行政院國家科學委員會專題研究計畫 成果報告

老人骨質健康影響因素之探討—飲食及生活型態因子與維生素 D 營養狀況之影響(II)
研究成果報告(精簡版)

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中文摘要

本計畫乃前一年度之「老人骨質健康影響因素之探討—飲食及生活型態因子與維生素D營養狀況之影響」之延伸研究，以先前研究當中所取得之個案血液樣本和分離之DNA檢體進行四氫甲基葉酸還原酶 (methylenetetrahydrofolate reductase, MTHFR) 677 C→T之單核苷酸多型性及維生素D受器 (vitamin D receptor, VDR)三種限制酵素切除位置之基因型，探討上述之基因型與老人血清維生素D營養指標、副甲狀腺素濃度、骨質代謝指標以及超音波骨密度測量結果是否有關。分析發現，四種基因型中僅 VDR 之 *Apa*I 限制酶切除位置之基因型與血清蝕骨作用指標 ICTP 有關。VDR限制酵素切除位置之基因型與血清25(OH)D濃度間並無顯著統計關聯；而 MTHFR C677T 之基因多型性與骨代謝指標或超音波骨密度測量值間亦無顯著相關性。然而血漿中全同半胱胺酸(total homocysteine, tHcy)濃度與血清 ICTP 濃度在男性個案呈現顯著相關，而血中同半胱胺酸濃度乃一心血管疾病之危險指標，因此老年男性是否因葉酸營養狀況而影響同半胱胺酸之代謝及/或骨代謝速率值得進一步探討。

Study Results & Discussion

The current study was a continuation to our previous project in which we have collected blood samples, bone measurement by quantitative ultrasound, and questionnaire information in 202 subjects residing in Chu-Tung and Pu-Tze. The aliquots of blood and previously isolated DNA samples were analyzed to further explore if the vitamin D status, bone measurement and/or bone turnover markers may be related to genotypes and/or serum levels of parathyroid hormone or homocysteine.

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The distributions of *Apa*I, *Taq*I and *Bsm*I restriction sites of VDR genotypes and MTHFR C677T SNP genotype are shown in Table 1. The allele frequencies were marginally in Hardy-Weinberg equilibrium. The distributions of genotypes are similar to those previously reported in Chinese in the *Apa*I, *Taq*I, *Bsm*I restriction sites (Chen *et al*., 2001).

Table 2 shows the results of the anthropometric measurements as well as the biochemical indicators and BUA of the studied subjects in three VDR polymorphic types. Subjects in the three *VDR* genotype groups were similar in age, height, weight and BMI. The results of ANOVA and post-hoc comparison showed that there was a relationship of *Apa*I genotypes to serum ICTP (p=0.04) such that the group of heterozygous genotype Aa had ICTP levels

lower than the homozygous group of aa (p=0.05). There were also relationships of *Taq*I and *Bsm*I genotypes to serum intact parathyroid hormone (iPTH) (p=0.01 for both genotypes, respectively). The results of ANOVA and post-hoc comparison may be biased, however, as there was only one subject genotyped as tt and one subject genotyped as BB as well. Biochemical indicators other than serum iPTH and ICTP did not significantly differ in *Apa*I, *Taq*I and *Bsm*I genotypic subgroups.

Similar analyses were also performed to compare subjects according to their MTHFR C677T SNP polymorphism. As shown in Table 3, there were no significant differences in all parameters between the *MTHFR* genotypic groups. There appeared to be trends toward significant relationships of MTHFR genotypic groups to plasma total homocysteine (tHcy) and serum 25(OH)D, respectively (p=0.06 for both)

The Pearson correlation coefficients for bone indicators (BUA and serum bone markers), serum 25(OH)D and plasma tHcy and serum iPTH are shown in Tables 4 and 5, respectively. The only significant correlation was observed between serum ICTP and plasma tHcy (Table 4).

In general, the results of our analyses showed an association between the VDR genotypes at *ApaI* restriction site and serum level of ICTP. There were no significant associations between any of the genetic polymorphisms examined in our study and bone measurement of BUA. It is possible that the subjects were relatively homogenous in bone density measured by quantitative ultrasound, a less precise device of which the measurement is mostly used to predict fracture risk, that is unable to reflect the difference in bone density of different genotypic groups if there is any.

At present we are trying to put together the information obtained in the current study for a manuscript, which is expected to be submitted to an international journal in the next 1-2 months. For better understanding of the factors influencing the bone health status of the elderly, the data is also being further analyzed for the relationships between the biochemical indicators and the interaction effect of genotypes, and for the effects of interaction between lifestyle factors and genotypes on bone parameters. The results of the above analyses are expected to generate at least another two manuscripts.

References

Chen HY, Chen WC, Hsu CD, Tsai FJ, Tsai CH, Li CW. Relation of BsmI vitamin D receptor gene polymorphism to bone mineral density and occurrence of osteoporosis in postmenopausal Chinese women in Taiwan. Osteoporos Int 2001; 12:1036-1041.

TABLE1. Distribution of vitamin D receptor (VDR) and methylenetetrahydrofolate reductase (MTHFR) genotypes in subjects

Gene		Distribution of Genotypes (n & %)				
VDR						
ApaI	AA	14 (7.1%)	Aa	94 (47.9%)	Aa	88 (44.8%)
TaqI	TT	182 (92.8%)	Tt	13 (6.6%)	tt	1 (0.5%)
BsmI	BB	1 (0.5%)	Bb	15 (7.6%)	bb	180 (91.8%)
MTHFR	CC	95 (48.4%)	CT	92 (46.9%)	TT	9 (4.5%)

TABLE2. Characteristics of subjects by VDR genotypes[1,2]

	*Apa*I				*Taq*I				*Bsm*I			
Characteristic	AA (n=14)	Aa (n=94)	aa (n=88)	p	TT (n=182)	Tt (n=13)	tt (n=1)	p	BB (n=1)	Bb (n=15)	bb (n=180)	p
Age(y)	73.9±4.3	73.1±4.4	71.9±4.2	0.08	72.5±4.3	73.5±5.3	79.0	0.24	79.0	72.5±4.8	72.6±4.3	0.34
Area (1:2)[3]	6:8	47:47	47:41	0.73	93:89	7:6	0:1	0.58	0:1	8:7	92:88	0.58
Gender (M: F)	7:7	57:37	43:45	0.26	98:84	9:4	0:1	0.30	0:1	7:8	100:80	0.44
Height (cm)	157.1±9.1	158.8±6.9	157.7±7.8	0.52	158.3±7.4	157.9±8.4	146.0	0.26	146.0	155.6±7.0	158.5±7.5	0.09
Weight (kg)	63.1±14.6	62.1±9.8	61.4±10.1	0.79	61.9±10.4	62.1±9.5	52.5	0.66	52.5	58.3±10.1	62.2±10.3	0.24
BMI(Kg/㎡)	25.3±4.3	24.6±3.3	24.6±3.6	0.77	24.6±3.5	24.9±3.3	24.6	0.97	24.6	23.9±2.8	24.7±3.6	0.69
Serum iPTH(pg/mL)	12.5±9.1	12.5±7.2	12.3±7.2	0.98	12.4±7.1[a]	11.4±8.0[b]	32.9[ac]	0.01*	32.9[d]	13.7±6.2[e]	12.2±7.2[ef]	0.01*
Serum 25(0H)D (ng/mL)	19.1±7.5	19.9±8.1	20.0±8.0	0.92	19.9±8.0	20.1±8.4	16.5	0.90	16.5	19.1±6.8	20.0±8.1	0.84
Osteocalcin (ng/mL)	9.4±4.6	9.7±4.1	9.9±5.0	0.90	9.9±4.5	8.7±4.4	1.5	0.12	1.5	11.0±3.9	9.8±4.6	0.11
ICTP (ng/mL)	1.8±1.1	1.9±1.4[g]	2.5±1.6[h]	0.04*	2.2±1.5	2.0±1.0	0.3	0.42	0.3	2.1±0.9	2.2±1.5	0.44
BUA (dB/MHz)	61.6±14.3	62.8±18.2	61.6±17.3	0.89	61.7±17.4	68.6±19.7	66.0	0.37	66.0	62.7±18.4	62.1±17.5	0.96

[1] Values are mean± SD; * $p<0.05$

[2] AA, BB and TT denote homozygosity for the absence of the *Apa*I, *Bsm*I, and *Taq*I restriction sites, respectively; aa, bb and tt denote homozygosity for the presence of the *Apa*I, *Bsm*I and *Taq*I sites; and Aa, Bb and Tt denote heterozygosity

[3] Area: 1=Chu-Tung, 2=Pu-Tze

TABLE 3. Characteristic of subjects by methylenetetrahydrofolate reductase (MTHFR) C677T SNP genotype[1]

	MTHFR			
	CC (n=95)	CT (n=92)	TT (n=9)	*p*
Age(y)	72.5±4.1	72.4±4.6	75.4±4.4	0.13
Area (1:2)[2]	52:43	46:46	2:7	0.16
Gender (M: F)	55:40	47:45	5:4	0.64
Height (cm)	158.3±7.1	157.9±7.9	159.4±8.3	0.82
Weight (kg)	62.6±9.8	60.8±10.9	64.2±7.9	0.37
BMI(Kg/㎡)	24.9±3.4	24.3±3.7	25.3±2.6	0.40
Plasma tHcy (μmol/L)	13.6±4.2	14.4±3.9	16.5±4.5	0.06
Serum 25(0H) D (ng/mL)	21.2±8.2	18.6±7.7	18.4±7.3	0.06
Osteocalcin (ng/mL)	10.1±4.8	9.5±4.3	9.7±4.6	0.71
ICTP (ng/mL)	2.3±1.6	2.1±1.4	1.8±1.5	0.58
BUA (dB/MHz)	62.6±17.8	61.8±17.1	60.3±21.2	0.90

[1] Values are mean± SD

[2] Area: 1=Chu-Tung, 2=Pu-Tze

TABLE 4. Pearson correlation coefficients for bone indicators and plasma tHcy by sex

	Plasma tHcy(μmol/L)	
	male (n=107)	female(n=89)
Serum 25(0H) D (ng/mL)	-0.08	-0.09
Osteocalcin (ng/mL)	0.05	-0.08
ICTP (ng/mL)	0.34**	-0.07
BUA (dB/MHz)	0.09	0.10

** $p<0.01$

TABLE 5. Pearson correlation coefficients for bone indicators and serum iPTH by sex

	Serum iPTH (pg/mL)	
	male (n=107)	female(n=87)
Serum 25(0H) D (ng/mL)	0.03	0.09
Osteocalcin (ng/mL)	0.08	-0.02
ICTP (ng/mL)	-0.03	-0.16
BUA (dB/MHz)	0.10	0.07