

3'-UTR polymorphisms of *MTHFR* and *TS* contribute to osteoporotic vertebral compression fracture susceptibility in postmenopausal women

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Introduction



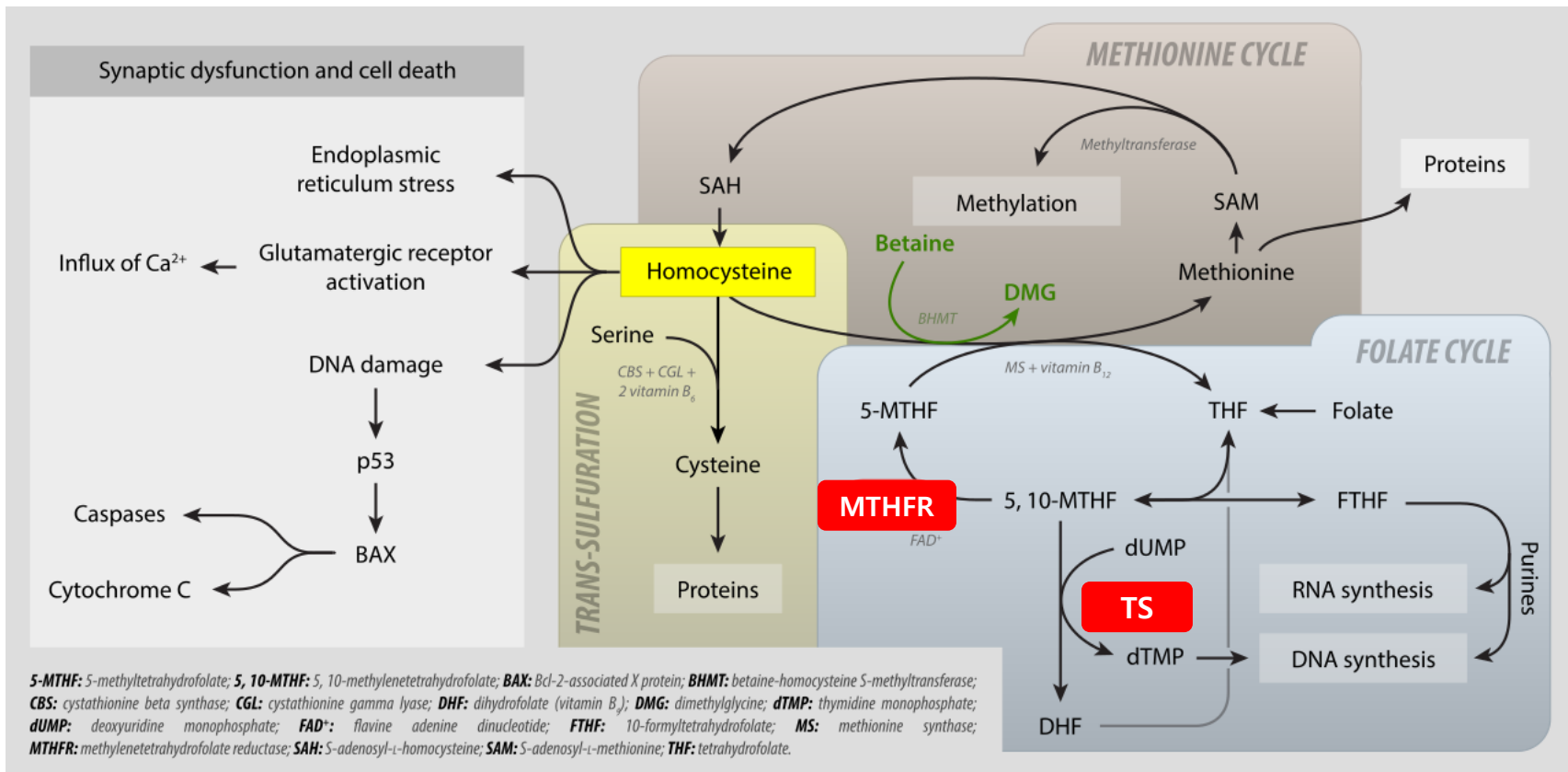
- **Osteoporosis**
 - Systemic skeletal disorder characterized by low bone mass and a deterioration of bone structure
 - Associated with bone fragility and increased fracture risk
 - Various susceptible genes are contributed to occurrence
- **Homocysteine (Hcy)**
 - Biosynthesized from methionine
 - An elevated plasma concentration of Hcy has been associated with an increased risk of osteoporotic fractures
 - Some *in vivo* studies : Hcy stimulates osteoclast differentiation and induces apoptosis of osteoblast-lineage cells

Introduction



- **Genes associated with Hcy/folate metabolism**

- Methylene tetrahydrofolate reductase (MTHFR) gene and thymidylate synthase (TS) gene



- **Previous studies about single nucleotide polymorphism (SNP) on osteoporosis**
 - Most genetic variants associated with osteoporosis are related to inflammation, extracellular matrix mechanisms, and vitamin D metabolism
 - SNPs in the 3'-UTR (untranslated region) can alter gene expression by microRNAs (miRNAs), which attenuate or degrade the transcription of the target messenger RNAs
 - Studies on the relationship between Hcy/folate metabolism and gene mutations are still insufficient
 - MTHFR gene only include 677C>T SNP studies (*Steer et al. 2009, Yang et al. 2008, Abrahamsen et al. 2005*) and TS genes have not been studied

Introduction



- **Purpose of the study**

- Four SNPs in MTHFR and TS 3'-UTR were identified by a database search
 - **MTHFR 2572C>A (rs4846049) and 4869C>G (rs1537514), and TS 1100T>C (rs699517) and 1170A>G (rs2790)**
- We investigated the genetic association between OVCF and the SNPs of MTHFR (rs4846049 and rs1537514) and TS (rs699517 and rs2790) genes in Korean postmenopausal women.

Material and Methods



- **Inclusion criteria**

- **301 postmenopausal Korean women**
- At least 50 years of age and had not menstruated for at least one year.
- **Diagnosis of osteoporosis (n=143)** : bone mineral density (BMD) < -2.5 SD
- **Osteoporotic vertebral compression fracture (OVCF, n=74)**
 - A progressive or newly generated compression fracture was identified after low-energy trauma

- **Exclusion criteria**

- Metabolic diseases such as diffuse idiopathic skeletal hyperostosis, pituitary diseases, hyperthyroidism, rheumatoid arthritis, or hyperparathyroidism
- Treatments associated with bone metabolism or blood coagulation
- No prior cancer diagnosis

Results



Baseline characteristics between controls and osteoporosis patients.

Characteristic	Controls (n=158)	Osteoporosis (n=143)	<i>P</i>	OVCF (n=74)	<i>P</i>	non-OVCF (n=69)	<i>P</i>
Age (years, mean ± SD)	69.36 ± 6.26	69.38 ± 7.25	0.983	70.23 ± 9.44	0.282	70.83 ± 6.53	0.207
Hypertension (%)	79	52	0.134	32	0.0007	20	<0.0001
SBP (mmHg, mean ± SD)	135.92±18.65	127.32±14.44	<0.0001	128.13±15.16	0.003	126.54±13.76	0.0002
DBP (mmHg, mean ± SD)	80.73±11.52	75.48±10.33	0.0001	76.57±10.38	0.012	74.42±10.24	0.0001
Diabetes mellitus (%)	22	26	0.392	9	0.05	17	0.645
FBS (mg/dL, mean ± SD)	111.78±28.12	123.56±43.20	0.006	132.56±54.01	0.0002	114.70±26.41	0.466
Hcy (μmol/L, mean ± SD)	9.74±3.04	9.83±4.09	0.83	10.11±3.84	0.441	9.54±4.35	0.69
Folate (ng/mL, mean ± SD)	9.51±6.48	8.28±4.87	0.074	6.57±4.11	0.0005	10.27±4.95	0.408
BMI (kg/m², mean ± SD)	24.52±3.11	23.48±3.81	0.045	21.37±6.98	0.005	23.88±2.77	0.173
HDL-chol (mg/dL, mean ± SD)	47.67±12.13	44.78±14.24	0.223	45.16±16.19	0.385	44.42±12.27	0.18
LDL-chol (mg/dL, mean ± SD)	130.30±44.64	107.31±39.55	0.002	120.64±42.64	0.281	95.18±32.38	<0.0001
TG (mg/dL, mean ± SD)	152.85±87.54	146.81±81.90	0.567	159.05±74.81	0.633	133.89±87.61	0.169
BMD (g/cm ² , mean ± SD)	-	-3.04±0.94	-	-2.93±1.21	-	-3.13±0.62	-

Genotype frequencies of *MTHFR* and *TS* genes polymorphisms in osteoporosis

Genotypes	Control (n=158)	Case (n=143)	AOR (95% CI) ¹	P	OVCF (n=74)	AOR (95% CI) ¹	P	non-OVCF (n=69)	AOR (95% CI) ¹	P
<i>MTHFR</i> 2572C>A										
CC	103 (65.2)	111 (77.6)	1.000 (reference)		62 (83.8)	1.000 (reference)		49 (71.0)	1.000 (reference)	
CA	52 (32.9)	29 (20.3)	0.494 (0.285 - 0.857)	0.012	11 (14.9)	0.382 (0.179-0.813)	0.013	18 (26.1)	0.682 (0.354-1.314)	0.253
AA	3 (1.9)	3 (2.1)	0.858 (0.163 - 4.512)	0.856	1 (1.4)	0.665 (0.067-6.590)	0.728	2 (2.9)	1.122 (0.160-7.862)	0.908
Dominant (CC vs CA+AA)			0.516 (0.303 - 0.880)	0.015		0.397 (0.191-0.825)	0.013		0.709 (0.375-1.340)	0.290
Recessive (CC+CA vs AA)			1.063 (0.207 - 5.470)	0.941		0.800 (0.081-7.868)	0.848		1.300 (0.196-8.645)	0.786
HWE-P	0.288	0.885								
<i>MTHFR</i> 4869C>G										
CC	135 (85.4)	132 (92.3)	1.000 (reference)		68 (91.9)	1.000 (reference)		64 (92.8)	1.000 (reference)	
CG	23 (14.6)	11 (7.7)	0.454 (0.201 - 1.026)	0.058	6 (8.1)	0.503 (0.181-1.395)	0.187	5 (7.2)	0.487 (0.173-1.367)	0.172
GG	0 (0.0)	0 (0.0)	N/A	N/A	0 (0.0)	N/A	N/A	0 (0.0)	N/A	N/A
Dominant (CC vs CG+GG)			0.454 (0.201 - 1.026)	0.058		0.503 (0.181-1.395)	0.187		0.487 (0.173-1.367)	0.172
Recessive (CC+CG vs GG)			N/A	N/A		N/A	N/A		N/A	N/A
HWE-P	0.324	0.632								
<i>TS</i> 1100C>T										
CC	92 (58.2)	95 (66.4)	1.000 (reference)		56 (75.7)	1.000 (reference)		39 (56.5)	1.000 (reference)	
CT	58 (36.7)	40 (28.0)	0.886 (0.314 - 2.497)	0.819	17 (23.0)	0.492 (0.252-0.961)	0.038	23 (33.3)	0.952 (0.503-1.804)	0.881
TT	8 (5.1)	8 (5.6)	0.625 (0.215 - 1.820)	0.389	1 (1.4)	0.249 (0.030-2.067)	0.198	7 (10.1)	2.417 (0.784-7.454)	0.125
Dominant (CC vs CT+TT)			0.781 (0.283 - 2.158)	0.634		0.462 (0.241-0.886)	0.020		1.114 (0.615-2.021)	0.721
Recessive (CC+CT vs TT)			1.355 (0.835 - 2.200)	0.219		0.303 (0.037-2.485)	0.266		2.433 (0.817-7.245)	0.110
HWE-P	0.497	0.280								
<i>TS</i> 1170A>G										
AA	64 (40.5)	104 (72.7)	1.000 (reference)		48 (64.9)	1.000 (reference)		56 (81.2)	1.000 (reference)	
AG	76 (48.1)	34 (23.8)	0.276 (0.165 - 0.460)	<0.0001	24 (32.4)	0.413 (0.221-0.774)	0.006	10 (14.5)	0.147 (0.068-0.320)	<0.0001
GG	18 (11.4)	5 (3.5)	0.168 (0.059 - 0.475)	0.001	2 (2.7)	0.086 (0.011-0.672)	0.019	3 (4.3)	0.137 (0.035-0.527)	0.004
Dominant (AA vs AG+GG)			0.255 (0.157 - 0.415)	<0.0001		0.354 (0.192-0.653)	0.001		0.146 (0.072-0.298)	<0.0001
Recessive (AA+AG vs GG)			0.281 (0.101 - 0.778)	0.015		0.129 (0.017-0.994)	0.049		0.275 (0.075-1.007)	0.051
HWE-P	0.520	0.267								

¹The adjusted odds ratio on the basis of risk factors, such as age, hypertension, diabetes mellitus.

Stratified effects of MTHFR and TS genes polymorphisms on osteoporosis risk

Variables	MTHFR 2572 CA + AA		MTHFR 4869 CG + GG		TS 1100 CT + CC		TS 1170 AG + GG	
	AOR(95% CI) ¹	P	AOR(95% CI) ¹	P	AOR(95% CI) ¹	P	AOR(95% CI) ¹	P
Hypertension								
No	0.417 (0.204 - 0.856)	0.017	0.426 (0.141 - 1.283)	0.129	0.620 (0.322 - 1.193)	0.152	0.238 (0.122 - 0.465)	<0.0001
Yes	0.773 (0.352 - 1.697)	0.521	0.649 (0.211 - 1.997)	0.451	1.042 (0.504 - 2.153)	0.912	0.252 (0.118 - 0.539)	0.0004
Diabetes mellitus								
No	0.597 (0.337 - 1.060)	0.078	0.567 (0.254 - 1.264)	0.165	0.724 (0.429 - 1.224)	0.228	0.262 (0.151 - 0.454)	<0.0001
Yes	0.381 (0.099 - 1.474)	0.162	N/A	N/A	1.169 (0.302 - 4.521)	0.821	0.142 (0.039 - 0.522)	0.003
Folate²								
>4.59 nmol/L	0.709 (0.404 - 1.245)	0.232	0.554 (0.240 - 1.280)	0.167	0.724 (0.426 - 1.231)	0.233	0.240 (0.138 - 0.417)	<0.0001
≤4.59nmol/L	0.057 (0.006 - 0.520)	0.011	N/A	N/A	1.339 (0.368 - 4.867)	0.658	0.204 (0.057 - 0.734)	0.015
Homocystein³								
≥12.68 μmol/L	0.306 (0.063 - 1.489)	0.143	1.052 (0.054 - 20.670)	0.973	0.852 (0.239 - 3.034)	0.805	0.278 (0.066 - 1.166)	0.08
<12.68 μmol/L	0.599 (0.338 - 1.062)	0.042	0.457 (0.193 - 1.082)	0.075	0.745 (0.439 - 1.265)	0.276	0.263 (0.155 - 0.446)	<0.0001
Age								
≥69	0.467 (0.231 - 0.945)	0.034	0.619 (0.210 - 1.820)	0.383	0.878 (0.449 - 1.717)	0.704	0.131 (0.064 - 0.269)	<0.0001
<69	0.669 (0.303 - 1.476)	0.319	0.407 (0.124 - 1.333)	0.138	0.646 (0.320 - 1.305)	0.224	0.509 (0.259 - 1.002)	0.051

Comparison of genotype frequencies of MTHFR and TS genes allele combination between the osteoporosis and control subjects

Haplotypes	Control (2n=316)	Case (2n=286)	OR (95% CI) ¹	P	OVCF (2n=148)	OR (95% CI) ¹	P	Non-OVCF (2n=138)	OR (95% CI) ¹	P
<i>MTHFR 2572/4869</i>										
C-C	258 (81.7)	216 (87.0)	1.000 (reference)		125 (84.2)	1.000 (reference)		116 (84.2)	1.000 (reference)	
A-C	35 (11.1)	23 (9.2)	0.785 (0.450-1.369)	0.393	18 (12.3)	1.061 (0.578-1.949)	0.876	17 (12.3)	1.080 (0.581-2.007)	0.873
A-G	23 (7.3)	8 (3.1)	0.416 (0.182-0.948)	0.032	5 (3.5)	0.449 (0.167-1.208)	0.140	5 (3.5)	0.484 (0.179-1.304)	0.199
<i>TS 1100/1170</i>										
C-A	74 (23.4)	49 (19.6)	1.000 (reference)		34 (22.8)	1.000 (reference)		31 (22.8)	1.000 (reference)	
T-A	130 (41.1)	161 (65.0)	1.870 (1.218-2.872)	0.004	95 (64.0)	1.578 (0.972-2.562)	0.072	88 (64.0)	1.604 (0.974-2.641)	0.066
T-G	112 (35.4)	38 (15.4)	0.512 (0.306-0.858)	0.011	19 (13.2)	0.373 (0.198-0.702)	0.003	18 (13.2)	0.387 (0.202-0.742)	0.006

Conclusion



- Our results suggest that the polymorphisms of MTHFR 2572C>A, TS 1100C>T, and TS 1170 A>G are significantly associated with a decreased risk of osteoporosis and/or OVCF.
- Our results suggest that the polymorphisms in the 3'-UTR of the MTHFR and TS genes have a significant association with osteoporosis and OVCF.
- Although these findings have a small impact on the complex pathogenesis for osteoporosis, the data described here could contribute to the pool of SNP variants needed for individual osteoporosis and OVCF risk assessment.

**I (and my co-authors) have
nothing to disclose.**