COX-2 POLYMORPHISMS, EPISTASIS AND THEIR ASSOCIATIONS WITH ASTHMA

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ABSTRACT

A total of 21 SNPs were identified in COX-2 gene in the Singapore Chinese population, of which only six have been reported in HapMap for CHB population. Eight of the SNPs were found to be in linkage disequilibrium. Two polymorphisms were selected for genotyping in a study population of 886 Singapore Chinese individuals consisting of 454 asthmatic cases and 432 non-asthmatic controls. A tag SNP, rs4648308 was selected for genotyping, with no significant association. A second polymorphism rs689466, or -1195G>A was prioritized for genotyping based on reported functional importance and was found to be associated to asthma (P=0.052). No gender association was found between the SNPs and the disease. Gene-gene interaction studies revealed that COX-2 interacts with two other genes, GSNOR and CTLA4.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways which is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (GINA 2008). In Singapore, the prevalence rate of asthma is 4.9% of the total population, with one of the highest mortality rate worldwide, 16.1 per 100,000 asthmatics (Masoli 2004).

The candidate gene under investigation in this study is prostaglandin-endoperoxide synthase 2 (PTGS2), or cyclooxygenase-2 (COX-2), a key enzyme in catalyzing the rate-limiting step in the formation of inflammatory prostaglandins from arachidonic acid (Malkowski et al. 2000).

We aim to provide a more complete picture of COX-2 gene and its relationship with asthma in our population, by identify SNPs in which are present in our population and genotyping, also, we hope to provide more insight into the interactions of COX-2 with other genes or environmental factors.

MATERIALS AND METHODS

Study population
A total of 886 unrelated ethnic Chinese subjects: 454 asthmatics and 432 controls were recruited in this study.

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Sequencing
Polymerase chain reaction (PCR) were performed in a 25μl reaction containing 100ng of amplified DNA, 5 nmol of each dNTP, 1U of pfu DNA polymerase (Fermentas, Glen Burnie, USA), 1X pfu buffer (200mM Tris-HCl pH 8.8 at 25°C, 100mM (NH4)SO4, 100mM KCl, 1% Triton X-100, 1mg/ml BSA, 20mM MgSO4), and 5 pmol each of forward and reverse primers. Cycle sequencing was done in a 20 μl reaction using BigDye® Terminator Cycle sequencing kit.

Genotyping by Allele-Specific PCR
Allele-specific PCR was performed in a 13 μl reaction mixture containing 40 ng of amplified DNA, 2.5 nmol of each dNTP, 0.6U of Taq DNA polymerase (Fermentas, Glen Burnie, USA), 0.6X Taq buffer (750mM Tris-HCl pH 8.8 at 25°C, 200mM (NH4)SO4, 0.1% Tween 20), 30 nmol of MgCl2, 2.5 pmol each of forward and reverse allele-specific primers, and 1.25 pmol of each forward and reverse internal control primers.

RESULTS
Identification of SNPs in COX-2
A total of 21 polymorphisms were identified, including 18 SNPs and 3 insertion-deletion polymorphisms. One of the SNPs has yet to be reported in the NCBI database before. When compared against SNPs reported in HapMap for Han Chinese population in Beijing, China (CHB). Only six of them were found to be reported, with comparable minor allele frequency.

Linkage disequilibrium of COX-2
There are 8 SNPs which are in linkage disequilibrium (LD), with $R^2$ at least 0.8 in the COX-2 gene. The distance between the two SNPs furthest away from each other (rs4648308 and rs4648250) is approximately 10 kb.

Genotype distribution and its relationship to asthma
Two SNPs, rs689466 and rs4648308 were prioritized for genotyping. The polymorphism rs689466 was found to be significantly associated with asthma, with borderline significance of $P = 0.052$, but no association was found for rs4648308. The AA homozygotes for rs689466 were overrepresented in the asthmatics (odds ratio = 1.45, 95% CI: 1.07-1.97, $P = 0.0175$).

Genotype distribution, gender and its relationship to asthma
When stratified the subjects according to sex, no statistically significance was found for female subjects for both the SNPs and male subjects for rs4648308. However, the p-value for rs689466 for males is close to significance ($P = 0.077$).

Gene-gene interaction of COX-2
Epistasis test based on SNP-SNP interaction was performed on COX-2 and several other in-house genes which have been studied or are currently under study.
Significant gene-gene interactions were found between COX-2 two other genes, GSNOR and CTLA4. Interaction was shown between COX-2 (polymorphism rs689466) and GSNOR (polymorphism rs1154404). The AA homoygotes of rs689466 on COX-2 were overrepresented in the cases when allele A of rs1154404 is also present.

When studied in isolation, no association was found for rs4648308 with asthma. However, epistasis of rs4648308 on COX-2 with CTLA4 (polymorphism rs16840252) showed significant association. The GG homozygotes of rs4648308 on COX-2 is overrepresented in the controls when allele T of rs16840252 is also present ($P=0.046$).

**DISCUSSION**

**Single nucleotide polymorphisms and linkage disequilibrium**

Although in general, most of the SNPs in the study population have minor allele frequencies which are comparable to the CHB population given in HapMap, some slight variations can be observed. This implies that even though the Singaporean Chinese may be highly similar to the CHB population, some slight deviations still exists.

**Genotype frequencies and their relationships with asthma**

Polymorphism rs4648308 is chosen as a tag SNP to represent seven other SNPs which are in LD with it, including rs20427, or -765G>C, a promoter polymorphism reported to be significantly associated to female asthmatics in the Polish population (Szczeklik *et al.* 2004). The minor allele (A) frequency of rs4648308 was found to be 0.06 in our controls, which is comparable to that reported by Koh *et al.* (2004) for -765G>C in Singapore Chinese. However, the C allele frequency of -765G>C was found to be 0.16 and 0.168 respectively in Polish and Australian population (Szczeklik *et al.* 2004; Shi *et al.* 2004), indicating that allele frequencies may be highly variable across different populations.

Another polymorphism studied is rs689466, or -1195G>A, which is not in LD with any other SNPs in COX-2. We found a significant association of -1195G>A with asthma ($P=0.052$). The allele frequency for A in the controls was 0.47, which is comparable to 0.49 reported by Zhang *et al.* (2005) but considerably different from that in the Australian population, which is reported to be 0.75 (Shi *et al.* 2008).

**Association of COX-2 with gender and asthma**

Our present study did not identify any significant association between gender and polymorphism association with asthma. The non-association may be attributed to real biological properties, but can also be caused by limitations in sample size.

**Gene-Gene interactions and association with asthma**

SNPs that failed to show association when studied in isolation may show significant association when epistasis effects were considered. The presence of risk alleles on multiple SNPs can have a magnified impact on the phenotype, as a smaller p-value of 0.039 was obtained when genotype of another SNP, rs1154404 on GSNOR was also considered. The protective effect of a certain
genotype may also be affected by alleles on another gene. For polymorphism rs4648308, the protective effect of GG homozygosity is only observed when allele T in rs16840252 of CTLA4 is co-existing.

CONCLUSION

A total of 21 SNPs were identified in COX-2 gene in the Singapore Chinese population, of which only six have been reported in HapMap for CHB population. Eight of the SNPs were found to be in linkage disequilibrium. Polymorphism rs689466, or -1195G>A was prioritized and was found to be associated to asthma ($P=0.052$).

REFERENCES:


