PGDS, epistasis and gene-environment association with asthma and atopy

Yow Y.M.I.\textsuperscript{1} and Chew F.T.\textsuperscript{2}

Department of Biological Sciences, The National University of Singapore
Blk S2, 14 Science Dr 4, Singapore 117543

ABSTRACT

Asthma is a common chronic disease characterized by symptoms of wheezing, cough, dyspnea and tightness in the chest. Atopy is the increased predisposition to produce Immunoglobulin E antibodies in response to exposure of allergens, an important risk factor for the development of allergic asthma. In this study, sequencing was done to identify Single Nucleotide Polymorphisms (SNPs) for PGDS gene, after which a Linkage Disequilibrium (LD) block was constructed. The SNPs were all in LD except for two SNPs with low Minor Allele Frequency (MAF). Two SNPs, in LD, were chosen for mass screening of 886 subjects. PGDS and associations with either asthma or atopy and various interactions were examined via adequate stratification. The results showed that PGDS-\textsuperscript{-}rs11097411 C/T was not associated with asthma or atopy but was found associated with asthma / atopy with obesity instead. PGDS - rs35744894 A/G, showed the reversed results. Epistasis interaction was taken into account but no significance for both SNPs. These demonstrated the level of complexity of interactions involved in asthma pathogenesis.

INTRODUCTION

Studies were never done for PGDS in association asthma or atopy in the Chinese population, so more must be done other than merely an association study with asthma for a Japanese population since 2002. Therefore, in this study, The aim of this project is for us to identify all common and novel SNPs, gradually determine the LD patterns that arise from the gene PGDS in the local Han Chinese population via the SNPs that are identified by sequencing the PGDS gene and thereafter selecting key representative tagged SNPs for the evaluation of the possible genetic associations, gene-gene interactions and gene-environmental influences in the development of asthma.

MATERIALS AND METHODS

There were a total of 886 Singaporean ethnic Chinese subjects in this project and they belonged to another ongoing genetic study in the National University of Singapore, Singapore. The identity of the subjects’ ethnicity was self-declared through the survey questionnaire following the International Study of Asthma and Allergies in Childhood (ISAAC).

Asthmatic cases are individuals with documented history of asthma with positive skin prick test whereby asthmatic controls are individuals without documented history of asthma. Skin prick test (SPT) was performed on the subjects with four allergens commonly found in Singapore.

DNA was extracted from subjects’ mouthwashes with saline solution. Polymerase Chain Reaction (PCR) sequencing was performed on 20 cases and 20 controls to identify SNPs present in the genes to generate LD block for the gene PGDS from 886 subjects. The technique of Allele Specific (AS) PCR was used in the screening for the genetic screening.

\textsuperscript{1}Student  
\textsuperscript{2}Supervisor
RESULTS

The sequencing results from the 40 cases and control revealed 18 SNPs and 1 insertion/deletion (indel) mutation, a total of 19 polymorphisms. These SNPs were compared to the SNP database and rsIDs were identified for all reported SNPs. Among the 18 SNPs found, 3 SNPs were not found in the SNP database. None of the SNPs were found to deviate from HWE so all the SNPs and indel were included into the construction of LD blocks. All the SNPs found in this project were in the intronic regions, strongly suggesting that the PGDS protein is well conserved.

There were a total of 42 SNPs from CHB population in HapMap, of which 10 were similar to those found in the sequencing of the 40 subjects. From the LD block generated from this project, the whole PGDS gene seemed to be in strong LD. HapMap might be incomplete due to the absence of a number of SNPs that were sequenced out from the ethnic Chinese population in Singapore.

Two SNPs were selected for the screening of the study subjects via AS PCR. After AS PCR, gel electrophoresis was ran to visualize and thus deduced from there if genotype of either homozygous for wild-type allele or homozygous for mutant allele or heterozygote alleles.

The samples were stratified into groups of cases and controls with accordance to asthma and atopy respectively. PGDS-rs35744894A/G showed significances with p= 0.0195 for asthma and p=0.05091 for atopy. Both the SNPs were significantly deviated from Hardy-Weinberg in asthma and atopy. PGDS-rs35744894A/G had a reduced heterozygosity in the population.

The ethnic Chinese population in Singapore had approximately 95.4% similarity to the Han Chinese population when compared to the HapMap database (In-house data, unpublished).

For gene-environment interactions, the criterion of obesity was set with a cut-off of 23 for BMI, so subjects with BMI over 23 would be deemed as obese. For PGDS-rs11097411C/T with either asthma or atopy and obesity, power of association was rather weak even though there was significance of p=0.017 for both atopy and asthma because of low number in samples after stratification.

PGDS-rs35744894A/G showed no significance with obesity at p=0.812 for both atopy and asthma.

Other asthma-associated risk factors such as smoking and gender were stratified with asthma and atopy but there were no significant gene-environment interactions.

Plink (http://pngu.mgh.harvard.edu/~purcell/plink/) was used to calculate the possible epistasis interactions between the two PGDS SNPs and other in-house SNPs that were done on the same samples from the same sample population. In summary, PGDS SNPs were omitted from the output and there were no significant epistasis interaction observed even though PTGDR and COX2 were in the same arachidonic pathway as PGDS.

DISCUSSION

A possible method to sequence out the large PGDS gene in a faster mode is to perform long-range PCR using Taq DNA polymerase for high process, coupled with Pwo polymerase for proofreading abilities to achieve PCR fragments of approximately 10kbp to 20kbp. In addition, to overcome the problematic GC-rich regions, dimethyl sulfoxide (DMSO) and 7-deaza-GTP can be incorporated into the long-range PCR (Liu, 1998) along with hot starts to reduce non-specific amplification.
Although the MAF for the SNPs from HapMap in concordance to those found in this project were relatively high, with most SNPs found to be in the intronic regions suggested a higher mutation rate in PGDS than other genes. Therefore a lower MAF cut-off, and a subsequent increase in number of cases and controls, would allow a better capture of other SNPs’ significances.

If the SNPs were not in HWE, then it could possibly be due to selection pressure on the ethnic Chinese population. Some models of migration such as the Wahlund effect accounted for the reduction of heterozygosity in a population. This could possibly be caused by geographic barriers to gene flow, followed by genetic drift in the subpopulations.

The visual similarity observed for the two LD blocks suggested the two populations shared ancestral mutation and recombination events as the CHB population LD corresponded well with the HapMap LD blocks, as shown from the similar SNPs shared in both LD blocks.

With a relative similarity between CHB and Japanese populations, this could lead to a hypothesis of a close relationship between PGDS and asthma in the ethnic Chinese population in Singapore, highly similar to the CHB population. As PGDS regulates inflammatory responses in the lungs and a recent study was done to show that high-fat feeding could possibly redirect local immune responses to allergen in the lungs and systematic responses in the spleen and serum. The conventional concept that dietary fats are associated with obesity and obesity correlates with asthma and attenuated airway function is being challenged.

In the stratification of the samples by obesity, there were not many significant differences in the genotypic and allelic distributions except some of the genotypes were observed to be conferring risk or protection in the two SNPS with either asthma or atopy. Genotype CT from PGDS-rs11097411C/T seemed to increase risk of asthma and atopy whereas genotype TT in controls could be over-represented and thought to confer a lower risk of asthma when number of controls was only a third of the number of cases. Obesity could be placing a multiplier effect on PGDS as it amplified the phenotypic strength of asthma. Therefore, other genes or SNPs that weakened the association of PGDS-rs11097411C/T with asthma or atopy could have masked the phenotypes of asthma since the SNP was found not to be associated directly with either asthma or atopy.

The genotype AG of PGDS-rs35744894A/G seemed to be conferring higher risk of atopic asthma whereas genotype AA conferred a protective effect on either asthma or atopy. This coincided with A. de Vries et al’s (2009) finding of high-fat diet able to redirect local immune responses to allergen in the lungs. This suggested that high-fat diet might be involved in a complex pathway such that the immune responses, part of the function of PGDS, could be suppressed to its lower than naturally occurring phenotypic strength as shown by the SNP’s associations with the diseases respectively. There seemed to be a masking effect on this gene, possibly suppressed by either another gene or SNP with respect to obesity. The genotype AG seemed to be conferring higher risk of atopic asthma whereas genotype AA conferred a protective effect on either asthma or atopy. This coincided with A. de Vries et al’s (2009) finding of high-fat diet able to redirect local immune responses to allergen in the lungs. This suggested that high-fat diet might be involved in a complex pathway such that the immune responses, part of the function of PGDS, could be suppressed to its lower than naturally occurring phenotypic strength as shown by the SNP’s associations with the diseases respectively. For PGDS - rs11097411 C/T, it could be under-represented in the local Chinese population. Hence, a need to stratify the local
Chinese population further before an association could be observed. Once again, a larger study group should be examined for further validation of these results.

REFERENCES