Allergol Immunopathol (Madr). 2015;43(1):92–100

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

REVIEW

Recent advance in investigation of gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease

M. Kurosawa[a,b,*], T. Yukawa[c], S. Hozawa[d], H. Mochizuki[e]

[a] *Department of Allergy and Respiratory Medicine, Sutoh Hospital, Annaka, Gunma, Japan*
[b] *Gunma Institute for Allergy and Asthma, Gunma, Japan*
[c] *Yukawa Clinic of Internal Medicine, Tochigi, Japan*
[d] *Hiroshima Allergy and Respiratory Clinic, Hiroshima, Japan*
[e] *Department of Pediatrics, School of Medicine, Tokai University, Kanagawa, Japan*

Received 17 March 2014; accepted 2 June 2014
Available online 12 September 2014

KEYWORDS
Gene polymorphisms;
AERD;
B2ADR;
IL-13;
IL-17A;
CYP2C19;
TBXA2R;
CRTH2;
HSP70

Abstract Aspirin-exacerbated respiratory disease (AERD) is a complex clinical syndrome characterised by severe asthmatic attack upon treatment with aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs). Genetic predisposition has been considered as a crucial determinant and candidate genes have concentrated especially on cysteinyl leukotrienes (LTs)-related genes as the inhibitory action of aspirin and NSAIDs on cyclooxygenase activity may cause overproduction of cysteinyl LTs. However, conflicting results have been reported, in parallel with replication studies in different ethnic groups. Thus, future areas of investigations need to focus on comprehensive approaches towards the discovery of other genetic biomarkers. Unfortunately, few papers have been reported about gene polymorphisms in Japanese patients with AERD. Here, we described on our recent genetic investigations on B2ADR, IL-13, IL-17A, CYP2C19, TBXA2R, CRTH2 and HSP70. This review indicates potential genetic biomarkers contributing to the early diagnosis of AERD, which may include CYP2C19 and HSP70 gene polymorphisms, and future validation studies in independent population are required to provide reassurance about our findings.

Introduction

Aspirin-exacerbated respiratory disease (AERD), so-called aspirin-intolerant asthma, is an acute asthmatic attack due to ingestion of aspirin and other non-steroidal

* Corresponding author.
E-mail addresses: motohiro@kl.wind.ne.jp, motohiroster@gmail.com (M. Kurosawa).

http://dx.doi.org/10.1016/j.aller.2014.06.004
0301-0546/

anti-inflammatory drugs (NSAID). However, the pathophysiological mechanisms underlying the development of this specific asthma phenotype have not yet been fully understood.

Because aspirin intolerance is found only in a specific population, genetic predisposition is considered a crucial determinant for the development of AERD. The inhibitory action of aspirin and NSAID on cyclooxygenase (COX) activity may cause diversion to the 5-lipoxygenase pathway, which leads to the overproduction of cysteinyl leukotrienes (LTs).[1] Therefore genetic association studies of LT-related genes have been undertaken to explore the genetic determinants of AERD. In fact, LTC_4 synthase promoter polymorphism has been reported to be associated with AERD.[2,3] Several investigations have shown that the genetic polymorphisms of 5-lipoxygenase promoter[4] and cysteinyl LT receptor 1 promoter[5] are risk factors for susceptibility to AERD. However, conflicting results have been reported[6,7] indicating that in parallel with replication studies in different ethnic groups, future areas of investigation should focus on the identification of genetic biomarkers for early diagnosis of AERD. In fact, Higashi et al.,[8] demonstrated that prostaglandin D_2 (PGD_2), a major prostanoid synthesised, among other cell types, by activated human mast cells, was overproduced during aspirin-intolerant bronchoconstriction, and no differences in the levels of lipoxygenase products have been found in blood from patients with AERD and those with aspirin-tolerant asthma (ATA).[9]

In this review, we report on the recent genetic investigations from our laboratory in Japanese patients with AERD,[10–14] which was performed with the approval of the Institutional Ethics Committee and with written informed consent from each individual prior to beginning the study. The target DNA sequence of each single-nucleotide polymorphism (SNP) was amplified using a set of primers as shown in each study, and allelic discrimination assay for the target SNP relating to the expression of each gene polymorphism was carried out as shown in the following section. Each study was carried out using the methods described below.

ß2-adrenergic receptor (B2ADR) genes analysis

B2ADR is encoded by intronless gene, which is located on chromosome 5q31-32.[15] It contains several reported SNPs,[16] including Arg16Gly (A46G, rs1042713), Gln27Glu (C79G, rs1042714) and Thr164Ile (C491T, rs1800888).[17–19] Although the B2ADR gene is not considered to be a major susceptibility gene for asthma, it has been suggested that its variant alleles may play a role in intermediate or asthma-associated phenotypes,[20] such as airway hypersensitivity,[21] asthma severity[22] and response to specific medications.[23]

As shown in the previous reports about the genotype frequencies of the B2ADR gene in Asian populations the allelic frequency of Gln27Glu polymorphism of the B2ADR gene is less prevalent among Japanese than in Caucasian population and only 7.5% of the subjects carried the polymorphism.[24] In fact, the frequency of Gln27Glu in a Japanese population is 2.3% in the dbSNP database of the National Centre for Biotechnology Information. On the other hand, the frequency of Arg16 allele is 53.8% in a Japanese population, which is similar to that observed in a Caucasian population.[24] So, we hypothesised that B2ADR gene polymorphisms might differ between patients with AERD and those with ATA.

DNA in the specimens (from 95 patients with AERD, 300 patients with ATA, and 100 normal controls) obtained by rubbing buccal mucosa with a cotton swab was extracted by using QIAamp 96 DNA blood kits (Qiagen, Hilden, Germany). The target DNA sequence of the B2ADR NM_000024.4 was amplified using a set of primers that were previously described[25,26] (forward, nucleotides 188–212: 5′-AGCCAGTGCGCTCAC-CTGCCAGACT-3′; reverse, nucleotides 406–383: 5′-GCTCGAACTTGGCAATGGC-TGTGA-3′) to generate an amplicon of 219 bp in length. Allelic discrimination assay for SNPs relating to the B2ADR expression (rs 1042713) was carried out using previously described SNPs detective system, sequence-specific thermal-elution chromatography.[27] All subjects and investigators remained unaware of the genotype until the final analysis. Allele frequencies were estimated by gene counting method. Significant departures of genotype frequency from the Hardy–Weinberg equilibrium were tested by the Chi-square analysis. Differences in minor allele (Gly) frequency in patients with AERD and control subjects were compared with that in patients with ATA by means of the Chi-square test and calculation of odds ratio (OR) with 95% confidence interval (CI). OR with 95% CI associated with ArgArg of patients with AERD was compared with that of patients with ATA. Polymorphisms related to the asthma phenotype were further examined by multivariable logistic regression analysis with adjustment for covariates. Statistical analyses were undertaken using SPSS for Windows version 17 (SPSS Inc, Chicago, IL, USA).

We[10] showed that the frequencies of wild-type ArgArg homozygote were significantly higher than those of variant-type ArgGly/GlyGly genotype in patients with AERD compared to those with ATA ($p < 0.001$), and the OR of patients with AERD associated with wild-type ArgArg homozygote to those with variant-type ArgGly/GlyGly genotype was 3.153 (95% CI = 1.789–5.558). In patients with AERD, frequencies of wild-type ArgArg homozygote in both female and male patients were significantly higher than those of variant-type ArgGly/GlyGly genotype in male patients compared with those with ATA ($p < 0.001$, OR = 5.128, 95% CI = 2.331–11.236 in female and $p = 0.007$, OR = 4.367, 95% CI = 1.495–12.821 in male, respectively). Also, in patients with AERD, frequencies of wild-type ArgArg homozygote in female patients were significantly higher than those of variant-type ArgGly/GlyGly genotype in female patients compared to those with ATA ($p = 0.002$, OR = 2.825, 95% CI = 1.453–5.495).

A study from Korea indicated a possible interaction of four loci including Arg16Gly genotype and cysteinyl LT receptor 1 promoter genotype in Korean subjects with AERD,[28] suggesting the possible interactions with B2ADR and overproduction of cysteinyl LTs in pathobiology of AERD. So, further studies are needed in Japanese people.

Cytokine genes analysis

Interleukin-13 (IL-13), mainly but not exclusively produced by T_H2 lymphocytes, is well known to be involved in eosinophilia and airway hyperresponsiveness.[29] On the other