected bipolar subjects

Total RNA of lymphoblast cell lines derived from bipolar probands was isolated using Trizol reagent according to the supplied protocol (Life Technologies, cat no. 15596-026), and 5 μg of total RNA was converted to first strand cDNA using the Preamplification System (Life Technology, cat no. 18089-011). The coding region of NEDD4L was amplified with RT–PCR from first strand cDNA using primers NEDD4L.F4 (5′ TCCAGCTGAACACTTTCCAG) and NEDD4L.R4 (5′ ACT-CAAGCTGGGACCAGGTTC). A second round PCR (nested PCR) was performed using primers NEDD4L.F5 (5′ TTAGT-CACCTTCCGACAGAAG) and NEDD4L.R5 (5′ CAAAAGTG-CAAGCAGAAC). The PCR product was purified with the Qiaquick PCR purification kit (Cat no. 28108, Qiagene) and sequenced in the Genetics Resources Core Facility (GRCF) at Johns Hopkins University. Base calling and sequence assembly were accomplished using the Phred/Phrap/Consed package.[25–27]

Results

Cloning of the full-length cDNA for *NEDD4L*

Exon trapping[18] was used to identify genes on chromosome 18 to search for candidate genes for bipolar disorder.[19,20] Sequences generated from trapped exons[21] were screened against the GenBank databases (nr, dbEST, Unigene) for sequence homologies using BLAST search algorithms.[23] Four of these exons (GenBank accession nos. AF149625, AF149431, AF149517, AF149521) showed identity to regions of three partial cDNA sequences published in GenBank (AB007899, BC000621, AL137469) and to a number of ESTs (for examples: R19489, T74302, R07229 and W87678). These exons also demonstrated strong homology (83–100% at amino acid sequence level) to *NEDD4* ubiquitin-protein ligase family genes identified in human, mouse, rat, Xenopus, and yeast.[2–7] Sequence alignment of AB007899, BC000621, and AL137469 indicated that they are from the same gene on chromosome 18q21, and have strong homology to the *NEDD4* gene family with the highest to mouse *Nedd4-2* (97% at amino acid level).

The sequences generated from the EST clone (W87678) and RACE product were used to constitute a 3246 bp full-length cDNA sequence (referred to as *NEDD4La*, submitted to GanBank in 1999) that contains 30 exons with an open reading frame (ORF) of 2562 nucleotides coding for a predicted polypeptide of 854 amino acids (Figure 1). The nucleotide sequence around the potential translation start site is in agreement with the conserved content of Kozak sequence.[28] The polyA adenylation site (AATAAA) is located 20 bp upstream to the end of the sequence. *NEDD4La* is identical from nucleotide (nt) 315–3246 or 168–3246 to the entire sequences of BC000621, and AL137469, respectively; and is identical from nt 136–3246 to the region of nt 244–3294 in AB007899 (KIAA0439). The sequence of first 243 bp of KIAA0439 is different from exon 1 (the first 136 bp) of *NEDD4La*. KIAA0439, BC000621 and AL137469 do not contain exon 12 of *NEDD4La*. In addition, KIAA0439 contains an extended 1585 bp 3′ UTR sequence not seen in *NEDD4La* and the other two sequences BC000621 and AL137469. No additional polyA adenylation site is seen in the extended 3′ UTR region of KIAA0439.

We observed that *NEDD4L* expressed a 3.4 kb mRNA species in heart and skeletal muscle, slightly longer than *NEDD4La*. A further 5′ RACE using a primer derived form exon 3 of *NEDD4La* was performed on cDNA templates derived from human brain, heart, and placenta polyA+ RNA. The expected amplification of a 240 bp fragment was seen in all three tissues, but an additional fragment about 480 bp was amplified only in heart (Figure 3B). Direct sequencing of the 480 bp 5′ RACE product showed that it shares exon 2 and down stream sequence with *NEDD4La*, but contains two additional exons in the most 5′ end that are different from exon 1 of *NEDD4La* and KIAA0439. The transcript expressed in heart is termed *NEDD4Lb*. When performing 3′ RACE with a primer in the last exon of *NEDD4La* (see Materials and methods), two fragments were amplified (Figure 3B). Sequencing analysis revealed that the shorter fragment (an expected amplification of 400 bp) is identical to the 3′ end of *NEDD4La* and AL137469; while the longer fragment has 130 bp extended from the 3′UTR of *NEDD4La*. This 3′UTR extension is also seen in sequence BC000621. *NEDD4Lb* was assembled to constitute the 130 bp extended 3′ UTR that shares the same polyA adenylation signal seen in *NEDD4La*.

The deduced polypeptide sequence of *NEDD4L* was predicted to contain 4 WW domains and an HECT ubiquitin-protein ligase domain (ScanProsite tool, http://www.expasy.ch/tools/scnpsite.html) highly conserved features in the *NEDD4* gene family. However, *NEDD4L* lacks the

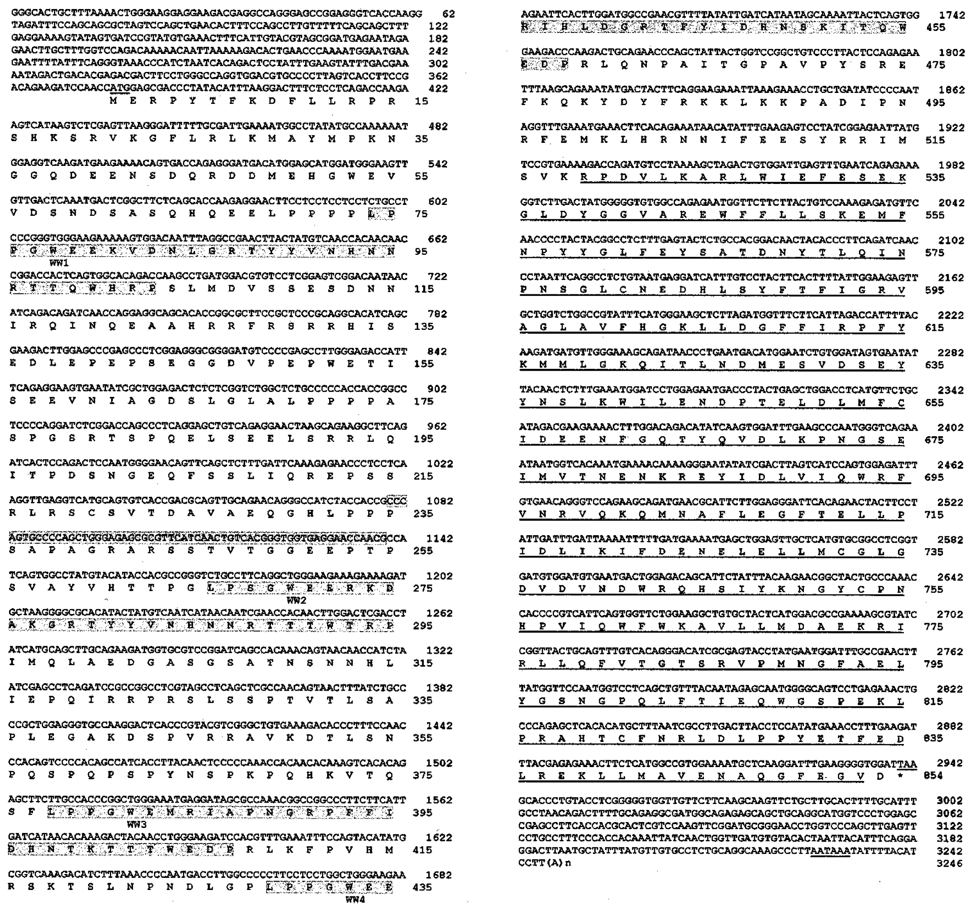


Figure 1 The nucleotide and predicted polypeptide sequences of *NEDD4La*. The predicted translation initiation site, the stop codon, and the polyA adenylation site are underlined. The 60 bp alternative spliced sequence are highlighted on the nucleotide sequence, also highlighted are the four potential WW domains. The ubiquitin-protein ligase homologous region in the C-terminus is underlined.

C2 domain presenting in *NEDD4*. BLAST search of GenBank databases showed that *NEDD4L* has 97 and 62% amino acid sequence identity to mouse Nedd4-2 and human *NEDD4*, respectively. It is also strongly homologous to *NEDD4* orthologues identified in other species. Figure 2 shows the amino acid sequence alignment of *NEDD4L* to selected other members of the *NEDD4* gene family from several species.

Expression analysis and alternative splicing of *NEDD4L*

Using the sequenced EST clone as a probe to hybridise a Northern blot indicated that this novel *NEDD4L* gene has transcripts of 3.2, 3.4, and 3.6 kb expressed differently in the tissues examined (Figure 3A). In heart and muscle, a 3.4 kb band was observed, while a 3.2 and/or a less intense 3.6 kb band were seen in other tissues examined. The 4.8 kb band corresponding to the size of KIAA0439 was not detected on the tissues examined (Figure 3A).

An alternative splicing event involving exon 12 of 60 bp was observed through sequencing analysis of 5′ end RACE and RT–PCR amplification (Figures 1 and 3C). Both alternative spliced forms of *NEDD4L* were expressed, however, the shorter allele without the 60 bp is predominantly present in several tissues (human foetal and adult brain, kidney, placenta, and lymphoblast cell lines), while the longer allele is predominantly expressed in kidney and placenta, but hardly detectable in foetal and adult brain (Figure 3C). Therefore, 18 forms of *NEDD4L* transcripts could potentially be produced to encode the novel human *NEDD4* like ubiquitin-protein ligase if all possible combinations of the three different 5′UTR and three different 3′ UTR together with the alternative events seen in *NEDD4La*, *NEDD4Lb*, and KIAA0439 (referred to here as *NEDD4Lc*) could occur in cells.

Chromosome localisation and genomic structure of *NEDD4L*

A trapped exon[21] (E2E11) of *NEDD4L* was mapped to 18q21 between markers D18S1117 and D18S64 using PCR amplification of the GeneBridge4 mapping panel.[24] BLAST database search identified a genomic sequence contig NT_010909.3 in the 18q21 region that has 486511 bp and contains all the

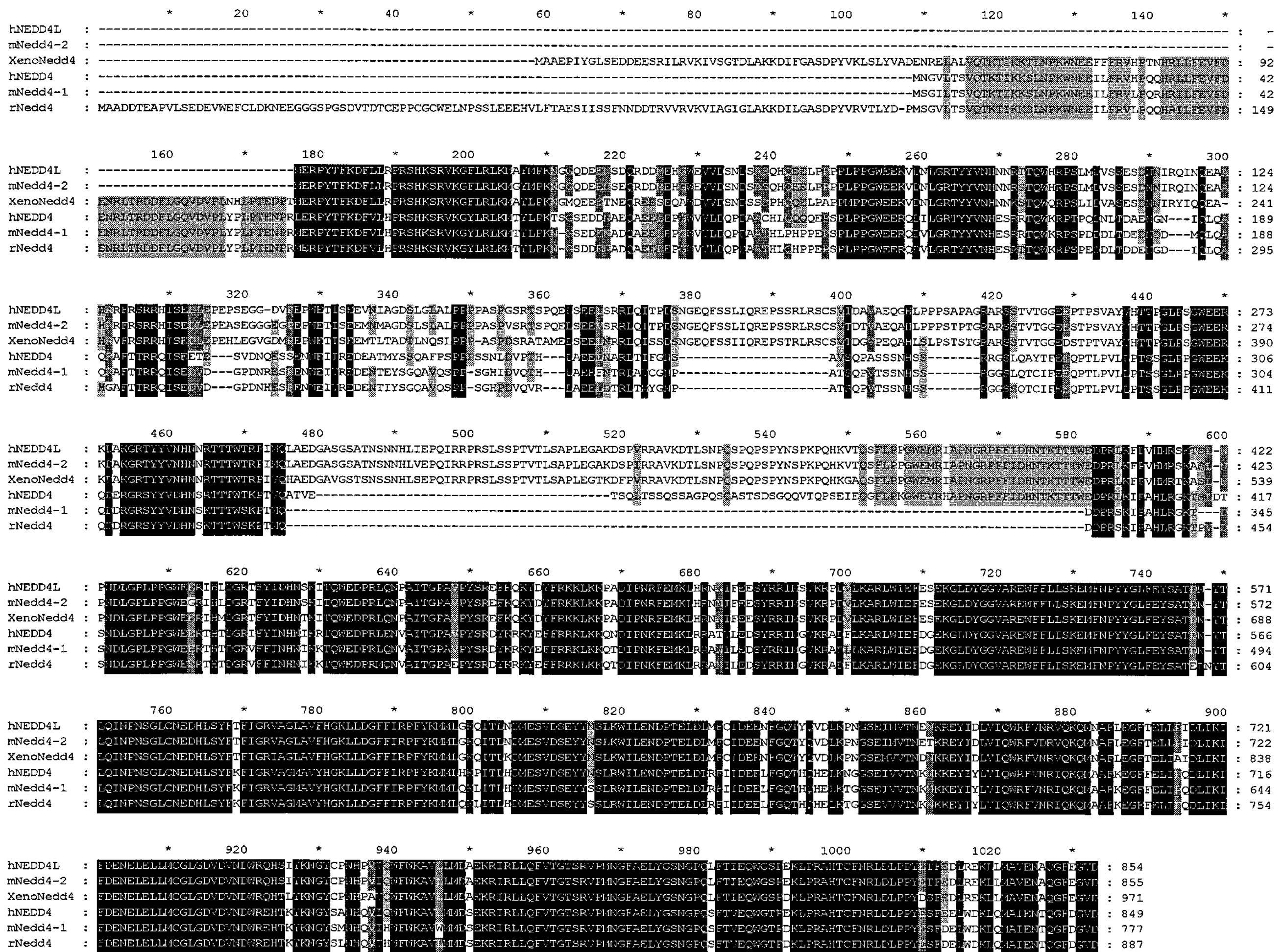


Figure 2 Multiple sequence comparison of *NEDD4L* with orthologues of *NEDD4* from different species. The alignment was generated using CLUSTAL W (http://www2.ebi.ac.uk/clustalw/) and was shaded using the Genedoc program.[34] Note: symbols hNEDD4L, mNedd4-2, XenoNedd4, hNEDD4, mNEdd4-1, and rNedd4 represent human NEDD4L, mouse Nedd4-2, Xenopus Nedd4, human NEDD4, mouse Nedd4-1, and rat Nedd4, respectively.

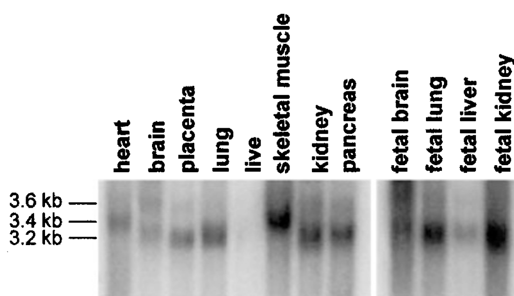
exons of *NEDD4La*. We observed that the first exon (exon 1a) of *NEDD4La* is identical to base pair 181446–181581 of NT_010909.3, while the 'first' exon (exon 1c) of *NEDD4Lc* is located 26 kb upstream of that of *NEDD4La*. The first exon (exon 1b) of *NEDD4Lb* is not contained in NT_001909.3, however, its first intron ends at nucleotide 125718 in NT_001909.3, and its second exon (exon 2b) is located at least 55 kb upstream of exon 1a in NT_001909. Table 1 lists the sizes of each exon, the exon-intron boundary sequences, and the intron ranges of *NEDD4L* in NT_010909.3.

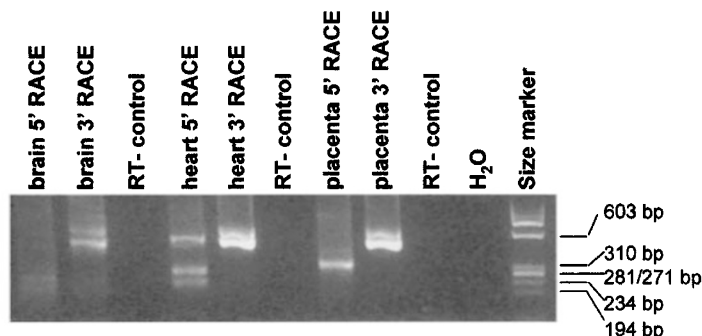
Mutation screen in bipolar subjects

Since *NEDD4L* maps to the region of 18q21 showing linkage evidence for a susceptibility locus for bipolar disorder,[19,20] three unrelated bipolar I probands and their parents were screened for mutation in the ORF of *NEDD4L*. RT–PCR was performed to amplify the cDNA coding sequence from total RNA isolated from lymphoblast cell lines derived from these individuals. Direct sequencing of the PCR products did not indicate variations (mutations) in the nine individuals tested. A semi-quantitative RT–PCR amplification analysis (normalised to the beta-actin amplification product) did not indicate an expression reduction of *NEDD4L* in lymphoblast cell lines derived from members of three families including subjects with bipolar disorder and unaffected relatives (data not shown).

Discussion

The full-length cDNA for a novel gene *NEDD4L* in the region of 18q21 has been characterised. The genomic structure of *NEDD4L* and its tissue expression pattern were also determined. The alternative splicing event of *NEDD4L* involving exon 12 has a tissue-specific pattern in that the





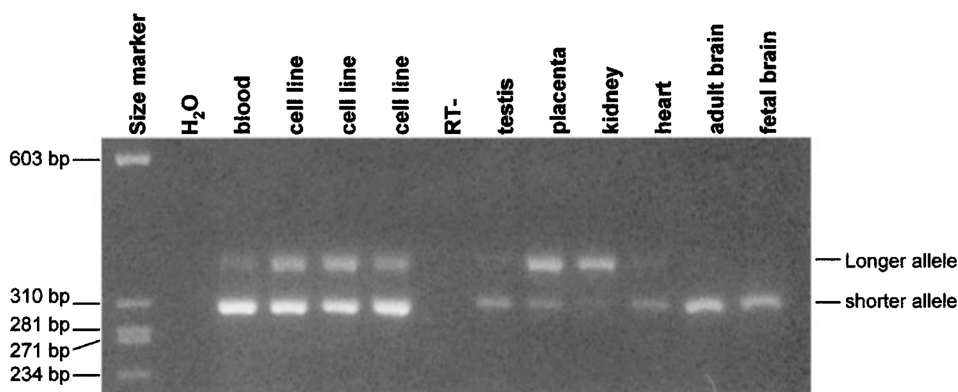


Figure 3 (**A**) Northern blot analysis. Northern blots (MTN, Clontech cat nos. 7760-1, 7756-1) were used to determine the expression pattern of *NEDD4L*. Each lane of the MTN blots contains 2 μg polyA$^+$ RNA. On the top of each blot the types of tissues are indicated. The arrows show the 3.2, 3.4, and 3.6 kb mRNA species detected with EST clone W87678 as hybridisation probe. (**B**) RACE amplification of the most 5′ and 3′ ends of *NEDD4L*. The tissue types, 5′ and 3′ RACE are shown on the top of the panel, RT− indicates a control sample used in reverse transcription without the addition of reverse transcriptase in the reaction. (**C**) RT–PCR analysis of exon 12 alternative splicing. Shown are analysed RNA samples from 10 different tissues. RT− indicates a reverse transcription negative control sample.

exon 12 minus form is predominantly present in brain and lymphocytes while the exon 12 plus form is strongly seen in kidney and placenta. Striking sequence homology to mouse *Nedd4-2* suggests that *NEDD4L* is the human homologue of the mouse Ubiquitin-protein ligase *Nedd4-2*.

The 30 exons of *NEDD4L* are distributed in at least 356 kb genomic region. Exons 2–30 are shared by *NEDD4La*, *b* and *c* except 12 involved in an alternative splicing event. The first exon (plus exon 2 in *NEDD4Lb*) has difference sequences in all these transcripts identified and is located in different upstream regions on genomic sequence. This observation indicates that there might be multiple promoters involved in *NEDD4L* transcription, and might explain the tissue-specific expression pattern of *NEDD4L*.

Ubiquitin-protein ligase *NEDD4* homologues have been demonstrated in human, mouse, rat, and Xenopus to interact, through their WW domains, with the proline-rich PY motifs (xPPxY) of the epithelial sodium channel *ENaC* and to down regulate the channel's activity.[5,10,12–14,29] Mutations or deletions of the PY motifs of *ENaC* prevent interaction of *NEDD4* with *ENaC*, and thus lead to Liddle's syndrome (an autosomal dominant inheritance form of hypertension).[5] In addition, the WW domains of *NEDD4* have been shown to bind to the phosphorylated serine and threonine residues of substrate proteins.[17] Therefore, the *NEDD4* gene family may have a wide range of functional interaction with proteins containing PY motifs and serine- and threonine-phosphorylated proteins.

Table 1 Genomic structure of NEDD4L. Sequences shown are flanking regions of exon-intron junctions. Capital letters indicate regions of exons, and subscript cases show splicing donor and acceptor sites. The intron ranges indicated refer to NCBI's contig sequence NT_010909.3

Exons in three transcripts					
NEDD4La	*NEDD4Lb*	*KIAA0439*	*Exon size bp*	*Exon-intron boundary sequence (exon/5′ intron 3′/exon)*	*Intron range in NT_010909.3*
–	1b	–	1–298	exon1b … TCTGTCCACTTTGTAGAAG/ ? … intron1b … TCCTTCTCACAG/GGAGAGTCCCGT … exon2b 5′	?–125713
–	2b	–	299–372	exon2b 3′ … CTTTGGAGCCAG/GTATGTTGGCTT … intron2b … ATTTTCTTACAG/TGATCCGTATGT … exon2a 5′	125788–205250
–	–	1c	1–244	exon1c … GCCTGGAATCTG/GTAAGTGCCACC … intronic … ATTTTCTTACAG/TGATCCGTATGT … exon2a 5′	155333–205250
1a	–	–	1–136	exon1a … GGAAAAGTATAG/GTAAGAACAAAG … intronia … ATTTTCTTACAG/TGATCCGTATGT … exon2a 5′	181582–205250
2a	2a	2a	137–218	exon2a 3′ … ACAATTAAAAAG/GTAGGTGTCGAT … intron2b … TTTCTCTTCCAG/ACACTGAACCCA … exon3 5′	205333–208722
3	3	3	219–257	exon3 3′ … TTTTATTTCAGG/GTAAGTTTTTCA … intron3 … TGTTCCTTATAG/GTAAACCCATCT … exon4 5′	208762–211824
4	4	4	258–311	exon 4 3′ … GAAAATAGACTG/GTAAGTGGATGC … intron4 … TCTTCCTAACAG/ACACGAGACGAC … exon5 5′	211879–275804
5	5	5	312–362	exon5 3′ … AGTCACCTTCCG/GTAAGGACAGTC … intron5 … TTTTCCCCACAG/TCATAAGTCTCG … exon7 5′	282310–283054
6	6	6	363–424	exon6 3′ … CAGACCAAGAAG/GTGAGGCTTGTG … intron6 … TTATGTGTGCAG/TCATAAGTCTCG … exon7 5′	282310–283054
7	7	7	425–527	exon7 3′ … GATGACATGGAG/GTACGTGGAGGG … intron7 … TTTATTCCGCAG/CATGGATGGGAA … exon8 5′	283158–284818
8	8	8	528–694	exon8 3′ … ACCAAGCCTGAT/GTGAGTACCGTG … intron8 … CTGCATGCTCAG/GGACGTGTCCTC … exon9 5	284986–288817
9	9	9	695–827	exon 8 3′ … GATGTCCCCGAG/GTACGATGTCCC … intron9 … CTTCTCTGAAAG/CCTTGGGAGACC … exon10 5′	288951–290560
10	10	10	828–1004	exon10 3′ … AGCTCTTTGATT/GTAAGTAGTGGC … intron10 … CTCTCCTTCCAG/CAAAGAGAACCC … exon11 5′	290738–293640
11	11	11	1005–1079	exon11 3′ … CATCTACCACCG/GTAACCCATGCT … intron11 … TATCATTCACAG/CCCAGTGCCCCA … exon12 5′	293716–295300
12	12	–	1080–1139	exon12 3′ … GAGGAACCAACG/GTAATGATCCAC … intron12 … TTGCACAAACAG/CCATCAGTGGCC … exon13 5′	295361–300860
13	13	13	1140–1271	exon13 3′ … CCTATCATGCAG/GTACGAAGATTG … intron13 … CCTCCAAAATAG/CTTGCAGAAGAT … exon14 5′	300993–301450
14	14	14	1272–1391	exon 14 3′ … GCCCCGCTGGAG/GTGAGACGGCTA … intron14 … GTTATTCTTTAG/GGTGCCAAGGAC … exon15 5′	301621–302728
15	15	15	1393–1589	exon15 3′ … ACTACAACCTGG/GTAAGGCTGCTG … intron15 … TTTTTCTTGCAG/GAAGATCCACGT … exon16 5′	302927–309359
16	16	16	1590–1667	exon16 3′ … GGCCCCCTTCCT/GTGAGTACACTG … intron16 … CCCGGATACTAG/CCTGGCTGGGAA … exon17 5′	309438–310813
17	17	17	1668–1722	exon17 3′ … ATATTGATCATA/GTAAGTAGGCGC … intron7 … AACATTTTCAGA/TAGCAAAATTAC … exon18 5′	310869–317016
18	18	18	1723–1781	exon 18 3′ … ATTACTGGTCCG/GTCAGTATTTTC … intron18 … TTTATCTTCCAG/GCTGTCCCTTAC … exon19 5′	317076–324090
19	19	19	1782–1847	exon19 3′ … TTAAAGAAACCT/GTGAGTAATCAT … intron19 … TCTTTTGTGTAG/GCTGATATCCCC … exon20 5′	324157–325821
20	20	20	1848–2077	exon20 3′GTACTCTGCCAC/GTAAGTATATGG … intron20 … TCTCCTCAAAAG/GGACAACTACAC … exon21 5′	326052–327568
21	21	21	2078–2199	exon21 3′ … AGCTCTTAGATG/GTAAGTCTTGAA … intron21 … ACCGTGTTTTAG/GTTTCTTCATTA … exon22 5′	327691–330219
22	22	22	2200–2270	exon22 3′ … ATGGAATCTGTG/GTAAGTAAATGC … intron22 … GTCATTCTGTAG/GATAGTGAATAT … exon23 5′	330291–332996
23	23	23	2271–2366	exon23 3′ … AACTTTGGACAG/GTACATGTGGGT … intron23 … TTGTAATTACAG/ACATATCAAGTG … exon24 5′	333093–343068
24	24	24	2367–2440	exon24 3′ … GGAATATATCGA/GTATGTATACAC … intron24 … TTTCTCCTGCAG/CTTAGTCATCCA … exon25 5′	343143–345348
25	25	25	2441–2501	exon25 3′ … GCCTTCTTGGAG/GTAAGCCATGCT … intron25 … TTTTTCTTCAG/GGATTCACAGAA … exon26 5′	345409–347261
26	26	26	2502–2561	exon 26 3′ … AATGAGCTGGAG/GTTTGTATTATA … intron26 … TTTTCTTCCTAG/TTGCTCATGTGC … exon27 5′	347322–348907
27	27	27	2562–2669	exon26 3′ … CGTCATTCAGTG/GTTCTGGAAGGT … intron27 … CTCTGTTCATAG/GTCTGTGCTACTC … exon28 5′	349016–350468
28	28	28	2670–2766	exon27 3′ … CCGAACTTTATG/GTGAGCAGGATA … intron28 … TTTCTTGTCTAG/GTTCCAATGGTC … exon29 5′	350566–351309
29	29	29	2767–2839	exon29 3′ … AGCTCACACATG/GTGAGTGACAAA … intron29 … TTCCTTTTGCAG/CTTTAATCGCCT … exon30 5′	351383–355989
30a	30b	30c	2840–3246*	exon30 3′ … TTTTACATCCTT	

*Indicates the range of exon 30 of NEDD4La, see text for details of exon 30 in NEDD4Lb and c.

Recently, the mouse *Nedd4-2* gene has been identified from a cell line derived from cortical collecting duct.[3] Unlike mouse Nedd4-1 that features having 3 WW, one C2, and one HECT domain, *Nedd4-2* contains 4 WW and a HECT domain, but lacks the C2 domain in the N-terminus. Both Nedd4-1 and 2 are expressed in the cortical collecting duct cells, however, it is thought that Nedd4-2 is the real regulator of the ENaC channel and a potential candidate for arterial hypertension since it is *Nedd4-2*, not *Nedd4-1* interacting with the *ENaC* when co-expressed in Xenopus oocytes.[3] The human novel *NEDD4L* gene showed 97% amino acid sequence identity to mouse *Nedd4-2*, and has similar protein structure modules as mouse *Nedd4-2* (containing one HECT and four WW domains, lacking the C2 domain). These properties suggest that *NEDD4L* is the human homologue of mouse *Nedd4-2*. A recombinant protein containing only the WW domains of KIAA0439 (*NEDD4Lc*) has recently been reported to act as a dominant negative mutant that can interfere with the Na^+-dependent feedback inhibition of the *ENaC* in whole-cell patch clamp experiments, which suggests *NEDD4* and *NEDD4L* either play a redundant role in *ENaC* regulation or function in a tissue- and/or signal-specific manner to down-regulate *ENaC*.[22]

There are several genetic disorders mapped to 18q21, including autosomal dominant orthostatic hypotensive disorder,[30] a locus for postural systolic blood pressure response,[31] IDDM6,[32] Grave's disease (GD),[33] and bipolar disorder.[19,20] The genes for these disorders are currently unidentified. Sequence analysis in bipolar subjects from families linked to 18q21[19,20] did not reveal variation in the protein-coding region of *NEDD4L*. Semi-quantitative RT–PCR analysis did not indicate a reduction of *NEDD4L* in the bipolar subject tested. However, to exclude the gene to be responsible for bipolar disorder, sequence variation in introns, splicing sites, and promoter region need to be further studied. Multi-point linkage analysis in 74 sib pairs of GD cases showed a peak NPL score of 3.09 (P=0.001) at marker D18S487.[33] In IDDM6, there is evidence for linkage disequilibrium at marker D18S487 as well.[32] Given that *NEDD4L* is located 6 cM distal to D18S487, it is unlikely that *NEDD4L* is responsible for IDDM6 or GD.

Does *NEDD4L* play a role in orthostatic hypotensive disorder? Linkage studies have mapped the autosomal orthostatic hypotensive disorder on 18q21 between markers D18S858 and D18S541.[30] There is also linkage evidence that a locus close to marker D18S858 may be responsible for postural systolic blood pressure response.[31] *NEDD4L* maps between these two markers and within 2 Mb proximal to 18S1367. This positional evidence in combination with its potential role in regulation of the epithelial sodium channel *ENaC* activity makes *NEDD4L* a candidate gene for mutation screen in orthostatic hypotensive disorder.

Acknowledgments

This study is supported by grants from the Theodore and Vada Stanley Foundation (JR De Paulo Jr, MG McInnis, and CA Ross), and from NARSAD, the National Alliance for Research on Schizophrenia and Depression (JR De Paulo Jr, MG McInnis, and H Chen). We thank D Avramopoulos for critical reading of the manuscript. We are grateful to Dr Paul McHugh and the Department of Psychiatry for the support of this work.

Accession number

The sequences reported here have GenBank nos. AF210730 (NEDD4La), AF385931 (NEDD4Lb). The LL18NC02 cosmid library was constructed in the Lawrence Livermore National Laboratory, and distributed by UK HGMP Resource Center.

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