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Genetic Susceptibility to Asthma and Genetic Interactions in the 5q31-q33 and 16p11 Regions in Sudanese Families

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Abstract

Background: Asthma is a complex disorder with heterogeneous phenotypes attributed to the interactions of many genes and the environment. Numerous genetic studies have mapped asthma susceptibility genes to a region on chromosome 5q31-q33. This study aimed to determine the association of 10 candidate polymorphisms in IL-4, IL-5, IL-9, IL-13 and *IL-4R* genes in 5q31-q33 region with susceptibility to asthma in Sudanese families.

Method: Fifty two multi cases families consisting of 141 known cases of asthma and 129 healthy individuals from Khartoum state were genotyped for seven SNPs on 5q31-33 region located in four candidate genes; IL4, IL5 IL13, IL9 and three SNPs in IL4Rα on chromosome 16p using multiplex PCR with Mass ARRAY system. Multiple logistic regression was used to test for association of asthma. P-value needed to achieve statistical significance taking multiple testing into account is P=0.005 (=0.05/10 (number of SNPs genotyped)). However, since SNPs within genes showed some degree of linkage disequilibrium and SNPs were selected as major SNPs for association with Asthma in other populations. Therefore, P ≤ 0.01 (=0.05/5 genes) can used as the P-value required to achieve correction for multiple testing.

Result: Genotype and allelic frequencies of all SNPs were similar in both asthmatics and healthy subjects. Stepwise logistic regression demonstrated that SNP IL-13 rs2069743 was markedly associated with Asthma (P=0.008) and same SNP added significant main effects to IL-4 rs2070874 or IL-9 rs31563, whereas the reverse was not true, indicating that the main effect for association with asthma in this population is most strongly tagged by SNP IL-13 rs2069743

Conclusion: There is strong genetic association of SNPs in 5q31-q33 and 16p11 region and asthma.

Keywords: Asthma; Sudanese; 5q31-q33; 16p11; SNPs

Introduction

Asthma is a chronic disease characterized by reversible airway obstruction, bronchospasm, resulting in episodes of cough, wheeze and chest tightness [1]. The disease affects 7.4% of adults and 8.6% of children in the Unites states resulting in significant morbidity [2]. In Sudan the prevalence of asthma is high, 9.2% to 17.9% of school children in some areas are affected [3]. Atopic asthma is an allergic condition mediated by inflammatory cells (eosinophils, mast cells), cytokines (IL-4, IL-5, IL-9), and antibodies towards innocent environmental antigens [4,5]. The level of serum IgE and eosinophil count correlate with the severity of asthma [6].

Asthma is a multifactorial disorder, caused by the interaction of hereditary and environmental factors [7]. The genetic component of asthma complex with multiple genes interacting with each other (polygenic inheritance) [8] and different combinations of allele variants contributing to a range of phenotype in different families (genetic heterogeneity) [9]. Genome wide association studies have linked many chromosomal regions to the susceptibility of asthma including human chromosomes 5, 6, 7, 11, 14, and 12 [10–12], but the most interesting is chromosome 512. The human chromosomal 5q31-33 region has been implicated as a susceptibility locus for several immune-mediated diseases including asthma and asthma related traits in several populations [13,14]. The region contains genes that code for inflammatory cytokines such as IL-4 IL-5, IL-9, IL-13 and CD14 [15]. These cytokines are well known mediators in the pathogenesis of asthma [16]. Genetic polymorphisms of those cytokine and their receptors are the focus of many genetic association studies [12-15]. Associations of these cytokines with susceptibility to asthma have been found in many different populations around the world but none was done in Sudan. The objective of this study was to investigate candidate polymorphisms in genes on 5q31-33 and 16p11 chromosomal regions, principally IL-4, IL-5, IL-9, IL-13, and IL-4R and their relationship to asthma in Sudanese families. We investigated both single variants and interactions of multiple variants in association with asthma, since the latter more likely underline the genetic role in susceptibility to asthma.

Materials and Methods

Study design and population

This study was a family based association study. A total of 52 Sudanese multi cases families, were included based on sample of nuclear and extended families consisting of 141 cases and 129 controls, the studied pedigrees were all together families' with sib pairs, complete sets of parents, and incomplete or missed parents from different ethnic backgrounds, but all living in Khartoum state. The subjects were asthmatic patients visiting respiratory outpatient clinic located in Khartoum area, selected by identifying at least in each case a proband (parent or children) with variable severity of asthma diagnosed by at least two chest physicians. The diagnosis of asthma was based on clinical symptoms and the criteria of the Global Initiative for Asthma (GINA, 2010) (Available from: www.ginasthma.com). Five ml of Blood was obtained from each participant for total IgE, and eosinophil count and for DNA analysis. DNA was extracted using guanidine chloride method as was mentioned in [17].

Eosinophil counts

A full-blood picture was performed on each subject; this includes an automated white cell count in order to establish a total blood eosinophil count. (Total white blood cell counts were performed with a Coulter counter) Blood smears from peripheral samples were stained (Wright-Giemsa stain) and evaluated under a microscope. A 200-count white blood cell differential was performed, and the percentages and absolute eosinophil counts were calculated accordingly.

Measurement of total IgE in serum sample

Serum total IgE levels were measured using ELISA technique with a commercially available kit (Monobind, INC, Costa Mesa, and CA92627 reagents, USA).

Skin prick tests were done using different allergens found to be common for asthmatic patients in Sudan (A panel of 13 inhalant allergens were used in this study).

Genotyping of SNPs

We selected 10 single-nucleotide polymorphisms (SNPs) based on their previously reported association with asthma and minor allele frequency. The SNPs are located in promoter and coding regions of five candidate genes. Four of these genes (containing seven SNPs), located in order on chromosome 5q31-q33: rs2070874 (promoter) in IL-4, rs2069812 (promoter) *in IL-5*, tow SNPs; rs2069743 (promoter) and rs20541 (exon 4) in IL-13, and three SNPs; rs31563 (promoter), rs2069885 (exon 5), and rs1859430 (intronic) in IL-9. Three more SNPs were selected from *IL4R* gene: rs2057768 (promoter), rs1805010 (exon 5), and rs1805015 (exon 11) located on chromosome 16 p11. The reference sequences used were GeneBankwww.ncbi.nlm.nih.gov/genbank. Genotyping were obtained for the ten SNPs using a multiplex PCR (SEQUENOM Mass ARRAY matrix MALDI-TOF mass spectrometry), (Sequenom Inc., San Diego, USA) (Appendix 5: MALDI-TOF/Mass ARRAY) Primers for PCR and single base extension were designed by using the Assay Designers software version 3.0 (Sequenom). Genotype calling was performed in real time with Mass ARRAY RT software version 3.0.0.4 and analyzed by using the Mass ARRAYTyper software version 3.4.

Data analysis

Demographic data and Phenotypic data were analyzed using SPSSA version 20. All SNPs were tested for Hardy-Weinberg equilibrium. Linkage disequilibrium (D') between SNPs was determined using Haploview 4.1. (www. broadinstitute.org/haploview/haploview). Analyses to determine associations between SNPs and asthma were performed (under which model using logistic regression within PLINK (http://pngu.mgh.harvard.edu/purcell/plink). The association between polymorphisms and asthma risk was estimated by odds ratio (OR) and 95% confidence intervals (95% CI), using logistic regression analysis adjusted for covariates (gender) using the statistical software

P-value needed to achieve statistical significance taking multiple testing into account is P=0.004 (=0.05/10 (number of SNPs genotyped)). However, since SNPs within genes showed some degree of linkage disequilibrium and SNPs were selected as major SNPs for association with Asthma in other populations. Therefore, P<0.01 (=0.05/5 genes) can used as the P-value required to achieve correction for multiple testing.

Ethical consideration

The study was approved by the ethical committee of the Institute of Endemic Diseases, University of Khartoum. All patients and their families were signed an informed consent showing their agreement to participate in the study.

Results

Total serum IgE level was found to be high among asthmatics 106 (75.2%), in comparison to non-asthmatics 33 (25.5%) and the difference was statistically significant (P-value <0.001). There was marked elevation in eosinophil count in asthmatics 73 (51.8%) compared to healthy subjects 30 (23.3%), the difference was also statistically significant (P-value <0.001), Figures 1A and 1B..

Skin prick test results showed that six out of 13 aeroallergens used in this study were significantly more reactive in asthmatic patients. These antigens were: house dust (P=0.001), D. Peterenyssinus (P=0.01), mixed molds (P=0.01), mixed ragweed (P=0.01), standardize grass pollens (P=0.01), cat hair (P=0.02) and mixed feathers with marginally significant (P=0.05).

Differences in allele and genotype frequencies for all SNPs studied were statistically non-significant, However, tests for interaction between different genetic variants showed that; all SNPs of five genes were significantly associated with asthma or related phenotypes. The three SNPs (rs2057768, rs1805010, rs1805015) of *IL-4R* gene showed significant association with asthma when interacting-with minor alleles of all other studied SNPs are shown in Figure 2. Two SNPs rs2069743 in IL13 and rs2057768 in IL4R were excluded from the analysis for significant deviation from HWE which probably results from genotyping error.

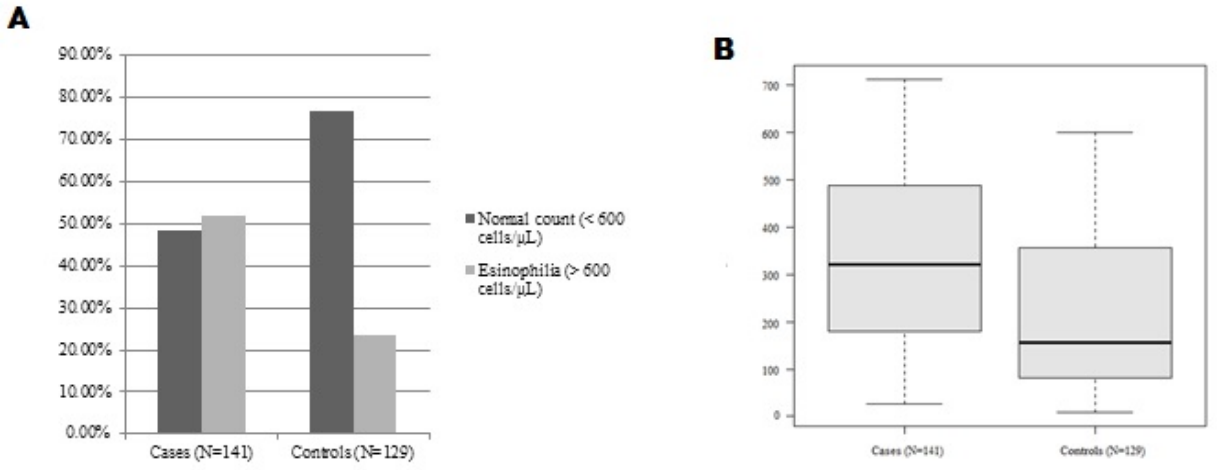

Figure 1: A) Proportion of normal and high eosinophil counts; **B)** Serum IgE level (IU/µL) in both asthmatics (N=141) and non-asthmatic (N=129) individuals in Sudanese families.

	IL4 rs2070874	IL5 rs2069812	IL13 rs20541	IL9 rs2069885	IL9 rs1859430	IL9 rs31563	IL4R rs1805010	IL4R rs1805015
IL4 rs2070874		OR:0.54, P:0.05					OR:2.08, P:0.03	OR:1.43, P:0.002
IL5 rs2069812	OR:0.75, P:0.02							OR:2.95, P:0.002
IL13 rs20541				OR:0.39, P:0.05				OR:0.92, P:0.01
IL9 rs2069885			OR:1.84, P:0.03					OR:0.73, P:0.026
IL9 rs1859430								OR:1.37, P:0.0001
IL9 rs31563				OR:1.56, P:0.02			OR:0.5, P:0.02	OR:1.88, P<0.001
IL4R rs1805010								
IL4R rs1805015								

Figure 2: SNP-SNP interactions in 5q31-q33 and 16p11 regions associated with asthma in Sudanese asthmatic patients (N=141).

Discussion

Asthma is a polygenic trait and interaction of multiple genes rather than single gene effect is responsible for disease phenotype. This study aimed to investigate the association of 10 candidate polymorphisms in genes on 5q31-33 and 16p11 chromosomal regions principally IL-4, IL5, IL9, IL13, and IL4R with asthma in Sudanese families. We failed to detect significant association between any of these variants in isolation with susceptibility to asthma; however the interaction of these variants with each other rather than single locus effect was significantly associated with asthma at least in studied families. The strongest association was between SNPs in IL9 and IL4R (P-value<0.0001). Our results are consistent with previous studies which identified gene-gene interactions as a major component in the pathogenesis of asthma [18]. These results can be explained by the fact that these interleukins share many signaling pathways and some can cross activate others' receptors [19].

Association between serum IgE level and eosinophilia has been found before and our study confirmed that. There was significant association between serum IgE level and eosinophiles in Sudanese asthmatic patients. IgE and eosinophiles are essential actors in allergic and hypersensitivity reactions including atopic asthma. The results of skin prick test is consistent with Magzoub et al. [20].

This study is unique because it is the first study to look at the 5q31-33 region in Sudanese families with asthma. This region is interesting and previous studies have linked it to susceptibility and or/ resistance to both malaria and visceral leishmaniasis [21,22] with the possible mechanism of over dominance [23]. Much of the altered genotypes in 5q31 region are driven by these two major infectious diseases, however with current control measures asthma is expected to play a greater role in shaping this region in Sudanese population in the future.

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