Association of Genetic Variants of Oxidative Stress Responsive Kinase 1 (OXSR1) with Asthma Exacerbations in Non-Smoking Asthmatics

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Abstract

Background: Asthma exacerbation threatens patient's life. Several genetic studies have been conducted to determine the risk factors for asthma exacerbation, but this information is still lacking. We aimed to determine whether genetic variants of Oxidative Stress Responsive Kinase 1 (OXSR1), a gene with functions of salt transport, immune response, and oxidative stress, are associated with exacerbation of asthma.

Methods: Clinical data were obtained from 1,454 asthmatics and single nucleotide polymorphisms (SNPs) of OXSR1 were genotyped. Genetic associations with annual exacerbation rate were analyzed depending on smoking status.

Results: Eleven SNPs were selected using Asian data in the International HapMap database. The common allele of rs1384006 C>T of OXSR1 showed a significantly higher annual exacerbation rate than the rare allele in non-smoking asthmatics (CC vs. CT vs. TT: 0.43 ± 0.04 vs. 0.28 ± 0.03 vs. 0.31 ± 0.09, P=0.004, Pcorr=0.039). The frequent exacerbators had a significantly higher frequency of the common allele of rs1384006 C>T than did the infrequent exacerbators (74.4% vs. 55.2%, P=0.004, P corr=0.038).

Conclusion: The common allele of rs1384006 C>T of OXSR1 was associated with the asthma exacerbation rate and a higher risk of being a frequent exacerbator, indicating that non-smoking asthmatics who carry common alleles may be vulnerable to asthma exacerbations.

Introduction

Asthma is a heterogeneous disease of chronic airway obstruction with a wide variety of symptoms, which develops in response to genetic and environmental influences.[1] Recent cluster analyses have demonstrated an exacerbation-prone phenotype in a certain number of asthmatics. [2, 3] Because asthma exacerbation is a potentially life-threatening condition, risk factors for exacerbation-prone asthma have been under intense research to assist in early diagnosis and the development of new treatment strategies. During the past decade, hypothesis-driven and hypothesis-free approaches about genetic factors and gene-environment interactions have been applied and many possibly associated genetic variants have been identified, including several single nucleotide polymorphisms (SNPs).[1] For example, a mutant allele of rs1800925 on IL13 was associated with emergency room (ER) visits or hospitalizations of Costa Rican children with asthma,[4] and those of rs1805011 and rs1801275 on IL4RA were associated with intensive care unit (ICU) care, ER visits or hospitalizations in two cohorts of US adult asthma patients.[5] The mutant allele of rs4950928 on CHI3L1 was also associated with asthma-related hospital admissions in adult and pediatric asthmatics.[6] The SNP rs726389 of ORMEL3 was associated with exacerbation of asthma of children between the ages of 1–6 years.[7] Variants of CD14 SNP rs2915863 and LY96 SNP rs17226566 were also related to the risk of acute severe exacerbations induced by environmental endotoxin exposure.[8] However, additional genetic factors associated with asthma exacerbation should be elucidated for in-depth understanding of the genetic pathogenesis and the improvement of diagnostic accuracy.

Patients with severe asthma, including exacerbation-prone asthma, have current unmet needs in terms of a lack of effective treatments, such as corticosteroids.[9] Corticosteroid insensitivity is a clinical feature of severe asthma and COPD, as characterized by the reduced effect of dexamethasone in inhibiting the release of proinflammatory cytokines from monocytes and macrophages.[10] Activation of p38 mitogen activated protein kinase (MAPK) may alter corticosteroid responsiveness in response to oxidative stress and enhanced oxidative stress is one of the main triggers inducing chronic airway inflammation.[11] Excessive generation of reactive oxygen species (ROS) has been shown to activate multiple protein kinases, such as extracellular signal-regulated kinase (ERK)1/2, protein kinase B (PKB), and protein tyrosine kinases (PTKs).[12, 13] Oxidants-induced mucin production from epithelial cells was accompanied by p38 MAPK activation resulting from a decrease in function of the tyrosine phosphatase Src homology region 2 domain-containing phosphatase-1 (SHP-1).[14]

One of the important clinical manifestations of exacerbation is an increase in the production of and alterations of the nature of mucus. A recent quantitative pathology analysis of fatal asthmatics found that more than 98% of their airways were occluded to some extent by mucus.[15] In addition, acute exacerbation with airway obstruction is usually caused by a mucus plug in the large and medium-sized bronchi, and even in the small airways.[16] The nature of mucus is regulated by the dilution of bronchial epithelial lining fluids. The transport of anions such as Cl− and HCO3− in the airway epithelium is recognized as one of the most important factors to regulate airway surface hydration and mucociliary clearance.[17] ROS induces lipid peroxidation of cell membranes and the oxidation of amino acids to inactivate membrane-bound receptors.[18, 19] This damage may modify the functions of membrane molecules, such as cystic fibrosis transmembrane conductance regulator (CFTR) and solute carrier family 26 member 4 (Slc26a4).[20]

The WNK-SPAK/OXSR1 kinase complex is composed of the kinases WNK (with no lysine) and SPAK (SPS1-related proline/alanine-rich kinase) or the SPAK homolog OXSR1 (oxidative stress responsive kinase 1). The WNK family senses changes in intracellular Cl− concentration, extracellular osmolarity, and cell volume and transduces this information to Na+, K+, and Cl− cotransporters (collectively referred to as CCCs [cation-chloride cotransporters]) and ion channels to maintain cellular and organismal homeostasis. WNK1 phosphorylates and activates two related kinases, OXSR1 and STK39, which in turn phosphorylate and activate the Na+K+Cl− co-transporters: SLC12A2 (NKCC1) and SLC12A1, SLC26A3, SLC26A6, SLC26A9 [21], CFTR [22], and the Cl− and/or HCO3− transporters NBCe1-B.[23, 24].

OXSR1 is ubiquitously expressed in most tissues, with high levels in the lung, especially the bronchial epithelium (https://www.proteinatlas.org/). In addition, OXSR1 is also thought to play an important role in regulation of immune response and oxidative stress.[25, 26] These data prompted us to study the association of genetic variants of OXSR1 with the risk of asthma exacerbation.

Materials And Methods

Study subjects
The study subjects were Korean asthma patients who met the following diagnostic criteria: physician-diagnosed asthma with airway reversibility (more than 12% and 200mL increase in forced expiratory volume in one second (FEV1), more than 20% change in peak expiratory flow rate), 20% or more of FEV1 improvement after asthma treatment for 2 weeks, or provocative concentration 20 (PC20) < 10mg/mL on methacholine bronchial provocation test. They were followed up to for longer than 1 year after enrollment at 3 tertiary hospitals.

DNA from 1,454 asthmatic patients who met these conditions were obtained from the biobank of Soonchunhyang University Bucheon Hospital, Korea and written informed consent was obtained at the time of DNA collection. The number of exacerbations was measured for the initial 1 year after enrollment, and asthma exacerbation was defined as the definition used by the American Thoracic Society/European Respiratory Society, which includes both severe and moderate exacerbation.[27] Severe exacerbation was defined as a condition that needs the addition of systemic corticosteroids (> 0.5mg of prednisolone/kg of body weight for more than 3 days) and consideration of a hospitalization or emergency room (ER) visit, and moderate exacerbation was defined as an exacerbation that is improved by increasing other asthma medications, such as inhaled corticosteroids (ICS), or by adding a rescue bronchodilator without using systemic corticosteroids.[27] Pulmonary function was measured at baseline and then every three months, and the ICS and systemic corticosteroids used were expressed as fluticasone equivalent dose (mcg/day) and prednisone equivalent dose (mg/year) as previously described.[28] The protocol was approved by the Ethics Committee of Soonchunhyang Bucheon Hospital (SCHBC_2014_07_028).

Selection of single nucleotide polymorphisms and genotyping

Single nucleotide polymorphisms (SNPs) in OXSR1 were selected using the Asian population database from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) and NCBI (http://www.ncbi.nlm.nih.gov/snp) databases as follows: first, candidate SNPs were extracted from the intragenic region including 2 kb of the 5’ region of the gene using Asian population data in the International HapMap database, and then linkage disequilibrium structures of each gene were analyzed using SNPs with > 5% minor allele frequencies (MAF). A representative SNP was selected in the case of absolute LD (|D′| = 1 and r2 > 0.95) between the SNPs. Finally, 11 SNPs were selected and genotyped using the GoldenGate assay with VeraCode microbeads (Illumina, San Diego, CA, USA) as previously described.[29] These were scanned using the BeadXpress® system (Illumina).

Statistics

Fisher’s exact test was used to compare the observed number of each genotype with those expected for a population in Hardy–Weinberg equilibrium (HWE). Haplotypes of each individual were inferred using the PHASE algorithm (ver. 2.1).[30] A type III univariate general linear model was applied to the continuous variables (number of exacerbations) and multiple logistic regression to the discrete variables (presence of frequent exacerbation). In the logistic regression analysis, the odds ratios (ORs) and 95% confidence interval were calculated for each genotype and haplotype. The data were analyzed using SAS ver. 9.1 (SAS, Cary, NC, USA) and SPSS ver. 12.0 (SPSS, Chicago, IL, USA). To correct the P-values for multiple comparisons, the effective number of independent SNPs (Meff) of OXSR1 was calculated using SNP spectral decomposition (http://genepi.qimr.edu.au/general/daleN/SNPSpD).[31] The calculated Meff value for the 11 SNPs of OXSR1 was 10.035. Corrected P (Pcorr) values < 0.05 were considered significant.

Results

Clinical characteristics of the study subjects

A total of 1,454 asthma patients were enrolled (Table 1). Their clinical characteristics were compared depending on their exacerbation frequency: frequent exacerbators (FE) were defined as subjects experiencing exacerbations 2 or more times in the first year of follow-up.
Association of SNPs and haplotypes on OXSR1 with annual number of exacerbations in the first year

In non-smokers, we performed analyses on each group of never-smokers (NS = 955) and current and ex-smokers (n = 499), because smoking itself is a confounding factor affecting asthma exacerbations. [32] Clinical parameters of the two groups are presented in supplement Table 3. In the analysis using the univariate general linear model, rs9839010, rs1392283, rs74919163, rs1384006, rs2011, ht1c, ht4c, and ht2 in block1 showed differences (P < 0.05) in the annual number of exacerbations depending on the genotypes in the non-smoker group (Table 2). Even after correction for multiple comparisons, patients with the common allele of rs1384006 C > T consistently showed higher annual exacerbation numbers (0.43 ± 0.04 vs. 0.28 ± 0.03 vs. 0.31 ± 0.09 in the dominant mode, Pcorr = 0.039, Table 2). In the smoker group, there was no significant association between SNP variants and number of asthma exacerbations (Table S4).
Table 2
Association of SNPs and haplotypes of *OXSR1* with the number of exacerbations during the 1st year of follow up in non-smoker subjects

| Locus          | No. of exacerbations, Mean ± SE (N) | Codomiant | Dominant | Recessive |
|----------------|----------------------------------|-----------|----------|-----------|
|                | CC                               | CR        | RR       | P*        | Pcorr     | P*        | Pcorr     | P*        | Pcorr     |
| rs9839010      | 0.41 ± 0.03 (600)                 | 0.28 ± 0.03 (308) | 0.34 ± 0.11 (47) | 0.038 | 0.383 | 0.011 | 0.114 | 0.268 | 1.000 |
| rs61005484     | 0.38 ± 0.03 (845)                 | 0.25 ± 0.06 (108) | 0.5 ± 0.5 (2) | 0.445 | 1.000 | 0.218 | 1.000 | 0.881 | 1.000 |
| rs4955408      | 0.31 ± 0.04 (342)                 | 0.44 ± 0.04 (462) | 0.28 ± 0.05 (150) | 0.081 | 0.811 | 0.090 | 0.905 | 0.411 | 1.000 |
| rs1392283      | 0.39 ± 0.03 (758)                 | 0.27 ± 0.04 (183) | 0.15 ± 0.1 (13) | 0.045 | 0.453 | 0.013 | 0.128 | 0.597 | 1.000 |
| rs112221585    | 0.37 ± 0.03 (817)                 | 0.33 ± 0.07 (131) | 0.33 ± 0.33 (3) | 0.846 | 1.000 | 0.566 | 1.000 | 0.875 | 1.000 |
| rs74919163     | 0.31 ± 0.03 (481)                 | 0.46 ± 0.04 (389) | 0.21 ± 0.07 (66) | 0.041 | 0.413 | 0.031 | 0.307 | 0.532 | 1.000 |
| rs1384006      | 0.43 ± 0.04 (541)                 | 0.28 ± 0.03 (356) | 0.31 ± 0.09 (58) | 0.015 | 0.153 | 0.004 | 0.039 | 0.476 | 1.000 |
| rs156260       | 0.37 ± 0.03 (736)                 | 0.36 ± 0.06 (206) | 0 ± 0 (13)    | 0.635 | 1.000 | 0.928 | 1.000 | 0.344 | 1.000 |
| rs9880223      | 0.38 ± 0.03 (845)                 | 0.25 ± 0.06 (108) | 0.5 ± 0.5 (2) | 0.445 | 1.000 | 0.218 | 1.000 | 0.881 | 1.000 |
| rs2298417      | 0.35 ± 0.03 (683)                 | 0.4 ± 0.05 (247) | 0.58 ± 0.15 (24) | 0.120 | 1.000 | 0.131 | 1.000 | 0.080 | 0.802 |
| rs2011         | 0.4 ± 0.03 (634)                  | 0.3 ± 0.04 (284) | 0.24 ± 0.09 (37) | 0.067 | 0.673 | 0.020 | 0.201 | 0.490 | 1.000 |
| Block1_ht1     | 0.34 ± 0.05 (216)                 | 0.42 ± 0.04 (506) | 0.28 ± 0.04 (233) | 0.120 | 0.360 | 0.077 | 0.231 | 0.646 | 1.000 |
| Block1_ht2     | 0.3 ± 0.06 (108)                  | 0.44 ± 0.04 (435) | 0.3 ± 0.03 (412) | 0.029 | 0.087 | 0.025 | 0.075 | 0.513 | 1.000 |
| Block2_ht2     | 0.47 ± 0.14 (34)                  | 0.37 ± 0.05 (309) | 0.36 ± 0.03 (612) | 0.768 | 1.000 | 0.814 | 1.000 | 0.469 | 1.000 |

*adjusted for age, sex, serum total IgE level, predicted FEV1% at the first visit, and total ICS and systemic steroid dose in the 1st year of visit as covariates.

CC, common allele homzygote; CR, heterozygote; RR, minor allele homzygote; SE, standard error of mean; Pcorr, corrected P value for multiple comparisons.

Pcorr: Corrected P values using the effective number of independent marker loci (*M*<sub>effLi</sub>) calculated by SNPSpD for each SNP (*M*<sub>effLi</sub> = 10.03501), and using the number of haplotypes (n = 3) for each haplotypes.
| Locus     | Exacerbation | Genotype (N, %) | Codominant | Dominant | Recessive |
|-----------|--------------|----------------|------------|----------|-----------|
|           |              | CC | CR | RR | Total | OR | P | PCorr | OR | P | PCorr | OR | P | Pc |
| rs9839010 | Exa < 2      | 544 | 293 | 44 (5%) | 881 (100%) | 0.48 [0.086] | 0.013 | 0.131 | 0.41 [0.2-0.84] | 0.015 | 0.151 | 0.29 [0.06-1.47] | 0.136 | 1.0 |
|           | Exa ≥ 2      | 56 | 15 | 3 (4%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs61005484| Exa < 2      | 778 | 101 | 2 (0.2%) | 881 (100%) | 0.86 [0.32-2.27] | 0.759 | 1.000 | 0.87 [0.32-2.33] | 0.779 | 1.000 | 0.0 [0-0] | 0.999 | 1.0 |
|           | Exa ≥ 2      | 67 | 7 | 0 (0%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs4955408 | Exa < 2      | 319 | 420 | 141 (16%) | 880 (100%) | 1.01 [0.65-1.59] | 0.957 | 1.000 | 1.12 [0.59-2.13] | 0.720 | 1.000 | 0.84 [0.34-2.05] | 0.702 | 1.0 |
|           | Exa ≥ 2      | 23 | 42 | 9 (12%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs1392283 | Exa < 2      | 693 | 175 | 12 (1.4%) | 880 (100%) | 0.53 [0.23-1.23] | 0.140 | 1.000 | 0.43 [0.17-1.1] | 0.078 | 0.784 | 2.03 [0.2-20.44] | 0.547 | 1.0 |
|           | Exa ≥ 2      | 65 | 8 | 1 (1.4%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs112221585| Exa < 2     | 755 | 119 | 3 (0.3%) | 777 (100%) | 1.28 [0.2-81] | 0.541 | 1.000 | 1.32 [0.59-2.95] | 0.506 | 1.000 | 0.0 [0-0] | 0.999 | 1.0 |
|           | Exa ≥ 2      | 62 | 12 | 0 (0%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs74919163 | Exa < 2      | 450 | 351 | 63 (7.3%) | 864 (100%) | 1.22 [0.2-205] | 0.461 | 1.000 | 1.29 [0.7-2.37] | 0.422 | 1.000 | 1.09 [0.24-4.93] | 0.912 | 1.0 |
|           | Exa ≥ 2      | 31 | 38 | 3 (4.2%) | 72 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs1384006 | Exa < 2      | 486 | 341 | 54 (6.1%) | 881 (100%) | 0.47 [0.0-83] | 0.009 | 0.094 | 0.36 [0.18-0.72] | 0.004 | 0.038 | 0.58 [0.15-2.26] | 0.435 | 1.0 |
|           | Exa ≥ 2      | 55 | 15 | 4 (5.4%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs156260  | Exa < 2      | 681 | 187 | 13 (1.5%) | 881 (100%) | 1.3 [0.69-2.43] | 0.413 | 1.000 | 1.51 [0.77-2.98] | 0.229 | 1.000 | 0.0 [0-0] | 0.999 | 1.0 |
|           | Exa ≥ 2      | 55 | 19 | 0 (0%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs9880223 | Exa < 2      | 778 | 101 | 2 (0.2%) | 881 (100%) | 0.86 [0.32-2.27] | 0.759 | 1.000 | 0.87 [0.32-2.33] | 0.779 | 1.000 | 0.0 [0-0] | 0.999 | 1.0 |
|           | Exa ≥ 2      | 67 | 7 | 0 (0%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs2298417 | Exa < 2      | 636 | 225 | 19 (2.2%) | 880 (100%) | 1.87 [1.13-3.09] | 0.015 | 0.155 | 1.96 [1.08-3.66] | 0.035 | 0.353 | 3.41 [0.97-12.01] | 0.056 | 0.5 |
|           | Exa ≥ 2      | 47 | 22 | 5 (6.8%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rs2011    | Exa < 2      | 575 | 270 | 36 (4.1%) | 881 (100%) | 0.43 [0.22-0.84] | 0.013 | 0.131 | 0.38 [0.17-0.81] | 0.013 | 0.132 | 0.24 [0.03-2.05] | 0.192 | 1.0 |
|           | Exa ≥ 2      | 59 | 14 | 1 (1.4%) | 74 (100%) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
|           | Exa ≥ 2      | 0 (0%) | 9 | 65 | 74 | 100% |

Table 3

Risk of a frequent exacerbator with the SNPs and haplotypes of OXSR1 in the non-smoker group.
immune responses by interacting with TNF receptor protein kinase C-θ relationship between asthma through these mechanism.

Therefore, the concentration, ASL uid layer, and mucociliary clearance, suggesting that genetic variants of OXSR1 gene, which plays a role in regulating salt, water and cell volume by an ionic mechanism, is likely to play an important role in the mucus gel.

This risk was significant even after correction for multiple comparisons (Pcorr: 0.038, Table 3). However, this SNP was not associated with the risk of frequent exacerbator in smokers (OR:0.93 [0.48–1.79], P = 0.825). In the analysis of all patients, common allele variants of rs1392283, rs1384006 and rs2011 were found to be associated with an increased risk of frequent exacerbators. However, these differences lost their significance after correction for multiple comparisons (Table S5).

**Association of SNPs and haplotypes of OXSR1 with risk of frequent exacerbation**

SNPs associated with the risk of frequent exacerbations were analyzed by logistic regression (Table S5). In the non-smoker group, the common allele homozygotes of rs1384006 C > T had significantly increased numbers of frequent exacerbators (CC vs. CR vs. RR: 74.3% vs. 20.3% vs. 5.4%, P = 0.004, OR: 2.7%). This mucociliary clearance is an important innate defense mechanism that cleans up inhaled allergens and other harmful stimuli.[33, 34] The mucus gel is placed on a uid layer called an airway-surface liquid (ASL), and the efficacy of mucociliary clearance depends on the ion transport pathways to maintain the depth of ASL.[35, 36]

Epithelial cells serve to protect airways from inhaled toxic substances and microorganisms. Airway secretory cells secrete mucin as the core glycoproteins of the mucus gel.

In previous studies, asthma was associated with reduced mucociliary clearance, especially during exacerbation. In B-epithelial Na+ channel (Scnnlb) transgenic mice, mucociliary clearance is reduced due to dehydration and thickened mucus.[39] In addition, the Scnnlb transgenic juvenile mice exhibit type 2 airway inflammation such as IL-13, airway eosinophilia, and alternative macrophage activation with reduced mucociliary clearance.[40, 41] In chronic lung diseases including asthma, epithelial Na+ channel blockers, amiloride, or hypertonic saline can restore mucociliary clearance by improving hydration of the airway surfaces. These facts support that there is a close association between the Na+ channel, mucociliary clearance, and asthma.[42, 43] Therefore, the OXSR1 gene, which plays a role in regulating salt, water and cell volume by an ionic mechanism, is likely to play an important role in the mucus concentration, ASL fluid layer, and mucociliary clearance, suggesting that genetic variants of OXSR1 are presumed to be related to frequent exacerbation of asthma through these mechanisms.

Recently, oxidative stress and its pathways have been thought to contribute significantly to severe asthma and asthma exacerbations.[44, 45] However, the relationship between OXSR1, a gene related to oxidative stress, and asthma exacerbation has never been studied. OXSR1 is also involved in the regulation of immune responses by interacting with TNF receptor protein kinase C-θ (PKCθ), which is expressed by lymphoid tissues.[25, 34] OXSR1 and WNK1 kinase, an

**Discussion**

In this study, we were the first to demonstrate that the common allele homozygotes of rs1384006 C > T of the OXSR1 gene were significantly associated with a higher exacerbation rate and the risk of FE in the nonsmoking asthmatics. OXSR1 has rarely been studied with regard to respiratory diseases, although it plays a role as a salt transportation, and cell volume control through ionic mechanisms and ion transport by bronchial epithelial cells is essential for healthy airways. Imbalance of the transport system is closely related to the pathophysiology of asthma such as dysfunction of epithelial cells and smooth muscles. [35, 36]

Previous studies found that asthma is associated with reduced mucociliary clearance, especially during exacerbation. In B-epithelial Na+ channel transgenic mice, mucociliary clearance is reduced due to dehydration and thickened mucus.[39] In addition, the Scnnlb transgenic juvenile mice exhibit type 2 airway inflammation such as IL-13, airway eosinophilia, and alternative macrophage activation with reduced mucociliary clearance.[40, 41] In chronic lung diseases including asthma, epithelial Na+ channel blockers, amiloride, or hypertonic saline can restore mucociliary clearance by improving hydration of the airway surfaces. These facts support that there is a close association between the Na+ channel, mucociliary clearance, and asthma.[42, 43] Therefore, the OXSR1 gene, which plays a role in regulating salt, water and cell volume by an ionic mechanism, is likely to play an important role in the mucus concentration, ASL fluid layer, and mucociliary clearance, suggesting that genetic variants of OXSR1 are presumed to be related to frequent exacerbation of asthma through these mechanisms.

Recently, oxidative stress and its pathways have been thought to contribute significantly to severe asthma and asthma exacerbations.[44, 45] However, the relationship between OXSR1, a gene related to oxidative stress, and asthma exacerbation has never been studied. OXSR1 is also involved in the regulation of immune responses by interacting with TNF receptor protein kinase C-θ (PKCθ), which is expressed by lymphoid tissues.[25, 34] OXSR1 and WNK1 kinase, an
upstream activator of OXSR1, are hardly detectable at basal activity, which may mean that WNK-OXSR1 signaling is regulated tightly in normal physiological conditions.

Interestingly, this genetic effect of rs1384006 C > T was not found in the smoker asthmatics. The reason for this finding could be explained by smoking itself being a strong inducer to exacerbate asthma.[32] Cigarette smoking is associated with accelerated decline of lung function in asthmatics,[46] resulting in worsening of asthma severity,[47] reduction of responsiveness to glucocorticoids,[48] poor asthma control, and a higher hospital admissions.[49]

The most important mechanism that may explain the relative corticosteroid resistance in smokers with asthma and COPD is a reduction in the expression of the enzyme histone deacetylase 2 (HDAC2). A reduction in HDAC activity and HDAC2 expression may account for the amplified inflammation and resistance to the actions of corticosteroids. The p38 mitogen-activated protein kinase (MAPK) pathway is also thought to play a role in corticosteroid insensitivity.[50] Thus, rs1384006 C > T of OXSR1 might not exert any genetic effect in the enhanced MAPK- and reduced HDAC-induced airway inflammation in smokers.

There are some limitations to this study. First, there is still little information about the function of the OXSR1 gene in asthma. According to functional estimation of the SNPs linked with rs1384006 in Asian populations (SNPinfo Web Server, https://snpinfo.niehs.nih.gov/), rs1384006 did not affect transcription factor binding, splicing sites, splicing regulation, or miRNA molecular functions. Additionally, since there is no known CpG island near rs1384006, this SNP is not likely to have an allele-specific effect on methylation of the OXSR1 gene as a CpG-SNP. On the contrary, since little is known about it, it is worthy as an original discovery to be the subject of future genetic studies about asthma exacerbations.

Second, we could not confirm the causal relationship between the minor allele variant of rs1384006 in the OXSR1 gene and asthma exacerbations in this study because it is only a genetic correlation study and a causal relationship needs a functional study. Third, because normal subjects were not included in the study, we cannot compare the SNP frequency with those of non-asthmatic controls. Therefore, further functional experiments are needed to identify its pathophysiology in asthma compared to normal controls.

Conclusions

We have newly discovered that variants of the OXSR1 gene, which is involved in the regulation of salt and cell volume, immune response, and oxidative stress, may affect asthma exacerbation. This will provide an opportunity to highlight a new genetic mechanism related to asthma exacerbation.

Declarations

Ethics approval and consent to participate

The protocol was approved by the Ethics Committee of Soonchunhyang Bucheon Hospital (SCHBC_2014_07_028). DNA from 1,454 asthmatic patients who met inclusion criteria were obtained from the biobank of Soonchunhyang University Bucheon Hospital, Korea and written informed consent was obtained at the time of DNA collection and the study protocol was carried out in accordance with the Declaration of Helsinki.

Consent for publication

All authors agreed to publication.

Availability of data and materials

Not applicable.

Competing interests

There is no competing interests.

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Author Disclosure:

All listed authors declare that they have no relevant conflicts of interest.

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