Article Title: *C9orf72* Hypermethylation Protects Against Repeat Expansion Associated Pathology in ALS/FTD

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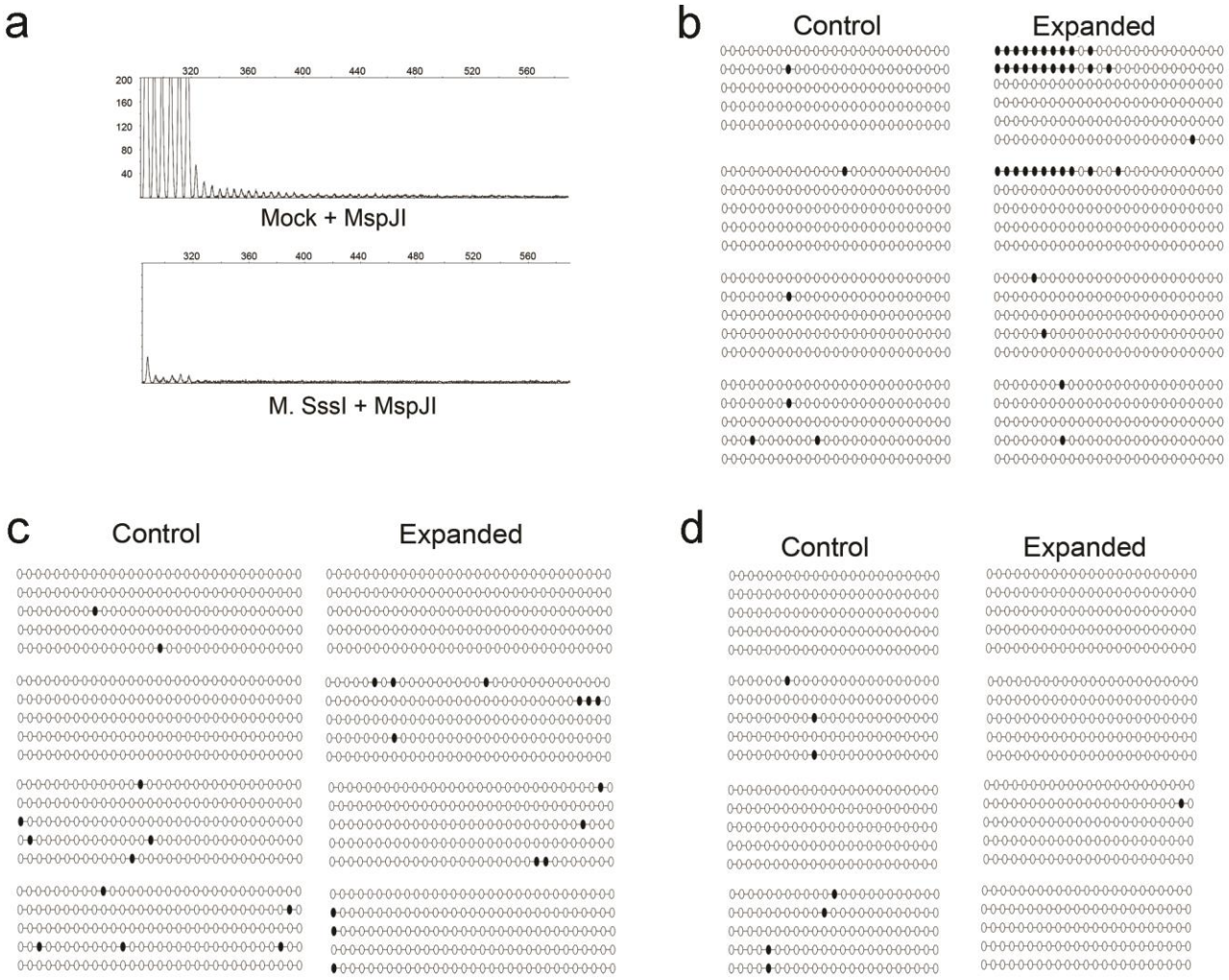
Authors: Elaine Y. Liu[1,2,4], Jenny Russ[1,2,4], Kathryn Wu[1,2,4], Donald Neal[2,4], Eunran Suh[2,4], Anna G. McNally[1,2,4], David J. Irwin[2,3,4], Vivianna M. Van Deerlin[2,4], Edward B. Lee[1,2,4]

Affiliations:
1. Translational Neuropathology Research Laboratory
2. Department of Pathology and Laboratory Medicine
3. Department of Neurology
4. Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Corresponding Author: Edward B. Lee, M.D., Ph.D.
Corresponding E-mail: edward.lee@uphs.upenn.edu



Supplemental Figure 1: Repeat primed PCR and bisulfite cloning of *C9orf72*

(A) DNA from a repeat expanded case was in vitro methylated using MSssl methyltransferase and digested with MspJI. Digested material was subject to repeat primed PCR and representative electropherograms are shown.

(B) Bisulfite cloning results for each sequenced clone for amplicon A (panel B), amplicon B (panel C) and amplicon C (panel D) are shown. Four non-expanded controls and four expanded cases were assessed. Each oval represents a single CpG dinucleotide where unfilled ovals represent unmethylated CpG dinucleotides and filled ovals represent methylated CpG dinculeotides. Sequencing results are presented in groups of 5-6 clones which correspond to results from each case. Background non-CpG cytosines showed a conversion rate of >95%.

Supplemental Tables

Description	Forward Oligonucleotide	Reverse Oligonucleotide
C9orf72 V2 mRNA (exon 2 to 3)	CGGTGGCGAGTGGATATCTC	TGGGCAAAGAGTCGACATCA
C9orf72 Total mRNA (exon 3 to 4)	ACTGGAATGGGGATCGCAGCA	ACCCTGATCTTCCATTCTCTCTG TGCC
C9orf72 V3 mRNA (exon 1b to 3)	GCAAGAGCAGGTGTGGGTTT	TGGGCAAAGAGTCGACATCA
C9orf72 intron (intron 1)	GCAAGAGCAGGTGTGGGTTT	TCTTGTTCACCCTCAGCGAG
C9orf72 SNP allele (sequencing)	GACCAAAAGACGCAAGGTTT	CTTTTACGTGGGCGGAACT
C9orf72 SNP allele (PAGE)	GACCAAAAGACGCAAGGTTT	TTTGTGCTTGGTAGGCAGTG
C9orf72 promoter	CAGTGTGAAAATCATGCTTGAGA GA	TTTGTGCTTGGTAGGCAGTG
C9orf72 CpG Island 1	GGAGTTGTTTTTTATTAGGGTTTG TAGT	TAAACCCACACCTACTCTTACT AAA
C9orf72 CpG Island 2, first half (outer)	GTGAGTGAGGAGGYGGTATTTT	AATCACTTCCTTTAAACAAAT
C9orf72 CpG Island 2, first half (inner)	GTGAGTGAGGAGGYGGTATTTT	AAACCTCTCAATACCCRAAACT
C9orf72 CpG Island 2, second half	AGTTGTTTTTTTTTYGGGGATT	AATCACTTCCTTTAAACAAAT
C9orf72 Southern Blot	TTGCGATGACTTTGCAGGGGACC	CAGCGAGTACTGTGAGAG
GPS mRNA (control)	AAGATGCTGGACGAGATGAAGGA	ACGGTTGCGAATCTGGGTGTA
BACT mRNA (control)	GCCCTGAGGCACTCTTCCA	ATGCCACAGGACTCCATGC
GA Repeat Fragment	TATGGGTGCGGGAGCTGGAGCTG GTGCAGGTGCAGGTGCAGGAGCA GGTGCTGGAGCAGGAGCTGGTGC AGGTGCAGGAGCAGGAGCCGGAG CCTAAG	AATTCTTAGGCTCCGGCTCCTG CTCCTGCACCTGCACCAGCTCC TGCTCCAGCACCTGCTCCTGCA CCTGCACCTGCACCAGCTCCAG CTCCCGCACCCA
GP Repeat Fragment	TATGGGTCCTGGACCTGGACCTGG ACCAGGGCCAGGGCCGGGACCTG GACCTGGACCAGGACCTGGACCA GGGCCAGGACCAGGACCTGGACC ATAAG	AATTCTTATGGTCCAGGTCCTG GTCCTGGCCCTGGTCCAGGTCC TGGTCCAGGTCCAGGTCCCGGC CCTGGCCCTGGTCCAGGTCCAG GTCCAGGACCCA

Table 1: Primer Sequences

Diagnosis	MND	Dementia	Gender	Age of Onset	Age of Death	Figure	C9ORF72 Expansion
ALS	Y	N	Female	54	55	2, 6	Y
ALS	Y	N	Female	55	59	1, 2, 5, 6	Y
ALS	Y	N	Female	73	76	2, 5, 6	Y
ALS	Y	N	Female		56	2, 6	Y
ALS	Y	N	Male	56	58	2, 5, 6	Y
ALS	Y	N	Male	56	58	1, 2, 5, 6	Y
ALS	Y	N	Male	62	64	1, 2, 5, 6	Y
ALS	Y	N	Male		54	2, 5, 6	Y
ALS	Y	N	Male		55	2	Y
ALS	Y	N	Male	51	56	1	Y
ALS-dementia	Y	Y	Female	63	70	2	Y
ALS-dementia	Y	Y	Male	46	48	1, 2, 5, 6	Y
ALS-dementia	Y	Y	Male	52	54	1, 2, 5, 6	Y
ALS-dementia	Y	Y	Male	55	58	2, 5, 6	Y
ALS-dementia	Y	Y	Male		63	1, 2	Y
ALS-dementia	Y	Y	Male		76	2, 5, 6	Y
FTD	N	Y	Female	59	62	2, 6	Y
FTD	N	Y	Female	66	76	1, 2, 5, 6	Y
FTD	N	Y	Female		72	2, 6	Y
FTD	N	Y	Male	47	54	2, 6	Y
FTD	N	Y	Male	63	76	1, 2, 5, 6	Y
FTD	N	Y	Male	63	67	1	Y
AD	N	Y	Female	57	70	1, 2, 6	N
AD	N	Y	Male	72	79	2	N
AD	N	Y	Male		83	2	N
ALS	Y	N	Female	50	53	1, 2, 6	N
ALS	Y	N	Female	81	82	1, 2	N
ALS	Y	N	Male	76	77	1, 2	N
DLB, AD	N	Y	Female	72	84	1, 2, 6	N
FTD	N	Y	Female	67	76	1, 2	N
normal	N	N	Female	n/a	72	2	N
normal	N	N	Female	n/a	78	2	N
normal	N	N	Female	n/a	82	2	N
normal	N	N	Male	n/a	47	2	N
normal	N	N	Male	n/a	74	2	N
normal	N	N	Male	n/a	76	2	N
PD with MCI	N	Y	Male	83	98	1, 2, 6	N
vascular dementia	N	Y	Male	58	65	1, 2, 6	N

Table 2: List of cases

FTD= frontotemporal degeneration with TDP-43 inclusions; AD= Alzheimer's disease; MCI= mild cognitive impairment; DLB= dementia with Lewy bodies; PD= Parkinson's disease

Cell line	Cell Type	Other Type
A549	Epithelial	
HAOEC	Epithelial	
HELAS3	Epithelial	
HEPG2	Epithelial	
HMEPC	Epithelial	
HPIEPC	Epithelial	
HSAVEC	Epithelial	
HUVEC	Epithelial	
MCF7	Epithelial	
NHEK	Epithelial	
AG04450	Fibroblastic	
BJ	Fibroblastic	
HAOAF	Fibroblastic	
HVMF	Fibroblastic	
IMR90	Fibroblastic	
NHDF	Fibroblastic	
CD20	Hematopoietic	
CD34 mobilized	Hematopoietic	
GM12878	Hematopoietic	
K562	Hematopoietic	
MONOCD14	Hematopoietic	
SKNSH	Neuronal	
SKNSH RA treated	Neuronal	
H1HESC	Other	Embryonic stem cell
HCH	Other	Chondrocytes
HFDPC	Other	Hair follicle dermal papilla cells
HMSCAT	Other	Mesenchymal stem cell from adipose
HMSCBM	Other	Mesenchymal stem cell from bone marrow
HMSCUC	Other	Mesenchymal stem cells from umbilical cord
HOB	Other	Osteoblasts
HPCPL	Other	Pericytes
HWP	Other	White preadipocytes
NHEMFM2	Other	Melanocytes
NHEMM2	Other	Melanocytes
SKMC	Other	Striated muscle

Table 3: ENCODE CAGE-seq Cell Lines