SUPPLEMENTARY DATA

APOE 5'UTR Methylation Pattern Analysis in Blood and Brain Tissue from Alzheimer's Disease Affected Patients

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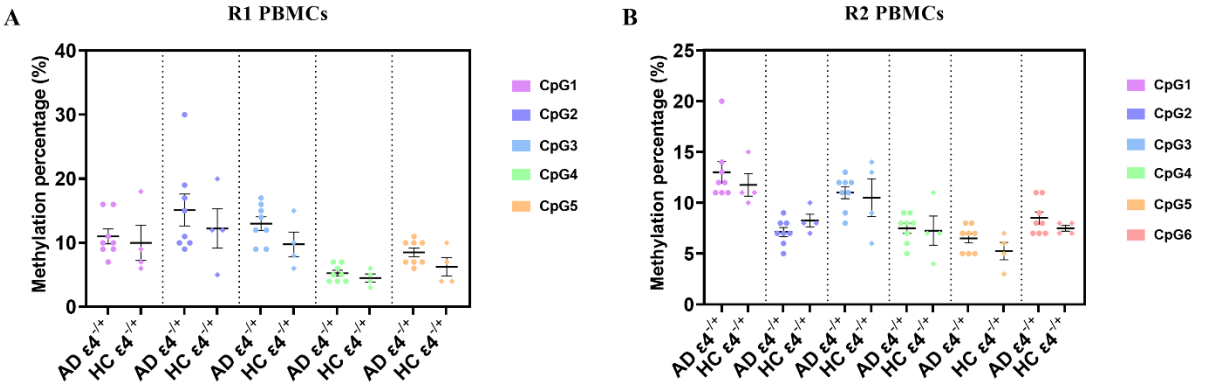
SUPPLEMENTARY DATA

SUPPLEMENTARY MATERIALS:

Supplementary figures



Supplementary Figure 1. *APOE* ε4$^{-/+}$ genotype effect on methylation level of single CpG sites in *APOE* 5'UTR in PBMCs. (**A**) R1 methylation level in AD ε4$^{-/+}$ (n=8) and HC ε4$^{-/+}$ (n=4) PBMCs. (**B**) R2 methylation level in AD ε4$^{-/+}$ (n=8) and HC ε4$^{-/+}$ (n=4) PBMCs. X axis: condition and *APOE* genotype. Single CpG sites are differentiated by color. AD and HC groups are differentiated by symbol (● = AD; ♦ = HC). Statistical analysis was performed using the one-way ANOVA Kruskal-Wallis test comparing AD ε4$^{-/+}$ with HC ε4$^{-/+}$ methylation percentage site by site and data are expressed as mean ± SEM. Data are not significant (Supplementary Table 6).

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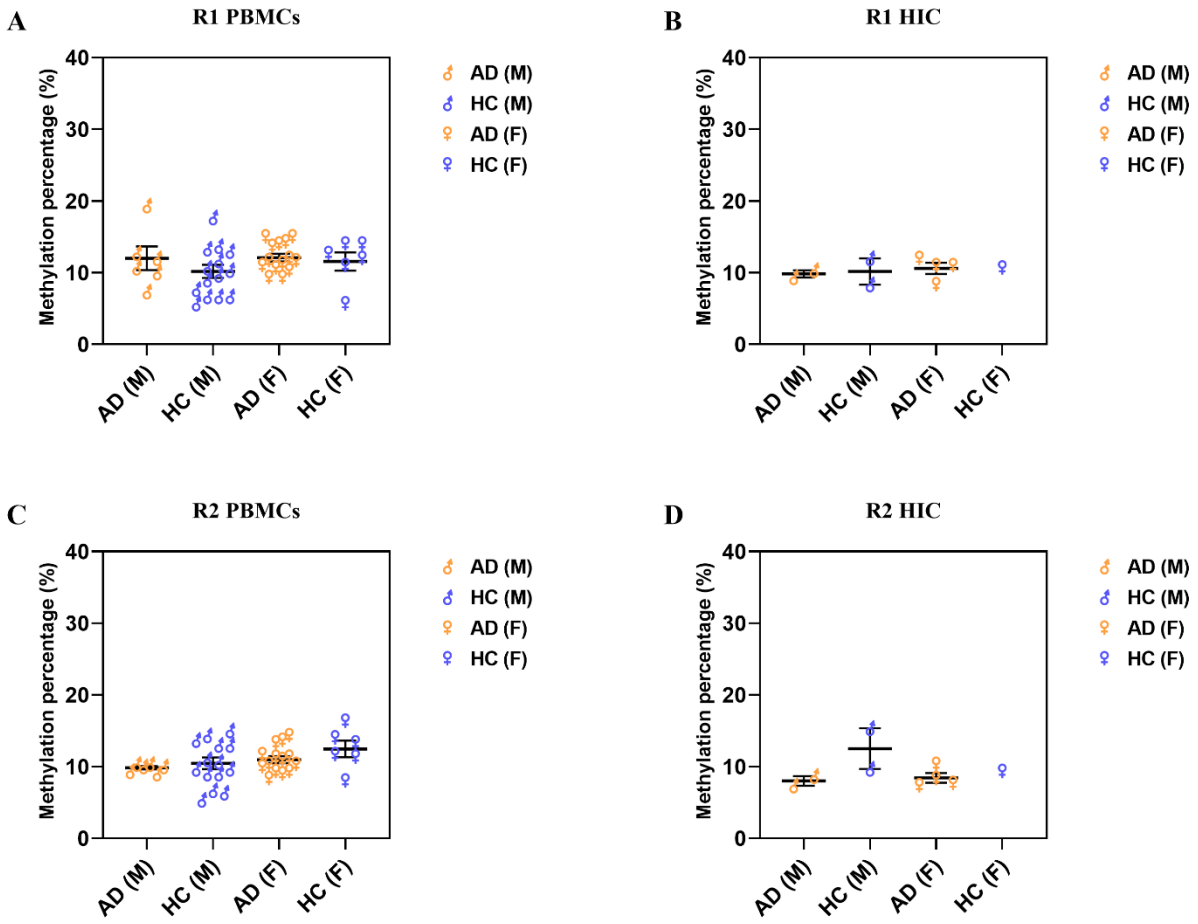

Supplementary Figure 2. Sex effect on the average methylation level of the two regions comprised in *APOE* 5'UTR of both PBMCs and HIC brain tissue. (**A**) R1 average methylation level in AD (M n=6; F n=16) and HC (M n=16; F n=6) PBMCs. (**B**) R1 average methylation level in AD (M n=2; F n=6) and HC (M n=2; F n=1) HIC brain tissue. (**C**) R2 average methylation level in AD (M n=6; F n=16) and HC (M n=16; F n=6) PBMCs. (**D**) R2 average methylation level in AD (M n=2; F n=6) and HC (M n=2; F n=1) HIC brain tissue. X axis: condition and sex; Y axis: average methylation percentage of the first three CpG sites. Females and males are differentiated by symbol (♀ = female; ♂ = male). AD and HC are differentiated by color. Statistical analysis was performed using the one-way ANOVA Kruskal-Wallis test comparing all variables and data are expressed as mean ± SEM. Data are not significant (Supplementary Table 7).

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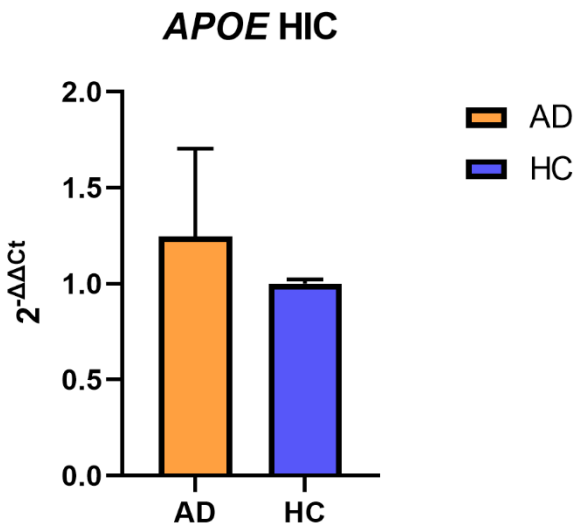

Supplementary Figure 3. *APOE* expression analysis by Real Time PCR in HIC brain tissue of AD patients (n=6) and HC (n=3). X axis: condition; Y axis: Fold-expression indicated as $2^{-\Delta\Delta Ct}$. Statistical analysis was performed using the Student's t test and data are expressed as mean ± SEM. Data are not significant (Supplementary Table 8).

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Supplementary Tables

Blood sample (IRCCS Mondino Foundation, Pavia)

						CSF			
ID	***Dx***	***Age***	***Sex***	***MMSE***	***APOE genotype***	***tTAU (pg/mL)***	***pTAU181 (pg/mL)***	***Aβ42 (pg/mL)***	***Aβ42/Aβ40***
ADP1	AD	77	F	19	3//4	1064	159	286	0,031
ADP2	AD	80	F	NA	2//3	657	117,5	335	0,035
ADP3	AD	50	F	23,3	3//3	1431	210,2	671	0,045
ADP4	AD	68	F	26,5	3//4	1964	382,6	534	0,032
ADP5	AD	72	M	21	3//4	1402	207,5	510	0,036
ADP6	AD	69	M	20	3//3	709	232,5	270	0,045
ADP7	AD	56	M	26	3//3	NA	NA	NA	NA
ADP8	AD	84	M	19	3//3	NA	NA	NA	NA
ADP9	AD	77	F	14	3//4	271	42,5	255	0,039
ADP10	AD	80	F	12	3//3	509	71,3	284	0,044
ADP11	AD	65	F	10	3//4	636	81,9	513	0,053
ADP12	AD	76	F	28,8	3//4	632	99,2	471	0,045
ADP13	AD	77	M	29,2	3//3	489	83,5	587	0,058
ADP14	AD	77	M	19	3//4	432	60,3	628	0,059
ADP15	AD	68	F	15,9	3//4	761	119,3	366	0,042
ADP16	AD	84	F	16	3//3	NA	NA	NA	NA
ADP17	AD	72	F	17	3//3	600	89	623	0,057
ADP18	AD	81	F	23	3//3	555	101	429	0,046
ADP19	AD	69	F	20	3//3	705	114,3	298	0,037
ADP20	AD	67	F	17	2//3	212	34,8	267	0,050
HCP1	HC	69	M	NA	3//3	NA	NA	NA	NA
HCP2	HC	62	M	NA	3//3	NA	NA	NA	NA
HCP3	HC	41	F	NA	3//3	NA	NA	NA	NA
HCP4	HC	60	M	NA	3//3	NA	NA	NA	NA
HCP5	HC	60	M	NA	3//4	NA	NA	NA	NA
HCP6	HC	51	F	NA	3//4	NA	NA	NA	NA
HCP7	HC	64	M	NA	3//3	NA	NA	NA	NA
HCP8	HC	61	M	NA	3//3	NA	NA	NA	NA
HCP9	HC	59	F	NA	2//3	NA	NA	NA	NA
HCP10	HC	63	M	NA	3//3	NA	NA	NA	NA
HCP11	HC	64	M	NA	3//3	NA	NA	NA	NA
HCP12	HC	67	F	NA	3//3	NA	NA	NA	NA
HCP13	HC	58	M	NA	3//4	NA	NA	NA	NA
HCP14	HC	65	F	NA	3//3	NA	NA	NA	NA
HCP15	HC	65	M	NA	3//4	NA	NA	NA	NA
HCP16	HC	60	M	NA	3//3	NA	NA	NA	NA
HCP17	HC	55	F	NA	3//3	NA	NA	NA	NA
HCP18	HC	57	M	NA	3//3	NA	NA	NA	NA
HCP19	HC	57	M	NA	3//3	NA	NA	NA	NA
HCP20	HC	64	M	NA	3//3	NA	NA	NA	NA

Supplementary Table 1. Clinical parameters of IRCCS Mondino Foundation recruited subjects. ID = sample identification number (ADP = Alzheimer's Disease PBMCs; HCP = Healthy Control PBMCs). Dx = diagnosis (AD = Alzheimer's Disease; HC = Healthy Control). MMSE = Mini-Mental State Examination. F = female. M = male. CSF = cerebrospinal fluid (tTAU = total TAU; pTAU181 = TAU protein phosphorylated at residue 181). NA = not available.

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Hippocampus tissue samples (Golgi Cenci Foundation, Abbiategrasso)

ID	*Dx*	*Age*	*Sex*	*MMSE*	*APOE genotype*	*Braak stage*	*Thal phase*
ADH1	AD	80	M	4	3//3	VI	5
ADH2	AD	80	M	0	3//3	V	4
ADH3	AD	89	F	0	3//4	V	5
ADH4	AD	85	F	2	3//3	V	5
ADH5	AD	78	F	0	2//3	IV-V	4
ADH6	AD	82	F	20,7	3//4	III	4
HCH1	HC	81	M	28	2//3	0	0
HCH2	HC	71	F	30	3//3	I+	0
HCH3	HC	79	M	25,3	3//3	I	1

Supplementary Table 2. Clinical parameters of Golgi Cenci Foundation recruited subjects. ID = sample identification number (ADH = Alzheimer's Disease Hippocampus; HCH = Healthy Control Hippocampus). Dx = diagnosis (AD = Alzheimer's Disease; HC = Healthy Control). F = female. M = male. MMSE = Mini-Mental State Examination.

Methylation analysis of single CpG sites in the two regions comprised in APOE 5'UTR of both PBMCs and HIC brain tissue (Figure 1)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
1A	1^{CpG1} AD vs HC	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	1^{CpG2} AD vs HC		NS	>0,9999
	1^{CpG3} AD vs HC		NS	>0,9999
	1^{CpG4} AD vs HC		NS	>0,9999
	1^{CpG5} AD vs HC		NS	>0,9999
1B	1^{CpG1} AD vs HC	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	1^{CpG2} AD vs HC		NS	>0,9999
	1^{CpG3} AD vs HC		NS	>0,9999
	1^{CpG4} AD vs HC		NS	>0,9999
	1^{CpG5} AD vs HC		NS	>0,9999
1C	2^{CpG1} AD vs HC	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	2^{CpG2} AD vs HC		NS	>0,9999
	2^{CpG3} AD vs HC		NS	>0,9999
	2^{CpG4} AD vs HC		NS	>0,9999
	2^{CpG5} AD vs HC		NS	>0,9999
	2^{CpG6} AD vs HC		NS	>0,9999
1D	2^{CpG1} AD vs HC	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	2^{CpG2} AD vs HC		NS	>0,9999
	2^{CpG3} AD vs HC		NS	>0,9999
	2^{CpG4} AD vs HC		NS	>0,9999
	2^{CpG5} AD vs HC		NS	>0,9999
	2^{CpG6} AD vs HC		NS	>0,9999

Supplementary Table 3. Details on the statistical analysis with relative p-values. AD = Alzheimer's disease; HC = Healthy Control; $CpG^{\#}$ = CpG number #; NS = not significant.

SUPPLEMENTARY DATA

Average methylation level of the two regions comprised in APOE 5'UTR of both PBMCs and HIC brain tissue (Figure 2)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
2A	AD vs HC	Student's t Mann-Whitney test	NS	0,1889
2B	AD vs HC	Student's t Mann-Whitney test	NS	>0,9999
2C	AD vs HC	Student's t Mann-Whitney test	NS	0,5506
2D	AD vs HC	Student's t Mann-Whitney test	NS	0,0833

Supplementary Table 4. Details on the statistical analysis with relative p-values.
AD = Alzheimer's disease; HC = Healthy Control; NS = not significant.

APOE ε4 allele effect on average methylation level of the two regions in PBMCs (Figure 3)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
3A	AD $\varepsilon4^{-/-}$ vs AD $\varepsilon4^{-/+}$	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	AD $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/-}$		NS	>0,9999
	AD $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/+}$		NS	>0,9999
	AD $\varepsilon4^{-/+}$ vs HC $\varepsilon4^{-/-}$		NS	0,7518
	AD $\varepsilon4^{-/+}$ vs HC $\varepsilon4^{-/+}$		NS	0,7565
	HC $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/+}$		NS	>0,9999
3B	AD $\varepsilon4^{-/-}$ vs AD $\varepsilon4^{-/+}$	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	AD $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/-}$		NS	>0,9999
	AD $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/+}$		NS	>0,9999
	AD $\varepsilon4^{-/+}$ vs HC $\varepsilon4^{-/-}$		NS	>0,9999
	AD $\varepsilon4^{-/+}$ vs HC $\varepsilon4^{-/+}$		NS	>0,9999
	HC $\varepsilon4^{-/-}$ vs HC $\varepsilon4^{-/+}$		NS	>0,9999

Supplementary Table 5. Details on the statistical analysis with relative p-values.
AD = Alzheimer's disease; HC = Healthy Control; NS = not significant.

SUPPLEMENTARY DATA

APOE ε4$^{-/+}$ genotype effect on methylation level of single CpG sites in APOE 5'UTR in PBMCs (Supplementary Figure 1)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
S1A	1^{CpG1} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	1^{CpG2} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	1^{CpG3} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	1^{CpG4} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	1^{CpG5} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
S1B	2^{CpG1} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	2^{CpG2} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	2^{CpG3} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	2^{CpG4} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	2^{CpG5} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999
	2^{CpG6} AD ε4$^{-/+}$ vs HC ε4$^{-/+}$		NS	>0,9999

Supplementary Table 6. Details on the statistical analysis with relative p-values.
AD = Alzheimer's disease; HC = Healthy Control; CpG$^{\#}$ = CpG number #; NS = not significant.

Sex effect on the average methylation level of the two regions comprised in APOE 5'UTR of both PBMCs and HIC brain tissue (Supplementary Figure 2)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
S2A	AD (M) vs HC (M)	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	AD (M) vs AD (F)		NS	>0,9999
	AD (M) vs HC (F)		NS	>0,9999
	HC (M) vs AD (F)		NS	0,4191
	HC (M) vs HC (F)		NS	>0,9999
	AD (F) vs HC (F)		NS	>0,9999
S2B	AD (M) vs HC (M)	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	AD (M) vs AD (F)		NS	>0,9999
	AD (M) vs HC (F)		NS	>0,9999
	HC (M) vs AD (F)		NS	>0,9999
	HC (M) vs HC (F)		NS	>0,9999
	AD (F) vs HC (F)		NS	>0,9999
S2C	AD (M) vs HC (M)	One-way ANOVA Kruskal-Wallis test	NS	>0,9999
	AD (M) vs AD (F)		NS	>0,9999
	AD (M) vs HC (F)		NS	0,3312
	HC (M) vs AD (F)		NS	>0,9999
	HC (M) vs HC (F)		NS	0,7159
	AD (F) vs HC (F)		NS	>0,9999
S2D	AD (M) vs HC (M)	One-way ANOVA Kruskal-Wallis test	NS	0,4893
	AD (M) vs AD (F)		NS	>0,9999
	AD (M) vs HC (F)		NS	>0,9999
	HC (M) vs AD (F)		NS	0,6052
	HC (M) vs HC (F)		NS	>0,9999
	AD (F) vs HC (F)		NS	>0,9999

Supplementary Table 7. Details on the statistical analysis with relative p-values.
AD = Alzheimer's disease; HC = Healthy Control; NS = not significant.

SUPPLEMENTARY DATA

APOE expression analysis by Real Time PCR in HIC brain tissue (Supplementary Figure 3)

Figure	*Comparison*	*Statistical test*	*Significance*	*Adjusted p-value*
S3	AD vs HC	Student's t Mann-Whitney test	NS	0,7143

Supplementary Table 8. Details on the statistical analysis with relative p-values.
AD = Alzheimer's disease; HC = Healthy Control; NS = not significant.