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Supplementary information

Autoimmune response to C9orf72 protein in amyotrophic lateral sclerosis

In the format provided by the authors and unedited

Supplementary Information

Supplementary Tables

Supplemental Table S1: Results of the screen of all peptides within the C9Orf72 protein.

Peptide Residues	Sequence	Responders/Tested	Average Magnitude	Number of predicted HLA bound
1-15	MSTLCPPPSPAVAKT	1/23	9.6	0
1-15	MSTLCPPPS*PAVAKT	0/23	0	N/A
6-20	PPPSPAVAKTEIALS	0/23	0	5
6-20	PPPS*PAVAKTEIALS	1/23	8.7	N/A
11-25	AVAKTEIALSGKSPL	2/23	143.9	2
16-30	EIALSGKSPLLAATF	2/23	70.3	3
21-35	GKSPLLAATFAYWDN	2/23	63.5	5
26-40	LAATFAYWDNILGPR	1/23	74.8	19
31-45	AYWDNILGPRVRHIW	0/23	0	1
36-50	ILGPRVRHIWAPKTE	1/23	42	8
41-55	VRHIWAPKTEQVLLS	3/22	95.8	6
46-60	APKTEQVLLSDGEIT	0/22	0	1
51-65	QVLLSDGEITFLANH	1/22	27.9	3
56-70	DGEITFLANHTLNGE	7/22	1215.9	27
61-75	FLANHTLNGEILRNA	3/22	462.1	0
66-80	TLNGEILRNAESGAI	2/22	388.3	4
71-85	ILRNAESGAIDVKFF	1/22	30	2
76-90	ESGAIDVKFFVLSEK	4/22	119.4	5
81-95	DVKFFVLSEKGVIIV	5/22	307.6	15
86-100	VLSEKGVIIVSLIFD	0/22	0	0
91-105	GVIIVSLIFDGNWNG	0/20	0	5
96-110	SLIFDGNWNGDRSTY	1/20	867.5	3
101-115	GNWNGDRSTYGLSII	0/20	0	4
106-120	DRSTYGLSIILPQTE	1/20	50.5	10
111-125	GLSIILPQTELSFYL	0/20	0	1
116-130	LPQTELSFYLPLHRV	1/20	6	6
121-135	LSFYLPLHRVCVDRL	1/20	15.7	1
126-140	PLHRVCVDRLTHIIR	3/20	133.3	2
131-145	CVDRLTHIIRKGRIW	1/20	16	3
136-150	THIIRKGRIWMHKER	2/20	680.5	3
141-155	KGRIWMHKERQENVQ	2/27	58.9	15

146-160	MHKERQENVQKIILE	0/27	0	0
151-165	QENVQKIILEGTERM	1/27	7.7	7
156-170	KIILEGTERMEDQGQ	1/27	5.9	6
161-175	GTERMEDQGQSIIPM	0/27	0	1
166-180	EDQGQSIIPMLTGEV	0/27	0	2
171-185	SIIPMLTGEVIPVME	1/27	696.9	4
176-190	LTGEVIPVMELLSSM	0/27	0	1
181-195	IPVMELLSSMKSHSV	1/27	176	7
186-200	LLSSMKSHSVPEEID	1/27	642.5	8
191-205	KSHSVPEEIDIADTV	0/22	0	5
196-210	PEEIDIADTVLNDDD	0/22	0	26
201-215	IADTVLNDDDIGDSC	0/22	0	4
206-220	LNDDDIGDSCHEGFL	0/22	0	0
211-225	IGDSCHEGFLLNAIS	0/22	0	0
216-230	HEGFLLNAISSHLQT	0/22	0	10
221-235	LNAISSHLQTCGCSV	0/22	0	0
226-240	SHLQTCGCSVVVGSS	0/22	0	0
231-245	CGCSVVVGSSAEKVN	0/22	0	5
236-250	VVGSSAEKVNKIVRT	0/22	0	1
241-255	AEKVNKIVRTLCLFL	1/25	28.8	0
246-260	KIVRTLCLFLTPAER	2/25	682	0
251-265	LCLFLTPAERKCSRL	1/25	14.8	0
256-270	TPAERKCSRLCEAES	0/25	0	0
261-275	KCSRLCEAESSFKYE	2/25	691.7	0
266-280	CEAESSFKYESGLFV	3/25	165.5	6
271-285	SFKYESGLFVQGLLK	1/25	78.8	5
276-290	SGLFVQGLLKDSTGS	4/25	86.7	5
281-295	QGLLKDSTGSFVLPF	4/25	272.5	4
286-300	DSTGSFVLPFRQVMY	1/25	55.3	2
291-305	FVLPFRQVMYAPYPT	2/23	339.9	7
296-310	RQVMYAPYPTTHIDV	2/23	173.5	19
301-315	APYPTTHIDVDVNTV	2/23	201.7	3
306-320	THIDVDVNTVKQMPP	2/23	181.4	5
311-325	DVNTVKQMPPCHEHI	2/23	147.7	1
316-330	KQMPPCHEHIYNQRR	2/23	123.8	0
321-335	CHEHIYNQRRYMRSE	2/23	147.1	7
326-340	YNQRRYMRSELTAFW	1/23	37	2
331-345	YMRSELTAFWRATSE	1/23	39.1	3
336-350	LTAFWRATSEEDMAQ	1/23	7.5	20
341-355	RATSEEDMAQDTIIY	2/20	19.3	0

346-360	EDMAQDTIIYTDESF	0/20	0	6
351-365	DTIIYTDESFTPDLN	2/20	24	13
356-370	TDESFTPDLNIFQDV	2/20	52.5	11
361-375	TPDLNIFQDVLHRDT	3/20	106.5	17
366-380	IFQDVLHRDTLVKAF	6/20	180.5	3
371-385	LHRDTLVKAFLDQVF	2/20	21.7	3
376-390	LVKAFLDQVFQLKPG	3/20	50.3	7
381-395	LDQVFQLKPGLSLRS	3/20	469.5	18
386-400	QLKPGLSLRSTFLAQ	2/20	21.8	2
391-405	LSLRSTFLAQFLLVL	3/19	568.1	2
396-410	TFLAQFLLVLHRKAL	4/19	631.6	5
401-415	FLLVLHRKALTLIKY	4/19	657	1
406-420	HRKALTLIKYIEDDT	3/19	471.9	7
411-425	TLIKYIEDDTQKGKK	3/19	253	24
416-430	IEDDTQKGKKPFKSL	2/19	73	0
421-435	QKGKKPFKSLRNLKI	4/19	768.6	20
426-440	PFKSLRNLKIDLDLT	3/19	870.7	10
426-440	PFKSLRNLKIDLDLT*	5/19	500.4	N/A
431-445	RNLKIDLDLTAEGDL	3/19	374.6	8
431-445	RNLKIDLDLT*AEGDL	5/19	717.7	N/A
436-450	DLDLTAEGDLNIIMA	1/26	131.8	3
436-450	DLDLT*AEGDLNIIMA	1/26	147.4	N/A
441-455	AEGDLNIIMALAEKI	2/26	157.7	15
446-460	NIIMALAEKIKPGLH	1/26	136.9	9
451-465	LAEKIKPGLHSFIFG	2/26	159	1
456-470	KPGLHSFIFGRPFYT	1/26	129.5	2
461-475	SFIFGRPFYTSVQER	1/26	119.2	2
466-480	RPFYTSVQERDVLMT	1/26	91.3	15
467-481	PFYTSVQERDVLMTF	1/26	64.1	2

Boxes indicated mesopools #1-10. For each peptide, table lists sequence position, peptide sequence, number of positive responders, average magnitude, and number of predicted HLA binding events. S* and T* indicates a phospho-serine and phospho-threonine.

Supplementary Table S2. HLA typing information

	Donor-1	Donor-2	Donor-3	Donor-4	Donor-5
HLA-A Allele 1	A*02:01	A*01:01	A*03:01	A*01:01	A*02:01
HLA-A Allele 2	A*68:02	A*68:01	A*23:01	A*29:02	A*02:01
HLA-B Allele 1	B*14:02	B*35:02	B*07:02	B*07:02	B*07:02
HLA-B Allele 2	B*50:01	B*58:01	B*49:01	B*45:01	B*18:01
HLA-C Allele 1	C*06:02	C*03:02	C*07:01	C*06:02	C*07:01
HLA-C Allele 2	C*08:02	C*04:01	C*07:02	C*07:02	C*07:02
HLA-DPA1 Allele 1	n/a	n/a	n/a	n/a	n/a
HLA-DPA1 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DPB1 Allele 1	DPB1*04:01	DPB1*04:01	DPB1*02:01	DPB1*03:01	DPB1*04:01
HLA-DPB1 Allele 2	DPB1*04:02	DPB1*04:02	DPB1*04:01	DPB1*04:01	DPB1*04:02
HLA-DQA1 Allele 1	DQA1*01:01	DQA1*01:03	DQA1*01:03	DQA1*01:02	DQA1*01:02
HLA-DQA1 Allele 2	DQA1*05:01	DQA1*05:01	DQA1*05:01	DQA1*03:01	DQA1*01:02
HLA-DQB1 Allele 1	DQB1*02:01	DQB1*03:01	DQB1*03:01	DQB1*03:01	DQB1*05:02
HLA-DQB1 Allele 2	DQB1*05:01	DQB1*06:03	DQB1*06:03	DQB1*06:02	DQB1*06:02
HLA-DRB1 Allele 1	DRB1*01:02	DRB1*11:04	DRB1*11:01	DRB1*04:01	DRB1*15:01
HLA-DRB1 Allele 2	DRB1*03:01	DRB1*11:04	DRB1*13:01	DRB1*15:01	DRB1*15:01
HLA-DRB3 Allele 1	DRB3*01:01	DRB3*02:02	DRB3*01:01	n/a	n/a
HLA-DRB3 Allele 2	n/a	DRB3*02:02	DRB3*02:02	n/a	n/a
HLA-DRB4 Allele 1	n/a	n/a	n/a	DRB4*01:01	n/a
HLA-DRB4 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DRB5 Allele 1	n/a	n/a	n/a	DRB5*01:01	DRB5*01:01
HLA-DRB5 Allele 2	n/a	n/a	n/a	n/a	DRB5*02:02

	Donor-6	Donor-7	Donor-8	Donor-9	Donor-10
HLA-A Allele 1	A*03:01	A*02:01	A*01:01	A*02:07	n/a
HLA-A Allele 2	A*24:02	A*68:01	A*01:01	A*24:02	n/a
HLA-B Allele 1	B*07:02	B*38:01	B*08:01	B*40:01	B*38:02
HLA-B Allele 2	B*35:01	B*40:01	B*35:02	B*46:01	B*38:02
HLA-C Allele 1	C*04:01	C*03:04	C*04:01	C*01:02	C*07:02
HLA-C Allele 2	C*07:02	C*12:03	C*07:01	C*07:02	C*07:02
HLA-DPA1 Allele 1	n/a	n/a	n/a	n/a	n/a
HLA-DPA1 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DPB1 Allele 1	DPB1*04:02	DPB1*03:01	DPB1*01:01	DPB1*05:01	n/a
HLA-DPB1 Allele 2	DPB1*09:01	DPB1*04:01	DPB1*02:01	DPB1*05:01	n/a
HLA-DQA1 Allele 1	DQA1*01:01	DQA1*01:02	DQA1*05:01	DQA1*01:01	DQA1*01:01
HLA-DQA1 Allele 2	DQA1*01:01	DQA1*01:03	DQA1*05:01	DQA1*03:01	DQA1*01:01
HLA-DQB1 Allele 1	DQB1*05:01	DQB1*06:03	DQB1*02:01	DQB1*03:03	DQB1*04:01
HLA-DQB1 Allele 2	DQB1*05:03	DQB1*06:04	DQB1*03:01	DQB1*05:02	DQB1*05:01
HLA-DRB1 Allele 1	DRB1*01:01	DRB1*13:01	DRB1*03:01	DRB1*09:01	DRB1*15:02
HLA-DRB1 Allele 2	DRB1*14:01	DRB1*13:01	DRB1*11:04	DRB1*14:01	DRB1*15:02
HLA-DRB3 Allele 1	DRB3*02:02	DRB3*01:01	DRB3*01:01	DRB3*02:02	n/a
HLA-DRB3 Allele 2	n/a	DRB3*03:01	DRB3*02:02	n/a	n/a
HLA-DRB4 Allele 1	n/a	n/a	n/a	DRB4*01:01	n/a
HLA-DRB4 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DRB5 Allele 1	n/a	n/a	n/a	n/a	DRB5*01:02
HLA-DRB5 Allele 2	n/a	n/a	n/a	n/a	DRB5*01:02

	Donor-11	Donor-12	Donor-13	Donor-14	Donor-15
HLA-A Allele 1	A*23:01	A*01:01	A*03:01	A*02:01	A*02:01
HLA-A Allele 2	A*29:02	A*02:01	A*24:02	A*24:02	A*03:01
HLA-B Allele 1	B*44:03	B*08:01	B*07:02	B*07:02	B*07:02
HLA-B Allele 2	B*44:03	B*44:02	B*55:01	B*44:02	B*08:01
HLA-C Allele 1	C*02:02	C*05:01	C*03:03	C*07:02	C*07:01
HLA-C Allele 2	C*16:01	C*07:01	C*07:02	C*07:04	C*07:02
HLA-DPA1 Allele 1	n/a	n/a	n/a	n/a	n/a
HLA-DPA1 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DPB1 Allele 1	DPB1*04:01	DPB1*04:01	DPB1*02:01	DPB1*02:01	DPB1*04:01
HLA-DPB1 Allele 2	DPB1*11:01	DPB1*04:01	DPB1*04:02	DPB1*04:01	DPB1*04:01
HLA-DQA1 Allele 1	DQA1*01:01	DQA1*05:01	DQA1*01:02	DQA1*01:02	DQA1*01:02
HLA-DQA1 Allele 2	DQA1*02:01	DQA1*05:01	DQA1*01:02	DQA1*03:01	DQA1*01:02
HLA-DQB1 Allele 1	DQB1*02:02	DQB1*02:01	DQB1*06:02	DQB1*03:02	DQB1*06:02
HLA-DQB1 Allele 2	DQB1*05:01	DQB1*03:01	DQB1*06:04	DQB1*05:02	DQB1*06:02
HLA-DRB1 Allele 1	DRB1*01:02	DRB1*03:01	DRB1*13:02	DRB1*04:01	DRB1*15:01
HLA-DRB1 Allele 2	DRB1*07:01	DRB1*11:01	DRB1*15:01	DRB1*16:01	DRB1*15:01
HLA-DRB3 Allele 1	n/a	DRB3*01:01	DRB3*03:01	n/a	n/a
HLA-DRB3 Allele 2	n/a	DRB3*02:02	n/a	n/a	n/a
HLA-DRB4 Allele 1	DRB4*01:01	n/a	n/a	DRB4*01:01	n/a
HLA-DRB4 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DRB5 Allele 1	n/a	n/a	DRB5*01:01	DRB5*02:02	DRB5*01:01
HLA-DRB5 Allele 2	n/a	n/a	n/a	n/a	DRB5*01:01

	Donor-16	Donor-17	Donor-18	Donor-19	Donor-20
HLA-A Allele 1	A*02:05	A*02:01	A*02:01	A*11:01	A*02:01
HLA-A Allele 2	A*24:02	A*03:01	A*68:01	A*26:01	A*24:02
HLA-B Allele 1	B*07:02	B*15:01	B*08:01	B*38:01	B*18:01
HLA-B Allele 2	B*14:02	B*44:02	B*40:01	B*57:01	B*44:02
HLA-C Allele 1	C*07:02	C*03:04	C*03:04	C*06:02	C*05:01
HLA-C Allele 2	C*08:02	C*05:01	C*07:01	C*12:03	C*12:03
HLA-DPA1 Allele 1	n/a	n/a	n/a	n/a	n/a
HLA-DPA1 Allele 2	n/a	n/a	n/a	n/a	n/a
HLA-DPB1 Allele 1	DPB1*04:02	DPB1*03:01	DPB1*01:01	DPB1*04:01	DPB1*04:02
HLA-DPB1 Allele 2	DPB1*06:01	DPB1*04:01	DPB1*04:01	DPB1*04:01	DPB1*05:01
HLA-DQA1 Allele 1	DQA1*01:02	DQA1*03:01	DQA1*05:01	DQA1*01:02	DQA1*01:03
HLA-DQA1 Allele 2	DQA1*05:01	DQA1*03:01	DQA1*05:01	DQA1*02:01	DQA1*05:01
HLA-DQB1 Allele 1	DQB1*03:01	DQB1*03:02	DQB1*02:01	DQB1*03:03	DQB1*03:01
HLA-DQB1 Allele 2	DQB1*06:02	DQB1*03:02	DQB1*02:01	DQB1*06:02	DQB1*06:03
HLA-DRB1 Allele 1	DRB1*11:01	DRB1*04:01	DRB1*03:01	DRB1*07:01	DRB1*11:04
HLA-DRB1 Allele 2	DRB1*15:01	DRB1*04:04	DRB1*03:01	DRB1*15:01	DRB1*13:01
HLA-DRB3 Allele 1	DRB3*02:02	n/a	DRB3*01:01	n/a	DRB3*01:01
HLA-DRB3 Allele 2	n/a	n/a	DRB3*01:01	n/a	DRB3*02:02
HLA-DRB4 Allele 1	n/a	DRB4*01:01	n/a	DRB4*01:01	n/a
HLA-DRB4 Allele 2	n/a	DRB4*01:01	n/a	n/a	n/a
HLA-DRB5 Allele 1	DRB5*01:01	n/a	n/a	DRB5*01:01	n/a
HLA-DRB5 Allele 2	n/a	n/a	n/a	n/a	n/a

	Donor-21	Donor-22	Donor-23
HLA-A Allele 1	A*01:01	A*01:01	A*01:01
HLA-A Allele 2	A*03:02	A*02:01	A*74:01
HLA-B Allele 1	B*08:01	B*08:01	B*07:02
HLA-B Allele 2	B*49:01	B*44:02	B*07:02
HLA-C Allele 1	C*07:01	C*05:01	C*07:02
HLA-C Allele 2	C*07:01	C*07:01	C*15:05
HLA-DPA1 Allele 1	n/a	n/a	n/a
HLA-DPA1 Allele 2	n/a	n/a	n/a
HLA-DPB1 Allele 1	DPB1*04:01	DPB1*02:01	DPB1*02:01
HLA-DPB1 Allele 2	DPB1*11:01	DPB1*03:01	DPB1*14:01
HLA-DQA1 Allele 1	DQA1*03:01	DQA1*03:01	DQA1*01:01
HLA-DQA1 Allele 2	DQA1*05:01	DQA1*05:01	DQA1*01:02
HLA-DQB1 Allele 1	DQB1*02:01	DQB1*02:01	DQB1*05:01
HLA-DQB1 Allele 2	DQB1*03:02	DQB1*03:01	DQB1*06:02
HLA-DRB1 Allele 1	DRB1*03:01	DRB1*03:01	DRB1*01:01
HLA-DRB1 Allele 2	DRB1*04:01	DRB1*04:01	DRB1*15:01
HLA-DRB3 Allele 1	DRB3*01:01	DRB3*01:01	n/a
HLA-DRB3 Allele 2	n/a	n/a	n/a
HLA-DRB4 Allele 1	DRB4*01:01	DRB4*01:01	n/a
HLA-DRB4 Allele 2	n/a	n/a	n/a
HLA-DRB5 Allele 1	n/a	n/a	DRB5*01:01
HLA-DRB5 Allele 2	n/a	n/a	n/a

n/a = allele not present. In cases where only a single allele for a locus is provided, a second allele was not detected. This may be due to true homozygosity however blanks or an undetected allele is not absolutely excluded. HLA genotyping has been performed by DNA sequencing of the exon 2-3 region of HLA class I and HLA-DQB1, and only exon 2 region of HLA-DRB1.

Supplementary Table S3. Antibodies using in study

Spectral Flow cytometry panel for broad immunophenotyping

Target	Fluorochrome	Cat. No.	Dilution
CD4	APC-eF780	47-0049-42	1:640
CD8	BUV496	612942	1:640
CD20	BUV563	748456	1:640
CD56	APC	362504	1:640
gdTCR	BV421	744870	1:320
CCR4	PE Cy-7	557864	1:320
CD16	PE Cy 5	MA5-38692	1:320
CD14	BV480	746304	1:320
HLA-DR	AF700	56-9956-42	1:320
CD161	BV650	563864	1:160
CXCR3	PE	353706	1:160
CD19	BV605	302244	1:160
CD38	PerCP-Cy5.5	551400	1:160
CD3	BUV805	612895	1:160
CCR7	BV785	353216	1:160
CCR6	BUV395	743356	1:160
CD26	FITC	302704	1:160
CD127	PEFire700	351365	1:160
CD45	PE Dazzle594	982308	1:160
CD25	BV711	356137	1:80
CD45RA	BV570	304132	1:80

Intracellular cytokine staining panels

Extracellular panel

Target	Fluorochrome	Cat. No.	Dilution
CD4	APC-ef780	47-0049-42	1:100
CD3	Alexa Fluor 700	56-0038-42	1:50
CD8	BV650	301042	1:100
Live/Dead Aqua	eFluor 506	65-0866-14	1:1000

Intracellular panel

Target	Fluorochrome	Cat. No.	Dilution
IL-10	APC	506807	1:40
IL-4	BV421	500826	1:40
IFNg	FITC	11-7319-82	1:40
IL-17	BV785	512338	1:40

Flurospot antibodies

Plate coating antibodies

Target	Fluorochrome	Cat. No.	Dilution
mouse anti-human IFN-g	-	FSP-010708-10	1:200
mouse anti-human IL-5	-	FSP-010708-10	1:200
Mouse anti-human IL-10	-	FSP-010708-10	1:100

Detection antibody cocktail

Target	Conjugate	Cat. No.	Dilution
mouse anti-human IFN-g	BAM	FSP-010708-10	1:200
mouse anti-human IL-5	WASP	FSP-010708-10	1:200
mouse anti-human IL-10	Biotin	FSP-010708-10	1:500

Fluorophore conjugated antibodies

Target	Fluorochrome	Cat. No.	Dilution
anti-BAM	490 (FITC)	FSP-010708-10	1:200
anti-WASP	640 (Cy3)	FSP-010708-10	1:200
Streptavidin	550 (Cy5)	FSP-010708-10	1:200