Investigation of the SMAD3 Haplotype Structure and Allelic Distribution of two Candidate SNPs

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BACKGROUND

The present study is an extension of two previous gene-environment interaction analyses on asthma that identified two single nucleotide polymorphisms (SNPs), rs2118610 and rs9302242, located on chromosome 15 within the SMAD3 gene. These SNPs were found to modify the association between outdoor air pollutants and two asthma-related outcomes, fractioned exhaled nitric oxide (FeNO) (rs2118610) and the Symptom frequency and anti-asthmatic Treatment intensity Score (STS score) [1] [2] (rs9302242), in adult patients with asthma from the general Italian population (Gene Environment Interaction in Respiratory Diseases - GEIRD [3]).

AIM

This analysis aims at investigating the haplotype structure of SMAD3 gene, in order to assess the distribution of the alleles of the two SNPs across the most common haplotypes of SMAD3 gene.

METHODS

GEIRD is an Italian multicentre (multi)case-control study investigating the role of genetic and modifiable factors in asthma, COPD, chronic bronchitis, and allergic rhinitis, with cases and controls identified from pre-existing cohorts and new population samples through a two-stage process involving a screening questionnaire followed by clinical examination. Participants' addresses were geocoded and linked to daily outdoor air pollution estimates using BIGEPI [4] exposure models, including three-year (2013–2015) averages of PM2.5, NO2, and O3, as well as summer (April–September) O3 levels. Respiratory symptoms and use of anti-asthmatics treatment were combined into the STS score [1] [2], which is a valid and replicable continuous measure of asthma severity in adults.

We performed quality check steps on 997 subjects and 384 SNPs from the GEIRD [3] study using PLINK [5]. Individuals with more than 10% missing genotype data and SNPs having more than 5% missing data were filtered out. We removed SNPs deviating from Hardy Weinberg equilibrium (p-value $< 1 \times 10^{-6}$), subjects with excessive heterozygosity level, and closely related individuals. In addition, we performed a Principal Component Analysis (PCA) to exclude population outliers based on genetic ancestry, using the 1000 Genomes Project (GRCh37) [6] as reference panel. Genotype phasing was conducted using Eagle v2.4.1 [7] [8] on a subset of 321 patients with asthma who passed the quality checks and on 15 genotyped SNPs located on chromosome 15. Genotype imputation was carried out using Minimac4 [9], based on the 1000 Genomes Project [6] reference panel. Haplotype frequency was estimated using imputed data.

RESULTS

The two polymorphisms (rs2118610 and rs9302242) are located within the intronic region of SMAD3 gene. SNP

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rs2118610 lays within an active regulatory region characterized by 11-zinc finger protein (CTCF) and RAD21 Cohesin Complex Component (RAD21) binding [10]. Similarly, rs9302242 overlaps a strong regulatory element marked by transcription factor binding peaks for Homeobox Containing 1 (HMBOX1), and RE1-Silencing Transcription factor (REST) [10]. Both SNPs are in very low linkage disequilibrium (LD; R2 = 0.016) and are located on different haplotype blocks. Both alleles in the two SNPs are uniformly distributed among the common haplotypes.

CONCLUSION

This haplotype analysis suggests that the two SNPs may influence asthma-related outcomes independently in response to environmental exposures, in adult patients with asthma from the general Italian population.

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