Associations of Thromboxane A2 receptor and IL-5 gene polymorphisms, with atopic asthma in Singapore ethnic Chinese Population

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ABSTRACT

Atopic asthma is a chronic disease whose incidence is rising in developed nations such as Singapore. The exact aetiology of asthma has yet to be elucidated as it is a complex multifactorial disease caused by interactions between both genetic and environmental factors. The Single Nucleotide Polymorphisms (SNPs) of asthma candidate genes Thromboxane A2 receptor (TBXA2R\textsubscript{T924C}) and Interleukin-5 (IL-5\textsubscript{T-746C}) had previously been found in a different population to be significantly associated with asthma when assessed in combination. This study analysed the two SNPs for associations with atopic asthma in the Singapore ethnic Chinese population, taking into account not only possible gene-gene interactions but also gene-environment interactions. It was done by mass screening of 868 subjects for both SNPs and then statistically analysing the SNP genotypes together with that of other genes, and with various environmental factors. None of the two SNPs was found to be directly associated with asthma when assessed individually or in combination. However, preliminary analyses reveal promising associations with asthma when the two SNPs are analysed in combination with other interacting factors. Of note, IL5\textsubscript{T-746C}, in combination with a SNP in Adrenergic beta-2 receptor gene (ADRB2\textsubscript{A45G}) was found to be significantly associated with asthma.

INTRODUCTION

Atopic asthma is a chronic disease whose incidence is rising in developed nations such as Singapore. The exact aetiology of asthma has yet to be elucidated as it is a complex multifactorial disease caused by interactions between both genetic and environmental factors. The Single Nucleotide Polymorphisms (SNPs) of asthma candidate genes Thromboxane A2 receptor (TBXA2R\textsubscript{T924C}) and Interleukin-5 (IL-5\textsubscript{T-746C}) had previously been found in a different population to be significantly associated with asthma when assessed in combination. This study analysed the SNPs from the TBXA2R and IL-5 genes for associations with atopic asthma in the Singapore ethnic Chinese population, taking into account not only possible gene-gene interactions but also gene-environment interactions.

MATERIALS AND METHODS

For this study, 868 adult ethnic Chinese subjects were recruited. The subjects were grouped into atopic asthmatic cases (N=454) and non-atopic healthy controls (N=414). The subjects

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filled in a survey questionnaire and also underwent a Skin Prick Test (SPT) to determine if they are atopic. DNA extraction and amplification, for use in genotyping, was carried out using buccal cells collected from gargling of saline solution by the subjects. Large-scale allele-specific genotyping of all subjects was done for the TBXA2R_T924C and IL-5_T-746C SNPs. Genotyping results of each SNP, individually and in combination with other SNP genotypes and environmental factors, were statistically analysed for association with asthma.

RESULTS AND DISCUSSION

Association of TBXA2R_T924C with asthma

The samples were stratified into cases and controls with their genotypic and allelic distributions for TBXA2R_T924C. The genotypes were found to be in Hardy-Weinberg Equilibrium (HWE) ($\chi^2$ values less than 3.14). Chi-square goodness-of-fit test was done to evaluate differences in TBXA2R_T924C genotypic distributions between cases and controls, but no significant differences were observed (p-value=0.382). Then, z-score was calculated for the allele frequencies, and again, no significant differences was seen (p-value=0.285).

The results show that for the Singapore ethnic Chinese population, there is no direct association between atopic asthma and TBXA2R_T924C polymorphism. This is in line with the results of a similar study done on Korean children (Hong et al., 2005).

However, in a similar study on the Japanese population, significant direct association of the SNP with asthma was seen (p-value=0.030) (Unoki et al., 2000). This is despite the allele frequencies of their cases and control, and sample size being comparable to those in this Singapore study. This implies that there are other interacting genetic and/or environmental factors, which are different between the Japanese and Singapore ethnic Chinese population.

More fascinating is that in a similar study on Hong Kong Chinese children (Leung et al., 2002), direct association between TBXA2R_T924C and atopic asthma was also seen, (p-value=0.044). This is despite the study having been done on ethnic Chinese, whom this study population is more genetically similar to. The difference in the Hong Kong findings with this study could be due to differences in sample size, with the Hong Kong study being smaller at 378 subjects, and the fact that their study population was children. Aside from that, considering that the Singapore and Hong Kong Chinese populations are genetically similar, the difference in findings may imply that differing environmental factors between these two populations have important interactions with the TBXA2R_T924C genotype that modify the propensity to develop asthma.

Association of IL5_T-746C with asthma

When the IL5_T-746C genotypes were analysed, it was found that in cases population, the genotypes are in HWE, but in the control population, the genotypes deviated from HWE to a significant extent (Case $\chi^2$ value=0.58, Control $\chi^2$ value=6.812). The deviation from HWE was detected due to over-representation of both the heterozygous (TC) and homozygous minor (CC) genotype in control population. No significant differences were seen between genotypes of cases and controls (p-value=0.276). Since the control population was not in HWE, the z-score for allelic frequencies was not calculated. Thus, there is no significant association between asthma and IL5_T-746C polymorphism. This is expected as although there is convincing evidence that IL-5 is important in asthma, no studies have found any direct association of IL-5 SNPs with asthma.
Gene-Gene Interactions in association with asthma

To investigate gene-gene interactions, the subjects were stratified according to their TBXA2R_T924C genotypes, followed by that of IL5_T-746C. No significant association of asthma with any combination of TBXA2R_T924C and IL5_T-746C genotypes (p-values=0.772, 0.164, 0.689) were seen.

The TBXA2R_T924C genotype was analysed in combination with the SNP genotypes of a variety of in-house candidate asthma genes. The same was done for IL5_T-746C. The genes are listed in Table 1. P-link software (Purcell et al., 2007) was first used to identify combinations of two SNPs that could possibly give a significant association with asthma. Table 2 shows the combinations that p-link returned as possibly giving a significant association with asthma.

| Table 1: In-house candidate asthma genes used for gene-gene interaction analysis. |
|---------------------------------|---------------------------------|----------------|
| 1 | Adrenergic beta-2 receptor | 2 | Alcohol Dehydrogenase 5 |
| 3 | Cyclooxygenase 2 | 4 | Cytotoxic T-Lymphocyte Antigen 4 |
| 5 | Defensin Beta 1 | 6 | Interferon-gamma |
| 7 | Interferon regulatory factor 1 | 8 | Interleukin-5 |
| 9 | Interleukin-13 | 10 | Prostaglandin-D2 Receptor |
| 11 | Prostaglandin-D2 Synthase | 12 | Thromboxane A2 Receptor |

| Table 2: Combinations of two SNPs that could possibly give a significant association with asthma. |
|------|-----------------|----------------|
| SNP ID | Associated SNP | P-link p-value |
| TBXA2R_T924C | COX2_rs4648308 (G/A) | 0.01198 |
| | IRF1_G1239A | 0.04661 |
| IL5_T-746C | ADRB2_A45G | 0.002187 |

However, upon manual stratification and calculation of chi-square values for the possible combination of genotypes, it was found that only the combination of Adrenergic beta-2 receptor SNP (ADRB2_A45G) and IL5_T-746C showed statistically significant association with asthma.

For the IL5_T-746C and ADRB2_A45G gene-gene interaction analysis, the subjects were stratified according to their IL5_T-746C genotypes, followed by that of ADRB2_A45G. It is seen that in subjects having genotypes IL5_T746C_TT, the genotypic distribution of ADRB2_A45G was significantly different between cases and controls (p-value=0.026). The ADRB2_A45G_AA seems to confer protection against asthma as the proportion of cases is significantly lower than controls for the combination of IL5_T746C_TT with ADRB2_A45G_AA phenotype.

The β2-adrenergic receptor (ADRB2) is the most common adrenergic receptor in the lung, and associations between ADRB2 polymorphisms and intermediate phenotypes of asthma have been reported (Migita et al., 2004). IL-5 plays an important role in airway hyper-responsiveness (Shi HZ et al., 1998). As such, the interaction between the two genes seen in the association
with asthma could be due to the combined effect of the two protein variants on lung function, leading to predisposition to airway hyper-responsiveness.

**Gene-Environment interactions in association with asthma**

The TBXA2R_T924C and IL5_T-746C genotypes were also analysed in combination with environmental factors based on answers to the survey questionnaire. Examples of these factors are frequency of exercise, gender and obesity. Some of these gene-environment combinations gave seemingly significant association with asthma. However, since simultaneous stratification for genotype and environmental factor greatly reduces the number of samples in each combination group, the power of the chi-square goodness-of-fit test is reduced greatly and thus the confidence for the significance obtained is ambiguous.

**CONCLUSION**

The Thromboxane A2 Receptor polymorphism (TBXA2R_T924C) and Interleukin-5 polymorphism (IL5_T-746C) do not have any direct association with atopic asthma in the Singapore ethnic Chinese population, and neither does the combined genotypes of TBXA2R_T924C and IL5_T-746C. IL5_T-746C in combination with ADRB2_A45G genotype is significantly associated with atopic asthma. Preliminary analysis of both TBXA2R_T924C and IL5_T-746C genotypes reveal promising association with asthma when genotypes are analysed in combination with other genes and environmental factors.

**REFERENCES**


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