Science Translational Medicine

AAAS

Supplementary Materials for

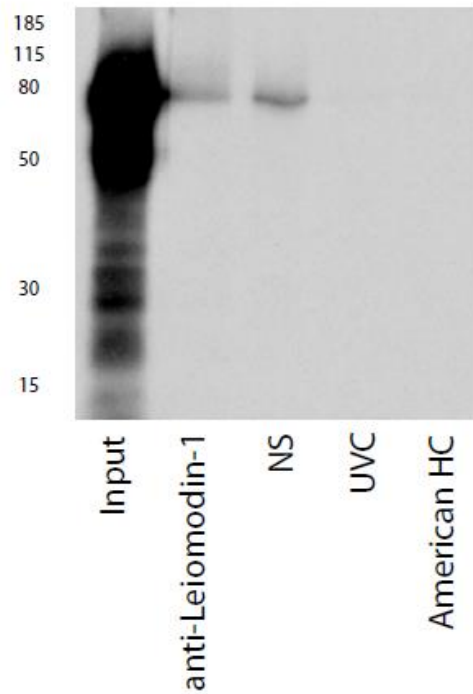## Nodding syndrome may be an autoimmune reaction to the parasitic worm *Onchocerca volvulus*

Tory P. Johnson, Richa Tyagi, Paul R. Lee, Myoung-Hwa Lee, Kory R. Johnson,
Jeffrey Kowalak, Abdel Elkahloun, Marie Medynets, Alina Hategan, Joseph Kubofcik,
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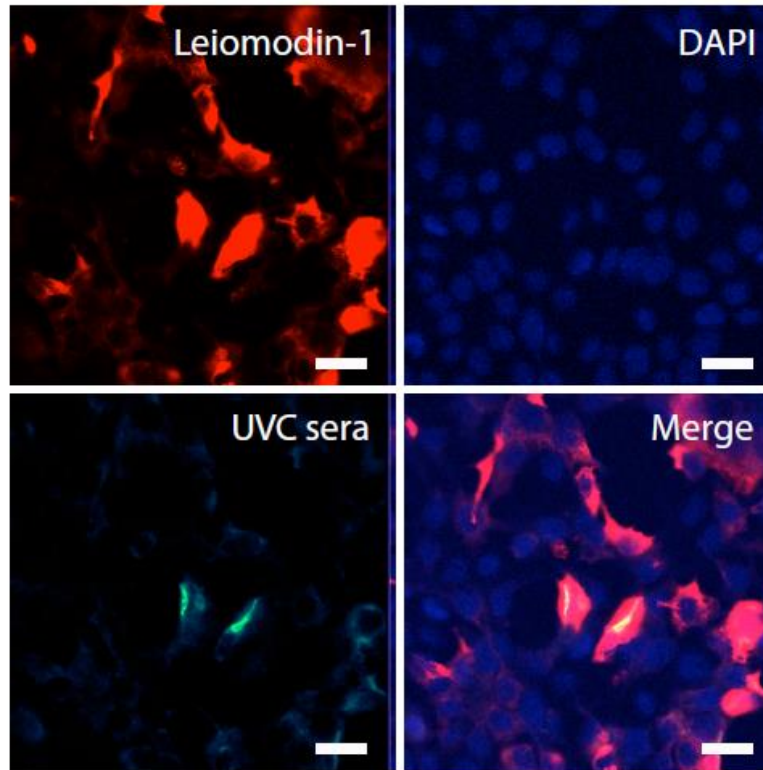
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Fig. S1. Representative image of immunoprecipitation experiments. Immunoprecipitation of leiomodin-1 IVTT product (input) by rabbit anti-leiomodin-1, sera from a patient with Nodding Syndrome (NS), sera from an unaffected village control (UVC) and an American healthy control (American HC).

Fig. S2. Subtyping of leiomodin-1 autoantibodies from patients with nodding syndrome. Antibodies to leiomodin-1 from patients with NS are IgG and IgM as determined by immunoblot. No IgE or IgA were detected.

Fig. S3. Immunofluorescence of leiomodin-1–transfected cells with sera from unaffected village controls. Unaffected village control (UVC) sera co-immunostained with leiomodin-1 (red) on leiomodin-1 transfected cells show limited reactivity to rare cells (UVC) and no co-labeling of leiomodin-1 (merge). Nuclei are stained blue with DAPI. Scale bar is 20 µM.

A.

B.

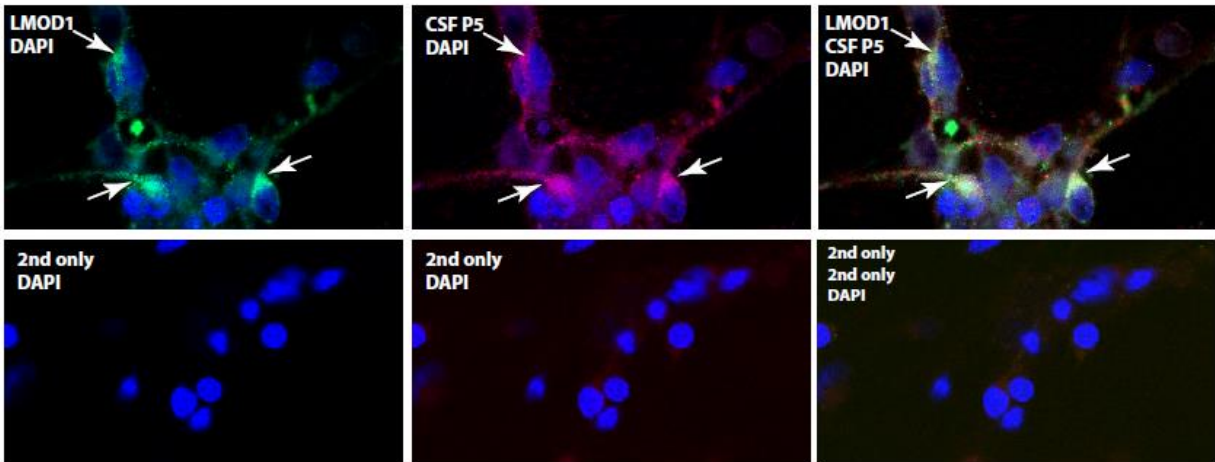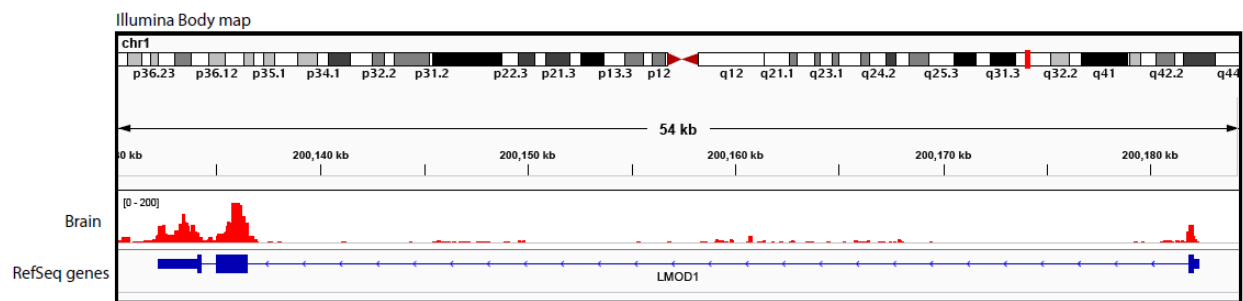**Fig. S4. Coimmunofluorescence of human neurons with leiomodin-1 and patient CSF.** (**A**) Human neuronal cultures were fixed with paraformaldehyde, permeabilized with acetone and then immunostained with rabbit polyclonal leiomodin-1 antibody (red) and human CSF from two patients with NS (#4 and #5) (green) or sera from a patient with NS (#14) as a positive control (green). Colocalization of rabbit anti-leiomodin-1 antibodies with antibodies in the CSF or serum in the cytoplasm and neurites was observed. (**B**) Higher magnification images of patient #5 demonstrate co-localization of CSF from patient with NS and rabbit polyclonal leimodin-1 antibody in the neuronal cytoplasm (arrow). Secondary antibody controls in A and B show absence of non-specific staining.

Fig. S5. Leiomodin-1 transcripts are expressed in the brain as detected by RNA sequencing. Illumina Body Map 2.0 publically available RNAseq data demonstrated the presence of leiomodin-1 transcripts in the brain. www.illumina.com/science/data_library.ilmn Data downloaded 7 July, 2013 (*37*).

Table S1. Proteins with enriched autoantibodies in patients with nodding syndrome.

ID	Ingenuity Gene Annotation	Fold change disease versus control
BC080187.1	LMOD1	32649.50
NM_007262.1	PARK7	753.14
BC057779.1	COX4I2	652.93
BC098117.1	C7orf10	155.43
BC004242.1	HNRNPUL1	6.77
NM_001002755.1	NFU1	5.48
NM_024821.1	CCDC134	4.98
NM_002824.4	PTMS	4.51
BC018732.1	CYB5R1	4.37
BC007957.1	DDRGK1	4.29
NM_001024631.1	ATXN3	4.11
NM_001011700.1	MCCD1	3.82
BC070290.1	TRDN	3.82
BC007872.1	TK1	3.80
BC028725.2	ELMOD1	3.75
NM_012249.2	RHOQ	3.55
NM_022465.2	IKZF4	3.53
BC057774.1	TRMT10B	3.51
BC010741.1	TRIT1	3.50
BC000809.1	TCEAL1	3.44
NM_007173.3	PRSS23	3.33
BC018206.1	MZT2A	3.26
BC017492.1	COG8	3.25
NM_020064.2	BARHL1	3.14
BC093700.1	PIN4	3.10
BC025278.1	AIG1	3.10
BC012183.1	UBQLNL	3.08
NM_033277.1	LACRT	3.08
BC058288.1	CCDC40	3.08
NM_012086.1	GTF3C3	3.08
BC008374.1	SH3YL1	3.07
BC017236.1	CSNK1G1	2.97
BC075800.1	PRKAR2B	2.95
BC053660.1	PNPT1	2.94
NM_004231.2	ATP6V1F	2.92
BC013039.1	GADD45GIP1	2.92
NM_145268.1	C7orf45	2.85
BC073866.1	PRMT6	2.83
NM_138353.1	DCAF15	2.81
BC051688.1	PNMAL1	2.78
BC042035.1	MGC72080	2.75
NM_006258.1	PRKG1	2.73
NM_021244.2	RRAGD	2.71
BC048217.1	SPATA5	2.71
BC012266.2	ATG12	2.71

BC011906.1	ISCU	2.70
BC014051.1	AIMP1	2.67
NM_002697.2	POU2F1	2.66
NM_183383.1	RNF13	2.66
NM_003146.2	SSRP1	2.66
NM_198287.1	ING4	2.66
NM_133640.3	MED22	2.66
NM_007041.2	ATE1	2.60
NM_000071.1	CBS	2.60
BC030237.1	SLC22A18AS	2.58
BC040339.1	NOD1	2.55
BC036450.1	SAMHD1	2.54
NM_012129.1	CLDN12	2.51
BC034483.1	HSPA1L	2.49
NM_206853.1	QKI	2.47
NM_000403.3	GALE	2.46
BC014225.2	HOPX	2.46
NM_000318.1	PEX2	2.45
BC025263.1	CDCA4	2.43
NM_024548.2	CEP97	2.40
BC009614.1	SPAG16	2.37
BC025930.1	NPLOC4	2.37
NM_152260.1	RPUSD2	2.36
BC004407.1	C9orf64	2.36
NM_153007.3	ODF4	2.35
BC051843.1	MAP4	2.34
BC013737.1	HDAC6	2.33
NM_012110.1	CHIC2	2.32
NM_152613.1	WBP2NL	2.31
BC008819.1	NR1H3	2.31
BC006148.1	OVOL2	2.29
BC004902.1	KIAA0947	2.27
BC014095.2	RELA	2.26
BC056872.1	NCOA5	2.25
NM_052820.1	CORO2A	2.25
BC032422.1	KIR2DL3 (includes others)	2.23
NM_152261.1	C12orf23	2.22
BC055427.1	TNIK	2.22
BC028954.1	PGBD3	2.22
NM_007019.1	UBE2C	2.21
BC002555.1	CLK3	2.19
NM_033064.1	ATCAY	2.19
NM_006454.2	MXD4	2.19
NM_005902.1	SMAD3	2.19
BC050456.1	THBS4	2.17
NM_022170.1	EIF4H	2.15
NM_015991.1	C1QA	2.15
BC008892.1	ALDH3A1	2.14

NM_080414.1	VPS16	2.14
NM_017563.1	IL17RD	2.13
NM_005207.1	CRKL	2.13
NM_002143.2	HPCA	2.12
BC065041.1	PLB1	2.12
NM_144602.1	C16orf78	2.12
BC017376.2	RNF31	2.11
BC028206.1	KIR3DL1	2.11
NM_201403.1	MOB3C	2.11
NM_058217.1	RAD51C	2.11
BC024187.2	DHX40	2.10
BC035055.1	GABRA4	2.09
NM_007066.3	PKIG	2.09
NM_174896.2	C1orf162	2.09
NM_174889.2	NDUFAF2	2.08
BC007206.1	SHD	2.08
NM_138612.1	HAS3	2.07
NM_016052.1	RRP15	2.07
NM_014042.1	ANAPC15	2.06
BC022189.2	C17orf47	2.06
BC002758.1	ADAT1	2.06
BC020630.1	CAMK2N1	2.06
NM_002823.2	PTMA	2.05
BC004885.1	TMEM234	2.05
NM_003732.1	EIF4EBP3	2.05
NM_152876.1	FAS	2.05
NM_017952.2	PTCD3	2.04
NM_138430.3	ADPRHL1	2.04
BC032124.1	BRD3	2.04
NM_006998.3	SCGN	2.03
NM_006244.1	PPP2R5B	2.03
BC019015.2	MED29	2.03
NM_014380.1	NGFRAP1	2.02
BC015358.1	HTATIP2	2.02
NM_000810.2	GABRA5	2.02
NM_153236.2	GIMAP7	2.02
BC002704.1	STAT1	2.01
NM_032955.1	AIF1	2.01
BC095406.1	H2AFY	2.00
BC009230.2	PAGE5	2.00
BC016821.1	MBIP	2.00
NM_017971.1	MRPL20	2.00
NM_003085.2	SNCB	2.00
NM_001003937.1	TSPYL6	2.00

Table S2. Top four proteins with enriched autoantibodies in patients with nodding syndrome.

Gene ID	Protein name	Function	Fold Change
LMOD1	Leiomodin 1	Actin capping	32649.50
PARK7	DJ1	Oxidative stress response	753.14
COX4I2	Cytochrome C oxidase subunit 4	Terminal enzyme of the mitochondrial ETC	652.93
C7orf10	CaiB/baiF CoA-transferase family protein C7orf10	Unknown, possible transferase	155.43

Table S3. Leiomodin-1 transcripts are expressed in the brain. Data shown are the leiomodin-1 copy number normalized to GAPDH copy number from three independent replicates from figure 2E.

Sample	Hela	NSC	Neuron	Brain 1	Brain 2	Muscle
Replicate 1	7.83E-20	1.13E-19	3.43E-20	2.67E-19	3.68E-20	8.86E-24
Replicate 2	9.17E-08	2.3E-07	3.11E-07	3.51E-06	2.89E-05	0.000115
Replicate 3	1.39E-07	3.48E-07	7.89E-07	6.35E-06	5.04E-05	0.000463

Table 4. Rabbit leiomodin-1 antibodies are neurotoxic. Differences in viability of neurons treated with rabbit anti-leiomodin-1, normal rabbit sera or saponin. Data shown are percent viability from cells from Fig 3A.

Well #	Rabbit anti-leiomodin-1	Normal rabbit sera	Saponin
1	62.28	30.39	32.28
2	72.61	34.91	37.13
3	66.94	32.72	35.56
4	60.32	32.05	34.23
5	59.77	31.85	33.34
6	90.23	52.34	50.94
7	87.56	52.70	51.18
8	83.39	48.01	46.87
9	78.88	46.59	46.28
10	92.75	48.37	50.55

Table S5. Antibodies in patient sera are neurotoxic. Differences in viability of neurons treated with either sera or antibody depleted sera from a single patient with NS. Data shown are percent viability from cells from Fig 3B.

Well #	Patient sera #1	Antibody depleted patient sera
1	12.93	64.57
2	15.03	67.93
3	15.12	74.39
4	15.19	90.7
5	15.2	92.13

Table S6. Leiomodin-1 antibodies from patients are neurotoxic. Differences in toxicity of patient sera or patient sera selectively depleted of leiomodin-1 auto-antibodies. Data shown are percent toxicity from cells from Fig 3C.

Patient ID #	Sera	Leiomodin-1 antibody depleted sera
1	68.87	45.68
4	79.1	54.33
10	32.54	17.95
17	30.34	14.68

Table S7. *O. volvulus* **proteins identified by mass spectrometry.**

Accession number	Entry name	Protein name
P30162	ACT1_ONCVO	Actin-1
J0DN48	J0DN48_LOALO	Glyceraldehyde-3-phosphate dehydrogenase
P46434	GST1_ONCVO	Glutathione S-transferase 1
O18532	O18532_ONCVO	22 upper
O44941	O44941_ONCVO	Peroxidoxin-2
O16006	O16006_DIRIM	Peroxidoxin-1
O46147	O46147_ONCVO	Putative heparan sulfate
O96906	O96906_ONCVO	Aspartic protease
Q25597	Q25597_ONCVO	Beta-galactoside-binding lectin
Q7YZX3	Q7YZX3_ONCVO	Enolase
A8PFE3	A8PFE3_BRUMA	Enolase, putative
Q95W51	Q95W51_ONCVO	Prolyl-4-hydroxylase alpha subunit-like protein
Q9U9R9	Q9U9R9_ONCVO	Fructose-bisphosphatealdolase
Q25632	TPM_ONCVO	Tropomyosin
P11503	HSP70_ONCVO	Heat shock 70 kDa protein
Q26357	Q26357_ONCVO	Heat shock 70 kDa protein
P27541	HSP70_BRUMA	Heat shock 70 kDa protein
J9FG14	J9FG14_WUCBA	Heat shock 70 protein
H2B652	H2B652_9MAXI	Heat shock protein 70
Q0RAH1	Q0RAH1_ONCVE	60 kDachaperonin
P29691	EF2_CAEEL	Elongation factor 2
A8PJV1	A8PJV1_BRUMA	Translation elongation factor aEF-2, putative
Q54NB6	FKBP4_DICDI	FK506-binding protein 4
A8QHE7	A8QHE7_BRUMA	FKBP-type peptidyl-prolylcis-trans isomerase-59, BmFKBP59
A8P3D2	A8P3D2_BRUMA	Glycosyl hydrolase family 20, catalytic domain containing protein
A8PGN9	A8PGN9_BRUMA	Glycosyl hydrolases family 18 protein
A8NGD6	A8NGD6_BRUMA	Phosphoglycerate kinase
F1L2P3	F1L2P3_ASCSU	Phosphoglycerate kinase
A8QGN5	A8QGN5_BRUMA	TPR Domain
A0B5P0	SYR_METTP	Arginine--tRNA ligase
A9H9A8	ATPB_GLUDA	ATP synthase subunit beta
E1FVE7	E1FVE7_LOALO	Branched-chain-amino-acid aminotransferase
G0P5J3	G0P5J3_CAEBE	CBN-EGL-30 protein
B8FUN4	DNAK_DESHD	Chaperone protein DnaK
E1FT93	E1FT93_LOALO	Intermediate filament protein B
Q1NJR3	Q1NJR3_9DELT	Mannosyl-glycoprotein endo-beta-N-

		acetylglucosamidase
J9EH08	J9EH08_WUCBA	MFP2 family protein
J9EW18	J9EW18_WUCBA	Phosphoenolpyruvatecarboxykinase
J0DMD4	J0DMD4_LOALO	Pseudouridine synthase
E1GL29	E1GL29_LOALO	Sodium/potassium ATPase subunit beta
E1FQ19	E1FQ19_LOALO	TAG-320 protein
Q17172	TDX2_BRUMA	Thioredoxin peroxidase 2
Q87QI7	TORA_VIBPA	Trimethylamine-N-oxide reductase
P23232	GBB_LOLFO	Guanine nucleotide-binding protein subunit beta
A8Q0T1	A8Q0T1_BRUMA	Disorganized muscle protein 1, putative

Table S8. Leiomodin-1 antibodies cross-react with *O. volvulus* antigens. Differences in immune reactivity of leiomodin-1 antibodies competed with BSA or *Onchocerca volvulus* whole-cell lysates to immobilized leiomodin-1. Optical density for each immune reactivity is provided for data from Fig 4E.

Patient ID #	BSA competed	*O. volvulus* competed
3	108.87	85.930
14	154.48	11.280
20	364.22	14.110
16	319.05	201.890