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Clinical course of patients with aspirin-exacerbated respiratory disease: can we predict the prognosis?

Aim: This study aimed to identify prognostic factors of aspirin-exacerbated respiratory disease by comparing clinical and genetic data with the clinical course. **Patients & methods:** Patients were classified into two groups according to their response to inhalation rechallenge with lysine-aspirin after at least 1 year of regular treatment with antiasthmatic medications. **Results:** Forty eight patients (39.3%, group I) had negative responses, whereas 74 patients (60.7%, group II) had positive responses ($n = 23$) or were not rechallenged owing to persistent symptoms ($n = 51$). FEV₁ at diagnosis and follow-up were significantly lower in group II than in group I. The CCR3 polymorphism at -520T/G differed significantly between the two groups, whereas no difference was found in other SNPs. **Conclusion:** Baseline FEV₁ and lower lung function after treatment were clinical factors indicating a poor prognosis of aspirin-exacerbated respiratory disease. The G allele of CCR3 -520T>G was associated with persistent bronchial hypersensitivity to aspirin.

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KEYWORDS: aspirin-induced ■ asthma ■ bronchial provocation tests ■ genetic polymorphism ■ prognosis

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Aspirin-exacerbated respiratory disease (AERD) is a clinical syndrome characterized by chronic rhinitis, nasal polyps, asthma, and the precipitation of asthma and rhinitis attacks after the ingestion of aspirin (ASA) or nonsteroidal anti-inflammatory drugs (NSAIDs). Persistent eosinophilic inflammation is a key finding, resulting from the upregulation of IL-5 and other eosinophil activating cytokines and chemokines. The main pathologies of AERD are the overproduction of cysteinyl leukotrienes (CysLTs), increased CysLTR1 expression in the airway mucosa, and decreased lipoxin and PGE2 synthesis [1,2].

Genetic studies have shown that the polymorphism of genes related to the leukotriene and inflammatory pathways is associated with the development of AERD and the response to medication. *DPB1*0301* is a strong genetic marker of the AERD phenotype and responsiveness to leukotriene receptor antagonist (LTRA) in the Korean population [3,4]. The *ALOX5* [5], *ALOX15* [6], *CysLTR1* [7], *LTC4S* [8] and *TBXA2R* [9] genes are associated with the AERD phenotype. IL-10 [10] and IL-13 [11] play important roles in airway inflammation; in particular, CCR3 [12] and CRTH2 [13] are associated with eosinophilic inflammation. Most studies have identified a genetic susceptibility to the disease or relevant genes that underlie the mechanism. However, no data show the effect

of AERD-related genetic variants on the clinical course of AERD.

AERD patients present with severe clinical symptoms and frequent exacerbations [14]. Despite the subsequent avoidance of ASA and NSAIDs, the patient's respiratory inflammatory process persists and often requires systemic corticosteroids to control respiratory symptoms. Nevertheless, in practice, some patients are well controlled, without the severe, intractable clinical features of typical AERD. Studies of the natural history of the disease have focused on describing patients from the first manifestation to a clear diagnosis of AERD and have collected clinical information. To our knowledge, only one publication has evaluated the clinical course of AERD after a diagnosis was made and treatment initiated [15].

Therefore, this study compared the clinical and genetic factors associated with AERD according to clinical course to identify prognostic factors in AERD patients.

Patients & methods

■ Patients

The study enrolled 122 patients with AERD from January 2001 to January 2010 at Ajou University Hospital, Suwon, South Korea. The diagnosis of AERD was confirmed by a positive response to a lysine-ASA bronchoprovocation test (L-ASA BPT), which was performed using

a previous described method [16]. The change in the forced expiratory volume in 1 s (FEV₁) was followed for 5 h after the final dose of the ASA challenge. The ASA-induced change (%) in FEV₁ was calculated as the percentage of the postchallenge FEV₁ relative to the prechallenge FEV₁, and defined as a positive reaction if the FEV₁ level declined more than 20% from the baseline value. Methacholine bronchoprovocation tests were performed as described previously [16]. Each patient was treated with anti-asthmatic drugs for more than 1 year to achieve asthma control based on the Global Initiative for Asthma (GINA) guidelines [17]. The treatment regimen included inhaled corticosteroid with a long-acting β -agonist and other controller drugs, such as theophylline, LTRA or oral corticosteroids. The L-ASA BPT was followed in subjects with a baseline FEV₁ of at least 80% of the predicted value after withdrawing anti-asthmatic drugs. They were told to withdraw oral corticosteroids and LTRA 1 week before the challenge test, and antihistamines, long-acting β -agonists, and inhaled corticosteroids 3 days before, and short-acting β -agonists 6 h before according to the guidelines [18]. Subjects were classified into two groups based on the result of the follow-up L-ASA BPT. Group I comprised 48 patients showing negative conversion, and group II included 74 patients with positive responses or persistent asthma symptoms who could not perform a follow-up L-ASA BPT.

Atopy was defined as one or more positive reactions on skin prick testing using 12 common aeroallergens (Bencard, Brentford, UK), with histamine and saline controls. Serum was aspirated, separated and stored in aliquots at -20°C until analyzed at the first and follow-up visits. Total serum IgE was measured using the UniCAP system (Phadia, Valinge, Sweden), according to the manufacturer's instructions. The threshold cutoff value for a specific IgE level was 0.35 KU/l as measured by UniCAP. The presence of rhinosinusitis and nasal polyps was evaluated using paranasal sinus x-rays and rhinoscopy. Peripheral blood total eosinophil counts and eosinophil cationic protein were measured from the collected blood samples. Informed consent was obtained from all subjects, and the institutional review board of Ajou University Hospital (Suwon, Korea) approved the study.

■ SNP selection & genotyping

Based on our previous genetic studies, we selected 11 candidate genes related to the leukotriene and

inflammatory pathways. Ultimately, ten SNPs were assayed: *ALOX5*, *ALOX15*, *CCR3*, *CRTH2*, *CysLTR1*, *IL10*, *IL13*, *LTC4S*, *TGF β* , *TNF α* and *HLA DPB1*0301*, which were selected as significant risk alleles associated with the AERD phenotype [19]. Genomic DNA was prepared from peripheral blood samples using the PURE-GENE DNA purification kit (Gentra, MN, USA), according to the manufacturer's protocol. Genotyping was performed using the SNaPshot ddNTP primer extension kit (Applied Biosystems, CA, USA), as described previously [12].

■ Statistical analysis

We assessed the differences in genotype frequency between the two groups with the χ^2 test and by calculating the odds ratio with 95% CI. The distributions of the ten SNP genotypes were analyzed using logistic regression models that controlled for sex and age as covariates. Continuous variables in clinical characteristics were analyzed using Student's t-test and categorical variables were assessed using the χ^2 test. Logistic regression was used for the results of the secondary L-ASA inhalation challenges. Covariates were age, sex, smoking status, asthma duration, and length of follow-up duration. A p-value less than 0.05 denoted a statistically significant difference. The statistical analyses were completed using SPSS version 16 (SPSS, IL, USA).

Results

■ Clinical characteristics of the study subjects

The study enrolled 122 patients with AERD seen between January 2001 and January 2010 at our clinic. The clinical characteristics of the study subjects are presented in TABLE 1. Forty-eight patients (39.3%) showed negative conversions on the follow-up L-ASA BPT (group I), whereas 74 patients (60.7%) showed positive responses (n = 23) or persistent asthma symptoms with a decreased FEV₁ (<80%) and could not undergo the L-ASA BPT test (n = 51) despite antiasthmatic drug treatment (group II). Females predominated in both groups. There were no significant differences in the mean age, asthma duration, length of follow-up duration, smoking status or atopic tendency between the two groups. The presence of nasal polyposis tended to be lower in group I than in group II. The total dose of inhaled corticosteroids, oral corticosteroids, and number of hospital admissions per year tended to be lower in group I than in group II. There was a significant decrease

Table 1. Clinical characteristics of study subjects.

Variables	Group I (n = 48)	Group II (n = 74)	p-value
Sex, percentage female	66.7	63.5	0.722
Age, years (SD)	43.8 (12.5)	47.0 (14.0)	0.203
Duration of asthma, years (SD)	7.0 (6.6)	7.8 (8.7)	0.628
Length of follow-up duration, years (SD), range (min–max)	4.4 (3.7), 1.0–14.0	4.6 (3.4), 1.0–12.0	0.798
Smoking status			
– Ever (%)	13.8	15.1	0.575
– Never (%)	86.2	84.9	
Atopic status, percentage positive	47.9	43.2	0.612
Nasal polyp, percentage positive	46.8	54.9	0.387
Dose of inhaled corticosteroid, BD equivalent/ μ g/year (SD)	149,843.5 (117,844.8)	185,417.1 (170,258.0)	0.219
Dose of oral corticosteroid, PD equivalent/mg/year (SD)	442.1 (393.1)	537.6 (593.4)	0.424
Hospital admissions for asthma, n/case/year (SD)	0.1 (0.4)	0.1 (0.5)	0.614
Courses of oral corticosteroids for asthma exacerbations, n/case/year (SD)	3.8 (3.6)	6.0 (6.8)	0.021
Use of leukotriene modifier (%)	93.6	91.8	0.709
Use of long-acting β -agonist (%)	100.0	100.0	1.000
Severe asthma [†] , percentage positive	12.5	31.1	0.019

Group I: patients with negative conversions to follow-up lysine-aspirin bronchoprovocation test; Group II: patients with positive responses or persistent asthma symptoms.

[†]Severe asthma is defined from the American Thoracic Society workshop consensus on severe/refractory asthma [20].

BD: Budesonide; PD: Prednisolone; SD: Standard deviation.

in the annual number of steroid bursts due to exacerbation in group I patients compared with group II ($p = 0.021$). The frequency of severe asthma, which was defined using the American Thoracic Society (ATS) consensus criteria of [20], was significantly lower in group I than in group II ($p = 0.019$).

■ Comparison of pulmonary & immunological parameters at diagnosis & the follow-up study

The baseline FEV_1 was significantly higher in group I than in group II at both first diagnosis and follow-up ($p = 0.012$ and $p < 0.001$, respectively). However, there were no differences in terms of the change in FEV_1 after L-ASA BPT, cumulative dose of L-ASA, change in FEV_1 after methacholine bronchoprovocation test, and provocative concentration that caused a 20% fall in the FEV_1 (PC_{20}) value between the two groups at diagnosis. After treatment with antiasthmatic drugs for at least 1 year, the mean FEV_1 improved significantly in group I (85.7 ± 16.2 to 89.6 ± 18.8 , $p = 0.041$), while it decreased in group II (76.8 ± 20.9 to 73.6 ± 16.8 , $p = 0.173$). At the follow-up visit, the mean FEV_1 was significantly lower in group II than in group I ($p < 0.001$). The percentage decline in FEV_1 during the L-ASA

BPT was greater in group II than in group I ($p < 0.001$, TABLE 2). Immunological parameters including total IgE, eosinophil cationic protein, blood eosinophil count, and sputum differential counts at diagnosis and the follow-up visit were similar between the two groups (TABLE 3).

■ Genotype frequencies of SNPs of the candidate genes for AERD

Genetic associations for AERD were examined in 11 candidate genes, involved in the leukotriene and inflammatory pathways. All SNPs were in Hardy–Weinberg equilibrium in the subjects, except for the *CysLTR1* -634C>T polymorphism because of its location on the X chromosome. The allele and genotype frequency of *CCR3* (520T>G) differed significantly between the two groups ($p = 0.002$, odds ratio: 4.790 in a recessive analysis model). The frequency of the G allele was significantly lower in group I than in group II. However, there were no significant differences in the genotype frequencies of the SNPs in the other genes, including *ALOX5* 1708G>I, *ALOX15* 427G>A, *CRTH2* 466T>G, *CysLTR1* 634C>T, *IL10* 1082A>G, *IL13* 1055C>T, *LTC4S* 444A>C, *TGF β* 509C>T, *TNF α* 308G>A and *HLA DPB1* *0301 between the two groups (TABLE 4).

Table 2. Pulmonary parameters in subjects at diagnosis and follow-up visit.

Variables	Group I (n = 48)	Group II (n = 74)	p-value
At diagnosis			
FEV ₁ , percentage predicted (SD)	85.7 (16.2)	76.8 (20.9)	0.012
Percentage decline of FEV ₁ at L-ASA BPT (SD)	20.9 (8.7)	22.9 (11.2)	0.321
Cumulative dose of L-ASA, µg/ml (SD)	665.0 (227.8)	653.1 (256.2)	0.810
Percentage decline of FEV ₁ at Mch BPT (SD)	24.5 (7.5)	24.5 (6.2)	0.972
PC ₂₀ methacholine, mg/ml (SD)	6.2 (12.5)	3.8 (5.7)	0.274
At follow-up visit			
FEV ₁ , percentage predicted (SD)	89.6 (18.1)	73.7 (16.8)	<0.001
Percentage decline of FEV ₁ at L-ASA BPT (SD)	8.6 (7.1)	23.2 (7.3)	<0.001
Cumulative dose of L-ASA, µg/ml (SD)	810.7 (92.6)	792.9 (90.5)	0.470
Percentage decline of FEV ₁ at Mch BPT (SD)	19.3 (8.7)	22.3 (7.0)	0.350
PC ₂₀ methacholine, mg/ml (SD)	8.4 (7.0)	5.1 (4.5)	0.069

FEV₁: Forced expiratory volume in 1 s; L-ASA BPT: Lysine-aspirin bronchoprovocation test; Mch BPT: Methacholine bronchoprovocation test; PC₂₀ methacholine: Provocative concentration of methacholine that causes a 20% fall in the FEV₁; SD: Standard deviation.

■ Association of the AERD phenotype with CCR3 polymorphisms

Of the patients with the TT genotype, 61% showed negative conversions at follow-up L-ASA BPT, while 28.6% of the patients with GT or GG genotype showed negative responses. A significant association was noted between the CCR3 genetic polymorphism (520T>G) and nasal polyposis. The presence of nasal polyposis was significantly higher in the patients with the GG or GT genotype than in those with the TT genotype (63.6 vs 40.3%, respectively; $p = 0.028$). Females predominated in the GT or GG genotypes ($p < 0.012$). Other clinical characteristics, such as duration of asthma, atopic status and medication requirement were not associated with the CCR3 genetic polymorphism.

The change in FEV₁ after L-ASA BPT differed significantly according to CCR3 genotype. A greater decline in FEV₁ was found in the patients with the GG or GT genotype compared with those with the TT genotype at diagnosis, as well as in the follow-up study (25.3 ± 12.7 vs $20.4 \pm 6.0\%$, $p = 0.029$ at diagnosis, 17.3 ± 10.9 vs $10.9 \pm 8.7\%$, $p = 0.026$ at follow-up, FIGURE 1). After regular treatment with antiasthmatic drugs, there were significant improvements in the mean percentage decline in FEV₁ at L-ASA BPT in both groups: for the GT or GG genotype 25.3 ± 12.7 to $17.3 \pm 10.9\%$ and for the TT genotype, 20.4 ± 6.0 to $10.9 \pm 8.7\%$ ($p = 0.001$ and $p < 0.001$, respectively). The mean values of baseline FEV₁ were similar at the first and follow-up visits between the two groups: during the first visits we found the GT

or GG genotype values to be $80.5 \pm 21.1\%$ while TT values were $80.5 \pm 16.2\%$. At the follow-up visits we found the GT or GG genotype values to be $78.6 \pm 19.9\%$ while TT values were $81.5 \pm 17.9\%$. However, there were no significant differences in the PC₂₀ methacholine, total IgE or sputum eosinophil counts in AERD patients according to genotype.

Discussion

Previous studies by Szczeklik in Europe and Berge-Gimeno in the US provided important insights into the natural history of AERD [21,22]. The typical patients with AERD are middle-aged females who developed refractory chronic rhinosinusitis, followed by bronchial asthma, ASA or NSAID hypersensitivity, and nasal polyposis. These symptoms are usually moderate to severe and in some of the patients, adequate control of asthma can be achieved only with oral corticosteroids. However, previous approaches for studying AERD consisted mostly of cross-sectional studies, using uniform questionnaires and clinical data collection, and focused on the disease processes from the first manifestation to the time of diagnosis of AERD. In this study, we first evaluated clinical and genetic factors to predict the clinical course of AERD patients through a longitudinal study where the second L-ASA BPT was performed after more than 1 year of antiasthmatic treatment. Moreover, we compared pulmonary function and immunologic markers with pharmacologic treatment, and analyzed the candidate SNPs known to be risk alleles for AERD.

We performed a second L-ASA BPT to confirm the bronchial sensitivity to ASA, and collected blood and sputum samples in subjects after treatment with controller medications. To exclude a false-negative reaction, patients discontinued their antiasthmatic drugs before the challenge test according to the guidelines [18]. Forty-eight patients (39.3%, group I) showed negative responses on the follow-up L-ASA BPT. They had better lung function at baseline and showed significant improvement in FEV₁ during the treatment period compared with those who had persistent positive reactions at the follow-up study. In addition, the number of steroid bursts was significantly lower in group I than in group II ($p = 0.021$). The total doses of inhaled corticosteroids and oral corticosteroids and the number of hospital admissions per year tended to be lower in group I than in group II, albeit not significantly so.

When we compared clinical characteristics between the patients with positive results to the follow-up L-ASA BPT ($n = 23$), and the others presenting persistent asthma symptoms who could not do follow-up ASA challenges ($n = 51$) from group II, there were no significant differences in terms of baseline characteristics and immunologic parameters between the two subgroups. Instead, baseline and follow-up FEV₁ decreased in all patients from group II compared with group I, and the decline of lung function during the second L-ASA BPT was greater in group II than in group I. These findings indicate that the bronchial reaction after an ASA challenge can be reduced to some extent after regular antiasthmatic drug treatment; however,

persistent ASA hypersensitivity is associated with the severity of asthma in AERD patients.

There was some evidence of a blocking effect of controller medications of an oral ASA challenge test in AERD patients [23–25]. Patients with LTRA or inhaled corticosteroids showed milder bronchoconstriction and more naso-ocular reactions compared with those who received no controller medications having classic bronchial reactions. Another study showed that pretreatment with salmeterol attenuated the ASA-precipitated bronchoconstriction [26]. However, in our study, 74 patients (60.7%, group II) had persistent positive responses or did not achieve a controlled state despite regular treatment with antiasthmatic drugs, including inhaled corticosteroids, long-acting β -adrenoceptor agonists or LTRA. When we compared medication requirements between the two groups, although we found a tendency to higher medication requirements for asthma control, there were no significant differences, suggesting that individual variance in genetic susceptibility is involved in the clinical course. We genotyped ten different SNPs for the candidate genes related to leukotriene and inflammatory pathways and found a significant difference in genotype frequency of *CCR3* (520T>G) between the two groups.

Our previous report showed that *CCR3* mRNA expression increased after an ASA challenge in AERD patients in an *in vitro* functional study [12], indicating that AERD patients with the G allele exhibited increased expression of *CCR3* receptors on eosinophils, mast cells and other inflammatory cells in airway mucosa, allowing activation of eosinophil and inflammatory cells

Table 3. Immunological parameters in subjects at diagnosis and follow-up visit.

Variables	Group I (n = 48)	Group II (n = 74)	p-value
At diagnosis			
IgE, IU/l (SD)	452.3 (880.7)	378.4 (487.5)	0.553
ECP, μ g/ml (SD)	25.3 (22.1)	29.3 (38.7)	0.667
Blood eosinophils, per μ l (SD)	427.6 (385.3)	458.9 (415.0)	0.680
Sputum eosinophils, percentage of WBC (SD)	33.5 (39.3)	38.7 (36.7)	0.617
Sputum neutrophils, percentage of WBC (SD)	57.7 (35.6)	46.0 (30.6)	0.224
At follow-up visit			
IgE, IU/l (SD)	279.8 (376.5)	271.4 (316.7)	0.911
ECP, μ g/ml (SD)	33.8 (36.9)	31.1 (32.3)	0.743
Blood eosinophils, per μ l (SD)	329.0 (307.4)	393.5 (488.6)	0.439
Sputum eosinophils, percentage of WBC (SD)	45.4 (40.4)	41.4 (36.7)	0.806
Sputum neutrophils, percentage of WBC (SD)	52.4 (37.0)	53.6 (35.1)	0.930

ECP: Eosinophil cationic protein; SD: Standard deviation; WBC: White blood cells.

Table 4. Allele frequencies of SNPs in the aspirin-exacerbated respiratory disease candidate genes between the two groups.

Gene	Locus	Risk allele	Genotype	Group I (n = 48), n (%)	Group II (n = 74), n (%)	p-value	OR	95% CI
ALOX5	1708G>A	A	GG	20 (83.3%)	36 (92.3%)	0.315	0.427	0.081–2.250
			AG or AA	4 (16.7%)	3 (7.7%)			
ALOX15	427G>A	G	GG	24 (68.6%)	39 (81.3%)	0.223	0.527	0.188–1.476
			AG or AA	11 (31.4%)	9 (18.8%)			
CCR3	520T>G	G	TT	22 (57.9%)	14 (25.9%)	0.002	4.790	1.818–12.621
			GT or GG	16 (42.1%)	40 (74.1%)			
CRTH2	466T>G	T	TT	19 (51.4%)	24 (45.3%)	0.588	1.264	0.542–2.948
			CT or CC	18 (48.6%)	29 (54.7%)			
CysLTR1	634C>T	T	CC	14 (36.8%)	22 (40%)	0.984	0.991	0.405–2.425
			CT or TT	24 (63.2%)	33 (60%)			
IL10	1082A>G	G	AA	31 (86.6%)	45 (83.3%)	0.819	0.88	0.294–2.632
			AG or GG	7 (18.4%)	9 (16.7%)			
IL13	1055C>T	C	CC	23 (62.2%)	33 (61.1%)	0.869	1.076	0.452–2.563
			CT or TT	14 (37.8%)	21 (38.9%)			
LTC4S	444A>C	A	AA	24 (66.7%)	42 (76.4%)	0.304	0.610	0.238–1.563
			AC or CC	12 (33.3%)	13 (23.6%)			
TGFB	509C>T	T	CC	16 (42.1%)	15 (28.8%)	0.203	1.778	0.733–4.315
			CT or TT	22 (57.9%)	37 (71.2%)			
TNFa	308G>A	A	GG	31 (86.1%)	42 (84%)	0.640	1.343	0.391–4.605
			AG or AA	5 (13.9%)	8 (16%)			
HLA DPB1*0301	+	Others		10 (25%)	16 (27.1%)	0.803	0.888	0.348–2.264
				30 (75%)	43 (72.9%)			

A p-value of ≤ 0.05 was considered statistically significant.
OR: Odds ratio.

and increased bronchial sensitivity to leukotrienes. In the present study, patients with the G allele showed more severe asthmatic reactions during the L-ASA BPT at diagnosis as well as at the follow-up study compared with patients with the TT genotype.

CCR3 is a G-protein-coupled receptor that binds to several small chemoattractant proteins, known as CC-type chemokines. These include members of the eotaxin family, including eotaxin-1, -2 and -3, which mainly recruit eosinophils to sites of inflammation [27]. Intense eosinophilic infiltration was clearly identified in bronchial mucosa and nasal polyps of AERD patients [28]. Eotaxin mRNA and protein expression was increased significantly in nasal polyp tissue, which might be a source of eotaxin [29]. Our previous study showed that CCR3 mRNA increased significantly after ASA challenges in AERD patients. In particular, an intronic SNP,

-520T/G, of the CCR3 gene was capable of causing increased transcription of the CCR3 gene [12]. In this study, although the baseline FEV₁ in patients with the GG or GT genotype was similar to those with the TT genotype at diagnosis, the patients with the G allele showed a greater decline in FEV₁ during the second L-ASA BPT, which was performed after treatment with antiasthmatics, compared with those with the TT genotype (Δ FEV₁ = 17.3 $\pm$ 10.9% for GT or GG vs 10.9 $\pm$ 8.7% for TT; FIGURE 1). Moreover, they showed a higher prevalence of nasal polyps, which reflect chronic eosinophilic inflammation in the upper airways. When we reanalyzed subjects according the presence of nasal polyposis, we found that the GG and GT genotypes were significantly more prevalent in patients with nasal polyposis compared with those without polyps (71.4 vs 48.8%, p = 0.028). Clinically, patients with nasal

polyps required more doses of inhaled corticosteroids than others ($192,417.39 \pm 135,371.06$ vs $136,034.04 \pm 130,579.01$ budesonide equivalent/ $\mu\text{g}/\text{year}$, $p = 0.044$). These findings suggest that eosinophilic inflammation can be exacerbated by the genetic polymorphism of *CCR3* and active, ongoing inflammatory responses contribute to the recurrent development of severe nasal polyps, bronchial epithelial damage, fibroproliferation and consequently airway remodeling.

Thirty patients were found to have severe asthma [20]. The prevalence of severe asthma was significantly higher in group II than in group I. Nevertheless, six patients in group I were classified into the severe asthma category. They had distinct features contributing to severe asthma in addition to persistent eosinophilic inflammation; one patient had sulfite hypersensitivity and two patients showed a mixed inflammation pattern of both eosinophils and neutrophils in their sputum analysis. The others were current smokers. Five of the six patients had the G allele at *CCR3*, which was a poor prognostic genetic marker of AERD. By contrast, approximately one third of group II were classified as severe asthma. They required significantly higher doses of inhaled and oral corticosteroids, had a history of more frequent hospitalization, and decreased baseline lung functions compared with the non-severe asthmatics in group II (data not shown).

In contrast to group I, there were no significant differences in the frequencies of the G allele at *CCR3* between severe asthmatics and nonsevere asthmatics in group II. Therefore, we suggest that the G allele at *CCR3* is a genetic marker that predicts persistent ASA hypersensitivity in AERD patients. Consequently, it might predict the severe eosinophilic asthma phenotype. However, we lack evidence regarding whether the G allele at *CCR3* is a genetic marker in other types of severe asthma. To clarify this issue, further studies of the severe asthma cohort with different phenotypes are needed.

There were several limitations to this study. First, the subjects were heterogeneous in terms of length of follow-up duration, use of controller medications, and severity of asthma. However, to minimize the effects of these factors on the results of the L-ASA BPT, logistic regression was performed; no significant relationships of the results of L-ASA BPT with these factors were identified in the study subjects.

Second, this study had risks of false-positive or false-negative results. In group I, all subjects showed negative responses to rechallenge with L-ASA inhalation. Although they were told to withdraw antiasthmatic drugs according to the guidelines [18], they risk being false negative because inhalation challenges are slightly less sensitive than the oral test [30]. Group II comprised both those with a second positive

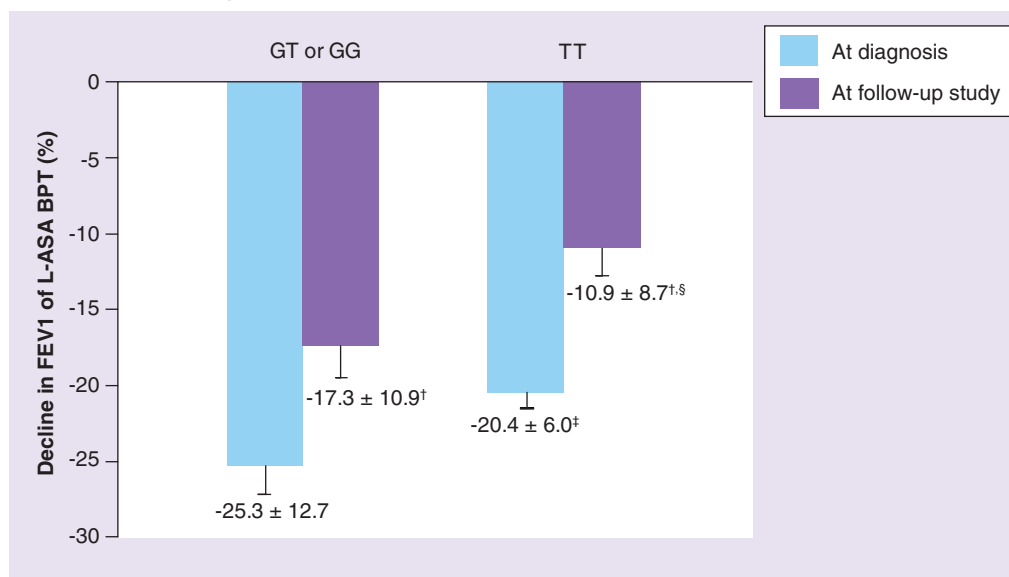


Figure 1. Effects of the *CCR3* -520T>G polymorphism on the decline in forced expiratory volume in 1 s of a lysine-aspirin bronchoprovocation test at diagnosis and follow-up study.

Data are mean values $\pm$ standard error.

[†] $p < 0.001$ at diagnosis and $p = 0.001$ at follow-up study in each genotype.

[‡] $p = 0.029$, GT or GG vs TT at diagnosis.

[§] $p = 0.026$ GT or GG vs TT at follow-up study.

FEV₁: Forced expiratory volume in 1 s; L-ASA BPT: Lysine-aspirin bronchoprovocation test.

reaction to L-ASA and the patients not tested owing to severe persistent asthma symptoms. During follow-up challenges, most patients needed full medications such as medium to high doses of inhaled corticosteroids with long active β -agonist and additional controller medications. When they were told to withdraw antiasthmatic drugs, their symptoms were too severe to perform rechallenge tests. For that reason, we determined the criteria of FEV₁ level should be more than 80% after stopping all antiasthmatic drugs prior to secondary inhalation challenges. In those patients, a nasal ASA challenge might be an alternative method of confirming ASA hypersensitivity, although its diagnostic sensitivity is lower than the L-ASA BPT. However, we did not perform the nasal ASA challenge in patients with severe asthmatic symptoms in group II – they were considered to have persistent ASA intolerance. Therefore, there might be a patient selection bias towards an increase in the number of patients in group II and those with persistent ASA hypersensitivity over time. However, the study was conducted in a practical, real-life setting that provided useful clinical markers, such as the baseline FEV₁ and degree of hypersensitivity of ASA, for predicting the prognosis of AERD.

This study demonstrated that 39.3% of AERD patients showed negative conversions at follow-up L-ASA BPT, suggesting that bronchial sensitivity to leukotrienes can be improved by antiasthmatic medications. The baseline FEV₁ and persistently low lung function were important clinical factors and the G allele at the *CCR3* -520 T>G polymorphism was a genetic factor that predicted a poor prognosis of AERD.

Conclusion & future perspective

Most AERD patients have moderate-to-severe phenotypes with significant mortality and morbidity. Abnormalities in the mediators and expression of arachidonic acid biosynthesis are related to the pathogenesis of AERD. Previous genetic studies of cysteinyl-leukotrienes have extended our understanding of the molecular mechanism of AERD and suggested genetic markers for predicting drug responses or hypersensitivity reactions. We evaluated the ability of both clinical and genetic factors to predict the clinical course of AERD using L-ASA rechallenge. Approximately one third of the study subjects showed negative conversions on rechallenge after treatment with antiasthmatic medications for at least 1 year, suggesting that AERD is not a homogenous disease when individual cases are analyzed carefully. In patients with positive responses or persistent asthmatic symptoms, the baseline lung functions were significantly lower than in patients with negative conversions, despite regular treatment. Therefore, the baseline FEV₁ and persistently worsening lung functions are important clinical factors, whereas the G allele of the *CCR3* -520 T>G polymorphism is a genetic factor related to persistent bronchial hypersensitivity to ASA and airway remodeling. These factors could guide individual therapy for AERD patients in clinical practice.

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Executive summary

- This study sought to identify prognostic factors in aspirin-exacerbated respiratory disease (AERD) by comparing clinical and genetic data with the clinical course.
- Patients with AERD were classified into two groups according to the results of a second lysine-aspirin bronchoprovocation test performed after 1 year of treatment; group I had negative conversions, and group II had positive results or were uncontrolled despite regular asthmatic medications. The frequencies of genetic polymorphisms in 11 candidate genes (*ALOX5*, *ALOX15*, *CCR3*, *CRTH2*, *CysLTR1*, *IL10*, *IL13*, *LTC4S*, *TGF β* , *TNF α* and *HLA DPB1*0301*) were compared between the two groups.
- Forty-eight patients (39.3%, group I) showed negative conversions, whereas 74 patients (60.7%) were in group II, which was composed of patients with positive responses ($n = 23$) or those not rechallenged owing to persistent symptoms ($n = 51$).
- Clinical parameters, including presence of nasal polyposis, smoking status and drug requirement, tended to be lower in group I than in group II, although this lacked statistical significance. However, there was a significant decrease in the annual number of steroid bursts in group I patients compared with group II ($p = 0.021$). The frequency of severe asthma was significantly lower in group I ($p = 0.019$).
- The baseline forced expiratory volume in 1 s (FEV₁) at diagnosis and follow-up were significantly higher in group I than in group II ($p = 0.012$ and $p < 0.001$, respectively).
- The *CCR3* polymorphism at -520T/G differed significantly ($p = 0.002$) between the two groups, whereas no difference was found in other genetic polymorphisms.
- The baseline FEV₁ and lower lung function after treatment were clinical factors that predicted a poor prognosis of AERD. The G allele of the *CCR3* -520T>G polymorphism was associated with persistent bronchial hypersensitivity to aspirin.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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