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High-level expression of *early growth response-1* and association of polymorphism with total IgE and atopy in allergic rhinitis adults

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ABSTRACT

Background: Early growth response-1 (Egr-1) is expressed in human airways and its polymorphisms have been associated with total IgE and atopy in asthmatic patients. We investigated the effects of Chinese-tagging single nucleotide polymorphism (SNP) of *Egr-1* and its mRNA expression on allergic rhinitis (AR) traits.
Methods: Among 214 Chinese AR adults and 259 controls, tag SNP −4071 A→G was genotyped and mRNA expression in peripheral blood was quantified by real-time PCR.
Results: Egr-1 mRNA expression was significantly higher in patients than controls (median of 0.23 *vs* 0.15 fold *GAPDH* expression; $p<0.001$). Its expression was not associated with −4071 polymorphism. However, significant correlations were found between −4071 A→G with increased plasma total IgE ($p=0.028$) and atopy ($p=0.030$) in patients. Logistic regression confirmed the association ($p=0.034$) with age and gender adjusted. Patients homozygous for the A allele had a 2.3-fold and 1.9-fold risks, respectively of having increased plasma total IgE and atopy than those G allele carriers.
Conclusions: We showed high levels of *Egr-1* mRNA expression and demonstrated a significant association of polymorphism with increased plasma total IgE and atopy in AR patients. It may be useful to explore the pharmacogenetics of Egr-1 inhibitors.

1. Introduction

Allergic rhinitis (AR) is a complex inflammatory disease of the upper airway characterized by sneezing, nasal pruritus, rhinorrhoea and nasal obstruction [1]. The pathogenesis of AR and other allergic diseases involves complex interactions of environmental exposure to allergens and respiratory irritants [2,3] as well as genetic predisposition of the individual patients [4,5]. Many susceptibility loci for AR and atopy have been described in the Western populations [4–6]. These genes are responsible not only for causing AR but also affect its severity [7,8]. However, there have only been a few small-scale studies on the AR susceptibility genes in our Chinese population [9]. In Hong Kong, the prevalence rate of school-aged children with AR is known at about 35% to 44% [10] and is increasing [11]. We used the candidate gene approach to study polymorphic markers in susceptibility genes of atopy and airway inflammation [7] in our Chinese adults with AR.

Early growth response-1 (*Egr-1*) is an immediate-early response gene that is rapidly and transiently expressed in many cell types in response to growth factors, cytokines, mechanical stimuli, and oxidative stress [12]. It is a zinc finger transcription factor that can modulate transcription through interactions with coactivators, corepressors, and other sequence-specific transcription factors [13,14]. Studies have been performed on the protein level of Egr-1 relating to asthma in human and mice. Hjoberg et al. [15] found induction of Egr-1 by platelet-derived growth factor (PDGF) in human airway smooth muscle. Silverman et al. [16] reported that the *Egr-1* knock-out mice had increased IgE concentrations at baseline and after allergen challenge compared with wild type mice. Recently, Li et al. [17] suggested that *de novo* synthesis of Egr-1 was required for the full responsiveness of mast cells to produce TNF and IL-13 upon IgE and antigen stimulation. We also first reported significant association of *Egr-1* polymorphisms with plasma total IgE

Abbreviations: AR, Allergic rhinitis; CT, Threshold cycle; Egr-1, Early growth response-1; ERK, Extracellular signal-regulated kinase; FcεRI, High-affinity IgE receptor; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HDM, House dust mite; IFN, Interferon; M-MLV, Moloney murine leukemia virus; PDGF, Platelet-derived growth factor; TGF, Transforming growth factor.

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and atopy in Chinese asthmatics children [18]. The aim of this study was to investigate the correlation between −4071 A→G polymorphism in the promoter region of the *Egr-1* gene and AR phenotypes, plasma total and allergen-specific IgE concentrations, as well as its mRNA expression in peripheral blood of Chinese AR adults.

2. Materials and methods

2.1. Study population

This study recruited unrelated patients aged over 18 years with AR from the Ear, Nose and Throat (ENT) clinics of the Prince of Wales Hospital, the Alice Ho Mui Ling Nethersole Hospital and the United Christian Hospital in Hong Kong. The diagnostic criteria were according to the guidelines defined by the Joint Task Force on Practice Parameters in Allergy, Asthma, and Immunology [19] and included watery rhinorrhea, nasal itch, sneezing and nasal blockage, with nasal endoscopy being used to exclude other nasal pathologies by experienced ENT specialists. Subjects with a present or past history of asthma or atopic dermatitis were excluded from this study. Patients were categorized as having intermittent, persistent, mild, or moderate-to-severe rhinitis according to the ARIA classification [20]. Both parents of these subjects are ethnic Chinese. Age- and sex-matched Chinese control subjects were recruited from the Hong Kong Red Cross when they came for blood donation. Subjects with any history of allergic diseases were excluded by using the standardized questionnaire. All subjects were free from any self-reported symptoms of infection for 4 weeks before study. Subjects gave written consent, and the Clinical Research Ethics Committee of our university approved this study.

2.2. Plasma total IgE and allergen-specific IgE assays

Plasma total IgE concentration was measured by chemiluminescence immunoassay using the Immulite Analyzer (Siemens Healthcare Diagnostics, Los Angeles, CA) and specific IgE antibodies to crude *Dermatophagoides pteronyssinus*, cat, dog, mixed cockroaches and mixed moulds were measured by fluorescent enzyme immunoassay (ImmunoCAP system, Phadia AB, Uppsala, Sweden). Plasma total IgE results were compared with our in-house upper limits of reference values to determine whether the concentrations were increased. Specific IgE concentration ≥0.35 kIU/l was considered positive. Subjects were classified as atopic if they were positive with at least one type of allergen-specific IgE antibody [5].

2.3. Genotyping of Egr-1 polymorphism

Genomic DNA was extracted from peripheral blood using High Pure Viral Nucleic Acid Kit (Boehringer Mannheim GmbH, Mannheim, Germany). Subjects were genotyped for −4071 A→G polymorphism by polymerase chain reaction (PCR) amplification of the promoter region of the *Egr-1* gene using the following primers: 5′-GTG ATT CTC ATT GGC CTG GT-3′ and 5′-TAC TAT TCC CCA GCC AGC AG-3′ according to the published methods [18]. Briefly, PCR reactions were conducted in a total volume of 25 μl containing 60 ng of DNA, 10 mmol/l Tris-HCl buffer (pH 8.3), 1.5 mmol/l $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 50 ng of each primer, and 0.5 U of Taq DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). PCR products were digested by 5 U of *Mse*I (New England Biolabs, Hitchin, Herts, UK) in each digestion. The digested PCR products were resolved on agarose gel containing ethidium bromide. Eight DNA samples of known genotypes were included in every restriction enzyme digestion as positive control together with 88 unknown samples in 96-well plates. Finally, the results of these RFLP assays were validated by direct sequencing of the polymorphisms using Big Dye Terminator Cycle sequencing kits with an ABI-3100 sequencer (Applied Biosystems, Foster City, CA) in 40 randomly selected samples.

2.4. mRNA extraction and quantitative real-time PCR

Total RNA was isolated from Trizol preserved whole blood using a commercial available kit (RNeasy Plus Mini Kit, Qiagen Inc., Valencia, CA) and stored at −70 ºC. Reverse transcription was performed with the First-Strand cDNA Synthesis Kit (Invitrogen Corporation, Carlsbad, CA) containing oligo (dT) primers and Moloney murine leukemia virus (M-MLV) reverse transcriptase. Primers for *Egr-1* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were listed in Table 1. Amplifications of *Egr-1* and *GAPDH* were performed in a 96-well reaction plate. The 25-μL reaction mixture in each well contained 1 μL of total cDNA, 200 nmol/l of sequence-specific primers, and 12.5 μL of qPCR Super Mix (SYBR® GreenER, Invitrogen Corporation, Carlsbad, CA, USA). A negative PCR control without template and a positive PCR control with a template of known amplification were included. All samples and controls were duplicated. Real-time PCR was performed using an iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). After amplification, the relative gene expression was determined for each sample. For each target gene, a threshold was set above the mean baseline value for fluorescence determined on the basis of the first 15 cycles. Amplification reactions in which the intensity of fluorescence exceeded the threshold were defined as positive reactions [21]. The threshold cycle (CT) is the PCR cycle at which an increase in reporter signal above the baseline signal can first be detected. A comparative CT method was used for quantification of *Egr-1* mRNA expression as previously reported [22]. Briefly, the *Egr-1* specific signal was normalized by constitutively expressed *GAPDH* signal using the formula $2^{-\text{delta CT}} = 2^{-(\text{CT, GAPDH-CT, Egr-1})}$, resulting in the evaluation of the samples as n-fold difference relative to that of *GAPDH* mRNA.

3. Statistical analysis

The results of plasma total IgE and allergen sensitization as risk factors for the development of AR between patients and controls were compared using Student's *t* test or χ^2 as appropriate. Hardy–Weinberg equilibrium for the *Egr-1* −4071 A→G polymorphism in each group and the associations between *Egr-1* genotypes with AR and increased plasma total IgE and atopy were analyzed using χ^2 test. Because only 6 patients and 6 controls with the GG genotype of −4071 A→G had allergen-specific IgE data available, AG and GG genotypes were combined into a single category for the statistical comparisons. Multivariate logistic regression analysis was used to evaluate the effects of different covariates on increased plasma total IgE concentrations. Kruskal–Wallis test was used for comparing *Egr-1* expression in all groups while subgroup analyses were followed by Dunn's Multiple Comparison test. All comparisons were made 2-sided. A $P<0.05$ was considered significant.

4. Results

4.1. Demographic data

Two hundred and fourteen AR patients and 259 non-allergic healthy control subjects were recruited. The mean (SD) ages in patients and controls were 39.2 (13.7) and 37.2 (11.3) years, respectively ($p=0.083$),

Table 1
Sequences of primers for quantitative real-time PCR.

Gene	PCR primers	References
Egr-1	F: 5′-CAC CTG ACC GCA GAG TCT TT-3′ R: 5′-GTG GTT TGG CTG GGG TAA CT-3′	NM_001964
GAPDH	F: 5′-ATG GGG AAG GTG AAG GTC G-3′ R: 5′-GGG GTC ATT GAT GGC AAC AAT A-3′	NM_002046

Abbreviation: F, foward primer; R, reverse primer.