Relationships among plasma folate, DNMT3A-448A>G polymorphism and urothelial carcinoma

Ping-Huan Tsai[1], Ssu-Ning Chien[1], Chao-Hsiang Chang[2,3], Chiu-Shong Liu[4], Chi-Jung Chung[1,5]

[1]Department of Health Risk Management, College of Public Health, China Medical University, Taichung, Taiwan
[2]Department of Urology, China Medical University Hospital, Taichung,Taiwan;
[3]Department of Medicine, College of Medicine, China Medical University Hospital ,Taichung, Taiwan
[4]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan
[5]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan

Objective

DNA methyltransferase (DNMT) 3A-448A>G polymorphism plays an important role in the development of embryogenesis and regulates the level of gene methylation in carcinogenesis. In addition, DNMT3 and folate are involved in the one-carbon metabolism pathway. In this study, we aim to evaluate the frequency of DNMT3A-448A>G genotype between urothelial carcinoma (UC) patients and healthy controls , and to explore the relationships among DNMT3A -448A>G, levels of plasma folate and the risk of UC.

Material and methods

We constructed a case-control study in China Medical University Hospital and recruited 168 UC patients and 332 healthy controls matched for age and gender. All study subjects completed the standard individual interview and collected the information of other UC-related risk factors. Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis was used to assess DNMT3A -448A>G gene polymorphism. Measurement of plasma folate was through competitive receptor-binding immunoassay. Chi-square test and multiple logistic regression were used to estimate the risks of UC.

Result

The distributions of -448A>G genotypes in 332 controls were GG 61.14%, AG 34.34%, AA 4.52%, and A allele frequency was 21.6%. Decreased risk of UC were observed in the individuals with increasing levels of plasma folic acid (p for trend = 0.0006). In addition, individuals with homogenous variant genotype of DNMT-448 and with high plasma folic acid were significantly 0.37-fold risk of UC (95%CI:0.20-0.66) compared to those with homogenous wild genotype and low plasma folic acid. There is a similar pattern for the combination of DNMT 3A genotype and daily vitamin intake. For cigarette smoking, individuals with homogenous variant genotype of DNMT-448 and with habits of smoking were significantly increased 3.14 -fold risk of UC (95%CI:1.61-6.13).

Conclusion

Our study shows that cigarette smoking, low plasma folic acid, low daily vitamin intake, and the polymorphism of DNMT3A-448 A>G would be important risk factors for increased UC risk.

Table1 Association between DNMT3A-448 gene polymorphism and UC

Variable		Control N=332 number（%）	Case N=168 number（%）	P-value[a]	OR[b] (95%CI)
DNMT3A -448 A>G	GG	203(61.14%)	114(67.86%)	0.305	1
	AG	114(34.34%)	49(29.17%)		0.76(0.51-1.14)
	AA	15(4.52%)	5(2.98%)		0.59(0.21-1.69)
	A allele	0.216	0.175		
DNMT3A -448 A>G	GG+AG	317(95.48%)	163(97.02%)	0.405	1
	AA	15(4.52%)	5(2.98%)		0.66(0.23-1.84)
DNMT3A -448 A>G	GG	203(61.14%)	114(67.86%)	0.141	1
	AG+AA	129(38.86%)	54(32.14%)		0.74(0.50-1.09)

a：Chi-square test
b：Adjust for age and sex

Table2 Association between folic acid and UC

Variable		Control N=332 number（%）	Case N=168 number（%）	P-value[c]	OR[b] (95%CI)
Folic Acid	(ng/ml)	12.04±7.85	13.88±16.3	0.237[a]	1.01(0.99-1.03)
Folic Acid					
	<3	8(2.41%)	10(5.95%)	0.044*	1
Folic Acid	≧3	324(97.59%)	158(94.05%)		0.38(0.148-0.99)*
Folic Acid	<6	62(18.67%)	54(32.14%)	0.0008*	1
	≧6	270(81.33%)	114(67.86%)		0.48(0.30-0.73)*
	<3	8(2.41%)	10(5.95%)	0.0023*	1
	3≦Folic	54(16.27%)	44(26.19%)		0.64(0.23-1.76)
	Acid<6	270(81.33%)	114(67.86%)		0.32(0.12-0.85)*
	≧6				P_{trend}：0.0006*

a：Student-t test , log
b：Adjust for age and sex
c：Chi-square test
*：P-value <0.05

Table3 The interaction between DNMT3A-448 gene polymorphism, plasma folic acid, smoking , vitamin and UC

Variable				Control N=332 number（%）	Case N=168 number（%）	OR[a] (95%CI)
DNMT3A-448 A>G	GG	Folic Acid (6ng/ml)	low	40(12.05%)	37(22.02%)	1
	AG+AA		low	22(6.63%)	17(10.12%)	0.87(0.40-1.19)
	GG		high	163(49.1%)	77(48.53%)	0.51(0.30-0.87)*
	AG+AA		high	107(32.23%)	37(22.02%)	0.37(0.20-0.66)*
						P_{trend}：0.0003*
DNMT3A-448 A>G	GG	Vitamin	no	102(30.72%)	83(49.40%)	1
	AG+AA		no	57(17.17%)	38(22.62%)	0.82(4.50-1.37)
	GG		yes	101(30.42%)	31(18.45%)	0.33(0.20-0.56)*
	AG+AA		yes	72(21.69%)	16(9.52%)	0.24(0.13-0.45)*
						P_{trend}：<0.0001*
DNMT3A-448 A>G	AG+AA	Smoking	no	94(28.31%)	33(19.64%)	1
	GG		no	148(44.58%)	72(42.86%)	1.42(0.87-2.23)
	AG+AA		yes	35(10.54%)	21(12.5%)	2.53(1.67-5.46)*
	GG		yes	55(16.57%)	42(25%)	3.14(1.61-6.13)*
						P_{trend}：0.0007*

a：Adjust for age and sex
*：P-value <0.05

Table4 Association between risk factor's number and UC

Variable			OR[a]
Risk factor	Number	Case/Control	(95%CI)
Smoling	0	8/55	1
Plasma folic acid	1	44/119	2.68(1.17-6.10)*
DNMT3A-448 gene polymorphism	2	55/92	5.24(2.27-12.08)*
Vitamin	3	46/53	8.31(3.45-20.03)**
	4	15/13	1.38(0.58-3.23)
			P_{trend}：<0.0001**

Reference group : No smoking、plasma folic acid>=6ng/ml、DNMT3A-448 AG+AA gene polymorphism and with vitamin
a： Adjust for age and sex
*：P-value <0.05
**：P-value <0.0001