

Supplementary Material

Table S1. Primer sequences, restriction-product characteristics and PCR conditions

Gene variants	Oligo sequence	Amplicon size (bp)	Restriction enzyme	Allele detected (bp)	PCR thermal profile
<i>DHFR ins/del</i> (rs70991108)					
Fw	5'-CCACGGTTCGGGGTACCTGGG-3'	113/94	--	(W) 113 (D) 94	94°C/5'; 94°C/55", 60°C/55", 72°C/25"; 72°C/5' (35 cycles)
Fw	5'-ACGGTCGGGGTGGCCGACTC-3'				
Rv	5'-AAAAGGGGAATCCAGTCGG-3'				
Fw Py	5'-CAAGAACGGGGACCTGCC-3'	133/114	--	--	94°C/5'; 94°C/30", 60°C/30", 72°C/30"; 72°C/5' (35 cycles)
Rv Py	5'-ATCCTCTCGCCGGGAGTC-3' [Bio]				
Sq	5'-GCCCACGGTTCGGGGT-3'				
<i>MTHFR C677T</i> (rs1801133)					
Fw Py	5'-ATCCCTATTGGCAGGTTACCC-3'	121	--	--	94°C/5'; 94°C/30", 54°C/30", 72°C/60"; 72°C/5' (35 cycles)
Rv Py	5'-AAAGAAAAGCTGCGTGATGATGA-3' [Bio]				
Sq	5'-GAAGGTGTCTGCGGG-3'				
Fw Rst	5'-CGAAGCAGGGAGCTTTGAG <u>T</u> C-3'	233	<i>Hinfl</i> (37°C)	(C) 216+17 (T) 176+40+17	94°C/5'; 94°C/45", 65°C/60", 72°C/90"; 72°C/5' (30 cycles)
Rv Rst	5'-AGGACGGTGCGGTGAGAGTG-3'				
<i>MTHFR A1298C</i> (rs1801131)					
Fw Py	5'-CTGCCCTCTGTCAGGAGTGT-3'	222	--	--	94°C/5'; 94°C/30", 57°C/15", 72°C/45"; 72°C/5' (35 cycles)
Rv Py	5'-CATCCGGTTTGGTTCTCC-3' [Bio]				
Sq	5'-GGAGCTGACCAGTGAA-3'				
Fw Rst	5'-GGGAGGAGCTGACCAGT <u>G</u> CAG-3'	138	<i>Fnu4HI</i> (37°C)	(A) 138 (C) 19+119	94°C/5'; 94°C/30", 59°C/13", 72°C/17"; 72°C/5' (30 cycles)
Rv Rst	5'-GGGGTCAGGCCAGGGGCAG-3'				

Fw and Rv, indicate the forward and reverse primer respectively; the underlined bold letter in the oligo sequence indicates the mutagenized forward primer; Sq, indicates the specific sequencing primer; Rst and Py, indicate restriction and Pyrosequencing technique respectively; [Bio], indicates the biotinylated primer; W and D, indicate the common and rare (polymorphic) *DHFR* allele respectively; C and T, indicate the common and rare (polymorphic) *MTHFR* 677 allele respectively; A and C, indicate the common and rare (polymorphic) *MTHFR* 1298 allele respectively

Table S2. Age of ALL onset according to different combinations of children and mothers genotype

Genotype Groups (n=169)	DHFR ins/del (children)			P ^a P ^b	Genotype Groups (n=169)	MTHFR 677 (children)			P ^a P ^b	Genotype Groups (n=169)	MTHFR 1298 (children)			P ^a P ^b			
	WW mean±SD median (n) range	WD mean±SD median (n) range	DD mean±SD median (n) range			CC mean±SD median (n) range	CT mean±SD median (n) range	TT mean±SD median (n) range			AA mean±SD median (n) range	AC mean±SD median (n) range	CC mean±SD median (n) range				
Onset in children with that genotype	4.8±3.6 3.78 (69) 0.003-16.6	6.4±4.5 4.82 (78) 0.04-16.8	7.2±4.7 5.44 (22) 2.1-16.9	0.007 0.002	Onset in children with that genotype	5.8±4.3 4.10 (50) 0.7-16.7	6.2±4.7 4.04 (82) 0.003-16.9	5.2±2.8 4.4 (37) 0.8-11.6	ns	Onset in children with that genotype	5.79±4.21 4.07 (71) 0.77-16.92	5.76±4.25 4.21 (85) 0.003-16.82	7.05±4.64 4.09 (13) 1.85-15.13	ns ns			
Mothers (n=169)					Mothers (n=169)					Mothers (n=169)							
DHFR ins/del	WW (n=62)	5.2±3.8 3.92 (42) 1.3-16.6	7.3±5.5 4.18 (20) 6.1-16.5	-	0.04	MTHFR 677	CC (n=46)	6.8±4.9 4.47 (26) 1.9-16.8	7.2±5.9 3.72 (20) 0.2-16.9	-	ns	DHFR ins/del	WW (n=62)	5.38±4.17 3.76 (28) 1.32-16.64	6.07±4.66 4.10 (28) 0.66-16.5	7.25±5.57 5.41 (6) 1.85-15.13	ns ns
	WD (n=81)	4.2±3.1 3.78 (27) 0.003-15.9	6.4±4.2 5.49 (41) 0.07-16.8	7.8±4.9 5.68 (13) 3.2-16.9	0.005 0.004		CT (n=89)	4.8±3.3 3.24 (24) 0.7-12.4	5.9±4.6 4.05 (46) 0.07-16.8	5.1±2.6 5.52 (19) 1.5-11.6	ns		WD (n=81)	6.28±4.22 4.24 (32) 2.37-16.92	5.53±4.04 4.51 (43) 0.003-16.82	6.54±4.43 4.05 (6) 3.15-12.43	ns ns
	DD (n=26)	-	5.5±4.2 3.40 (17) 0.4-15.1	6.4±4.6 5.21 (9) 2.1-14.3	ns		TT (n=34)	-	5.6±3.6 4.39 (16) 0.003-11.7	5.3±3.1 4.05 (18) 0.8-11.1	ns		DD (n=26)	5.4±4.5 3.4 (11) 0.77-15.1	5.84±4.27 3.56 (14) 2.03-14.28	8.94 (1)	-
MTHFR 677	CC (n=46)	6.05±4.6 4.15 (20) 0.17-15.9	7.15±5.4 4.66 (21) 1.3-16.5	10.1±6.7 10.3 (5) 3.1-16.9	0.061 0.08	DHFR ins/del	WW (n=62)	5.9±4.8 3.68 (22) 0.7-16.5	6.3±5.1 3.75 (27) 1.3-16.6	5.0±2.4 6.06 (13) 1.4-9.5	ns	MTHFR 677	CC (n=46)	8.72±5.64 7.86 (14) 1.82-16.91	5.97±4.96 3.77 (26) 0.17-16.5	7.37±5.46 5.84 (6) 1.85-15.13	ns ns
	CT (n=89)	4.17±2.9 3.36 (34) 1.3-16.6	6.3±4.4 5.09 (41) 0.07-16.8	6.1±3.8 4.79 (14) 2.1-16.3	0.02 0.007		WD (n=81)	6.0±3.9 4.77 (22) 2.3-16.7	6.0±4.7 4.04 (42) 0.003-16.9	5.6±2.8 4.4 (17) 2.8-11.1	ns		CT (n=89)	4.91±3.76 3.54 (34) 1.32-16.65	5.64±4.01 4.38 (48) 0.068-16.82	6.76±4.25 4.01 (7) 3.16-12.44	ns ns
	TT (n=34)	4.6±3.0 3.78 (15) 0.003-11.6	5.8±3.4 4.3 (16) 0.8-11.7	7.6±3.6 7.7 (3) 3.7-11.1	0.07 ns		DD (n=26)	5.0±3.8 2.72 (6) 2.14-10.7	6.8±4.7 5.86 (13) 2.0-15.1	4.5±3.8 3.05 (7) 0.8-11.6	ns		TT (n=34)	5.99±3.08 4.07 (23) 0.77-11.64	5.78±3.81 4.70 (11) 0.003-11.67	-	-
MTHFR 1298	AA (n=88)	4.79±3.6 3.7 (38) 0.003-16.6	6.0±3.8 5.2 (36) 0.77-15.1	8.4±4.8 6.7 (14) 3.05-16.9	0.0029 0.0166	MTHFR 1298	AA (n=88)	6.7±4.2 5.1 (18) 2.3-16.7	6.2±4.7 3.9 (43) 0.003-16.9	4.75±2.5 4.1 (27) 0.77-11.1	0.03 0.04	MTHFR 1298	AA (n=88)	5.9±4.1 4.5 (53) 0.77-16.9	5.8±4.0 4.2 (35) 0.003-14.7	-	-
	AC (n=67)	4.27±2.8 3.7 (26) 0.17-14.65	7.05±5.1 5.1 (35) 0.07-16.8	5.8±4.1 4.6 (6) 2.1-13.1	ns 0.009		AC (n=67)	4.8±4.1 3.2 (24) 0.66-16.5	6.46±4.8 4.51 (33) 0.07-16.8	6.4±3.2 6.4 (10) 1.3-11.6	ns ns		AC (n=67)	5.5±4.5 3.3 (18) 1.8-15.1	5.9±4.4 4.4 (40) 0.07-16.8	6.33±4.58 4.01 (9) 1.85-12.65	ns ns
	CC (n=14)	7.9±5.1 7.5 (5) 2.3-15.9	5.5±4.9 2.9 (7) 1.23-15.1	2.3±0.2 2.3 (2) 2.7-3.1	ns ns		CC (n=14)	6.74±4.3 6.1 (8) 2.7-15.1	4.98±5.4 2.99 (6) 1.29-15.9	-	-		CC (n=14)	-	4.9±4.4 2.99 (10) 1.29-15.93	6.65±5.03 8.26 (4) 2.93-15.13	ns ns

P^a, indicates comparisons performed between opposite genotype conditions (i.e. DHFR WW versus DD; MTHFR 677 CC versus TT and MTHFR 1298 AA versus CC); P^b, indicates comparisons performed between DHFR D-allele carriers versus WW-homozygotes (dominant model) and MTHFR 677 TT- or MTHFR 1298 CC-homozygotes versus the remaining genotypes (recessive model).

Table S3. *DHFR/MTHFR* genotype distribution stratified by ALL onset-age quartiles

Age of onset quartiles		1 st	2 nd	3 rd	4 th	2 nd + 3 rd + 4 th	
Genotype		% (n)	% (n)	% (n)	% (n)	% (n)	
Children whole cohort (n=235)	<i>DHFR</i> ins/del	WW	49.2 (29)	49.6 (23)	40.7 (24)	25.4 (15)	35.2 (62)
		WD	42.4 (25)	33.1 (25)	47.4 (28)	50.9 (30)	47.2 (83)
		DD	8.4 (5)	17.3 (10)	11.9 (7)	23.7 (14)	17.6 (31)
	<i>MTHFR</i> 677	CC	39.0 (23)	31.0 (18)	30.5 (18)	44.1 (26)	35.2 (62)
		CT	47.4 (28)	43.1 (25)	42.4 (25)	44.1 (26)	43.2 (76)
		TT	13.6 (8)	25.9 (15)	27.1 (16)	11.8 (7)	21.6 (38)
	<i>MTHFR</i> 1298	AA	44.1 (26)	43.1 (25)	42.4 (25)	35.6 (21)	40.3 (71)
		AC	50.8 (30)	41.4 (24)	52.5 (31)	42.4 (25)	45.5 (80)
		CC	5.1 (3)	15.5 (9)	5.1 (3)	22.0 (13)	14.2 (25)
Children dyads (n=169)	<i>DHFR</i> ins/del	WW	50.0 (21)	45.2 (19)	40.5 (17)	27.9 (12)	37.8 (48)
		WD	45.2 (19)	38.1 (16)	45.2 (19)	55.8 (24)	46.5 (59)
		DD	4.8 (2)	16.7 (7)	14.3 (6)	16.3 (7)	15.7 (20)
	<i>MTHFR</i> 677	CC	33.3 (14)	23.8 (10)	31.0 (13)	30.2 (13)	28.4 (36)
		CT	50.0 (21)	52.4 (22)	35.7 (15)	55.8 (24)	48.0 (61)
		TT	16.7 (7)	23.8 (10)	33.3 (14)	14.0 (6)	23.6 (30)
	<i>MTHFR</i> 1298	AA	42.9 (18)	45.2 (19)	40.5 (17)	39.5 (17)	41.7 (53)
		AC	54.8 (23)	42.9 (18)	54.8 (23)	48.8 (21)	48.8 (62)
		CC	2.3 (1)	11.9 (5)	4.7 (2)	11.7 (5)	9.5 (12)
Mothers (n=169)	<i>DHFR</i> ins/del	WW	40.5 (17)	35.7 (15)	31.0 (13)	39.5 (17)	35.4 (45)
		WD	35.7 (15)	54.8 (23)	54.8 (23)	46.5 (20)	52.0 (66)
		DD	23.8 (10)	9.5 (4)	14.2 (6)	14.0 (6)	12.6 (16)
	<i>MTHFR</i> 677	CC	23.8 (10)	33.3 (14)	21.4 (9)	37.2 (16)	30.7 (39)
		CT	61.9 (26)	45.3 (19)	61.9 (26)	41.9 (18)	49.6 (63)
		TT	14.3 (6)	21.4 (9)	16.7 (7)	20.9 (9)	19.7 (25)
	<i>MTHFR</i> 1298	AA	50.0 (21)	50.0 (21)	57.1 (24)	51.2 (22)	52.8 (67)
		AC	42.9 (18)	38.1 (16)	38.1 (16)	39.5 (17)	38.6 (49)
		CC	7.1 (3)	11.9 (5)	4.8 (2)	9.3 (4)	8.6 (11)

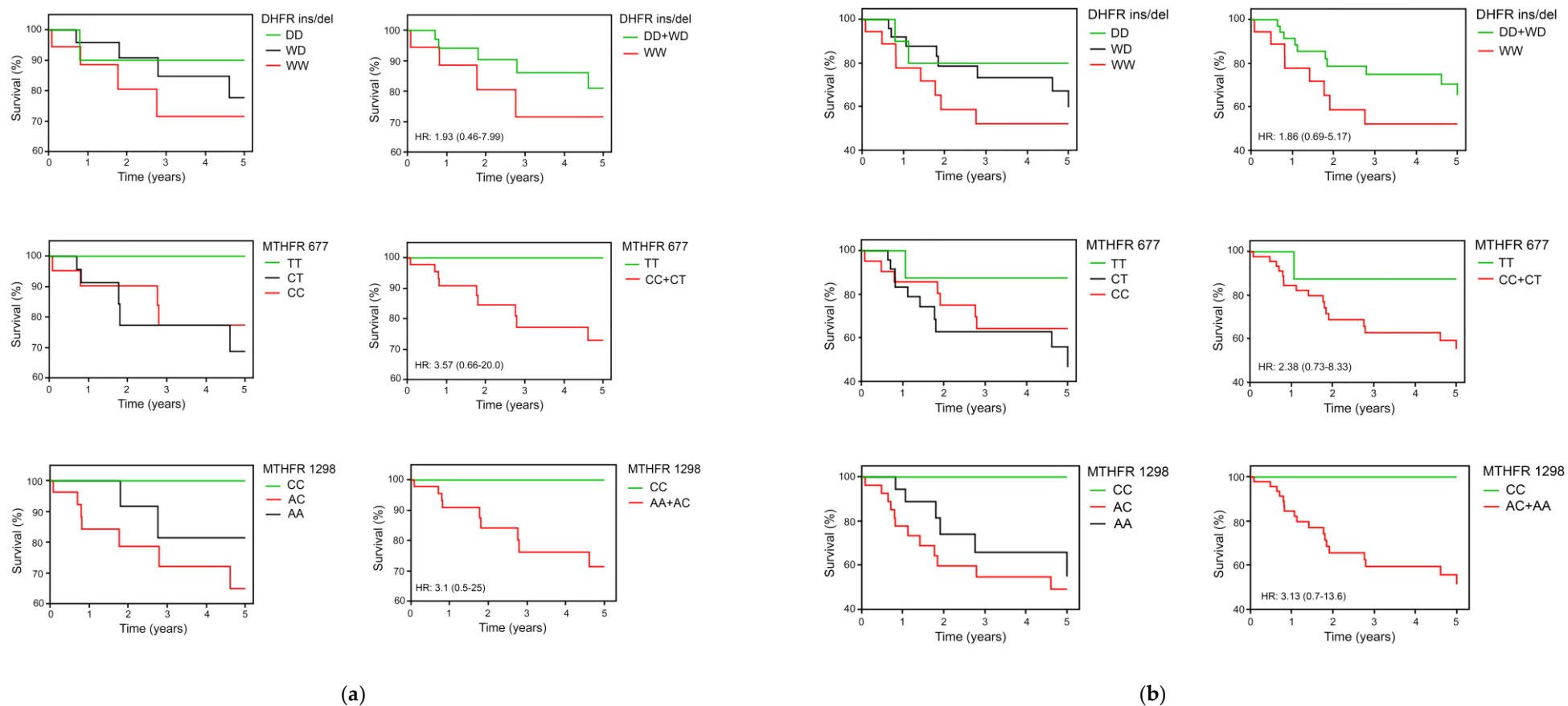


Figure S1. Event-free survival (EFS) among high-risk patients at 5-years survey in patients according to different *DHFR*/*MTHFR* variants. EFS curves are provided for each genotype of indicated gene variants. In **A**, EFS according to *DHFR* (**upper panels**) and *MTHFR* (**lower panels**). In **B**, EFS including prednisone response according to *DHFR* (**upper panels**) and *MTHFR* (**lower panels**). Associated HR with 95% CI are indicated. EFS: Event-free survival; HR: Hazard risk.