Genetic Variation of GSNOR in the Singapore Chinese Population

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ABSTRACT
The main objective of this project is to identify SNPs (Single Nucleotide Polymorphism) in the Singaporean Chinese population by sequencing GSNOR. Representatives SNPs would then be selected and evaluated for possible associations with asthma, with other genes or environmental factors such as gender. Through sequencing, novel SNPs are discovered. Two of the SNPs were then chosen for genotyping. Out of the two SNPs chosen for genotyping, one of them, rs1154404 A/T, showed significant association with gender and alcohol consumption. Furthermore, gene-gene interaction was shown between GSNOR and COX2.

INTRODUCTION
Asthma is a complex multifactorial disease that is characterized by reversible airway obstruction, airway hyperresponsiveness, and eosinophilic airway inflammation (Peden, 2000) The regulation of airway hyperresponsiveness in asthmatics has been found to be associated with the Nitric oxide (NO) signaling pathways. Comparing the NO levels in the exhaled air between asthmatics and unaffected individuals, NO levels are higher in asthmatics. The most common S-nitrosothiol in the airway is S-nitrosoglutathione (GSNO) and the enzyme that serves to catalyze its metabolism and thus controlling its intracellular levels is GSNOR. In a study performed by Que, et al, GSNOR gene knockout mice have increased lung S-nitrosothiols and are protected from airway hyperresponsiveness after allergen challenge, indicating that GSNOR is an essential modulator of airway tone. Furthermore, genetic variations in GSNOR are found to be associated with asthma.
RESULTS AND DISCUSSION

Sequencing results

The GSNOR gene was sequenced and despite the huge length of gene sequenced (17kb), it is observed that there are relatively few polymorphisms found for the gene within the population. This might be because the gene is evolutionary important and hence it is highly conserved. This suggests that they fulfil important housekeeping roles in cellular metabolism.

Genotyping results

Association of GSNOR with asthma

Doing statistical analysis from both the SNPs, it is found there is no direct association with the both the SNP with asthma in terms of genotypic and allelic distribution.

Association of GSNOR with environmental factors

As there were no associations of both SNPs with asthma, other possible associations were evaluated. First, we evaluated the presence of any associations of the SNPs with environment factors such gender, exposure to tobacco smoke, consumption of alcohol and activity level. There was no significant interaction found between either SNPs and the exposure to tobacco smoke and activity level. Near significant association was found for between the association of rs-1154404 with gender and the consumption of alcohol.

GSNOR vs Gender

From table 4, it is observed that the p-value for the fishers exact test was 0.087, showing near significance in terms of gender effects. Males having both copies of the major allele seemed to be at reduced risk for asthma. In the females, there is no significance difference in having any of the genotypes with the risk of getting asthma. This might be due to the effect of estrogen. Some studies have suggested that NO metabolism, particularly GSNOR expression, is influenced by estrogen.
**GSNOR vs alcohol consumption**

Having the A allele in asthmatics might cause the gene to be less active. With a reduction in gene activity in the airway, the levels of the nitrosothiols would remain relatively higher and not easily lead to airway hyperresponsiveness and therefore not lead to bronchodilation as easily as people who have the T allele.

**Gene-gene interaction**

Using plink, gene gene interactions were tested out between GSNOR and the other in-house genes that were genotyped. Gene-gene interaction with a p-value of <0.05 was considered to be significant. Out of all the genes that were tested against, only COX2 showed association with GSNOR.

However, the presence of the minor allele of AA for COX2 rs689466 SNP, seems to increase the risk for asthma as there is a difference in the numbers of case and control. In all the other genotype combinations, the number of cases are lesser then the number of controls. In contrast, there are more asthmatics with the genotype of TATT as compared with the people in the control group. Having the minor allele for AA of the GSNOR-rs1154404 and AA genotype of COX2 rs689466 SNP seemed to confer protection against asthma. None of the asthmatics have the homozygous minor alleles of both SNPs. This could be because there is some synergistic interactions between the NO pathway and prostaglandin systems. Apart from the prostaglandins, nitric oxide (NO) is a major small-molecule mediator of inflammation. Both GSNOR and COX2 are involved in the NO pathway. GSNOR breaks down the GSNO and controls the amount of intracellular levels of SNOs. If there is a decrease in GSNOR, there would be an increase in the GSNO and thus more GSNO could help in the s-nitrosylation of COX-2 and enhances the catalytic activity of COX-2, increasing the production of proinflammatory prostaglandins that may ultimately result in an exacerbated inflammatory response.
REFERENCES


Hao Wu, PhD,1 Isabelle Romieu, MD,3 Juan-Jose Sienra-Monge, MD,4 Blanca Estela del Rio-Navarro, MD,4 Daniel M. Anderson, BA,1 Charlotte A. Jenchura, BA,1 Huiling Li, MD,1 Matiana Ramirez-Aguilar, MD,3 Irma del Carmen Lara-Sanchez, BS,3 and Stephanie J. London, MD,1,2 Genetic Variation in S-nitrosoglutathione Reductase (GSNOR) and Childhood Asthma. Journal of allergic clinical immunology. (2007)

O’Byrne P et al, Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2007)