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Supplementary Data

Replication of the *MTHFD1 L* Gene Association with Late-Onset Alzheimer's Disease in a Northern Han Chinese Population

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GENOTYPING

Genomic DNA was isolated from peripheral blood leukocytes from by using the Wizard Genomic DNA Purification Kit (Promega, USA) following the supplier's instructions. The primer sequences used for the PCR reaction were: rs11754661, forward: TCAGGTAGGATGCTCCTTGAGAAA, reverse: TGAAACTGGAAGCATATTTTACTGTGG; rs2073067, forward: CTGGATTGCCGCATTTTAC CTT, reverse: CGGCTTACCTGGATAATTGCAA GA. The PCR reaction mixture (10 μl) contained 1× GC-I buffer (Takara.), 3.0 mM Mg2+, 0.3 mM dNTP, 1 U HotStarTaq polymerase (Qiagen Inc.), 1 μl of sample DNA and 1 μl of each primer. PCR amplification was performed under the following conditions: 95°C for 2 min and 11 cycle at 94°C for 20 s, 65°C for 40 s, 72°C for 1.5 min, and 24 cycles at 94°C for 20 s, 59°C for 30 s and 72°C for 1.5 min, and a final extension at 72°C for 2 min. PCR products were treated with 1U of Shrimp Alkaline Phosphatase and 1U of Exonuclease I to degrade excess dNTPs and primers.

Two allele-specific probes and one a fluorescently labeled probe for the LDR reaction are shown supplemental Table 3. LDR reaction was carried out in 1 μl of 10× binding buffer, 0.25 μl of thermostable Taq DNA ligase, 0.4 μl of 1 μM 5′ ligation primers mixture, 0.4 μl of 2 μM, 3'ligation primers mixture, 2 μl of multiplex PCR product, 6 μl of double distilled H_2O. The reaction mixtures were subjected to 38 cycles of 94°C for 1 min and 58°C for 4 min. and then 4°C. Half microliter of the reaction mixtures were denatured at 95°C for 5 min in 9 μl Hi-Di formamide along with 0.5 μl of the LIZ-500 size standard, and run on the ABI3130XL genetic analyzer. Data analysis was achieved using GeneMapper Software v4.0 (AppliedBiosystems, USA).

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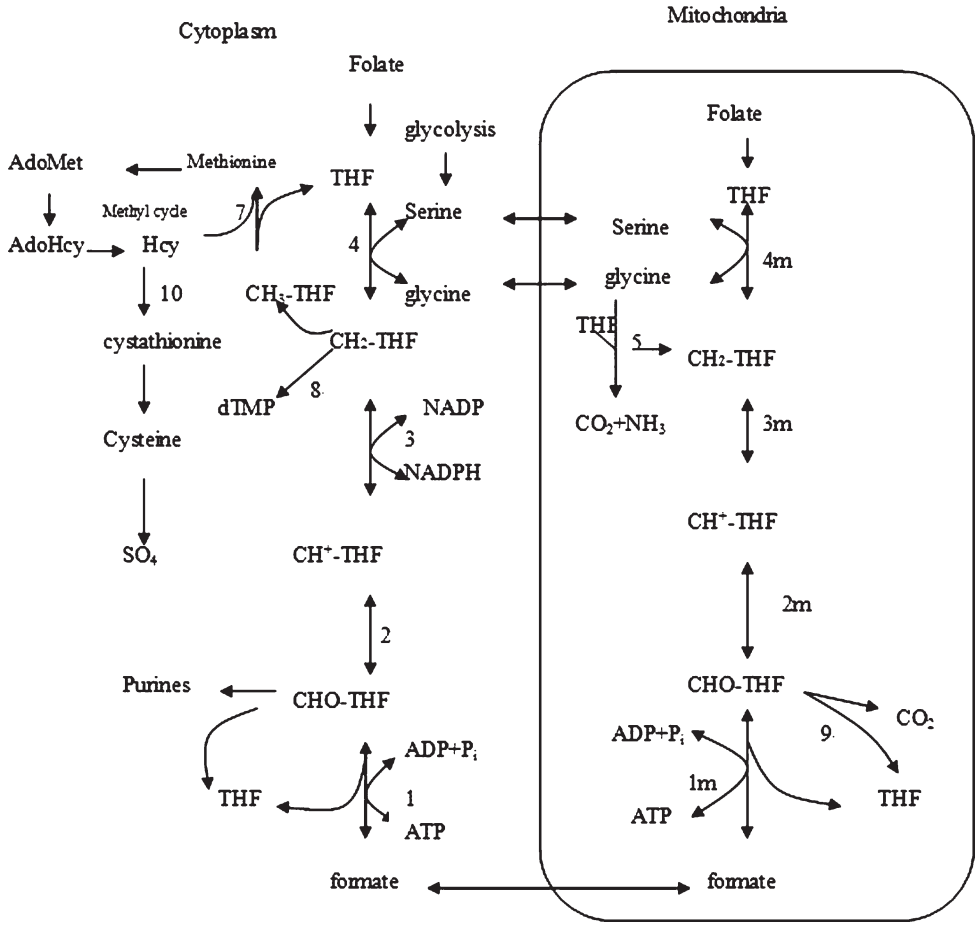

Supplementary Figure 1. Homocysteine and one-carbon metabolism. Reactions 1–3 are catalyzed by trifunctional C1-THF synthase. In mitochondria (m), reaction 1 m catalyzed by mono-functional C1-THF synthase (*MTHFD1L)*, and reactions 2 m and 3 m are catalyzed by bifunctional C1-THF synthase. The other reactions are catalyzed as follows: serine hydroxymethyltransferase (reactions 4 and 4 m), glycine cleavage system (reaction 5), 5, 10-methylene-THF reductase (reaction 6), methionine synthase (reaction 7), thymidylate synthase (reaction 8), and 10-formyl-THF dehydrogenase (reaction 9), cystathioninebeta-synthase (reaction 10). Hcy, homocysteine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine.

Supplementary Table 1
The distribution of the *MTHFD1 L* polymorphisms in Cases and Controls after stratification by gender

		Genotype				Allele		
rs11754661	*n*	AA (%)	AG (%)	GG (%)	*p*	A (%)	G (%)	*p*
Female								
Cases	298	2 (0.7)	34 (11.4)	262 (87.9)	0.011#,*	38 (6.4)	558 (93.6)	0.004*
Controls	284	0 (0.0)	16 (5.6)	268 (94.2)		16 (2.8)	552 (97.2)	
Male								
Cases	284	0	14 (4.9)	270 (95.1)	0.989	14 (2.5)	554 (97.5)	0.989
Controls	323	0	16 (5.0)	307 (95.0)		16 (2.5)	630 (97.5)	
rs2073067		GG (%)	GC (%)	CC (%)		G (%)	C (%)	
Female								
Cases	298	8 (2.7)	105 (35.2)	185 (62.1)	0.736	121 (20.3)	475 (79.7)	0.582
Controls	284	5 (1.8)	98 (34.5)	181 (63.7)		108 (19.0)	460 (81.0)	
Male								
Cases	284	9 (3.2)	84 (29.6)	191 (67.3)	0.3	102 (18.0)	466 (82.0)	0.121
Controls	323	15 (4.6)	109 (33.7)	199 (61.6)		139 (21.5)	507 (78.5)	

The frequencies of genotype and allele were compared between groups by using the Pearson's χ^2 tests, with the exception of those marked with #, which were determined by the Fisher's exact test; *Results remain significant as compared to controls.

Supplementary Table 2
Meta-analysis based on all available published data on the SNP rs11754661 in LOAD

Study	*n*		MAF		Association test	
	Cases	Controls	Cases	Controls	OR (95% CI)	*p* value
Naj et al. [1]	931	1103	0.07	0.05	2.10 (1.67 2.64)	1.90×10^{-10}
Ramírez-Lorc et al. [2]	1140	1327	0.07	0.07	1.04 (0.81 1.30)	0.69
Ren et al. [3]	191	189	0.26	0.16	1.83 (1.28 2.62)	1.00×10^{-3}
Present study	582	607	0.05	0.03	1.73 (1.10 2.70)	0.02
Meta-analysis	2844	3226			1.32 (1.15 1.52)	1.48×10^{-5}

OR: odds ratio; 95% CI: 95% confidence interval; MAF: minor allele frequency.

Supplementary Table 3
Sequence of probes in LDR reaction

Probes	Sequence for LDR
rs2073067RG	TTCCGCGTTCGGACTGATATGAGTAGAAAGGACTGACACAACACATTTTGTG
rs2073067RC	TACGGTTATTCGGGCTCCTGTGAGTAGAAAGGACTGACACAACACATTTTGTC
rs2073067RP	TGAAAGTTCCTTCCTCCAAGTGAA
rs11754661RG	TCTCTCGGGTCAATTCGTCCTTGATGGTCTTACTAGAAAAAGTTTCTAGGTATTCCTC
rs11754661RA	TGTTCGTGGGCCGGATTAGTGATGGTCTTACTAGAAAAAGTTTCTAGGTATTCCTT
rs11754661RP	TGTATCTCCCAAAAGWTGAGAAAAAKAAAATTTTTTTT

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