Genetic polymorphisms of the DNA repair gene *MPG* are associated with the susceptibility of rheumatoid arthritis.

Shih-Yin Chen,[1,2†] Lei Wan,[1–3†] Chung-Ming Huang,[4] Yu-Chuen Huang,[1,2] Jim Jinn-Chyuan Sheu,[1–3] Ying-Ju Lin,[1,2] Shih-Ping Liu,[5] Yu-Ching Lan,[6] Chih-Ho Lai,[7] Cheng-Wen Lin,[8] Chang-Hai Tsai,[2] and Fuu-Jen Tsai[3,9,10*]

[1]Genetic Center, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan. [2]Graduate Institute of Chinese Medical Science, College of Chinese Medicine, China Medical University, Taichung, Taiwan. [3]Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan. [4]Division of Immunology and Rheumatology, China Medical University Hospital, Taichung, Taiwan. [5]Center for Neuropsychiatry, China Medical University and Hospital, Taichung, Taiwan. [6]Department of Health Risk Management, China Medical University, Taichung, Taiwan. [7]Department of Microbiology, School of Medicine, China Medical University, Taichung, Taiwan. [8]Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan. [9]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan. [10]Department of Medical Genetics, China Medical University Hospital, Taichung, Taiwan.

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*Correspondence to: Dr. Fuu-Jen Tsai, Department of Medical Research, China Medical University Hospital, No. 2 Yuh Der Road, Taichung, Taiwan. E-mail: d0704@www.cmuh.org.tw

†SY Chen, Ph.D., and L Wan, Ph.D. are joint first authors and equal contribution in this study.

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease and can lead to deformities and severe disabilities, due to irreversible damage of tendons, joints, and bones. Previous study indicated that DNA repair system was involved in the pathology of RA. In this study, we investigated the association of four N-methylpurine-DNA glycosylase (*MPG*) gene polymorphisms (rs3176364, rs710079, rs2858056, and rs2541632) with the susceptibility to RA in 384 Taiwanese individuals (192 RA patients and 192 controls). Our data showed that statistically significant difference in genotype frequency distributions was found at rs710079 and rs2858056 SNPs between RA patients and control groups (p = 0.04, odds ratio [OR] = 2.06, 95% confidence interval [CI] = 1.02–4.16, at rs710079 SNP; p = 0.029, OR = 1.92, 95% CI = 1.06–3.46, at rs2858056 SNP). Our data also indicated that

individuals with the GG genotype at rs2858056 SNP may have a higher risk of developing RA. In addition, compared with the haplotype frequencies between case and control groups, individuals with G-C-G-C haplotype appeared to be a significant "at-risk" haplotype for RA progression (p = 0.003, odds ratio [OR] = 1.75; 95% confidence interval [CI] = 1.20-1.55). Our results suggest that rs710079 and rs2858056 polymorphisms and the haplotypes (G-C-G-C) in *MPG* gene are associated with the risk of RA progression and these may be used as molecular markers of RA.

KEY WORDS: Rheumatoid Arthritis (RA); N-methylpurine-DNA glycosylase (MPG); Single nucleotide polymorphisms (SNPs); Haplotypes.

RUNNING TITLE:

MPG polymorphisms and Rheumatoid Arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a joint inflammation disease combining several peripheral inflammatory conditions (Harris et al. 1990). It could involve chronic synovitis, inducing the demolition of joint tissue, especially containing bone and cartilage, and therefore, joint function is seriously impaired. The prevalence is about

1% with some variation among ethnic groups (Harris et al. 1990; Ziff et al. 1990; Feldmann et al. 1996). Its genetic contribution was well documented by multiple family studies, and multiple whole-genome sib-pair linkage studies have been reported with limited consistency among them (Yamada et al. 2005; Weyand et al. 1998; Lynn et al. 1995). The previous studies on RA-susceptible genes were published from a group based on a high-throughput single nucleotide polymorphism (SNP) genotyping facility that adopts case-control linkage disequilibrium (LD) mapping on a large scale as an initial survey method without using subjects that were used for preceding linkage studies (Ohnishi et al. 2001; Ozaki et al. 2002; Suzuki et al. 2003). One of them identified functionally relevant polymorphisms of peptidylarginine deiminase 4, an enzyme that catalyzes the post-translational citrullination of proteins, as a RA gene (Speckman et al. 2003; Worthington et al. 2003). Besides, the major histocompatibility (MHC) class II region is an important susceptibility factor, and the human leukocyte antigen (HLA)–DR4 has been associated with serious disease courses (van Zeben et al. 1991; Wagner et al. 1997).

Recently, Shao et al. reported that DNA repair system was involved in the pathology of RA (Shao et al. 2009). It had been implicated that T cells of RA patients failed to produce sufficient transcripts and protein of the DNA repair kinase ataxia telangiectasia (AT) mutated (ATM). Therefore, we decided to study the effect of

genetic polymorphism of DNA repair genes as modifiers of the risk for RA as well in this project.

As well know, DNA repair enzymes modulate free-radical production after DNA damage. The oxidized base 7,8-dihydroxy-8-oxoguanine (8-oxo-G) is one of the mutagenic products of oxidative DNA damage; however, misincorporated 8-oxo-G could be excised by base excision repair (BER) pathway, and N-methylpurine DNA glycosylase (*MPG*; MIM 156565) was involved in which removes a diverse group of damaged bases, including cytotoxic and mutagenic alkylation adducts of purine (Broderick et al. 2006).

In the present study, we aimed to identify genetic polymorphisms in potential candidate genes for RA, and we therefore investigated the association of *MPG* gene polymorphisms with RA in a Taiwanese population. Our findings are expected to help us understand the role of *MPG* gene polymorphisms in RA disease and its progression; this knowledge can point us toward possible management strategies for this common rheumatopathy.

MATERIALS AND METHODS

Study Population

The study subjects including a total of 192 patients with RA and 192 healthy

subjects were recruited from China Medical University Hospital in Taiwan. Patients with RA according to the revised America College of Rheumatology criteria (Arnett et al. 1988) were enrolled. Nephelometry was used to detect rheumatoid factor (RF). Values ≧ 30 IU/ml were defined as positive. The presence or history of extra-articular manifestations in patients with RA was recorded (Yen et al. 1995). The healthy control from the general population was selected from health examination. All individuals' samples were collected by venipuncture for genomic DNA isolation. Informed consent was from all participants and was approved by the local Ethics Committee.

Genomic DNA Extraction and Genotyping

Genomic DNA was prepared from peripheral blood according to standard protocols of the DNA extraction kit (Qiagen, Valencia, CA, USA). The *MPG* genetic polymorphisms were detected by polymerase chain reaction (PCR) using primers that amplified a short fragment of DNAcontaining the polymorphism (Table 1). All the *MPG* genetic polymorphisms used in this study were selected with an allele frequency >5% in the CHB (Han Chinese in Beijing, China) population as reported in the HAPMAP website. Polymorphic site identification was performed by incubating the PCR products with a restriction enzyme chosen to cut one of the two alleles, followed

by electrophoresis on 1-3% agarose gels. All samples were amplified and digested in parallel with the samples of a known genotype.

Statistical Analysis

Chi-square test or Fisher's exact tests will be used to determine statistically significant differences in allele/genotype frequencies between case and control groups. Allelic frequencies will be expressed as percentage of the total number of alleles. The Hardy–Weinberg equilibrium will be tested for each marker using χ^2-test. The haplotype combination at rs3176364, rs710079, rs2858056, and rs2541632 in *MPG* gene was estimated using Haploview version 4.1 (Barrett et al. 2005). The differences in the distribution of the haplotype frequencies between the two groups were assessed with a χ^2-test. Odds ratios [ORs] and 95% confidence intervals (95% CIs) were obtained using logistic regressions to determine associations between *MPG* alleles/genotypes/haplotypes and RA susceptibility. The Kaplan-Meier method was used to estimate cumulative survival. Differences in survival were analyzed with the log-rank test. All data were analyzed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

RESULTS

The genotypic and allelic frequencies of rs3176364, rs710079, rs2858056, and rs2541632 are shown in Table 2. Genotype distributions were in Hardy-Weinberg equilibrium. We observed the C allele to be the major one at rs3176364, rs710079, and rs2858056 polymorphisms both in RA patients (70.3%, 84.6%, and 65.1%, respectively) and controls (70.1%, 81.5%, and 71.6%, respectively). The T allele was the major one at the rs2541632 polymorphism in RA patients (74.7%) and controls (72.1%). When we compared the genotype distribution between RA patients and control groups, statistically significant differences in genotype frequency distributions were noted for the rs710079 and rs2858056 SNPs in RA patients and controls (p = 0.04 and 0.029, respectively). Our data indicated that individuals with the GG genotype at rs2858056 SNP may have a higher risk of developing RA (Table 1).

Haplotype frequencies were estimated using the rs3176364, rs710079, rs2858056, and rs2541632 SNPs with haplotype frequencies > 5% (Table 3). Four major haplotypes of the *MPG* were present in the study population. The C-C-C-T and G-C-G-C were the common haplotypes both in RA patients (51.8% and 22.0%, respectively) and health control (50.2% and 13.8%, respectively) groups. When we compared the overall distribution of haplotype frequencies between RA patients and health controls, a significant difference was observed (p = 0.003, by chi-square test

from a 4 x 2 contingency table). Our data showed that individuals with G-C-G-C haplotype appeared to be a significant “at-risk” haplotype for RA development (p = 0.003, odds ratio [OR] = 1.75; 95% confidence interval [CI] = 1.20-1.55). In addition, comparisons of the clinical features of RA patients with the different haplotypes were shown in Table 4. However, there were no significant differences results in gender distribution, rheumatoid factor accompaniment, incidence of extra-articular and bone erosion occurrence.

DISCUSSION

Currently, RA is considered to be a chronic inflammatory disease with tissue-destructive potential that occurs in genetically susceptible individuals. Polymorphic gene sequences of cytokines known to be involved in the pathogenesis of RA are potential markers of disease susceptibility. Previous studies have examined the relationship between cytokine gene polymorphisms and the incidence of RA, including urokinase, IL-6, IL-8, CD4 cells (Huang et al. 2004; Lo et al. 2008a; Lo et al. 2008b). Moreover, One effect involves a “snow-balling” mechanism of increased levels of cellular damage and death leading to more inflammation, which in turn produces more ROS (Meira et al. 2008). The question remains whether the defect in DNA damage repair functions to render individuals susceptible to RA or is a

consequence of disease. Accumulation of DNA damage may also have broader implications on impairing diverse cellular functions.

In this study, we focused on the variants of the *MPG* gene that had previously been investigated for breast cancer and lung cancer (Cerda et al. 1998; Rusin et al. 1999; Zienolddiny et al. 2006). We found a statistically significant association between RA and the rs710079 and rs2858056 polymorphisms. The non-TT genotype frequency at rs710079 and the non-TT genotype frequency at rs2858056 were significantly higher in RA than in the control participants (Table 1). Our results also indicated that the G-C-G-C haplotype of the *MPG* gene was estimated to be present in approximately 22.0% of RA patients. We observed that the G-C-G-C haplotype seems appeared to be an "at-risk" haplotype for RA progression (p = 0.003, odds ratio [OR] = 1.75; 95% confidence interval [CI] = 1.20-1.55) (Table 3).

As a result of previous study showed that the CD4+CD28-T cell compartment is closely associated with extra-articular RA (Weyand et al. 1998), but in our finding, neither the genotype of *MPG* gene (rs3176364, rs710079, rs2858056, and rs2541632) (data not show) nor the haplotypes showed significant difference with extra-articular in RA patients (Table 4). It has been suggested that extra-articular disease is a different dimension of RA with different pathomechanisms and is not just a more severe form of the disease. From a clinical point of view, the dissection of

phenotypic/genotypic variants of RA is critical for further exploration of more selective therapeutic treatment.

The interpretation of our study results is limited because the patients were recruited from just one center in Taiwan. Our results strongly suggest a significant role of *MPG* gene polymorphisms in the risk of developing RA of Taiwan. To the best of our knowledge, this is the first report on *MPG* gene polymorphisms in RA patients. However, the identification of *MPG* as genetic risk factors for RA susceptibility in Taiwan may be further evaluated as prognostic markers for predictive clinical testing in RA worldwide, especially in ethnically disparate populations.

In summary, our study firstly demonstrated the different genotype distribution between normal controls and patients with RA of *MPG* gene. The data show that *MPG* gene may be associated with renal deterioration in RA patients. *MPG* gene is one of an important inflammatory related gene; our observations suggest those polymorphisms contribute to the genetic background of RA pathogenesis.

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REFERENCES

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31: 315-324.

Barrett JC, Fry B, Maller J, Daly MJ, 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–5.

Broderick P, Bagratuni T, Vijayakrishnan J, Lubbe S, Chandler I, Houlston RS, 2006. Evaluation of NTHL1, NEIL1, NEIL2, MPG, TDG, UNG and SMUG1 genes in familial colorectal cancer predisposition. BMC Cancer 9;6:243.

Cerda SR, Turk PW, Thor AD, Weitzman SA, 1998. Altered expression of the DNA repair protein, N-methylpurine-DNA glycosylase (MPG) in breast cancer FEBS Lett 10;431(1):12-8.

Feldmann M, Brennan FM, Maini RN, 1996. Rheumatoid arthritis. Cell 85: 307-310.

Harris ED Jr, 1990. Rheumatoid arthritis. Pathophysiology and implications for therapy. N Engl J Med 322:1277–1289.

Huang CM, Chen CL, Tsai JJ, Tsai CH, Tsai FJ, 2004. Association between urokinase gene 3'-UTR T/C polymorphism and Chinese patients with rheumatoid arthritis in Taiwan. Clin Exp Rheumatol 22: 219-22.

Lo SF, Huang CM, Lin HC, Chen WC, Tsai CH, Tsai FJ, 2008a. Cytokine (IL-6) and chemokine (IL-8) gene polymorphisms among rheumatoid arthritis patients in Taiwan. Clin Exp Rheumatol 26: 632-7.

Lo SF, Wan L, Lin HC, Huang CM, Tsai FJ, 2008b. Association of CD4 enhancer gene polymorphisms with rheumatoid arthritis and systemic lupus erythematosus in Taiwan. J Rheumatol 35: 2113-8.

Lynn AH, Kwoh CK, Venglish CM, Aston CE, and Chakravarti A, 1995. Genetic epidemiology of rheumatoid arthritis. Am J Hum Genet 57: 150-159.

Meira LB, Bugni JM, Green SL, Lee CW, Pang B, Borenshtein D, Rickman BH, Rogers AB, Moroski-Erkul CA, McFaline JL, Schauer DB, Dedon PC, Fox JG, Samson LD, 2008. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest 118: 2516-2525.

Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y, 2001. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477.

Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Hori M,

Nakamura Y, Tanaka T, 2002. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet 32:650–654.

Rusin M, Samojedny A, Harris CC, Chorazy M, 1999. Novel genetic polymorphisms in DNA repair genes: O(6)-methylguanine-DNA methyltransferase (MGMT) and N-methylpurine-DNA glycosylase (MPG) in lung cancer patients from Poland. Hum Mutat 19;14(3):269-70.

Shao L, Fujii H, Colmegna I, Oishi H, Goronzy JJ, Weyand CM, 2009. Deficiency of the DNA repair enzyme ATM in rheumatoid arthritis. J Exp Med 206(6):1435-49.

Speckman RA, Wright Daw JA, Helms C, Duan S, Cao L, Taillon-Miller P, Kwok PY, Menter A, Bowcock AM, 2003. Novel immunoglobulin superfamily gene cluster, mapping to a region of human chromosome 17q25, linked to psoriasis susceptibility. Hum Genet 112:34–41.

Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakitani S, Teshima R, Nishioka Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K, 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase

4, are associated with rheumatoid arthritis. Nat Genet 34:395–402.

van Zeben D, Hazes JM, Zwinderman AH, Cats A, Schreuder GM, D'Amaro J, Breedveld FC, 1991. Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis. Results of a follow up study. Arthritis Rheum 34:822–830.

Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H, Kalden JR, Wassmuth R, 1997. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 40:341–351.

Weyand CM, Klimiuk PA, Goronzy JJ, 1998. Heterogeneity of rheumatoid arthritis: from phenotypes to genotypes. Springer Semin Immunopathol 20: 5-22.

Worthington J, John S, 2003. Association of PADI4 and rheumatoid arthritis: a successful multidisciplinary approach. Trends Mol Med 9:405–407.

Yamada R, Ymamoto K, 2005. Recent findings on genes associated with inflammatory disease. Mutat Res 573:136–151.

Yen JH, Chen JR, Tsai WJ, Tsai JJ, Liu HW, 1995. HLA-DRB1 genotyping in patients with rheumatoid arthritis in Taiwan. J Rheumatol 22: 1450-1454.

Zienolddiny S, Campa D, Lind H, Ryberg D, Skaug V, Stangeland L, Phillips DH, Canzian F, Haugen A, 2006. Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. Carcinogenesis 27(3):560-7.

Ziff M, 1990. Rheumatoid arthritis--its present and future. J Rheumatol 17: 127-133.

Table 1. Characteristics of the *MPG* genetic polymorphisms and PCR condition for genotyping analysis.

Gene	SNPs	rs number	Position	Alleles	Primers	PCR product size (bp)	PCR conditions (annealing temperature)	Restriction enzyme	DNA fragment size
MPG	MPG-323	rs3176364	16:67577	G/C	Forward:5'-TAGGGGCATGGATCTGACTT-3 Reverse:5'-GGGGAGTCCTTATCCAGGAA-3'	341	58℃	BstUI	C：173+65+103 G：238+103
	MPG-1973	rs710079	16:69223	C/T	Forward:5'-ACCAGCTCAGACGTTTGCTT-3' Reverse:5'-GCATCCGAATAGGAGACAGC-3'	358	61℃	BccI	C: 90+96+59+113 T: 90+155+113
	MPG-3644	rs2858056	16:70893	G/C	Forward:5'-AGAGCTGAGATCACGCCATT-3 Reverse:5'-AGGGCATCCACTAGGAGGTT-3'	328	60℃	HaeIII	C：29+112+86+101 G：29+169+130
	MPG-3860	rs2541632	16:71109	C/T	Forward:5'-GGCATCAGGGACCACAATAC-3 Reverse:5'-CAACACACACCCCTCTTCCT-3'	330	58℃	RsaI	C: 330 T: 210+120

Table 2. Genotypic and allelic frequencies of *MPG* genetic polymorphisms in RA patients and controls.

dbSNP ID		Patient with RA (N=192)	Control (N=192)	OR (95% CI)	*p* value
MPG-323 (rs3176364)	Genotype				
	GG	16 (8.3)	20 (10.4)	Ref	
	CG	82 (42.7)	75 (39.1)	--	
	CC	94 (49.0)	97 (50.5)	--	
	CC + CG	176 (91.7)	172 (89.6)	1.28 (0.64-2.55)	0.484 [a]
	Allele frequency				
	G	114 (29.7)	115 (29.9)	Ref	
	C	270 (70.3)	269 (70.1)	1.01 (0.74-1.38)	0.937
MPG-1973 (rs710079)	Genotype				
	TT	13 (6.8)	25 (13)	Ref	
	CT	33 (17.2)	21 (10.9)	--	
	CC	146 (76.0)	146 (76.0)	--	
	CC + CT	179 (93.2)	167 (86.9)	2.06 (1.02-4.16)	0.040 [b]
	Allele frequency				
	T	59 (15.4)	71 (18.5)	Ref	
	C	325 (84.6)	313 (81.5)	1.25 (0.86-1.82)	0.248
MPG-3644 (rs2858056)	Genotype				
	GG	35 (18.2)	20 (10.4)	Ref	
	CG	64 (33.3)	69 (35.9)	--	
	CC	93 (48.4)	103 (53.6)	--	
	CC + CG	157 (81.8)	172 (89.6)	1.92 (1.06-3.46)	0.029 [c]
	Allele frequency				
	C	250 (65.1)	275 (71.6)	Ref	
	G	134 (34.9)	109 (28.4)	1.35 (1.00-1.84)	0.052
MPG-3860 (rs2541632)	Genotype				
	CC	11 (5.7)	13 (6.8)	Ref	
	CT	75 (39.1)	81 (42.2)	--	
	TT	106 (55.2)	98 (51.0)	--	
	TT + CT	181	179	1.2 (0.52-2.74)	0.673 [d]
	Allele frequency				
	C	97 (25.3)	107 (27.9)	Ref	
	T	287 (74.7)	277 (72.1)	1.14 (0.83-1.57)	0.414

CI, confidence interval; OR, odds ratio.

[a]Compared with the GG and CC+CG genotype of rs3176364 SNP

[b]Compared with the TT and CC+CT genotype of rs710079 SNP

[c]Compared with the GG and CC+CG genotype of rs2858056 SNP

[d]Compared with the CC and TT+CT genotype of rs2541632 SNP

Table 3. Distribution of MPG haplotype frequencies in RA patients and controls.

Haplotype[a]	Patient with RA (%)[b] (n=192)	Control (%) (n= 192)	OR (95% CI)	*p* value
C-C-C-T	51.8%	50.2%	1.06 (0.80-1.41)	0.659
G-C-G-C	22.0%	13.8%	1.75 (1.20-1.55)	0.003
C-T-C-T	9.1%	9.6%	0.94 (0.58-1.53)	0.790
C-C-G-T	7.8%	6.2%	1.27 (0.73-2.22)	0.372

CI, confidence interval; OR, odds ratio.

[a]Order of single nucleotide polymorphisms comprising the MPG haplotypes: rs3176364, rs710079, rs2858056, and rs2541632

[b]Percentages may not sum to 100% because of the presence of the presence of rare haplotypes(<5%) not presented here.

Table 4. Association between clinical feature and haplotype distribution of MPG polymorphisms in RA patients.

Clinical Parameters	Patient with RA					
	male (n=41)			female (n=151)		
	GCGC (n=18)[a]	non GCGC (n=23)	*p* value	GCGC (n=60)[a]	non GCGC (n=91)	*p* value
Rheumatoid factor						
yes	15 (83.3)	15 (65.2)	0.291	43 (71.7)	68 (74.7)	0.677
no	3 (16.7)	8 (34.8)		17 (28.3)	23 (25.3)	
Extra-articular						
yes	5 (27.8)	11 (47.8)	0.192	30 (50)	45 (49.5)	0.947
no	13 (72.2)	12 (52.2)		30 (50)	46 (50.5)	
Erosion						
yes	10 (55.6)	9 (39.1)	0.295	35 (58.3)	43 (47.3)	0.182
no	8 (44.4)	14 (60.9)		25 (41.7)	48 (52.7)	

[a]With the diplotype containing GCGC