Supplemental Data

Table of contents

- ✓ **E-Methods**
  - Isolation of crude central nervous system synaptosomes
  - Immunoprecipitation assay
  - Electrophoresis and mass-spectrometry analysis
  - Differential proteomic and functional analysis
- ✓ **Table e-1.** Shortlisted immunoprecipitated proteins
- ✓ **Table e-2.** Identified precipitated peptides of Caspr2, DCC and UNC5A proteins
- ✓ **Figure e-1.** Detection of Caspr2, DCC or UNC5A antibodies with a HEK293T cell based-assay
- ✓ **Table e-3.** Immunohistochemistry of Caspr2, DCC and UNC5A proteins on thymomas and normal thymic tissues
- ✓ **Figure e-2.** Immunohistochemical studies on normal thymic tissues
- ✓ **References.**

E-Methods

Isolation of crude central nervous system synaptosomes. Crude synaptosme fractions were prepared from whole rat brain by differential centrifugation method with minimal modifications.[e1] One male Wistar rat (2 months, Charles River Laboratories, Saint-Germain-sur-l'Arbresle, FR) per experiment was euthanized with CO_2 in an appropriate chamber. Brain was quickly removed, dissected on ice, and homogenized in isotonic sucrose/EDTA buffer (0.32 M sucrose, 1mM EDTA, 5mM Tris pH 7.4 with protease inhibitor cocktail III [P8340; Sigma Labs, St.Louis, US]) using 15 mL teflon-glass tissue grinder at 4ºC. The homogenate was centrifuged at 1,000g 10 min at 4ºC using Sorvall SS34 fixed angle rotor. The supernatant (S1, *total brain homogenate*) was diluted to a final protein concentration of 5mg/ml with isotonic sucrose/EDTA buffer. Afterwards, the diluted S1 supernatant was centrifuged at 15,000g 30 min 4ºC, and the pellet (P2) containing the *crude*

synaptosome fraction isolated. This fraction contains the bulk of the synaptosomes and plasma membrane from nerves and glial cells, myelin and mitochondria.

Immunoprecipitacion assay. Crude synaptosome fractions were incubated with patient's or control serum (1:100) in isotonic sucrose/EDTA buffer (0.32 M sucrose, 1mM EDTA, 5mM Tris pH 7.4 with protease inhibitor cocktail III [all from Sigma Labs]) overnight at 4ºC. After the incubation, samples were washed three times with isotonic sucrose/EDTA buffer, and treated with lysis buffer (150mM NaCl, 1mM EDTA, 100mM Tris-HCl, 1% triton X-100 and 1% sodium deoxycholate pH 7.5 with 1:100 protease inhibitor cocktail III [all from Sigma Labs]). Lysed synaptosomes were then centrifuged at 21,300g 30 min at 4ºC and supernatant was retained and incubated with protein A/G agarose beads (20423; Pierce, Rockford, US) overnight at 4ºC.

Electrophoresis and mass-spectrometry analysis. Antibody coated protein A/G beads were washed three times with phosphate-buffered saline (PBS), and the pellet was resuspended in Laemmli buffer, boiled for 10 min and separated in 4-12% Bis-Tris polyacrylamide NuPAGE precast gels (Invitrogen). EZBlue gel staining (G1041; Sigma Labs) was used to visualize proteins. Because EZBlue gel staining did not identify specific protein bands, gel lanes were cut out and sent to the Proteomics Unit from the University of Valencia (member of Proteored, PRB2-ISCIII). Samples were digested using sequencing grade trypsin (Promega, Madison, WI, USA) as described elsewhere.[e2] The digestion mixture was dried in a vacuum centrifuge and resuspended in 10µL of 2% acetonitrile (ACN), 0.1% trifluoro acetic acid (TFA). Mass spectrometry analysis was performed by loading 5µL of tryptic peptides onto a trap column (NanoLC Column, 3µ C18-CL, 75µm x 15cm, Eksigent, Dublin,US), desalted with 0.1% TFA at 2µL/min for 10 min, and transferred afterwards onto an analytical column (LC Column, 3µm C18-CL, 75µm x 12cm; Nikkyo Technos, Tokyo, JP) equilibrated in 5% ACN 0.1% formic acid (FA). Elution was carried out with a linear gradient of 5 to 40% B in 45 min (A: 0.1% FA; B: ACN, 0.1% FA) at a flow rate of 300 nL/min. Mass spectrometry analysis was performed in a nanoESI qQTOF (5600 TripleTOF; ABSCIEX, Old Connecticut Path Framingham,USA) mass spectrometer operated in data-dependent

mode. Peptides were ionized applying 2.8kV to the spray emitter. Survey MS1 scans were acquired from 350-1250 m/z for 250 ms. The quadrupole resolution was set to ‘UNIT’ for MS2 experiments, which were acquired from 100-1500m/z for 50 ms in ‘high sensitivity’ mode. Following switch criteria were used: charge: 2+ to 5+; minimum intensity; 70 counts per second (cps). Up to 50 ions were selected for fragmentation after each survey scan. Dynamic exclusion was set to 15s.

NCBI database was searched with Mascot v2.3.02 (Matrix Science, London, UK) and ProteinPilot v4.5 (ABSCIEX) search engines using peak list files (mgf) generated with ProteiPilot from the instrument wiff files. Mascot search parameters included tryptic peptides with up to 1 missed cleavage, mass tolerance of 50 ppm in MS mode and 0.6 Da in MS/MS mode, carbamidomethylation of Cys as a fixed modifications, and no taxonomy restriction. ProteinPilot search parameters were trypsin specificity, cys-alkylation, no taxonomy restriction and the search effort set to through. A 95% protein confidence cut-off value was used (ProteinPilot, Unused>1.3; http://sciex.com/Documents/tech%20notes/ProteinPilot-Software-Overview-RUO-MKT-02-1777-A.pdf).

Differential proteomic and functional analysis. Protein abundance was estimated by a label-free approach based on peptide signal intensity.[e3] The signal of the three most intense peptides with confidence > 95% was used as a measure of the protein abundance in each sample. Immunoprecipitated proteins by patients with neuromyotonia and tymoma were compared with those identified with controls. All proteins identified with control sera were not considered for further analysis. Universal Protein Resource (UniProt; available at www.uniprot.org) and Online Mendelian Inheritance in Men (OMIM; available at www.omim.org) databases were used to evaluate subcellular location and functional analysis of the proteins respectively.

Table e-1. Shortlisted immunoprecipitated proteins (membrane proteins highlighted).

Protein name	Gene	Gi number	Subcellular location
60S ribosomal protein L18	Rpl18	gi|89573867	Cytoplasm
Adenylate cyclase type 5	Adyc5	gi|149060612	Membrane, cell projection (cilium)
Aquaporin-4	Aqp4	gi|6978531	Membrane
Capping protein (actin filament) muscle Z-line	N/A	gi|149065041	Cytoplasm
Choline O-acetyltransferase	Chat	gi|282154809	Cytoplasm, nucleus
Collapsin response mediator protein 3	Crmp3	gi|3122037	Cytoplasm
Collapsin response mediator protein 5	Crmp5	gi|6714522	Cytoplasm, dendrite
Contactin-associated protein-like 2	Caspr2	gi|7662350	Membrane, cell projection (axon)
Deleted in colorectal carcinoma	Dcc	gi|149064590	Membrane
Furry-like protein	Fry	gi|189342174	Cytoplasm
Golgin subfamily A member 3	Golga3	gi|157822655	Golgi apparatus
Leucine-rich repeats and immunoglobulin-like domains protein 2	Lrig2	gi|68299752	Membrane, cytoplasm
Malonyl-CoA decarboxylase	Mlycd	gi|67460103	Cytoplasm, mitochondrion and peroxisome matrix
Metabotropic glutamate receptor 3	Grm3	gi|157787068	Membrane
Metabotropic glutamate receptor 5	Grm5	gi|8393490	Membrane
Netrin receptor UNC5A precursor	Unc5a	gi|11559980	Membrane, membrane raft, cell projection
Neurexin-1	Nrnx1	gi|37620151	Membrane, cell junction (synapse)
Neurocan core protein	Ncan	gi|404312657	Extracellular matrix
Phospholipase C, beta 1	Plcb1	gi|149023393	Nucleus, cytoplasm
Protein furry homolog-like	Fryl	gi|293341689	Cell cortex
Septin 7	Sept7	gi|9789715	Cytoskeleton
Serine/threonine-protein kinase MRCK	Cdc42bpa	gi|16758474	Cytoplasm
Vacuolar protein sorting-associated protein 16	Vps16	gi|53850610	Lysosomal membrane

Sequence identifier GI number from National Centre for Biotechnology Information (NCBI) database available at:

http://www.ncbi.nlm.nih.gov/protein/. N/A: no data available.

Table e-2. Identified precipitated peptides of Caspr2, DCC and UNC5A proteins.

Protein name	Peptide sequence	Unused	GI number	%Cov	Specie
Contactin-associated protein-like 2	DAGFLSYKDHLPVSQVVVGDT-DRQGSEAK, VQFNHIAPLK, VIETGKIDQEIHKYNTPGFTGCLSR.	3.33	gi|7662350	4,8%	*Homo sapiens*
Deleted in colorectal carcinoma	GNIQTFTVFFSR, GVGPLSDPILFR, HHKPDEGLYQCEASLGDSGSIISR, VVVLPSGALQISR, NGDVVIPSDYFQIVGGSNLR, DVVPVLVSSR, ILSDPGLHR, VMVAGPLR, FLAYNR, DLTVITR, HGDGGYWPVDTNLIDR.	10.12	gi|6978755	11,7%	*Rattus norvegicus*
Netrin receptor UNC5A	STDSSSGLPTMEVR, FALVGEALSVAATKR, FQLSNGHLLSPLGSGR, GTSNMAYGTFNFLGGR, HTLHHSSPTSEAEDFVSR, IPFLIR, KKEGLDSDVADSSILTSGFQPVSIK PSK, LMIPNTGISLLIPPDAIPR, LSIHDVPSSLWK, NKPVLLVCK, QLGGQLIQEPR, QVDHVIER, VYCLHDTHDALKEVVQLEK.	1.59	gi|11559980	21,2%	*Rattus norvegicus*

Contactin-associated protein-like 2

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mqaapragcg aalllwivss clcrawtaps tsqkcdeplv sglphvafss sssisgsysp
gyakinkrgg aggwspsdsd hyqwlqvdfg nrkqisaiat qgrysssdwv tqyrmlysdt
grnwkpyhqd gniwafpgni nsdgvvrhel qhpiiaryvr ivpldwngeg riglrievyg
csywadvinf dghvvlpyrf rnkkmktlkd vialnfktse segvilhgeg qqgdyitlel
kkaklvlsln lgsnqlgpiy ghtsvmtgsl lddhhwhsvv ierqgrsinl tldrsmqhfr
tngefdyldl dyeitfggip fsgkpssssr knfkgcmesi nyngvnitdl arrkklepsn
vgnlsfscve pytvpvffna tsylevpgrl nqdlfsvsfq frtwnpngll vfshfadnlg
nveidltesk vgvhinitqt kmsqidissg sglndgqwhe vrflakenfa iltidgdeas
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tqlvysasmd qisaitdsae yceqyvsyfc kmsrllntpd gspytwwvgk anekhyywgg
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sgcsghctsy gtncenggkc leryhgyscd csntaydgtf cnkdvgaffe egmwlrynfq
apatnardss srvdnapdqq nshpdlaqee irfsfsttka pcillyissf ttdflavlvk
ptgslqiryn lggtrepyni dvdhrnmang qphsvnitrh ektiflkldh ypsvsyhlps
ssdtlfnspk slflgkviet gkidqeihky ntpgftgcls rvqfnqiapl kaalrqtnas
ahvhiqgelv esncgasplt lspmssatdp whldhldsas adfpynpgqg qairngvnrn
saiiggviav viftilctlv flirymfrhk gtyhtneakg aesaesadaa imnndpnfte
tideskkewl i
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Deleted in colorectal carcinoma

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menslgcvwv pklafvlfga sllsahlqvt gfqikpftsl hfvsepsdav tmrggnvlln
csaesdrgvp vikwkkdgli lalgmddrkq qlpngslliq nilhsrhhkp deglyqceas
lgdsgsiisr takvmvagpl rflsqtesit afmgdtvllk cevigdpmpt ihwqknqqdl
npipgdsrvv vlpsgalqis rlqpgdsgvy rcsarnpast rtgneaevri lsdpglhrql
yflqrpsnvi aiegkdavle ccvsgyppps ftwlrgeevi qlrskkysll ggsnllisnv
tdddsgtytc vvtyknenis asaeltvlvp pwflnhpsnl yayesmdief ecavsgkpvp
tvnwmkngdv vipsdyfqiv ggsnlrilgv vksdegfyqc vaeneagnaq ssaqlivpkp
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ysierlesss hyvislkafn nagegvplye sattrsitdp tdpvdyypll ddfptsgpdv
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ravivswqpp leangkitay ilfytldkni piddwimeti sgdrlthqim dlsldtmyyf
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ypgilpsptc gyphpqftlr pvpfptlsvd rgfgagrtvs egptaqqqpm lppaqpehps
seeapsrtip tacvrpthpl rsfanpllpp pmsaiepkvp ytpllsqpgp tlpkthvkta
slglagkars pllpvsvpta pevseeshkp tedpasvyeq ddlseqmasl eglmkqlnai
tgsaf
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Netrin receptor UNC5A

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mavrpglwpv llgivlaawl rgsgaqqsat vanpvpganp dllphflvep edvyivknkp
yllvckavpa tqiffkcnge wvrqvdhvie rstdsssglp tmevrinvsr qqvekvfgle
eywcqcvaws ssgttksqka yiriaylrkn feqeplakev sleqgivlpc rppegippae
vewlrnedlv dpsldpnvyi trehslvvrq arladtanyt cvaknivarr rstsaavivy
vnggwstwte wsvcsascgr gwqkrsrsct npaplnggaf cegqnvqkta catlcpvdgs
wsswskwsac gldcthwrsr ecsdpaprng geecrgadld trnctsdlcl htascpedva
lyiglvavav clflllalgl liycrkkegl dsdvadssil tsgfqpvsik pskadnphll
tiqpdlsttt ttyqgslcsr qdgpspkfql snghllsplg sgrhtlhhss ptseaedfvs
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pedvrlplag cqtllspvvs cgppgvlltr pvilamdhcg epspdswslr lkkqscegsw
edvlhlgees pshlyycqle agacyvfteq lgrfalvgea lsvaatkrlr lllfapvact
sleynirvyc lhdthdalke vvqlekqlgg qliqeprvlh fkdsyhnlrl sihdvpsslw
kskllvsyqe ipfyhiwngt qqylhctftl erinastsdl ackvwvwqve gdgqsfninf
nitkdtrfae llaleseggv palvgpsafk ipflirqkii asldppcsrg adwrtlaqkl
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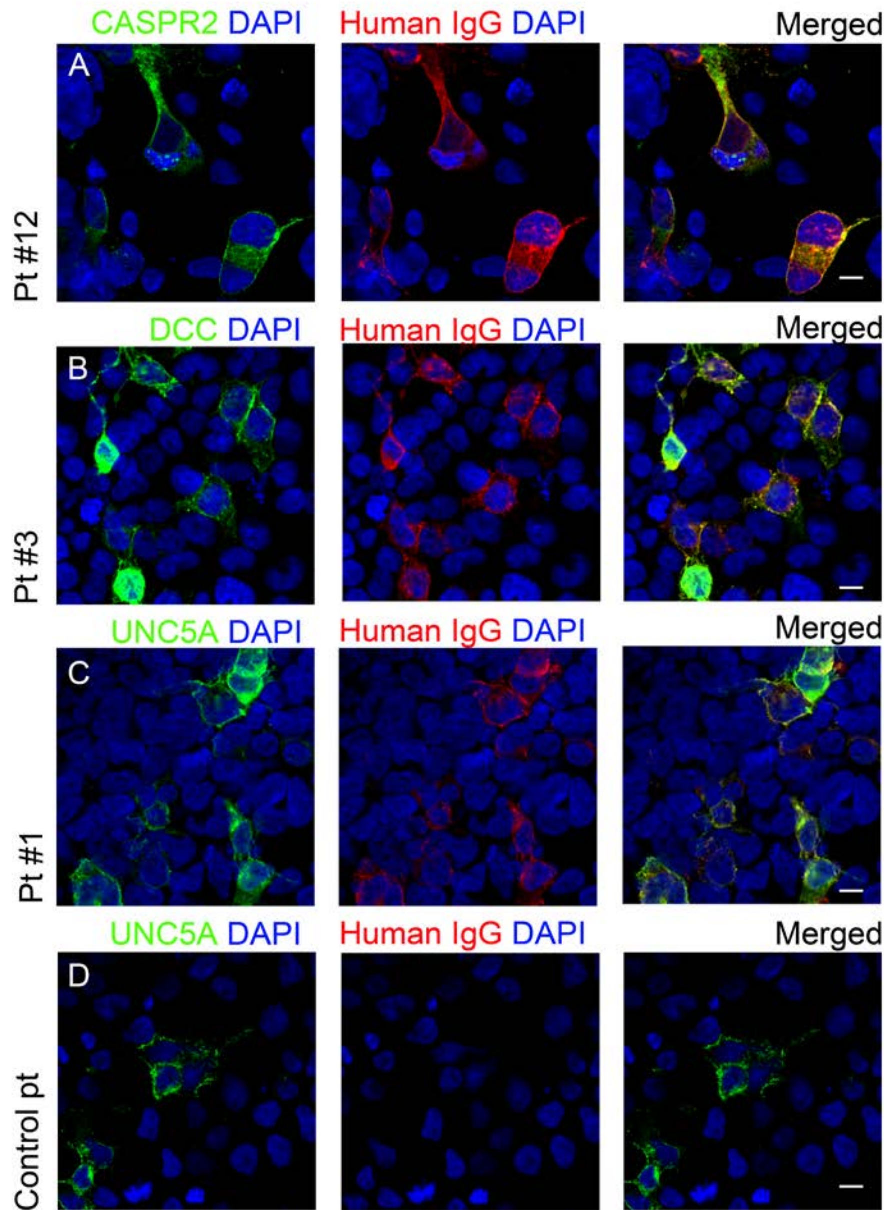

Figure e-1. Detection of Caspr2, DCC or UNC5A antibodies with a HEK293T cell based-assay. To test for antibodies against Caspr2, DCC or UNC5A, HEK293T cells transfected with the plasmids were exposed to patients' sera. Paraformaldehyde-prefixed cells were incubated with the corresponding commercial Caspr2 or DCC antibodies (A, B: green) and the serum of patients or controls (A-D: red). The UNC5A transfected cells were displayed with a green fluorescent UNC5A-tagged plasmid (C,D: green). The sera of patient, but not that of healthy controls, labeled the surface of cells that specifically expressed the antigens (A-D: merged images). Nuclei were counterstained with 4',6-diamino-2-phenylindole (DAPI). Scale bars: 10 µm.

Table e-3. Immunohistochemistry of Caspr2, DCC and UNC5A proteins in thymomas and normal thymic tissues.

	Thymoma			Normal thymus						
	patient #1	patient #2	patient #8	C1	C2	C3	C4	C5	C6	C7
Caspr2	+	+++	++	++	++	+	+	+++	+	+++
DCC	+	+++	+	+	+++	0	0	+	0	+
UNC5a	++	+	++	+	+	+	+	+	+	+
CD3	+	++	++	++	++	++	++	++	++	+++
CK	+++	++	++	++	+	+	+	+	+	+
CD20	0	++	+	+	+	+	+	0	0	0
HLA-DR	0	+	+	++	+	+	++	++	++	+

Score: 0, negative; 1+, weak immunoreactivity; ++, moderate immunoreactivity; and +++, strong immunoreactivity.

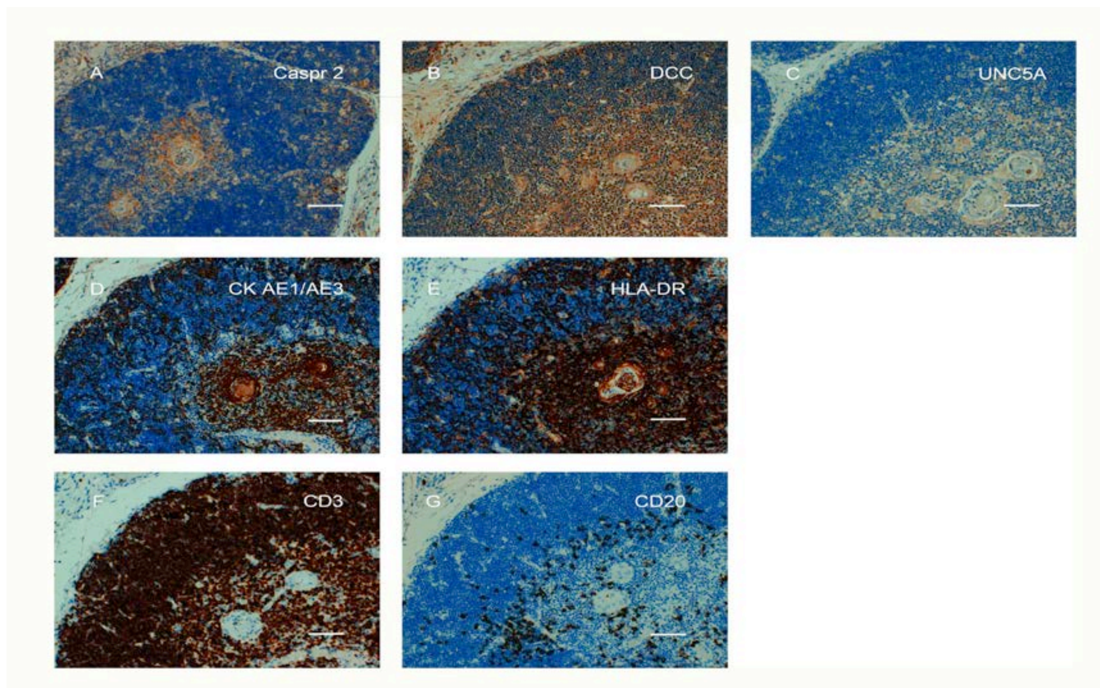

Figure e-2. Immunohistochemical studies on normal thymus. Sections of control #2 were immunolabeled with specific antibodies against Caspr2 (A), DCC (B), UNC5A (C), cytokeratin AE1/AE3 (D), HLA-DR (E), CD3 (F) and CD20 (G). Staining of cell surface neuronal antigens is observed in epithelial stromal cells contained in the thymic cortex and medulla. Staining is especially intense in areas surrounding the Hassall corpuscles. Scale bars: 100µm.

References

e1. Dunkley PR, Jarvie PE, Robinson PJ. A rapid Percoll gradient procedure for preparation of synaptosomes. Nat Protoc 2008;3:1718-1728.

e2. Shevchenko A, Jensen ON, Podtelejnikov AV, et al. Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad U S A 1996;93:14440-14445.

e3. Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ. Absolute quantification of proteins by LCMSE: a virtue of parallel MS acquisition. Mol Cell Proteomics 2006;5:144-156.