ORIGINAL ARTICLE

Matrix Metalloproteinase-2 Gene Polymorphisms in Nasal Polyps

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Objective: To systematically investigate the role of matrix metalloproteinase-2 (*MMP2*) tagging single nucleotide polymorphisms (SNPs) and promoter functional polymorphism in the development of nasal polyps in a Chinese population in Taiwan.

Design: We conducted a case-control study in 136 cases of chronic rhinosinusitis with bilateral nasal polyps and 136 controls. Seventeen SNPs were selected, including 16 tagging SNPs and 1 promoter functional SNP. The genotypes were determined by TaqMan technology. Hardy-Weinberg equilibrium was tested for each SNP, and genetic effects were evaluated according to 3 modes of inheritance. Subset analysis based on the recurrence of nasal polyps was also performed.

Setting: Medical university center hospital.

Results: All 17 SNPs were in Hardy-Weinberg equilibrium. When comparing the patients with recurrent nasal polyps and controls, none of the SNPs reached the significant level of $P < .05$ except rs857403. The *AT* genotype of rs857403 had an adjusted odds ratio of 2.07 (95% confidence interval, 1.09-3.95) ($P = .03$). However, the result became nonsignificant after including an additional 691 controls. Therefore, we considered that the initial significance was a false-positive finding. Neither haplotype analysis nor subset analysis yielded any significant result.

Conclusion: The *MMP2* gene does not play a crucial role in conferring risk for nasal polyps in a Taiwanese population.

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NASAL POLYP IS A RECALCITRANT disease in otorhinolaryngology. Medical treatment is usually not enough, and repeated surgical intervention is often necessary because of a high recurrence rate. The histological appearance of nasal polyp is characterized by massive stroma edema, infiltration of inflammatory cells, alteration of the overlying epithelium, and tissue remodeling. The tissue remodeling included basement membrane thickening and extracellular matrix accumulation.[1-3] Eosinophils are the most predominant inflammatory cells. Regarding the pathogenesis of nasal polyp, it has been reported that epithelial damage might be an essential step to initiate polyp formation.[4-6] The next steps require extracellular matrix accumulation[5,6] and protrusion through the initial epithelial defect.[7]

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases that are known to be important in remodeling of the extracellular matrix components.[3,8] Matrix metalloproteinase-2 (*MMP2*) (gelatinase A, 72-kDa gelatinase, type IV collagenase) (GenBank NM_004530) cleaves type IV collagen, the major structure component of basement membranes. Up-regulation of *MMP2* in the nasal polyp may damage the collagen of basement membranes of epithelia and blood vessels, causing increases in permeability and edema in the stroma. Elevated levels of MMP2 protein[1,2] and MMP2 messenger RNA (mRNA)[1] were found in nasal polyps. Therefore *MMP2* may play a role in the pathogenesis of nasal polyps. To our knowledge, there are no published data regarding the relationship between *MMP2* genetic polymorphisms and the risk of nasal polyps. We conducted a case-control study to systematically investigate the role of *MMP2* tagging single nucleotide polymorphisms (tSNPs) and promoter functional polymorphism in the development of nasal polyps in a Chinese population in Taiwan.

METHODS

SUBJECTS

We recruited 136 patients with chronic rhinosinusitis combined with bilateral nasal polyps and 136 controls at the Kaohsiung Medical Uni-

Table 1. Baseline Demographics of Study Subjects

Characteristic	Cases (n=136)	Controls (n=136)	*P* Value
Age, mean (SD) [range], y	42.5 (16.6) [9-76]	34.9 (12.1) [16-72]	<.001
Sex male/female, No. (male to female ratio)	96/40 (2.4:1)	108/28 (3.9:1)	.09
Nasal polyp recurrence, No. (%)			
Yes	70 (51.5)	NA	NA
No	66 (48.5)	NA	NA

Abbreviation: NA, not applicable.

versity Hospital in Kaohsiung, Taiwan, between October 2005 and December 2006. The diagnosis of nasal polyp was based on history review and findings from physical examination, nasal endoscopy, and sinus computed tomography. All the patients were followed up at least 3 months after surgery. If a patient had a recurrent nasal polyp after surgery, this patient was defined as a "recurrent patient." According to a previous report,[9] a patient was classified as "nonrecurrent" if he or she was disease-free for at least 3 months after removal of the polyp. Twenty-six recurrent patients underwent their first surgery at a hospital other than ours, and their diagnosis of recurrent nasal polyp was based on history review and findings from physical examination. The control subjects were patients with chronic hypertrophic rhinitis who underwent turbinectomy to relieve nasal obstruction. None of the controls had a history of cancer or nasal polyp based on history review and findings from physical examination and nasal endoscopy on enrollment. Information on demographic characteristics was collected. The study was approved by the institutional review board of Kaohsiung Medical University Hospital, and written informed consent was given by each subject or custodian (if the patient was younger than 18 years).

SNP SELECTION AND GENOTYPING

Genomic DNA was extracted from peripheral blood by a standard method. The tSNPs were selected from the HapMap Project,[10] and all of them have the minor allele frequency of 10% or higher in the Han Chinese population (http://www.hapmap.org). In addition, we also chose one commonly studied functional polymorphism (–1306 C→T, rs243865).[11] Some tSNPs at the *MMP2* gene indicated by the HapMap Project were replaced by our previously genotyped SNPs that were in strong linkage disequilibrium ($r^2 \geq 0.8$) with the indicated tSNPs. Genotyping was carried out by using TaqMan technology (ABI 7500 Real-Time PCR [polymerase chain reaction] System; Applied Biosystems, Foster City, California), and reactions were performed in 96-well microplates with ABI 9700 thermal cyclers (Applied Biosystems). Fluorescence was measured with the ABI 7500 Real-Time PCR System and analyzed with its SDS software, version 1.2.3. Every subject was typed for all SNPs.

STATISTICAL ANALYSIS

Continuous variables were analyzed by independent *t* test and were presented as means (SDs). The allele frequency was obtained by direct gene counting. The Hardy-Weinberg equilibrium (HWE) was tested in cases and controls separately by using the x^2 test. Since there were 34 (17 SNPs in cases and controls) HWE tests, we used $P=.01$ as the cutoff point to indicate Hardy-Weinberg disequilibrium. Multiple logistic regression analysis was performed to adjust for the effects of age and sex, while assessing the genetic effects. We examined the effect of the common allele in 3 genetic models (dominant, additive, and recessive). We also performed a subset analysis stratified by recurrence. Linkage disequilibrium was assessed for any pair of SNPs, and haplotype blocks were defined using the default setting of the Haploview software.[12] We used the Hap-Clustering program[13] to evaluate haplotype-phenotype association. SPSS version 13.0 (SPSS Inc, Chicago, Illinois) for Windows was used for statistical analysis. $P<.05$ (2-tailed) was considered statistically significant.

RESULTS

STUDY PARTICIPANTS

Table 1 gives the baseline characteristics of the subjects. The genotyping call rate for each SNP ranged from 96% to 100%. The mean (SD) age of the 136 cases (96 male and 40 female) was 42.5 (16.6) (range, 9-76 years). The mean (SD) age of the 136 control volunteers (108 male and 28 female) was 34.9 (12.1) (range, 16-72 years). The sex distribution for the subjects with nasal polyps was 2.4:1 (male to female), and this ratio is similar to what has been reported.[14] Among the nasal polyp cases, 51.5% were recurrent.

SINGLE SNP RESULTS

The distribution of *MMP2* genotypes was all in HWE in either cases or controls based on $P=.01$ as the cutoff point. We found that none of the tSNPs had a significant *P* value. The multivariate logistic regression model yielded an adjusted odds ratio of 2.07 (95% confidence interval, 1.09-3.95) ($P=.03$) for the genotype AT vs AA of rs857403, while the model was adjusted for age and sex. To confirm this result, we further added 691 controls (S.-H.H.J., unpublished data, 2007), who were normal subjects in our 2 independent endometriosis and myopia studies.[15,16] However, the result became nonsignificant after adding the additional controls ($P=.10$). We did not find significant results under dominant, additive, and recessive models for all other SNPs (**Table 2**).

LINKAGE DISEQUILIBRIUM AND HAPLOTYPE ANALYSIS

The 17 SNPs form 4 haplotype blocks (**Figure**). The low r^2 for every pair of SNPs indicated that these SNPs cannot be tagged by each other. As a result, haplotypes were created from all SNPs in each block. Haplotype analysis did not yield significant results for any block (global

Table 2. The 17 SNPs and Their Relationships With Nasal Polyps Under 3 Genetic Models (Dominant, Additive, and Recessive)

SNP	Genotype (No. of Subjects)			*P* Value		
				Dominant	Additive	Recessive
rs2438656				.26	.94	.77
Cases	GG (108)	GA (24)	AA (4)			
Controls	GG (105)	GA (30)	AA (1)			
rs857403				.47	.24	.27
Cases	AA (67)	AT (55)	TT (14)			
Controls	AA (73)	AT (52)	TT (11)			
rs1030868				.55	.31	.33
Cases	GG (82)	AG (46)	AA (8)			
Controls	GG (71)	AG (55)	AA (10)			
rs1477017				.35	.31	.42
Cases	AA (82)	AG (46)	GG (8)			
Controls	AA (71)	AG (52)	GG (11)			
rs1053605				.07	.23	.55
Cases	CC (93)	CT (34)	TT (9)			
Controls	CC (97)	CT (37)	TT (2)			
rs9302671				NA	.47	.47
Cases	GG (102)	GT (34)	TT (0)			
Controls	GG (96)	GT (40)	TT (0)			
rs2241145				.95	.88	.77
Cases	GG (38)	CG (65)	CC (33)			
Controls	GG (35)	CG (68)	CC (33)			
rs2241146				.78	.43	.40
Cases	GG (94)	AG (35)	AA (7)			
Controls	GG (85)	AG (44)	AA (7)			
rs243849				.60	.31	.13
Cases	CC (84)	CT (46)	TT (6)			
Controls	CC (92)	CT (33)	TT (9)			
rs12599775				.66	.29	.28
Cases	GG (114)	CG (19)	CC (3)			
Controls	GG (104)	CG (29)	CC (3)			
rs243847				.41	.27	.32
Cases	TT (53)	CT (59)	CC (23)			
Controls	TT (48)	CT (63)	CC (25)			
rs243844				.51	.29	.32
Cases	GG (72)	AG (51)	AA (12)			
Controls	GG (63)	AG (53)	AA (12)			
rs243840				.41	.71	.92
Cases	AA (53)	AG (58)	GG (25)			
Controls	AA (47)	AG (68)	GG (20)			
rs2287076				.14	.39	.81
Cases	TT (64)	CT (54)	CC (18)			
Controls	TT (64)	CT (63)	CC (9)			
rs11639960				.51	.98	.78
Cases	AA (76)	AG (50)	GG (10)			
Controls	AA (71)	AG (58)	GG (7)			
rs243832				.50	.77	.93
Cases	GG (53)	CG (64)	CC (19)			
Controls	GG (50)	CG (60)	CC (23)			
rs7201				.25	.55	.86
Cases	AA (78)	AC (50)	CC (7)			
Controls	AA (77)	AC (47)	CC (11)			

Abbreviations: NA, not applicable; SNPs, single nucleotide polymorphisms.

P values: *P* = .26 for block 1; *P* = .58 for block 2; *P* = .44 for block 3; and *P* = .53 for block 4).

COMMENT

We systematically investigated 17 SNPs of the *MMP2* gene and performed analyses based on 3 modes of inheritance in a Chinese population in Taiwan. The results did not show any of them to be significantly associated with nasal polyp. Although we found 1 *P* value of .03 for rs857403 in the recurrent patients, the significance could not be replicated using additional controls. Therefore, this initial "significant" result is likely to be false positive. We also performed subset analyses on subjects stratified by recurrence of the nasal polyp, but the results were still not significant for either group. Our study indi-

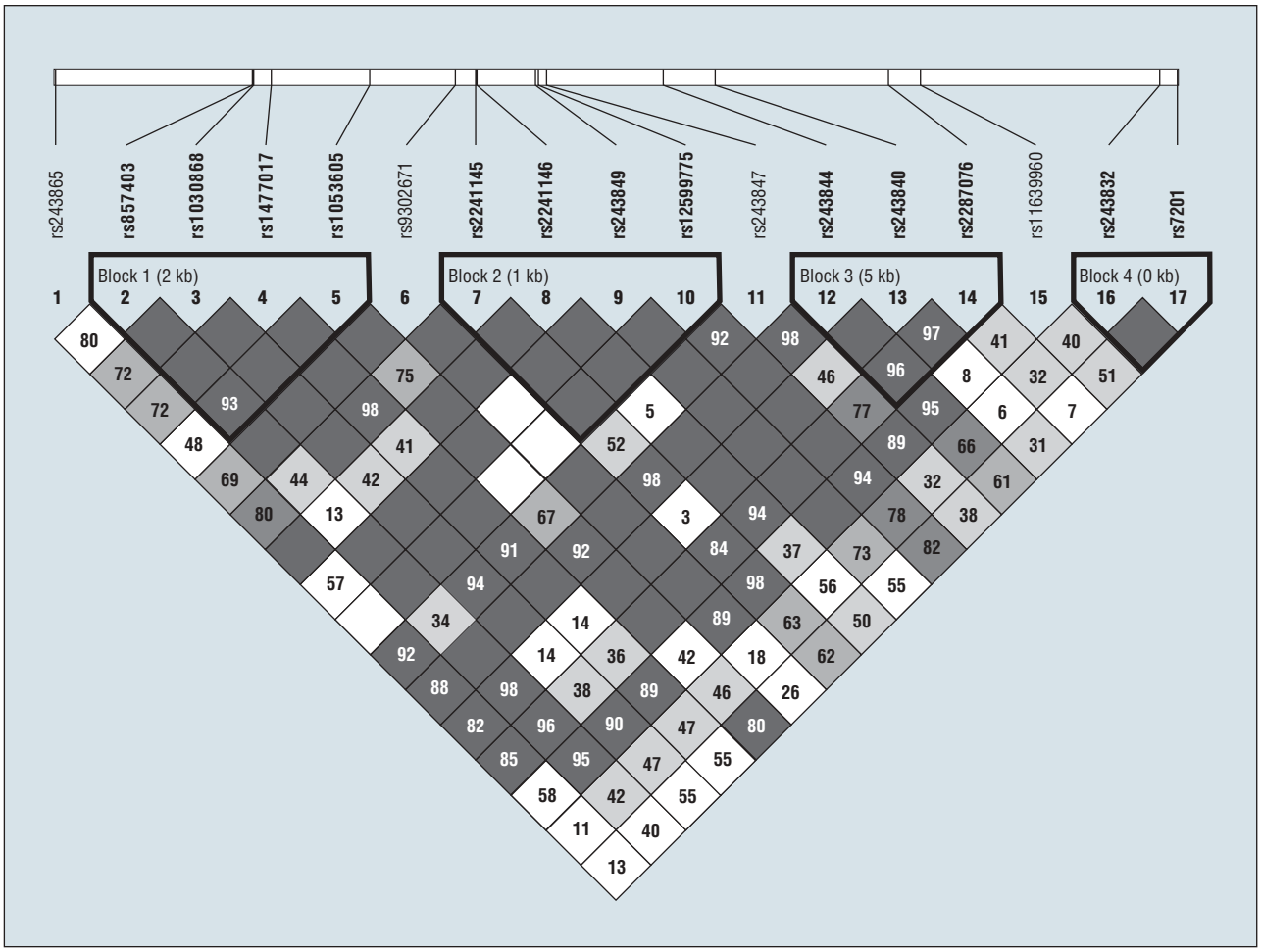

Figure. Seventeen matrix metalloproteinase-2 (*MMP2*) single-nucleotide polymorphisms form 4 haplotype blocks. The pairwise D' value (percentage) is displayed in each cell, but its value is not shown when D'=1. The cells in dark gray indicate strong linkage disequilibrium and log of odds (LOD) of 2 or greater and in white indicate uninformative with LOD lower than 2. The bar on the top is the *MMP2* gene structure and location of each single-nucleotide polymorphism; kb indicates kilobase.

cated that common genetic variants of the *MMP2* gene are unlikely to play a pivotal role in the development of nasal polyp.

Regulation of MMPs is complex. It is considered that regulation of MMP activity occurs at 3 levels: gene transcription, activation of the secreted proenzyme, and inhibition by specific and nonspecific inhibitors.[17] Given that MMP2 protein levels[1,2] and mRNA amount[1] were different between patients with nasal polyp and controls, our finding suggests that such difference may not be caused by the differential transcriptions due to genetic variants. Our study design has strengths and limitations. We used tSNPs plus additional SNPs to evaluate the overall *MMP2* effect rather than just focus on the commonly studied promoter SNP rs243865. We used additional controls to validate the initial "significant" finding for rs857403. This approach excluded false positivity and allowed us to prevent a report of spurious association. The sample size in the present study was relatively small, which may not provide sufficient power to detect a genetic variant with a small effect.

Our study had a power of 80% for a significance level of .05 under the following assumptions: a disease prevalence of 0.04, a risk allele frequency of 0.1, a tagging allele frequency of 0.1, a pairwise D' value (a parameter to measure the strength of linkage disequilibrium) of 0.8, and a dominant risk allele with relative risk of 2.0.

In conclusion, the *MMP2* gene does not play a crucial role in conferring risk for the nasal polyp in a Chinese population in Taiwan.

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Author Contributions: Dr Juo had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Wang, Chien, and Juo. *Acquisition of data:* Wang, Chien, Kuo, Tai, and Juo. *Analysis and interpretation of data:* Wang, Chien, Kuo, Tai, and Juo. *Drafting of the manuscript:* Wang, Chien, Kuo, Tai, and Juo. *Critical revision of the manuscript for important intellectual content:* Juo. *Statistical analysis:* Chien and Juo. *Obtained funding:* Wang, Kuo, and Tai. *Administrative, technical, and material support:* Chien, Kuo, Tai, and Juo. *Study supervision:* Wang, Chien, and Juo.

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