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Single Nucleotide Polymorphism and Asthma: From Conformational Variations to Structural Alternations

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Abstract

Background: Asthma is the main reason of disability, health resource exploitation and low quality of life for those who are affected. It is estimated that nearly 300 million people in the world are suffering from asthma. Studies have identified 18 genomic regions and more than 100 genes associated with asthma. Among these candidate genes, IL-17F plays a very interesting role in asthma. This study was conducted to predict the conformational and functional impact of asthma-associated IL-17F polymorphisms on protein product of the corresponding gene using Phyre2, PolyPhen2 and SIFT softwares.
Methods: In the present study, 10 significant missense SNPs (rs763780, rs144576902, rs11465553, rs368500268, rs141798304, rs2397084, rs146083682, rs200163061, rs376671742, and rs373228601) were taken from Ensembl Genome Browser database. Polymorphism-induced protein structural changes were predicted using Protein Homology analogY Recognition Engine V2.0 (PHYRE2) program. The possible impact of an amino acid substitution on the function of protein was analyzed using PolyPhen-2 (Polymorphism Phenotyping Version2) and SIFT (Sorting Intolerant From Tolerant) tools.
Results: The analysis revealed mutant proteins having structural changes in the number of atoms, H-bonds, turns and helices. While wild copy has 82 H-bonds, 5 helices and 20 turns, the mutant types show considerable changes. At functional level also, substantial changes were observed between the wild protein and the mutant one.
Conclusion: A single nucleotide polymorphism in the gene sequence can lead to the substantial structural and functional variations in the protein product of the gene, a process that may account for etiology of a number of complex diseases including asthma.

Keywords: Asthma, SNP, IL-17F, Phyre2, PolyPhen2.

Introduction

Asthma is a chronic inflammatory disorder of the airways affecting over 300 million people worldwide (Masoli et al., 2004). It is associated with bronchial hyperresponsiveness, chest tightness, airway remodeling, allergen-specific IgE (Immunoglobulin E) secretion and T cells infiltration into the airways (Larché et al., 2003). It has been shown that genetic components play an important role in asthma through their interactions with environmental factors (Mukherjee et al., 2011). Previous studies have identified 18 genomic regions and

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more than 100 genes associated with allergy and asthma in 11 different populations (Ober and Hoffjan, 2006). Our earlier studies on asthma in Indian population have promised that genetic studies contribute extensively towards the understanding of asthma (Bijanzadeh et al., 2010; Bijanzadeh et al., 2011; Mahdi et al., 2010; Davoodi et al., 2012). Among these genes the role of Interleukin 17F (IL-17F) in related to asthma has been supported by several studies (Akdis et al., 2011).

IL-17F, also called cytokine ML-1 or IL-24, is the last member of IL-17 family which is involved in the regulation of inflammatory responses (Kawaguchi et al., 2001; Starnes et al., 2001). It is expressed in allergen-specific T cells, basophils and mast cells which are shown to be involved in the inflammation of the asthmatic airways (Kawaguchi et al., 2001). It induces the expression of a panel of proinflammatory cytokines, chemokines, adhesion molecules and growth factors including IL-6, IL-8, IL-1β, CXCL1, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), transforming growth factor (TGF-β), IP-10, epithelial- cell derived neutrophil activating protein 78 (ENA-78) and intracellular adhesion molecule1 (Redington et al., 1997; John et al., 1998; Lummus et al., 1998; Cheung et al., 2008). The expression of these molecules in bronchial epithelial and endothelial cells recruit other inflammatory cytokines and chemokines which are involved in leukocyte activation and airway remodeling, commonly observed in patients with asthma (Kawaguchi et al., 2006). Further evidence comes from studies showing the role of IL-17F in airway neutrophilia, goblet cell hyperplasia, airway remodeling and pulmonary mucus hypersecretion (Liang et al., 2007; Akdis et al., 2011).

IL-17F has been discovered as the last member of IL-17 family, based on its sequence homology to IL-17A (Kawaguchi et al., 2001; Starnes et al., 2001). The gene encoding human IL-17F is 7742 bps in length (Ramsey et al., 2005), locating adjacent to IL-17A (human genomic sequence in clone RP11-935B23; DDBJ/EMBL/Genbank accession No. AL355513). It codes for a protein of 163 amino acids length, with 50% amino acid sequence homology to IL-17A. IL-17F adopts a monomer fold in which a knot is formed by four cysteine residues. Such structure provides a platform for homodimers in which each monomer form an interface that is covalently linked by a disulfide bond (Hymowitz et al., 2001). With 50 % sequence homology to IL-17A, heterodimers consisting of IL-17A and IL-17F monomers are quite expected (Liang et al., 2007; Chang et al., 2007).

IL-17F is located on chromosome 6p, a region

which is associated with asthma in a number of genome studies (Wjst et al., 1999; Kruse et al., 2000; Haagerup et al., 2002). Furthermore, gene expression studies have identified the IL-17F as a novel candidate gene in asthma-related studies. In this regard, investigating the polymorphisms in this gene may help us to link SNPs to their clinical implication by paving a way from conformational changes to functional impairments. As characterizing the functional impact of SNPs by direct testing is both costly and time consuming, availability of softwares as additional tools to predict the functional variants is quite promising (Shihab et al., 2014). Phyre2 (Protein Homology/AnalogY Recognition Engine v2.0) and PolyPhen2 (Polymorphism Phenotyping v2.0) are two common softwares for the prediction of the possible consequence of an amino acid substitution on the structure and function of the corresponding protein. The aim of this study was to predict the conformational and functional impact of 10 asthma-associated IL-17F polymorphisms on protein product of the corresponding gene using bioinformatics tools including Phyre2, PolyPhen2 and SIFT softwares.

Materials and Methods

SNP Selection

The following human IL-17F SNPs were selected from Ensembl Genome Browser database (http://www.ensembl.org/): rs763780, rs144576902, rs11465553, rs368500268, rs141798304, rs2397084, rs146083682, rs200163061, rs376671742, and rs373228601. All the selected variations were SNP, missense variant and referenced by NCBI dbSNP at http://www.ncbi.nlm.nih.gov/snp/.

Structural Variation Prediction

The protein sequence of wild type human IL-17F and selected SNPs were obtained from NCBI (National Center for Biotechnology Information) database. The Phyre2 web interface was accessed at http://www.imperial.ac.uk/phyre/. Each query protein sequence was submitted to Phyre2 software in FASTA format in an intensive modeling mode separately, where the results had been sent back to the authors through their email addresses. Additional structural features such as number of helices, turns, strands, groups, atoms, bonds and H-bond were obtained using RasMol (www.RasMol.org) program which is a computer program written for molecular graphics visualization.

Functional Variation Prediction in PolyPhen2

To predict the functional consequences of the selected SNPs, the PolyPhen2 web interface was accessed at http://genetics.bwh.harvard.edu/pph2/. Either SNP IDs or the quary protein sequences were

submitted to PolyPhen2 tools, along with additional information on the position of the substitutions, wild-type amino acid residues (AA1) and the substitution residues (AA2). Prediction outcome can be benign, probably damaging or possibly damaging.”Effect Score” is the probability of the substitution being damaging. The score ranges from 0 to 1. The corresponding prediction is "probably damaging", if it is within the range of 0.909 to 1; "possibly damaging”, if it is within the range of 0.447- 0.908; and "benign", if it is within the range of 0 - 0.446. Score cutoff for binary classification is 0.5, in the sense that the prediction is "neutral" , if the score is less than 0.5 and "deleterious" , if the score is more than 0.5 [Adzhubei et al., 2013].

Functional Variation Prediction in SIFT

The functional consequences of the SNPs were assessed using SIFT software at http://www.blocks.fhcrc.org/sift/SIFT.html.

The protein sequences and amino acid substitutions of the selected SNPs were submitted to the tools where in fully automated mode, SIFT will search for protein sequence homology to the query protein to calculate probability for each possible amino acid substitution. SWISS-PROT, SWISS-PROT/TrEMBL or NCBI protein databases can be selected by the user for SIFT to search among. The score is the probability that the amino acid substitution is tolerated. Substitutions with scores less than 0.05 are predicted as deleterious [Ng et al., 2003].

Results

A total of 10 IL-17F SNPs were selected for conformational and functional variation analysis. All the SNPs were missense variants in which the consequent SNPs resulted in a substitution in the wild type amino acid residue at a specific position. Chromosomal mapping, amino acid coordinate, resulting amino acid, along with other variant consequences, is presented in Table 1.

Table1. Human IL-17F missense SNPs consequences

ID	Chr:bp	Alleles	Resulting Amino Acid	Amino Acid Coordinate
rs763780	6:52236941	T/C	H/R	161
rs144576902	6:52236954	G/A	P/S	157
rs11465553	6:52236960	C/T	V/I	155
rs368500268	6:52237016	C/A	G/V	136
rs141798304	6:52237035	C/T	V/I	130
rs2397084	6:52237046	T/C	E/G	126
rs146083682	6:52237107	C/T	G/S	106
rs200163061	6:52237114	C/A	R/S	103
rs376671742	6:52238749	T/A	T/S	79
rs373228601	6:52238761	C/T	E/K	75

Structural variation analysis revealed mutant proteins having conformational changes in the number of atoms, bonds, H-bonds, helices, strands and turns. While the wild type protein has 1261 atoms, 1414 bonds, 92 H-bonds, 4 helices, 7 strands and 18 turns, the mutant types show lots of variations (Table 2, Fig. 1-3). Only two mutant proteins (rs144576902 and rs376671742 variants) had the same atom numbers as the wild type one (1261 atoms). The minimum number of atoms belonged to rs2397084 and rs200163061 variants with 1257 atoms and the maximum number of atoms belonged to rs368500268 variant with 1265 atoms. Comparing the number of bonds between the wild type and mutant proteins showed a significant decrease in the number of bonds in mutant proteins. While the wild type protein has 1414 bonds in its structure, the minimum number of bonds was seen in rs2397084 and rs200163061 variants, with 1283 bonds and the maximum number of bonds was observed in rs368500268 variant, with 1291 bonds. Furthermore, the number of H-bonds was decreased in mutant proteins as compared to the wild type bearing 92 H-bonds. The minimum number of H-bonds was 71 that was observed in rs373228601 variant and the maximum number of H-bonds was 89 which was observed in rs763780 and rs11465553 variants. The results on the number of helices revealed 4 helices in the wild type protein. Out of selected 10 SNPs, rs763780, rs146083682 and rs376671742 variants had the same number of helices as the wild type. The minimum number of helices was two which was observed in rs373228601 and rs141798304 variants and the maximum number of helices was five which was observed in rs144576902, rs368500268 and rs200163061 variants. The results on conformational variation analysis showed an increase in the number of strands in mutant proteins compared to the wild type. With the wild type protein bearing 7 strands, the minimum number of strands was 8 in rs141798304, rs146083682 and rs200163061 variants and the maximum number of strands was 10 in rs763780, rs144576902, rs11465553, rs368500268, rs2397084 and rs376671742 variants. Comparison of the number of turns between the wild type and mutant proteins showed an increase in the number of turns in mutant proteins with the exception of rs763780 with 17 turns. The maximum number of turns was 24 as in rs368500268 variants. Of all the structural features, only the number of groups was same between the wild type and mutant proteins.

Functional variation prediction of IL-17F SNPs in PolyPhen2 software showed different results (Table 3). Out of selected 10 SNPs, five SNPs (rs763780, rs141798304, rs200163061, rs376671742 and rs373228601) were predicted

as benign, two SNPs (rs11465553 and rs146083682) as possibly damaging and three SNPs (rs144576902, rs368500268 and rs2397084) as probably damaging.

Table 2. Structural variation prediction of human IL-17F missense variants in Phyre2 and RasMol programs.

Wild/Mutant IL-17F	Number of Residues	Number of atoms	Number of bonds	Number of H-bonds	Number of Helices	Number of strands	Number of turns
IL-17F	163	1261	1414	92	4	7	18
rs763780	163	1263	1288	89	4	10	17
rs144576902	163	1261	1286	87	5	10	21
rs11465553	163	1263	1289	89	3	10	22
rs368500268	163	1265	1291	81	5	10	24
rs141798304	163	1263	1289	79	2	8	19
rs2397084	163	1257	1283	77	3	10	20
rs146083682	163	1264	1290	87	4	8	20
rs200163061	163	1257	1283	77	5	8	19
rs376671742	163	1261	1287	86	4	10	19
rs373228601	163	1262	1288	71	2	9	22

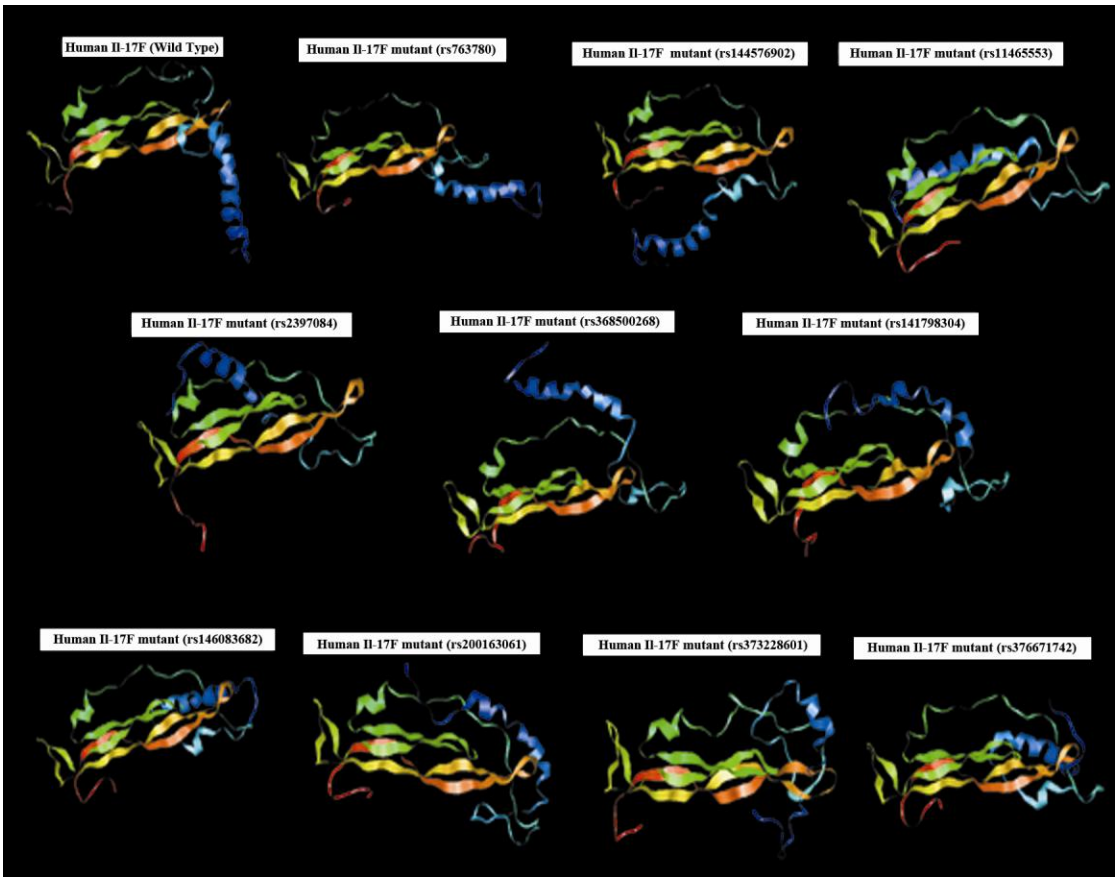

Figure 1 3D structural conformation of Human IL-17F wild type and missense variants (rs763780, rs144576902, rs11465553, rs368500268, rs141798304, rs2397084, rs146083682, rs200163061, rs376671742, and rs373228601) in RasMol software. Structural variation analysis revealed mutant proteins having conformational changes as compared to the wild type protein.

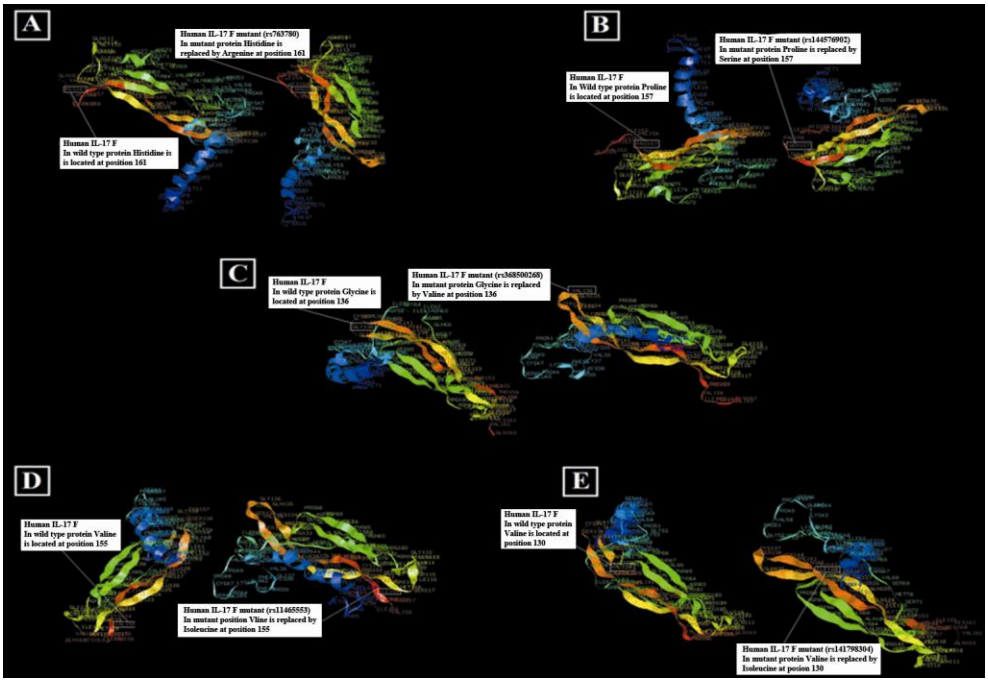

Figure 2 3D structural conformation of Human IL-17F wild type and missense variants (rs763780, rs144576902, rs11465553, rs368500268 and rs141798304) labeling the amino acid substitution residue and coordinate in RasMol software. A) In rs763780 missense variant histidine is replaced by argenine at position 161; B) In rs144576902 missense variant proline is replaced by serine at position 157; C) In rs368500268 missense variant glycine is replaced by valine at position 136; D) In rs11465553 missense variant valine is replaced by isoleucine at position 155; E) In rs141798304 missense variant valine is replaced by isoleucine at position 130.

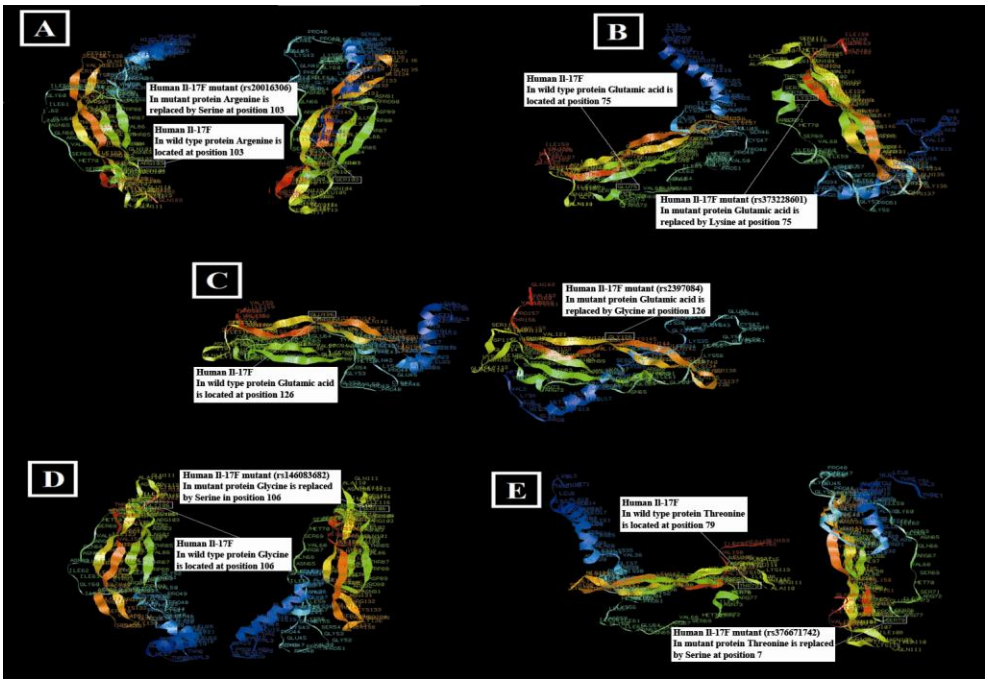

Figure 3 3D structural conformation of Human IL-17F wild type and missense variants (rs200163061, rs373228601, rs2397084, rs146083682 and rs376671742) labeling the amino acid substitution residue and coordinate in RasMol software. A) In rs200163061 missense variant argenine is replaced by serine at position 103; B) In rs373228601 missense variant glutamic acid is replaced by lysine at position 75; C) In rs2397084 missense variant glutamic acid is replaced by glycine at position 126; D) In rs146083682 missense variant glycine is replaced by serine at position 106; E) In rs376671742 missense variant threonine is replaced by serine at position 79.

Table 3. Functional variation prediction of human IL-17F missense variants in PolyPhen2 program.

rsID	Effect Score	Effect Type
rs763780	0.003	Benign
rs144576902	0.988	Probably Damaging
rs11465553	0.858	Possibly Damaging
rs368500268	0.982	Probably Damaging
rs141798304	0.001	Benign
rs2397084	0.992	Probably Damaging
rs146083682	0.864	Possibly Damaging
rs200163061	0.280	Benign
rs376671742	0.027	Benign
rs373228601	0.159	Benign

Table 4. Functional variation prediction of human IL-17F missense variants in SIFT program.

rsID	Prediction Score	Prediction Type
rs763780	0.09	Tolerated
rs144576902	0.03	Deleterious
rs11465553	0.0	Deleterious
rs368500268	0.01	Deleterious
rs141798304	0.09	Tolerated
rs2397084	0.0	Deleterious
rs146083682	0.16	Tolerated
rs200163061	0.08	Tolerated
rs376671742	0.27	Tolerated
rs373228601	0.08	Tolerated

The results on functional variation prediction in SIFT software predicted 6 SNPs (rs763780, rs141798304, rs146083682, rs200163061, rs376671742, rs373228601) as tolerated and four SNPs as deleterious (rs144576902, rs11465553, rs368500268, rs2397084). The results are presented in Table 4.

Discussion

In this study, the conformational and functional impacts of 10 asthma-associated IL-17F SNPs have been predicted using Phyre2 and PolyPhen2 softwares. Structural variation analysis revealed mutant proteins having conformational changes in the number of atoms, bonds, H-bonds, helices, strands and turns as compared to wild type. Functional variation prediction of IL-17F SNPs in PolyPhen2 software predicted five SNPs (rs763780, rs141798304, rs200163061, rs376671742 and rs373228601) as benign, two SNPs (rs11465553 and rs146083682) as possibly damaging and three SNPs (rs144576902, rs368500268 and rs2397084) as probably damaging. The results on functional variation prediction in SIFT software predicted 6 SNPs (rs763780, rs141798304, rs146083682, rs200163061, rs376671742, rs373228601) as tolerated and four SNPs as deleterious (rs144576902, rs11465553, rs368500268, rs2397084).

SNPs are the most common form of human DNA variations. Human exome bear around 20,000 SNPs, of which half of them belong to nonsynonymous SNPs (Bamshad et al., 2011).

Nonsynonymous SNPs, along with the mutations in the regulatory region of the genes, are shown to have the most impact on phenotypic variations among individuals. However, unlike completely penetrant mutations involved in Mendelian disorders, SNPs causing complex human disorders such as asthma or Parkinsonism are not the only component to define the phenotypic features, but their effects rely on several other environmental and genetic factors. Therefore, the effect size of each SNP may be defined as the difference in the SNP frequency between diseased and healthy control individuals. Nowadays association studies have been used widely as experimental tools to identify SNPs causing complex phenotypes; commonly human multifactorial disorders (Risch and Merikangas, 1996). Though whole genome scanning may be a hypothesis-free procedure, but still is too bothersome with the overwhelming number of markers needed to be screened (Lai et al., 1998; Risch et al., 2000). This problem may seem to be solved in candidate gene studies by taking the most associated SNPs for each disease. Even in the latter case, the results are not too promising if enormous number of candidate genes have been selected for the association studies. On the other hand, limitation of disease association studies is not only restricted to massive number of markers to be screened, but also their results are usually incontinent in the sense that the reports of previously published studies are not verified by the subsequent independent investigations (Risch et al., 2000, Emahazion et al., 2001, Zheng et al., 2014). Several false positive and negative associations have been reported. Furthermore, if the association of a gene variant to a specific disease is confirmed by different independent studies, the causative relationship between the disease and gene variant is not apparent in many cases (Johnson et al., 2000). A possible approach to solve the problem of having false associations and testing a huge number of marker SNPs is using computational softwares as an additional tools to costly and prolonged laboratory experimentations.

Bioinformatics tools such as PolyPhen2, Phyre2 and SIFT can be always used as independent evidences to reduce both the number of SNPs and false associations by separating neutral and functional and causal and noncausal SNPs (Ramensky et al., 2002). The effect of amino acid residue substitution on the structure and function of the corresponding protein can be predicted using multiple sequence alignments and protein 3D structure analysis. Such kind of analysis not only discloses the structural background of the disease, but also reveals the SNPs affecting actual phenotypes. This is mainly because the predictions correlate with the impact of natural

selection as an abundance of the rare alleles (Sunyaev et al., 2000; Sunyaev et al., 2001). As the aim of association studies is functional analysis of the high risk allele and identification of the molecular mechanism involved in the etiology of the disease phenotype, using bioinformatics servers can help us to identify the functional impact of truly-associated variants by excluding the false positives resulting from improper statistical analysis, study design and population option [Ramensky et al., 2002]. Though predictive tools are broadly used to investigate the etiology of complex diseases, occasional reporting of false positives and negatives may necessitate an additional work to improve the specifity and sensitivity of these softwares [Shihab et al., 2014].

Our results imply that even a SNP in the gene sequence can lead to the substantial structural and functional changes in the protein product of the corresponding gene, a process that account for etiology of a number of complex diseases including asthma.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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