Biochemical and Biophysical Research Communications 411 (2011) 56–61

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Bidirectional transcription from human *LRRTM2*/*CTNNA1* and *LRRTM1*/*CTNNA2* gene loci leads to expression of N-terminally truncated CTNNA1 and CTNNA2 isoforms

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ARTICLE INFO

Article history:
Received 27 May 2011
Available online 17 June 2011

Keywords:
LRRTM family
CTNNA family
Alternative promoter
Schizophrenia
Handedness

ABSTRACT

α-Catenins (CTNNAs) are essential for the regulation of cell–cell and cell–matrix interactions in tissues. All human *CTNNA* genes contain antisense oriented leucine rich repeat transmembrane (*LRRTM*) genes within their seventh introns. Recently, a haplotype upstream of one of the human *LRRTM* genes, *LRRTM1* that resides in *CTNNA2*, was shown to be associated with handedness and schizophrenia. Here, we show that both *CTNNA1* and *CTNNA2* contain alternative 5′ exons linked to bidirectional promoters that are shared with the antisense oriented *LRRTM2* and *LRRTM1* genes, respectively. We demonstrate that bidirectional activity of these promoters results in alternative *CTNNA1* and *CTNNA2* transcripts that are expressed at high levels in the nervous system and show that N-terminally truncated CTNNA1 and CTNNA2 proteins lacking the β-catenin interaction domain are produced from these alternative *CTNNA* mRNAs. In addition, our results indicate that the haplotype that affects *LRRTM1* expression and is associated with schizophrenia and handedness, could also influence the expression of brain-enriched alternative transcripts of *CTNNA2*.

1. Introduction

α-Catenins are involved in formation and maintenance of structural integrity of tissues by mediating cell–cell and cell–matrix interactions through coupling cadherin–β-catenin complexes to the cytoskeleton [1]. α-Catenins bind strongly either to the cadherin–β-catenin complex or actin, regulate actin filament assembly and control the dynamics of cadherin-dependent junctions [2,3].

In mammals there are three subtypes of α-catenins: αE-catenin (CTNNA1), expressed predominantly in non-neural tissues [4], αN-Catenin (CTNNA2), expressed widely in the central nervous system [5], and αT-catenin (CTNNA3), expressed primarily in the testis and heart [6]. Variability in α-catenin protein isoforms is generated by alternative splicing. Alternative mRNAs of *CTNNA1* or *CTNNA2* encode a C-terminally truncated isoform of αE-catenin [7] or an isoform of αN-catenin with in-frame insertion in the C-terminal region [8], respectively. In addition, transcripts of *CTNNA2* and *CTNNA3* that contain an alternative 5′ exon instead of the conventional initial exon encode isoforms with truncated N-termini [9,10]. With the exception of testis-specific N-terminally truncated CTNNA3 isoform that does not bind β-catenin [10], the functional significance of alternative α-catenin isoforms is unknown.

The *CTNNA1*, *CTNNA2* and *CTNNA3* genes contain antisense oriented leucine rich repeat transmembrane (*LRRTM) 2*, *LRRTM1* and *LRRTM3* genes, correspondingly, within their seventh intron [11]. The *LRRTM* genes encode transmembrane proteins that regulate presynaptic differentiation [12]. Importantly, *LRRTM1* has been associated with establishment of handedness and susceptibility to schizophrenia because of a specific haplotype upstream of *LRRTM1* [13]. However, it has not been thoroughly addressed whether the regulatory effect accompanied by this haplotype on *LRRTM1* expression, affects expression of *CTNNA2*. Here, we show that human *CTNNA1* and *CTNNA2* share bidirectional promoters with *LRRTM2* and *LRRTM1*, respectively, leading to the expression of N-terminally truncated CTNNA1 and CTNNA2 isoforms.

2. Materials and methods

All experiments with human tissues and all animal procedures were approved by the local ethics committee. Bioinformatic analysis, RNA isolation, mRNA expression analyses by RT-PCR, rapid amplification of cDNA 5′ ends (5′ RACE), RNase protection assay (RPA), cloning of cDNAs encoding full-length proteins, cell culture, DNA transfection, luciferase reporter assay, Western blotting and immunocytochemistry were performed according to standard procedures. Detailed description of materials and methods, including primers and antibodies, is provided in the Supplementary materials and methods.

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0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.06.085

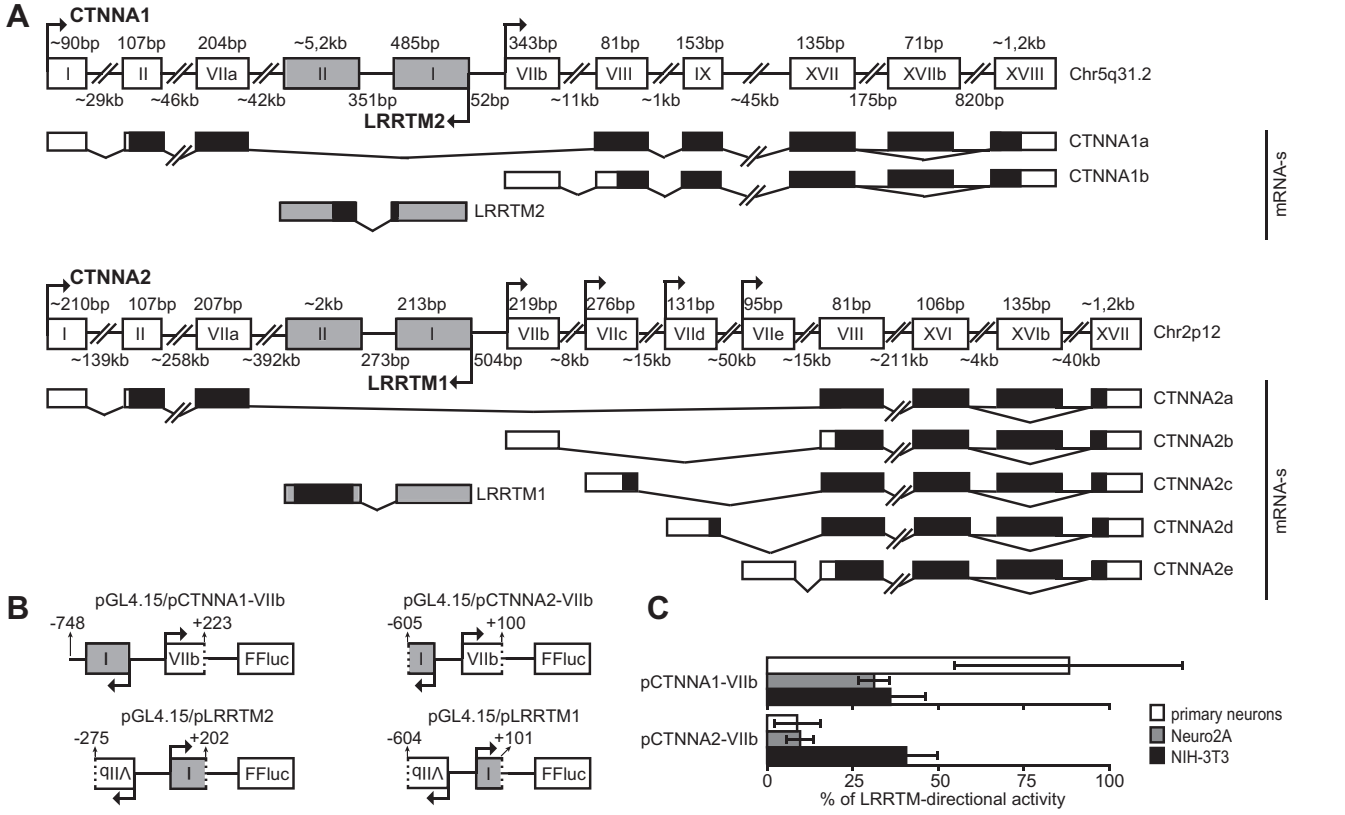

Fig. 1. Genomic structures of human *CTNNA1*/*LRRTM2* and *CTNNA2*/*LRRTM1* gene loci and bidirectional activities of the *CTNNA1*-VIIb/*LRRTM2* and *CTNNA2*-VIIb/*LRRTM1* promoters. (A) Structural organization of human *CTNNA1*, *LRRTM2*, *CTNNA2* and *LRRTM1* exons and introns was determined by analyzing genomic and mRNA sequence data using bioinformatics and RT-PCR. White and gray boxes represent *CTNNA* and *LRRTM* exons, respectively. Black lines represent introns. The orientation of transcription is shown by arrows. Arabic numerals above the exons and below the introns indicate their sizes. Exon numbers are shown in roman characters. White and gray boxes of mRNAs represent non-coding regions of exons. Black boxes designate translated regions of exons. (B) FFluc reporter constructs used for determining the bidirectional activity of the *CTNNA1*-VIIb/*LRRTM2* and *CTNNA2*-VIIb/*LRRTM1* promoters. Construct names indicate the direction of promoter activity analyzed. Numbers indicate the lengths of the cloned promoters in bps relative to the most 5′ TSS on the sense strand. (C) Promoters in front of *CTNNA1* exon VIIb and *CTNNA2* exon VIIb activate transcription bidirectionally. Percent of *LRRTM2*- and *LRRTM1*-directional promoter activity, respectively, is shown in rat primary cultured cortical neurons, Neuro2A neuroblastoma and NIH-3T3 fibroblast cells. Results of two independent experiments measured in triplicates and normalized to thymidine kinase (TK) promoter-dependent renilla luciferase (Rluc) signals derived from cotransfected TK-Rluc constructs are shown. Error bars represent SD.

3. Results

3.1. Identification of novel alternative 5′ exons of human CTNNA1 and CTNNA2 genes

Using bioinformatics we identified novel potential alternative 5′ exons in the seventh intron of both human *CTNNA1* and *CTNNA2*. In *CTNNA1* there is one such exon that we have named exon VIIb (Fig. 1A). In *CTNNA*2 we identified four potential 5′ exons in the seventh intron that are named here VIIb, VIIc, VIId and VIIe (Fig. 1A). Hereafter the conventional full-length *CTNNA* transcripts are referred to as *CTNNA1a* and *CTNNA2a*. Transcripts containing the novel 5′ exons are named according to the designation of the alternative seventh exon: *CTNNA1b* and *CTNNA2b*, *CTNNA2c*, *CTNNA2d* or *CTNNA2e* (Fig. 1A, GenBank IDs: HQ589335, HQ589336, BX537769, DC331874 and DC340948, respectively). Exon VIIb of *CTNNA1* and exon VIIb of *CTNNA2* are located in head-to-head orientation with antisense oriented *LRRTM2* and *LRRTM1* gene, respectively, and could share a common bidirectional promoter with the corresponding *LRRTM*. In order to support this hypothesis and to verify that these exons are used as 5′ exons, we applied the 5′ RACE method. We used human hippocampal cDNA and primers targeting exon IX of *CTNNA1* or *CTNNA2* and the 5′ exon of *LRRTM2* or *LRRTM1*. Firstly, our results showed that exon VIIb of *CTNNA1* has at least 11 different transcription start sites (TSSs) spanning 271 bp (Supplementary Fig. 1). We detected two TSSs separated by 28 bp for *LRRTM2* and found that the distance between the most 5′ TSS of *CTNNA1* exon VIIb and the most 5′ TSS of *LRRTM2* is only 52 bp (Supplementary Fig. 1). Secondly, we found that usage of only two of the four *CTNNA2* alternative seventh exons is detectable in the human hippocampus. With *CTNNA2* RACE primers the obtained clones contained predominantly exon VIIb of *CTNNA2* and on rare occasions usage of exon VIId was detected. None of the RACE clones comprised *CTNNA2* exon VIIc or VIIe, indicating that transcription of these exons is infrequent and below the detection limit of our assay. *CTNNA2* exon VIIb has at least five TSSs spanning 16 bp and *LRRTM1* has two TSSs that are 4 bp apart (Supplementary Fig. 1). The distance between the most 5′ TSS of *CTNNA2* exon VIIb and the most 5′ TSS of *LRRTM1* is 504 bp. For *CTNNA2* exon VIId we mapped two adjacent nucleotides as TSSs (Supplementary Fig. 1). Collectively, these results corroborated that *CTNNA1* exon VIIb and *CTNNA2* exon VIIb and VIId are used as initial exons and transcribed into *CTNNA* mRNAs that lack exons I-VIIa.

3.2. Bidirectional activities of CTNNA1-VIIb/LRRTM2 and CTNNA2-VIIb/LRRTM1 promoters

We used luciferase reporter assay to analyze transcription-promoting activities of the putative bidirectional promoters of *CTNNA1* exon VIIb and *LRRTM2* (*CTNNA1*-VIIb/*LRRTM2*) and of *CTNNA2* exon VIIb and *LRRTM1* (*CTNNA2*-VIIb/*LRRTM1*), respectively. We transfected mouse fibroblast NIH-3T3 and neuroblastoma Neuro2A cells