

Journal title: Hypermethylation of repeat expanded C9orf72 is a clinical and molecular disease modifier

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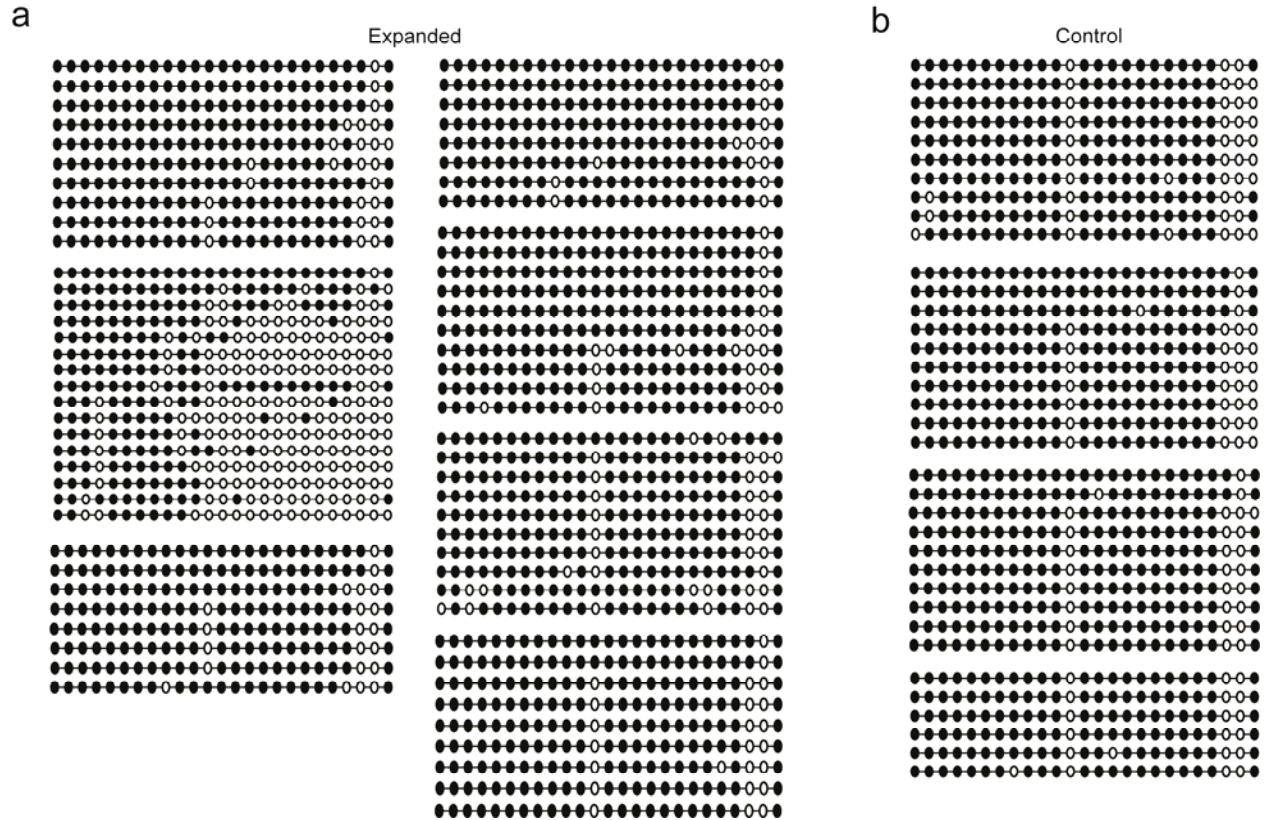


Figure S1: Bisulfite cloning results of *HhaI* digested genomic DNA.

Genomic DNA from (a) 7 *C9orf72* expansion carriers, including 5 cerebella and 2 patient derived lymphoblastoid cell lines, and (b) 4 control cerebella was used. Each oval represents a CpG site, where black ovals are methylated CpG dinucleotides and white ovals are unmethylated CpG dinucleotides. The 2nd CpG is the site assayed in the *HhaI*-qPCR assay. Six to sixteen clones of each case were included in the analysis. Background conversion rate of non-CpG cytosines for each case was >95%.

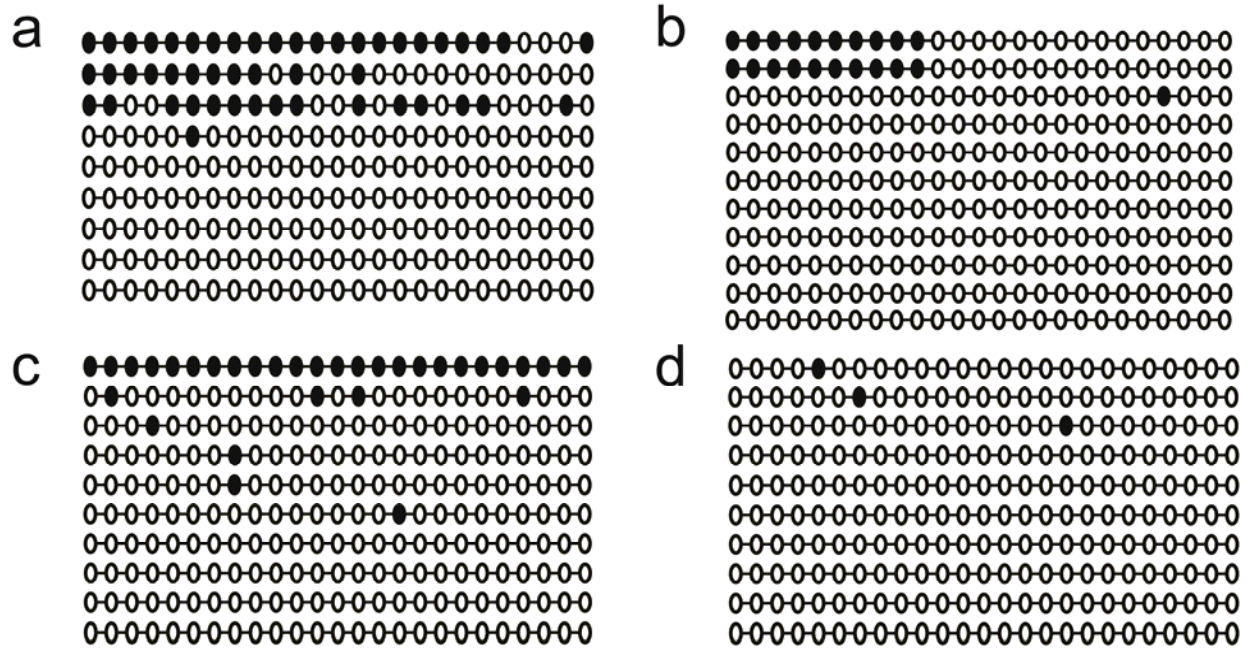


Figure S2: Bisulfite cloning results of *C9orf72* promoter from four repeat expansion carriers.

Each oval represents a CpG site, where black ovals are methylated CpG dinucleotides and white ovals are unmethylated CpG dinucleotides. A total of nine to eleven clones were sequenced for each case. Clones with at least 10 methylated CpG sites were counted as hypermethylated. Background conversion rate of non-CpG cytosines for each case was >95%. Methylation levels assessed by *HhaI* resistance were: (a) 29.92%, (b) 10.4%, (c) 6.93%, and (d) 6.26%.

Table S1: Clinical characteristics of *C9orf72* repeat expansion carriers (four disease subtypes)

	ALS	ALS-MCI	ALS-FTD	FTD
Blood, n	27	16	13	27
Deaths	22	10	8	8
Gender, no. male (%)	18 (66.67)	9 (56.25)	9 (69.23)	15 (55.56)
Age at onset, yr (IQR)	57.63 (52.03-66.08)	54.06 (50.81-62.50)	53.75 (47.51-60.60)	58.01 (50.51-62.05)
Age at death, yr (IQR)	62.10 (57.08-70.26)	57.47 (54.01-63.69)	59.58 (50.05-68.38)	63.14 (57.06-67.42)
Disease duration, yr (IQR)	2.56 (1.93-3.57)	2.62 (1.88-3.16)	2.50 (2.18-2.77)	5.45 (4.15-9.35)
Methylation, pct (IQR)	5.51 (1.73-9.17)	8.60 (2.93-19.68)	4.98 (1.58-12.27)	8.18 (1.47-30.38)

Data are shown as median and interquartile range (IQR) unless otherwise stated.

Table S2: Peripheral blood *C9orf72* promoter methylation and age at onset, age at death and disease duration (four disease subtypes).

	Age at onset (n = 77)			Age at death (n = 47)			Disease duration (ln transformed, n=47)		
	β (SE)	95% CI	p-value	β (SE)	95% CI	p-value	β (SE)	95% CI	p-value
Intercept	57.84 (2.34)	53.16 to 62.51	<0.001	3.25 (1.98)	-0.77 to 7.26	0.110	0.97 (0.55)	0.03 to 2.04	0.084
Diagnosis (ALS-MCI)	-4.01 (3.90)	-11.80 to 3.77	0.307	-0.31 (1.09)	-2.52 to 1.90	0.778	-0.03 (0.30)	-5.90 to 4.51	0.925
Diagnosis (ALS-FTD)	-5.85 (3.67)	-13.18 to 1.47	0.115	-1.30 (0.87)	-3.06 to 0.45	0.141	-0.25 (0.24)	-5.90 to 4.51	0.299
Diagnosis (FTD)	-0.45 (3.13)	-6.70 to 5.81	0.887	1.46 (1.09)	-0.75 to 3.67	0.190	0.45 (0.30)	-0.32 to 0.65	0.140
Gender (Male)	2.89 (1.95)	-1.00 to 6.78	0.143	0.01 (0.52)	-1.04 to 1.06	0.989	-0.08 (0.14)	-0.41 to 0.15	0.585
Age at onset	n.a.	n.a.	n.a.	0.99 (0.03)	0.93 to 1.06	<0.001	0.00 (0.01)	-0.02 to 0.02	0.945
Methylation	-0.18 (0.13)	-0.45 to 0.09	0.179	-0.01 (0.03)	-0.07 to 0.05	0.652	-0.01 (0.01)	-0.02 to 0.01	0.433
Methylation*Diagnosis (ALS-MCI)	0.18 (0.27)	-0.35 to 0.72	0.496	-0.00 (0.08)	-0.16 to 0.15	0.975	-0.00 (0.02)	-5.90 to 4.51	0.995
Methylation*Diagnosis (ALS-FTD)	0.29 (0.27)	-0.24 to 0.82	0.284	0.22 (0.06)	0.10 to 0.34	<0.001	0.05 (0.02)	-5.90 to 4.51	0.006
Methylation*Diagnosis (FTD)	0.08 (0.17)	-0.26 to 0.42	0.628	0.11 (0.05)	0.02 to 0.20	0.021	0.02 (0.01)	0.01 to 0.05	0.084
R ²		0.088			0.971			0.497	

SE – standard error, CI – confidence interval, ln – natural log