Author's response to reviews

Title: Genetic effect of CysLTR2 Polymorphisms on its mRNA synthesis and stabilization

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Author's response to reviews: see over
Reviewer's report

Title: Genetic effect of CysLTR2 Polymorphisms on its mRNA synthesis and stabilization

Version: 2 Date: 2 March 2009
Reviewer: Mayumi Tamari

Reviewer's report:
Shin and colleagues reported genetic effect of CysLTR2 polymorphisms on its mRNA synthesis, stabilization, and protein expression.

I have following comments.

C1. In introduction, the author described that CYSLTR2 is expressed in lung interstitial macrophage, pulmonary vascular smooth muscle, endothelium, eosinophils, and mast cells. However, in the present study, mRNA expression, DNA binding affinity, and luciferase analyses were conducted in B cell lines. The authors should describe the role of CYSLTR2 in B cells in aspirin hypersensitivity in asthmatics. Functional studies using monocytes or macrophages may strengthen the genetic effects of the variants.

R1. We added as following sentences in line 79 of “Introduction” section;
CYSLTR2 has been documented to be expressed in lung interstitial macrophage [10], pulmonary vascular smooth muscle, endothelium [11-13], eosinophils [14], and mast cells [15], B lymphocytes and T lymphocytes [24, 25]. Particularly, CysLTR2 may have important role in remodeling and fibrosis pathways in B lymphocytes.

C2. In discussion session, several descriptions overlap with those in result session.

R2. Thanks for your point. We condensed the overlapped sentences in discussion.


We demonstrated protein expression (Figure 1) and mRNA expression (Figure 2) using flow cytometry and RT–PCR in EBV-infected B cell lines. We then demonstrated that the B cell lines having $ht2^{+/+}$ had a higher expression level of CysLTR2 protein than those having $ht1^{+/+}$, but with similar positive rates of CysLTR2 expression in the two groups (Figure 1). At the transcriptional levels, the expression levels of CysLTR2 mRNA by B cell lines of $ht2^{+/+}$ were significantly higher than those of $ht1^{+/+}$, especially when stimulated (Figure 2). These results indicate that the genetic differences affect the amount of protein and mRNA expression in the CYSLTR2 gene.

We demonstrated that B cell lines having $ht2^{+/+}$ had higher translational and transcriptional expression levels of CysLTR2 than those having $ht1^{+/+}$, especially when stimulated (Figure 1, 2). These results indicate that the genetic differences affect the amount of protein and mRNA expression in the CYSLTR2 gene.

In the evaluation of the sequence variance of the 3′-UTR (c.2078C>T and c.2534A>G) on CYSLTR2 gene expression, the former was chosen since the c.2534A>G is located 124 bp downstream from the polyadenylation site, and would not be present in mature mRNA. In the 293T cell lines transfected with EGFP–CYSLTR2 3′-UTR fusion constructs, the expression level of EGFP containing the c.2078T type of 3′-UTR was significantly higher than that of c.2078C (Figure 5). This indicates that mRNA degradation of the T type of CYSLTR2 3′-UTR was much slower than that of the C type.

In the evaluation of the sequence variance of the 3′-UTR (c.2078C>T and c.2534A>G) on CYSLTR2 gene expression, the former was chosen since the c.2534A>G is located 124 bp downstream from the polyadenylation site, and would
not be present in mature mRNA. The expression level of fluorescence containing the c. 2078T type was significantly higher than that of c. 2078C (Figure 5). This indicates that mRNA degradation of the T type of CYSLTR2 3′-UTR was much slower than that of the C type.

C3. To demonstrate the generalizability of the study results, it would be better to explain about linkage disequilibrium information among the three SNPs with actual D’ and r^2 values and allele frequencies in several ethnic populations.

R3. Following sentences are added in line 89 of “Introduction” session;

In previously study, Five haplotypes (GCAA, TTGA, TCGG, TCAA and others) were constructed. The frequencies of these five SNPs in the Korean population (N=642), African-American (N=50) and Caucasian (N=50) were: 0.495, 0.14, 0.44 (ht1), 0.346, 0.02, 0.23 (ht2), 0.083, 0.17, 0.23 (ht3), 0.07, 0.6, 0.09 (ht4) and 0.006, 0.07, 0.01 (others) in Korean, African-American and Caucasian, respectively. Significant differences in the frequencies of the SNPs and haplotypes were observed among the three ethnic groups. The linkage disequilibrium coefficients (ID’I) and r^2 among the SNPs were calculated for all of the study subjects. Strong LDs were noted between SNPs (ID’I > 0.97) in Korean. Complete LDs were observed between SNPs (c.-819 G>T, c.2078 C>T and c.2534 A>G) in Caucasian (ID’I > 0.90). In African-American, between SNPs (c.2534 A>G and c.2545+297A>G) were observed complete LDs (ID’I > 0/92) [18].

In the reference 18,

Figure 1.
(c) LDs among CYSLTR2 polymorphisms in three ethnics

C4. Explanations for the roles of CYSLTR2 in other diseases would be helpful for readers.

R4. Thanks for your points. Following sentences were added in line 82 of “Introduction” session.

Previously sentence;
Furthermore, CYSLTR2 is located on chromosome13q14.2-21.1, near a locus known to be associated with the risk of asthma in various populations [16, 17].

Revised sentence;
Furthermore, CYSLTR2 is located on chromosome13q14.2-21.1, near a locus known to be associated with the risk of asthma in various populations. CysLTR2 play a disease-regulating role in glands and epithelium of rhinosinusitis, particularly aspirin-sensitive disease, predominantly [16]. Cysteinyl-LT signaling may provide a key balance between a release of endothelium-dependent relaxant and constricting factors in endothelium dysfunction [17]. In Myocardial Ischemia, the endothelial CysLTR2 is rapidly down-regulated by pro-inflammatory stimuli [18]. A role for
CysLTR2 is mediated the coronary constriction in patients with coronary artery disease [19]. CysLTR2 may contribute to neurological inflammation in the human brain and the adrenal glands of neuroendocrine system [20].

C5. Addition of rs numbers to the three SNPs would be helpful for readers.

R5. As reviewer’s recommendation, the sentence “rs number” has been inserted into line 91 as following;

We previously identified four sequence variants of the CYSLTR2 gene: one in the promoter (c.–819G>T, rs7324991), two in the 3’-flanking region (c.2078C>T and c.2534A>G, novel and rs912278), and one downstream of the gene (c.2545+297A>G, rs2407249) [18].

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
**Reviewer's report**

**Title:** Genetic effect of CysLTR2 Polymorphisms on its mRNA synthesis and stabilization

**Version:** 2  **Date:** 26 February 2009

**Reviewer:** Emiko Noguchi

**Reviewer’s report:**

The paper by Shin et al. performed functional analysis of CYSLT2 SNPs that have been shown to be associated with aspirin hypersensitivity in asthmatics. They found that these SNPs had some influence on the transcription levels. I have following comments.

**Major Compulsory Revisions**

**C1.** They found that haplotypes of CYSLT2 SNPs were associated with aspirin hypersensitivity. In order to investigate the effects of the haplotype, the authors should construct the vectors containing both 5’- and 3’- flanking regions of CYSLT2.

**R1.** Thanks for your point. According to your point, we performed the additional experiment using construct the vectors containing both 5’- and 3’-flanking regions of CYSLTR2. The expression level of luciferase containing the ht2 was significantly higher than that of ht1, especially when stimulated. These results indicate that the genetic differences affect the amount of protein and mRNA expression in CYSLTR2 gene.
In the flow cytometric analysis, approximately half of B cell are positive for CYSLT2R and the fluorescente intensity was significantly higher in B cells of the subjects with ht2+/+ than in those with ht1+/+. Heterogeneity of EBV-transformable human B lymphocyte populations has been reported, and not all B lymphocyte subsets are transformed equally well (Chan et al., J Immunol, 1986). Are there any characteristic features in CYSLT2 positive cells compared to these in negative cells? Please state these in the manuscript.

R2. Thanks for your points. Following sentences were added in line 321 of results section as following;
On flow cytometry analysis, side scatter and forward scatter were not different between the two cell types.

Minor Essential Revisions

C1. SNP should be described as reference SNP number (rsXXX) throughout the manuscript.

R1. As reviewer’s recommendation, the sentence “rs number” has been inserted into line 91 as following;
We previously identified four sequence variants of the CYSLTR2 gene: one in the promoter (c.–819G>T, rs7324991), two in the 3′-flanking region (c.2078C>T and c.2534A>G, novel and rs912278), and one downstream of the gene (c.2545+297A>G, rs2407249) [18].

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.