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CONTRIBUTION OF GENETIC VARIANTS OF DNA REPAIR GENES XRCC1 AND APE1 IN PEDIATRIC PATIENTS WITH FOOD AND AIR BORN ALLERGEN ASTHMA

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ABSTRACT

INTRODUCTION: Allergic asthma in children occurs in response to an allergic trigger major. In the pathophysiology of asthma, genetic variations in many loci and genes play a crucial role. Genes of DNA repair pathways have been well characterized in association with clinical pathologies of different forms of allergic asthma with conflicting outcomes. This study reports the role of single nucleotide polymorphisms (SNPs) in X-ray cross-complementing group 1 (XRCC1) and apurinic/apyrimidinic endonuclease 1 (APE1) polymorphisms in pediatric patients with food and air born allergen asthma.

METHODS: 125 pediatric asthma patients and age and gender matched 164 controls were enrolled. Blood samples were collected after confirming the food and air allergen related asthma. Genomic DNA was isolated from whole blood and genotyping was done for *XRCC*1 Arg399Gln and *APE*1 Asp148Glu using ARMS-PCR.

RESULTS: The frequency of genotype Arg399Gln (heterozygous) of *XRCC*1 gene was significantly higher in patients with allergic asthma than the controls (odds ratio [OR] 2.75; 95% confidence interval [CI] 1.69 -4.47; p=0.006). 'A' allele of *XRCC*1 gene was found to be predominant in pediatric asthma group compared to controls. Similarly the genotype TG frequency of *APE*1 Asp148Glu showed statistically significant change in allergic asthma patients compare to controls (OR 2.93; 95% CI 1.77-4.85; p<0.001). 'G' allele of *APE*1 gene was found predominantly in pediatric asthma group compared to controls.

CONCLUSION: Polymorphisms in *XRCC*1 Arg399Gln and *APE*1 Asp148Glu significantly increased the risk of allergic asthma in pediatric patients.

KEYWORDS : DNA repair genes; XRCC1 Arg399Gln; APE1 Asp148Glu; allergic asthma

INTRODUCTION

Asthma is a heterogeneous chronic inflammatory disorder of the lungs characterized by bronchial hyper-responsiveness to stimuli in the forms of allergens, infections, and environmental irritants. Asthma presents with different type of phenotypes depending on age, gender, and genetic background (**Stein et al. 1997**). Asthma and allergic diseases are believed to be complex genetic diseases, which result from the interaction of multiple genes and environmental stimuli including allergens, pollutants, and infectious agents (**Sandford et al. 1996**).

Initial allergic sensitization occurs on exposure to an allergen breaching the epithelial barrier due to disruption or dysfunction, which may have genetic and/or environmental causes. Specifically, disruption of the epidermal barrier is considered to be the first step in the development of eczema. In both children and adults, the most common infections of the airways may be associated with the development of chronic asthma (**Micillo et al. 2000**).

Food allergy (FA), defined as an immunoglobulin (Ig) E-mediated hypersensitivity reaction to food, is emerging as a major clinical and public health problem worldwide (**Sampson, 2004; Gupta et al. 2007**). It affects approximately 5-8% of children and 1-5% of adults (**Bock, 1987; Sicherer and Sampson, 2006; Vierk et al. 2007**). Atopic children have a genetic predisposition to expand immunoglobulin E (IgE) antibodies to a mixture of dietary and inhalant allergens to which they are exposed (**Chad, 2001**). Genetic predisposition, the allergen city of food and the timing of exposure will determine the foods to which the atopic child develops allergy. Young infants are especially prone to the development of food allergies. In younger children, the most common foods implicated are milk and eggs. These allergies are self-limited and most of these will resolve within a few years with appropriate elimination diets. In older children and adults few allergenic foods (such as nuts, fish and seafood) tend to persist throughout life (**Chad, 2001**). Despite this, our current understanding on the etiology and biological mechanisms of FA is still incomplete.

A study by **Litonjua et al. (2002)** has shown that exposure to the endotoxin in house such as dust is associated with wheezing in children below 5 years which was rapidly declined within a few years. **Rizzo et al. (1997)** has also shown the direct relationship between inhaled endotoxin levels and the exacerbation of preexisting asthma symptoms. Around 176 families have shown the direct association between asthma in parent and offspring (**Gerrad et al. 1976; Dold et al. 1992; Gua et al. 2019**).

The genetic variants may play role in the development of the asthma phenotype in children (**Gua et al. 2019**). Candidate gene studies have targeted immune related genes postulated to be involved in the mechanisms of food allergy. Additionally, given that there are shared genetic risk factors among asthma, allergic rhinitis and eczema (**Portelli et al. 2015; Ferreira et al. 2017**). However, compared to other allergic diseases, the genetic basis of food allergy remains relatively under explored. Investigators suggested that genes involved in the development and function of regulatory T cells, specifically IL2RA, TLR2, TGFBR2, and FOXP3, are associated with atopy and asthma by gene-gene interaction (**Juhn et al. 2010**).

Organisms have evolved complex and sometimes redundant ways to repair a variety of DNA damage using a number of different pathways. The importance of these DNA repair genes is evident from consequential disease associated with a particular DNA repair gene dysfunction (**Bernstein et al. 2002; Hoeijmakers, 2009**). Polymorphisms have been identified in several DNA damage repair genes. X-ray repair cross-complementing group 1 (XRCC1) gene is one of the most important DNA repair genes (**Lee et al. 2009**). XRCC1 also participates in single-strand DNA break (SSB) repair pathway for the repair of DNA destruction which occurs very frequently in mammals and base excision repair (BER) pathway which operates on small lesions caused normally by endogenous substances or xenobiotics. Moreover, it is reported that DNA repair function could be modified by genetic polymorphisms (**Lee et al. 2003**).

These polymorphisms may affect DNA repair capacity and modulate asthma susceptibility. Deficiencies of many genes in DNA repair pathways have been characterized and often result in other clinical pathologies (**Riballo et al. 1999; Abbaszadeh et al. 2010**). This study is an attempt to identify the role of repair gene polymorphisms in pediatric asthma which could provide an insight into the genetic diagnosis, risk stratification and clinical management of the condition.

MATERIALS AND METHODS

Study population

A total of 125 confirmed cases of pediatric asthma were enrolled in the present study. All the patients were confirmed case of food and air allergen related asthma. Study was approved from Institutional Ethics committee, Deccan College of Medical Sciences, Kanchanbagh, Hyderabad. Diagnosis of pediatric asthma was based as per the conventional clinical criteria. A total of 164 healthy individuals as control were included in the present study who were referred for routine general medical check-up at Owaisi Group of Hospitals, Hyderabad. Written consent forms were taken from all the parents of the subjects. The details about family history, number of affected individuals and the information on other co-morbid conditions were also obtained from each participant.

Sample Collection

2 ml venous blood in K3-EDTA tube was collected from all the study participants. DNA samples were analyzed for genotyping of selected polymorphic genes (*XRCC*1 and *APE*1).

Genotyping of DNA repair gene polymorphisms by ARMS-PCR

Genomic DNA from blood-EDTA samples was extracted by salting out methods. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was adopted for genotyping of *XRCC*1 and *APE*1 as previously described using specific oligonucleotide primers. Amplification for assessing SNP (*XRCC*1 codon 399 and *APE*1 codon 148) was performed as described previously (**Ito et al. 2004**), with minor modifications (change in annealing temperature). Amplicons were analyzed by electrophoresis along with DNA ladder (50 bp) on 2.0% agarose gel containing 10 µg/ml ethidium bromides (EtBr) under UV transilluminator. Photographic gel images were documented using Quantity One software in a digital Gel Doc system (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) was calculated in the total samples as well as separately in cases and controls. The odds ratios (ORs) and 95% confidence intervals (CIs) for each SNP were calculated using multiple logistic regression. A p value <0.05 was considered statistically significant.

RESULTS

Distribution of genotypes and frequency of alleles of T148G polymorphisms in *APE*1 gene

The frequency of '**G**' allele of *APE*1 gene was found to be predominant in pediatric asthma group compared to controls (38% *vs* 24%) (**Table 1**).

Table 1: *APE*1 genotype and allele frequency distribution in controls and pediatric asthma samples

Model	Genotype	Control	Patients	OR (95% CI)	*p* value
Codominant	T/T	88 (53.7%)	35 (28%)	1.00	**<0.001**
	T/G	72 (43.9%)	84 (67.2%)	**2.93 (1.77-4.85)**	
	G/G	4 (2.4%)	6 (4.8%)	3.77 (1.00-14.18)	
Dominant	T/T	88 (53.7%)	35 (28%)	**2.98 (1.81-4.89)**	**<0.0001**
	T/G-G/G	76 (46.3%)	90 (72%)		
Recessive	T/T-T/G	160 (97.6%)	119 (95.2%)	2.02 (0.56-7.31)	0.28
	G/G	4 (2.4%)	6 (4.8%)		
Overdominant	T/T-G/G	92 (56.1%)	41 (32.8%)	**2.62 (1.61-4.25)**	**<0.002**
	T/G	72 (43.9%)	84 (67.2%)		
Allele	T	248 (76%)	254 (62%)	0.8535 (0.6048-1.2045)	0.3817
	G	80 (2%)	96 (38%)		

TG genotypic frequency was found to be predominant in pediatric asthma group (67.2%) compared to controls (43.9%) with the difference being statistically significant (p<0.001). Based on the dominant model, combination of TG+GG genotype frequency were 72.0% in pediatric asthma and 46.3% in control (OR 2.98, 95% CI 1.81-4.89, p<0.0001). Overdominant model revealed TG (Compare with TT+GG genotype) genotype association with pediatric asthma (OR 2.62, 95% CI 1.62-4.25, p=0.002) (**Table 1**).

Distribution of genotypes and frequency of alleles of G399A polymorphisms in *XRCC*1 gene

The frequency of '**A**' allele of *XRCC*1 gene was found to be predominant in pediatric asthma group compared to controls (78% *vs*18%) (**Table 2**).

Table 2: *XRCC*1 genotype and allele frequency distribution in controls and pediatric asthma samples

Model	Genotype	Control	Patients	OR (95% CI)	*p* value
Codominant	G/G	108 (65.8%)	51 (40.8%)	1.00	**0.006**
	G/A	54 (32.9%)	70 (56%)	**2.75 (1.69-4.47)**	
	A/A	2 (1.2%)	4 (3.2%)	4.24 (0.75-23.88)	
Dominant	G/G	108 (65.8%)	51 (40.8%)	**2.80**	**<0.000**
	G/A-A/A	56 (34.1%)	74 (59.2%)	**(1.73-4.53)**	**1**
Recessive	G/G-G/A	162 (98.8%)	121 (96.8%)	2.68 (0.48-14.86)	0.24
	A/A	2 (1.2%)	4 (3.2%)		
Overdominant	G/G-A/A	110 (67.1%)	55 (44%)	**2.59**	**0.002**
	G/A	54 (32.9%)	70 (56%)	**(1.60-4.19)**	
Allele	G	270 (82%)	172 (69%)	**0.4737**	**0.0001**
	A	58 (18%)	78 (31%)	**(0.3207-0.6996)**	

Heterozygotes (GA) were found to be predominant in the pediatric asthma group compared to controls (56.0% vs 32.9%, p=0.006) with 2.75 folds increased risk for pediatric asthma, which was statistically significant (OR 2.75, 95% CI 1.69-4.47, p=0.006) (**Table 2**). Based on the dominant model, combination of GA+AA genotypes was observed to be associated with high risk for pediatric asthma(OR 2.80, 95% CI 1.73-4.53, p=0.0001). In recessive model the AA genotype (compared with GG+GA) did not reveal any risk to pediatric asthma (OR 2.68, 95% CI 0.48-14.86, p= 0.24) (**Table 2**).

Of the all haplotypes obtained, one haplotype carrying the recessive allele of XRCC1 and APE1 polymorphism, GA were found to be significantly predominant in the disease group than controls with a 3.22 fold significant increase (OR 3.22, 95% CI 1.93–5.39, p <0.0001) risk of allergic asthma in pediatric patients (**Table 3**).

Table 3: Haplotype association between *APE*1 and *XRCC*1 gene with response

S. No.	*APE*1	*XRCC*1	OR (95% CI)	*p* value
1	T	G	1.00	---
2	G	A	**3.22 (1.93 - 5.39)**	**<0.0001**
3	G	G	**2.13 (1.10 - 4.09)**	**0.025**
4	T	A	2.68 (0.90 - 8.02)	0.079

DISCUSSION

Asthma is a common chronic and complex inflammatory respiratory disease characterized by recurrent episodes of wheezing, shortness of breath, chest tightness, and coughing. As the incidence and mortality of asthma are increasing, so is its negative impact on modern society. While the exact mechanisms underlying the development and progression of asthma has not yet been fully uncovered, both genetic and environmental factors are known to be involved.

Several studies have investigated the genetic associations of asthma, but few of them have been successfully replicated across multiple populations. Among these studies, many have identified ORMDL3 as a potential asthma candidate gene and the single- nucleotide polymorphism (SNP) rs7216389 as a major susceptibility locus (**Moffatt et al. 2007**).

Defective DNA repair has been reported to be a risk factor for various malignancies. Genetic polymorphisms of DNA repair genes are

thought to result in different phenotypic features compared to the wild type. Genetic polymorphisms in XRCC1 and APE1 genes can alter the protein structure, which may lead to defective functioning of DNA Polbeta, PARP and LIG3 enzymes resulting in defective DNA repair and increased risk of various clinical conditions in childhood.

Liu et al. (2019) conducted a three-center case-control study to evaluate the association between APEX1 polymorphisms (rs1130409 T>G, rs1760944 T>G, and rs3136817 T>C) and neuroblastoma risk in Chinese children, consisting of 469 cases and 998 controls. OR and 95% CIs were calculated to evaluate the associations (**Liu et al. 2019**). No significant association with neuroblastoma risk was found for the studied APEX1 polymorphisms in the single locus or combination analysis.

Our study has demonstrated that frequency of '**G**' allele of *APE*1 gene is predominant in pediatric asthma group compared to controls (38% *vs* 24%). TG genotype frequency was found to be predominant in pediatric asthma group (67.2%) compared to controls (43.9%) with the difference being statistically significant (p<0.001). Based on the dominant model, combination of TG+GG genotype frequency were 72.0% in pediatric asthma and 46.3% in control (p<0.0001). Overdominant model revealed TG (Compare with TT+GG genotype) genotype was observed to be associated with pediatric asthma (p=0.002).

XRCC1 399 polymorphism may increase the risk of childhood acute lymphoblastic leukemia (ALL). Different ethnic groups with some gene polymorphism have different disease risks (**Du et al. 2013**). **Zhang et al. (2018)** conducted a case-control study with 393 neuroblastoma patients and 812 controls to explore the association of XRCC1 gene polymorphisms (rs1799782 G>A, rs25487 C>T, rs25489 C>T and rs915927 T>C) with neuroblastoma risk. **Zhang et al. (2018)** showed that none of the studied polymorphisms was associated with neuroblastoma risk. However, individuals with 2 risk genotypes seemed to be at significantly higher risk for neuroblastoma compared with those without risk genotype.

Our study also demonstrated that frequency of '**A**' allele of *XRCC*1 gene was found to be predominant in pediatric asthma group compared to controls (78% *vs* 18%). Heterozygotes (GA) were found to be predominant in the pediatric asthma group compared to controls (56.0% vs32.9%, p=0.006) with 2.75 folds increased risk for pediatric asthma, which was statistically significant (p=0.006). Based on the dominant model, combination of GA+AA genotypes were also observed to be associated with high risk for pediatric asthma (p=0.0001). In recessive model AA genotype (compared with GG+GA) did not reveal any risk to pediatric asthma (p=0.24).

Several haplotypes were obtained, one haplotype carrying the recessive allele of XRCC1 and APE1 genes. GA was found to be significantly predominant in the disease group than controls with a 3.22 fold increase (p<0.0001) risk of allergic asthma in pediatric patients.

Further studies on the epistatic interactions can be done to elucidate their possible underlying mechanisms. Since different populations have distinct genetic backgrounds, it is necessary to validate or replicate such associations with independent samples collected from other ethnic groups/populations. Overall risk, age of onset, and severity are influenced by numerous biological pathways that interact with environmental exposures. It is not surprising, therefore, that the associated genes have diverse functions and act in multiple pathways. Although the number of genes that contribute to any one of these related phenotypes is still unknown, it is likely to be quite large.

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Conflict of Interest: None

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