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Identification and characterization of heart-specific splicing of human neurexin 3 mRNA (NRXN3)

Gianluca Occhi, Alessandra Rampazzo, Giorgia Beffagna, and Gian Antonio Danieli*

Department of Biology, University of Padua, Via U. Bassi 58/B, Padua I-35131, Italy

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Abstract

Three neurexin (NRXN) genes are known in humans, each transcribed from two promoters and extensively spliced at five canonical positions, thus generating thousands of isoforms. For NRXN3, only neuronal expression was reported so far. We reported here on the expression of NRXN3 in additional tissues (lung, pancreas, heart, placenta, liver, and kidney) and on the identification and characterization of heart-specific splicing variants of NRXN3. Cardiac isoforms of NRXN3 probably participate in a complex involving dystroglycan and proteins of extracellular matrix, involved in intercellular connections.

Keywords: Neurexins; Alternative splicing; Heart transcript; Expression pattern

Neurexins belong to a family of highly polymorphic neuronal-specific cell surface proteins, whose architecture suggests a role in cell adhesion and cell recognition [1–3]. Three genes, designated as NRXN1 (2p16.3), NRXN2 (11q13.2), and NRXN3 (14q24.3–q31.1), were identified so far in the human genome. Complete nucleotide sequence and genomic organization of the three human neurexin genes were recently reported [4].

Each NRXN gene has two promoters, one for a longer transcript, encoding α-neurexin, and the second, for the shorter β-neurexin. Thus, three NRXN genes produce six primary transcripts, which are then subjected to alternative splicing at five positions [1,2,4]. Alternative splicing sites 1–3 are specific for α-neurexin, whereas 4 and 5 are common to both α- and β-neurexins. Since each site of alternative splicing is used independently, up to 2250 different isoforms are theoretically possible [4]. Alternative splicing at site 2 regulates NRXN–dystroglycan binding [5], whereas site 4 is involved in binding of NRXN to neuroligin, dystroglycan, and α-latrotoxin [5–8]. In neurexin 3α and 3β, some of the insert sequences in splice sites 5 contain stop codons, thus producing proteins lacking of a transmembrane domain, which are secreted [2,3].

In this paper we report on the characterization of a cardiac isoform of NRXN3, one of the largest human genes, spanning 1,826,818 nt [4].

Materials and methods

Expression analysis of NRXN3. Two micrograms mRNA from human male heart (Stratagene) was reverse-transcribed in volumes of 25 μl using 1 μg random hexamer primers, 25 nmol of each deoxyribonucleotide triphosphate (Gibco-BRL), and 30 U RNasin (Promega) under the following conditions: incubation at 70° for 5 min, reverse transcription with 30 U AMV reverse transcriptase (Promega) at 37 °C for 1 h. Primers used for reverse transcriptase PCR were: NRX14F, 5′-GAGTGGCAAGGCTCAGGTAG-3′; NRX15R, 5′-TGGCACCATTGATAACCTGA-3′; NRX1βF, 5′-CCCATCTGTAGTGGTCCCGT-3′; NRX18R, 5′-CCAAGATGCCATCCTTCACA-3′.

RT-PCR experiments were carried out in a reaction buffer (12.5 μl) containing 2 μl cDNA, 5 pmoles oligonucleotide primers, 100 μM each of dGTP, dATP, dCTP, and dTTP, 67 mM Tris–HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, and 0.2 U RTB Taq polymerase (Experteam). All polymerase chain reactions were carried out in a PCR Express (Hybaid) thermal cycler, under the following conditions: 1 denaturation step at 94 °C for 3 min, and 33 cycles of denaturation (at 94 °C for 1 min), annealing (at 65 °C for 1 min), and extension (at 72 °C for 1 min). Aliquots of the amplified products were separated by 2% agarose gel electrophoresis and stained by ethidium bromide. RT-PCR products were then subjected to direct DNA sequencing.

* Corresponding author. Fax: +39-049-8276209.
E-mail address: danieli@bio.unipd.it (G. Antonio Danieli).

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Expression of neurexin in different human tissues was assessed by RT-PCR amplification of MTC (Multiple Tissue cDNA) panel (Clontech), including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. Amplification was performed by using the primers mentioned above. PCR products were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining.

Molecular characterization of NRXN3 mRNA, cardiac isoform. Based on the known sequences of Rattus norvegicus (L14851), Bos taurus (L27869) and on the human NRXN3 brain-isoforms recently published [4], we designed PCR primers by PRIMER3 (http://www-genome.wi.mit.edu) to determine the sequence of the NRXN3 cardiac isoform mRNA through RT-PCR. The RT-PCRs were performed as described above. Each RT-PCR product was purified (PCR Product Pre-Sequencing kit; USB) and sequenced using the BIG DYE dideoxy-terminator chemistry (Perkin–Elmer) on an ABI 377 DNA sequencer (PE Applied Biosystems). Chromas 1.5 software (Technelysium) and LASERGENE package computer programs (DNASTAR) were used to edit, assemble, and translate sequences. To determine the genomic structure of NRXN3, the cDNA sequence corresponding to the cardiac isoform was compared with genomic sequences by using the BLAST algorithm.

Protein structure prediction. The amino acidic sequence from the cardiac isoform of NRXN3 was used for secondary structure prediction analysis. Protein motifs and profiles searches were performed with Pfam (http://pfam.wustl.edu/hmmsearch.shtml) [9] and SMART (http://smart.embl-heidelberg.de) [10,11].

Results and discussion

Non-neuronal expression pattern of NRXN3

It was previously reported that expression of the neurexins was brain-specific [1]. By hybridizing probes from neurexin 3α to Northern blots of various rat tissues (adrenal gland, brain intestine, kidney, liver, spleen, and testis) it was found that neurexin 3α is expressed exclusively in brain [2].

We searched Unigene database for neurexin 3 EST clusters. Moreover, we performed BLAST homology search of the NRXN3 cDNA against human dbEST database. We detected 76 ESTs derived from several

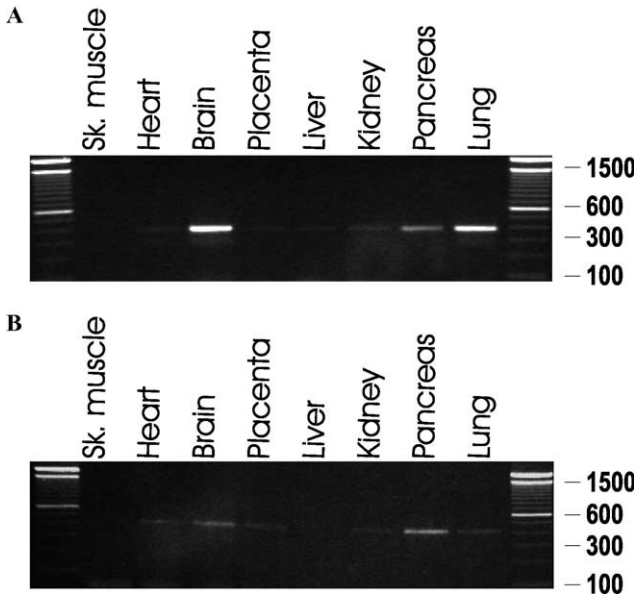

Fig. 1. Distribution of α- (A) and β-neurexin (B) in human tissues.

Table 1
Oligonucleotide primers used to characterize NRXN3 cardiac isoforms

Primer	Forward	Primer	Reverse
NRX1F	CCCAAAGAGGAAACATACCC	NRX2R	CAGGTAGAAACAGGCCAAGGA
NRX2F	ACCCTTGATGGAGTTCAGGC	NRX6Ra	ACGCTGCCAGGTCTTAAAGG
NRX3F	AGAAGATGTCAGTCAAGATCCAG	NRX6R	GGTGATTTCATCACTGCTGC
NRX4F	GCCTCTCCCACCTCATGATG	NRX6Ra	ACGCTGCCAGGTCTTAAAGG
NRX6F	CATTGTGGAGCCAGTGAATG	NRX8R	TGAAGTTGTTGCTGACAGGG
NRX8F	GGCTCGGACGACTTCTTCTA	NRX9R	TGGCTTTCTTCTGAGTGGCT
NRX9F	CAAGAGAGGAAGGATGCTCG	NRX10R	CTGTCACACTGCTTGGCACT
NRX10F	GACCTATTCATTGATGGGCG	NRX11R	CAGAGTCCCTGGAGGTCGTA
NRX11F	GGTCATGCATACTGAGGCAG	NRX13R	TAAGGCTTTTTCCTCTCCGC
NRX13F	ACCTTGTATGCAGGGCAGAA	NRX14R	GAGTGGCAAGGCTCAGGTAG
NRX14F	ATTGTGAGCTGAAGGCTCGT	NRX15R	TGGCACCATTGATAACCTGA
NRX15F	AATGACAACCAGTGGCACAA	NRX16R	ATCATTGATGAGGTCTGGCA
NRX16F	AGGCATGTACAGCAACCTCC	NRX17R	TCATTGCACTGGTTTCCAGA
NRX17F	AGTACCACCTGCCAGGAAGA	NRX18Ra	TGGAGGAAGTCACCAAGTCC
NRX1βF	CCCATCTGTAGTGGTCCCGT	NRX18R	CCAAGATGCCATCCTTCACA
NRX18F	TCTTTGGGAAAAGTGGTGGG	NRX19R	TCCTGGTGAAGCGTACCACA
NRX19F	TGGCACAGTTGACATCTCCAT	NRX20R	CCACTCCTCTACAGGCCGAT
NRX20F	TGAACGCTTCCAAATGGTAAAA	NRX21R	GACATTTCTGGTGGCATGGA
NRX21F	GTGGAAAGGACAAAGGACGC	NRX22aR	CTCGGCTCACATTCAACAAA
NRX21Fa	CACAACCCGTAAGAATCGCT	NRX23aR	ATGTCCATGTAAGGGCGG
NRX22aF	TGCTGAATGTTCAAGTGATGATG	NRX24cRb	CCAAAGATTCATAGGCGTGG
NRX22aFa	TTTGTTGAATGTGAGCCGAG	NRX23bR	AACAAATTGCCCGCATGT
		NRX24aR	CTCAGAGGTCATGGACAGGG
		NRX24bR	GTGCTTTGTAGCCACCTTCG
		NRX24cR	TCGTCCACTTGATAGGACCC

cDNA sources (adrenal gland, aorta, bladder, bone, brain, breast, central nervous system, eye, germ cell, head neck, heart, kidney, lung, pancreas, pituitary, prostate, skin, stomach, testis, uterus, and whole embryo), included in the Unigene cluster Hs.247837 and containing part of the NRXN3 mRNA sequence. This suggested that the expression pattern of NRXN3 was not limited to neuronal tissues. Only an EST clone IMAGE:1706801 (GenBank Accession No. AI146439) from heart overlapped part of the 3′-UTR of the NRXN3 gene. Because this could be due to experimental artefacts in EST cloning, we decided to design primers to bridge exons 14–15 (NRXN3α) and exons β1–18 (NRXN3β) by RT-PCR using heart cDNA (Table 1).

Results of the amplifications showed that NRXN3α is highly expressed in brain, lung, and pancreas; a lower level of expression is detectable in heart, placenta, liver, and kidney, whereas no expression can be observed in skeletal muscle. Differently, NRXN3β showed a reduced level of expression in brain and lung. Furthermore in liver NRXN3β is almost undetectable (Fig. 1).

Identification of NRXN3 heart isoform

NRXN3 contains 25 exons [4], NRXN3α transcript contains 24 exons (1–24) while NRXN3β transcript is generated by connecting exons β1 to exons from 18 to 24. Exons 3a, 4, and 5 are alternatively spliced at site 1,

Table 2
Splice variants of the five alternative splice sites in NRXN3

Splice site	Amino acid sequence	Exons	Heart
1	CSEDVSQDPGLSHLMMSEQGSRKARE	2]3a,4,5[6	–
	CSEDVSQDPGLSHLMMSEQ ARE	2]3a,4[6	–
	CSEDVSQDP GRSKARE	2]3a,5[6	–
	CSE GLSHLMMSEQGRSKARE	2]4,5[6	+
	CSEDVSQDP ARE	2]3a[6	–
	CSE GLSHLMMSEQ ARE	2]4[6	–
	CSE GRSKARE	2]5[6	–
	CSE ARE	2][6	+
2	RQHSGIGHAMVNKLHCLVT	6]7b[8	–
	RQHSGIGHAM VT	6]7a[8	–
	RQ VT	6] [8	+
3	VNLDCIRINCNSSKGP	11]12[13b	–
	VNL GKGP	11][13b	+
4	PTGNTDNERFQMVKQKIPFKYNRPVEEWLQEKGRQ	19]20[21	+
	PT GRQ	19][21	–
5		21]22b,23a,24a	–
	SIQPTSDDLVSSAECSSDDEDFVECEPSTGRSARSSN/ /CVVHS*	21]22b,23a,24b	–
		21]22b,23a,24c	–
		21]22a,23a,24a	+
	SIQPTSDDLVSSAECSSDDEDFVECEPST ARSSN/ /CVVHS*	21]22a,23a,24b	+
		21]22a,23a,24c	+
		21]22b,23b,24a	–
	SIQPTSDDLVSSAECSSDDEDFVECEPSTGRSDILLKSF*	21]22b,23b,24b	–
		21]22b,23b,24c	–
		21]22a,23b,24a	–
	SIQPTSDDLVSSAECSSDDEDFVECEPST DILLKSF*	21]22a,23b,24b	–
		21]22a,23b,24c	–
	SIQPTSDDLVSSAECSSDDEDFVECEPSTGRSGGELV/ /FSLVTD KSLS/ /NVPTANPTEPG/ /REYYV*	21]22b,24a	+
	SIQPTSDDLVSSAECSSDDEDFVECEPST GGELV/ /FSLVTD KSLS/ /NVPTANPTEPG/ /REYYV*	21]22a,24a	+
	SIQPTSDDLVSSAECSSDDEDFVECEPSTGRS D KSLS/ /NVPTANPTEPG/ /REYYV*	21]22b,24b	+
	SIQPTSDDLVSSAECSSDDEDFVECEPST D KSLS/ /NVPTANPTEPG/ /REYYV*	21]22a,24b	+
	SIQPTSDDLVSSAECSSDDEDFVECEPSTGRS NPTEPG/ /REYYV*	21]22b,24c	+
	SIQPTSDDLVSSAECSSDDEDFVECEPST NPTEPG/ /REYYV*	21]22a,24c	+

At each splice site, modules are either present or absent. Presence or absence of the specific transcript in the heart is reported in the last column.

exons 7a/b at site 2, whereas exons 12 and 20 are alternatively spliced at sites 3 and 4, respectively. The most complex alternative splicing occurs at site 5, where exons 22b, 23a/b, and 24a/b/c are alternatively spliced [4].

Having detected transcription of NRXN3 in human heart, we decided to reconstruct the sequence of such cardiac isoform.

By using RT-PCR and primers derived from cDNA sequence of the brain isoform (see Table 2), we

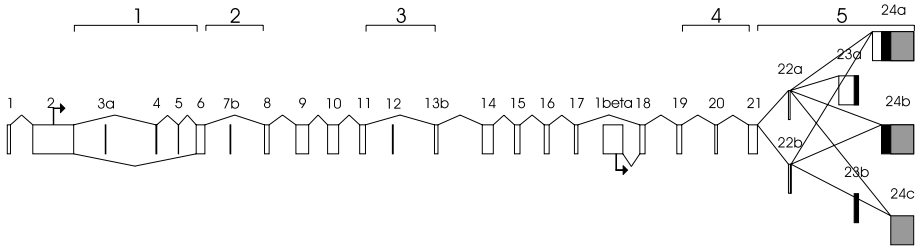

Fig. 2. Genomic organization of NRXN3 gene, producing cardiac isoforms. As demonstrated by several authors and confirmed by our results, NRXN3 gene shows two initiation sites located on exon 2 and on exon 1β, which generate a longer α- and a shorter β-neurexin, respectively. The five groups of exons involved in the alternative splicing are indicated. At site 1 only two of the eight possible alternative patterns were found in the heart. At site 5, nine different combinations of exons 22, 23, and 24 were detected in heart tissue. Exons 3a, 7b, 12, and 23b were never observed in cardiac transcripts, although they were detected in brain transcripts.

MSSTLHSVFFTLKVSILLGSLLGLCLGLEFMGLPNQWARYLRWDAS**TRSDLSFQ**
FKTNVSTGLLLYLDDGGVCDFLCLSLVDGRVQLRFSMDCAETAVLSNKQV
NDSSWHFLMVSRDRLRTVLMLDGEGQSGELQPQRPYMDVVSDLFLGGVPTDIR
PSALTLDGVQAMPGFKGLILDLKYGNSEPRLLGSRGVQMDAEGPCGERPCENG
GICFLLDGHPTCDCSTTGYGGKLCSEDVSQDPGLSHLMMSEQGRSKAREENVA
TFRGSEYLCYDLSQNPIQS**SSDEITLSFKTWQRNGLILHTGKSADYVNLALKD**
GAVSLVINLGSGAFEAIVEPVNGKFNDNAWHDVKVTRNLRQHSGIGHAMVN
KLHCLVTISVDGILTTTGYTQEDYTMLGSDDFFYVGGSPSTADLPGSPVSNNFM
GCLKEVVYKNNDIRLELSRLARIADTKMKIYGEVVFKCENVATLDPINFETPEA
YISLPKWNTK**RMGSISFDFRTTEPNGLILFTHGKPQERKDARSQKNTKVDFF**
AVELLDGNLYLLLDMGSGTIKVKATQKKANDGEWYHVDIQRDGRSGTISVNS
RRTPFTASGESEILDLEGDMYLGGLPENRAGLILPTELWTAMLNYGYVGCIRDL
FIDGRSKNIRQLAEMQNAAGVKSSCSRMSAKQCDSYPCKNNAVCKDGWNRFIC
DCTGTGYWGRTCEREASILSYDGSMYMKIIMPMVMHT**EAEDVSFRFMSQRAY**
GLLVATTSRDSADTLRLELDGGRVKLMVNLDCIRINCNSSKGPETLYAGQK
LNDNEWHTVRVVRRGKSLKLTVDDDVAEGTMVGDHTRLEFHNIETGIMTEKR
YISVVPSSFIGHLQSLMFNGLLYIDLCKNGDIDYCELKARFGLRNIIADPVTFKTK
SSYLSLATLQAY**TSMHLFFQFKTTSPDGFILFNSGDGNDFIAVELVKGYIHYV**
FDLGNGPNVIKGNSDRPLNDNQWHNVVITRDNSNTHSLKVDTKVVTQVINGA
KNLDLKGDLYMAGLAQGMYSNLPKLVASRDGFQGCLASVDLNGRLPDLINDA
LHRSGQIERGCEGPSTTCQEDSCANQGVCMQQWEGFTCDCSMTSYSGNQCNDP
GATYIFGKSGGLILYTWPANDRPST**RSDRLAVGFSTTVKDGILVRIDSAPGLGD**
FLQLHIEQGKIGVVFNIGTVDISIKEERTPVNDGKYHVVRFTRNGGNATLQVD
NWPVNEHYPTGNTDNERFQMVKQKIPFKYNRPVEEWLQEKGRQLTIFNTQAQI
AIGGKDKGRLFQGQLSGLYYDGLKVLNMAAENNPNIKINGSVRLVGEVPSILGT
TQTTSMPPEMSTTVMETTTTMATTTTRKNRSTASIQPTSDDLVSSAECSSDDEDF
VECEPSTGRSARSSNAARSLRAALTWTWRLTYTFTPIIFISCVVHS– DILLKSF–
GGELVIPLLVEDPLATPPIATRAPSITLPPTFRPLLTIIETTKDSLSMTSEAGLPCLS
DQGSDGCDDDGLVISGYGSGETFDSNLPPTDDEDFYTTFSLVTDKSLSTSIFEGG
YKAHAPKWESKDFRPNKVSETSRTTTTSLSPELIRFTASSSSGMVPKLPAGKMN
NRDLKPQPDIVLLPLPTAYELDSTKLKSPLITSPMFRNVPTANPTEPGIRRVPGAS
EVIRESSSTTGMVVGIVAAAALCILILLYAMYKYRNRDEGSYQVDETRNYISNSA
QSNGTLMKEKQQSSKSGHKKQKNKDREYYV–

Fig. 3. Domains and motifs of human NRXN3α. Bold-faced and underlined letters indicate the LNS- and EGF-domains, respectively. The transmembrane region is double underlined, whereas the putative band 4.1 homologues' binding motif is showed by a shaded background. The amino acidic sequence of exons 23a and 23b, which is present in secreted NRXN3 isoforms, is boxed.

succeeded in amplifying full-length transcript of the NRXN3 cardiac isoform. DNA sequencing of the PCR amplicons and subsequent alignment of such sequences with those of NRXN3 gene enabled us to determine the NRXN3 cardiac mRNA sequence and its genomic organization. As shown in Fig. 2 and summarized in Table 2, we observed only few of the several possible splice variants. At the first alternative splice site we identified only 2 of the 8 possible patterns: in one, exons 2 and 6 are directly connected, whereas in the second exons 4 and 5 are included in the transcript. No RT-PCR products including exons 7a/b and 12 at sites 2 and 3, respectively, were observed, while at site 4 the only transcript we could amplify included exon 20.

At splicing site 5, pattern is more complicated: we detected in the heart at least nine different variants; only exon 23b seems to be not included in any cardiac transcript. DNA sequences of cardiac α- and β-neurexin three isoforms were submitted to GenBank (Accession Nos. AJ316284 and AJ493127, respectively).

Protein structure of NRXN3 cardiac isoform

α-Neurexins have been described as proteins with a typical N-terminal signal peptide followed by a large extracellular sequence containing six laminin/neurexin/sex-hormone binding globulin domains (LNS), also known as laminin G domains, separated by three interspersed epidermal growth factor-like domains (EGF). β-Neurexins shows, after an atypical cleaved signal peptide, only the sixth LNS domain of α-neurexin. After the repeats described above, at the C-terminus of the molecule, α- and β-neurexins contain an O-linked sugar domain, a transmembrane region, and a short cytoplasmic tail [1,12].

Analysis of the amino acidic sequences of cardiac neurexins 3α/β by SMART (Simple Modular Architecture Research Tool) enabled us to detect in exon 24c a putative band 4.1 homologue binding motif (4.1 m), localized within exon 24c (see Fig. 3). According to SMART, this motif is shared by syndecans and glycophorin C intracellular C-termini, while it cannot be detected by the Pfam software.

Possible role of neurexin 3 cardiac isoforms

In brain, neurexin 1 forms a stoichiometric complex with dystroglycan, an ubiquitously expressed transmembrane protein linking cytoskeletal actin to extracellular matrix. Immunoblotting experiments revealed interaction between α-dystroglycan extracted from brain, heart, and skeletal muscle and neurexin 1 and it was also suggested that such interaction might extend to other members of the neurexins family [5].

Data reported in this paper suggest that cardiac isoforms of NRXN3 could participate in a complex involving dystroglycan and possibly some proteins of the extracellular matrix, thus playing a role in intercellular connections.

Acknowledgments

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References

[1] Y.A. Ushkaryov, A.G. Petrenko, M. Geppert, T.C. Südhof, Neurexins: synaptic cell surface proteins related to the α-latrotoxin receptor and laminin, Science 257 (5066) (1992) 50–56.
[2] Y.A. Ushkaryov, T.C. Südhof, Neurexin 3α: extensive alternative splicing generates membrane-bound and soluble forms, Proc. Natl. Acad. Sci. USA 90 (14) (1993) 6410–6414.
[3] B. Ullrich, Y.A. Ushkaryov, T.C. Südhof, Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons, Neuron 14 (3) (1995) 497–507.
[4] L. Rowen, J. Young, B. Birditt, A. Kaur, A. Madan, D.L. Philipps, S. Qin, P. Minx, R.K. Wilson, L. Hood, B.R. Graveley, Analysis of the human neurexin genes: alternative splicing and the generation of protein diversity, Genomics 79 (4) (2002) 587–597.
[5] S. Sugita, F. Saito, J. Tang, J. Satz, K. Campbell, T.C. Sudhof, A stoichiometric complex of neurexins and dystroglycan in brain, J. Cell Biol. 154 (2) (2001) 435–445.
[6] S. Sugita, M. Khvochtev, T.C. Sudhof, Neurexins are functional α-latrotoxin receptors, Neuron 22 (3) (1999) 489–496.
[7] K. Ichtchenko, T. Nguyen, T.C. Sudhof, Structures, alternative splicing, and neurexin binding of multiple neuroligins, J. Biol. Chem. 271 (5) (1996) 2676–2682.
[8] K. Ichtchenko, Y. Hata, T. Nguyen, B. Ullrich, M. Missler, C. Moomaw, T.C. Sudhof, Neuroligin 1: a splice site-specific ligand for β-neurexins, Cell 81 (3) (1995) 435–443.
[9] A. Bateman, E. Birney, L. Cerruti, R. Durbin, L. Etwiller, S.R. Eddy, S. Griffiths-Jones, K.L. Howe, M. Marshall, E.L. Sonnhammer, The Pfam protein families database, Nucleic Acids Res. 30 (1) (2002) 276–280.
[10] J. Schultz, F. Milpetz, P. Bork, C.P. Ponting, SMART, a simple modular architecture research tool: identification of signaling domains, Proc Natl. Acad. Sci. 95 (1998) 5857–5864.
[11] I. Letunic, L. Goodstadt, N.J. Dickens, T. Doerks, J. Schultz, R. Mott, F. Ciccarelli, R.R. Copley, C.P. Ponting, P. Bork, Recent improvements to the SMART domain-based sequence annotation resource, Nucleic Acids Res. 30 (1) (2002) 242–244.
[12] Y.A. Ushkaryov, Y. Hata, K. Ichtchenko, C. Moomaw, S. Afendis, C.A. Slaughter, T.C. Sudhof, Conserved domain structure of β-neurexins. Unusual cleaved signal sequences in receptor-like neuronal cell-surface proteins, J. Biol. Chem. 269 (16) (1994) 11987–11992.