Original article

IL-13 R130Q single nucleotide polymorphism in asthmatic Egyptian children

Background: *Asthma and its associated phenotypes are under a substantial degree of genetic control. The common variant IL-13 gene polymorphism R130Q is reported to be associated with the risk of development of asthma in some populations.*

Objective: *We sought to study the association of IL-13 genetic variant R130Q with bronchial asthma in Egyptian children and its relation to various clinical and laboratory phenotypes of the disease.*

Methods: *IL13 gene polymorphism (R130Q) was detected by PCR amplification followed by sequencing using pure script total DNA in 20 asthmatic patients in acute exacerbation. The results were compared to 20 healthy age and sex matched children.*

Results: *Asthmatic children had significantly higher frequency of distribution of R130Q genotype (50%) than controls (15%). The serum total IgE as percent of high normal for age was significantly higher in asthmatic patients as compared to controls with a mean of 208.77 ±237.06% and 14.21 ± 8.08% respectively. No significant difference was observed in the mean AEC(as a percent of high normal for age) of both groups (80.85 ± 116.4% and 82.50 ± 81.4% respectively). No significant differences were observed between patients with IL-13 polymorphism R130Q and those without such polymorphism as regards family history, relation of exacerbations to upper respiratory tract infections, history of food allergy or asthma grading. Serum total IgE was significantly higher in asthmatics with GA genotype as compared to those with GG genotype with a mean of 373.25 ± 238.11% and 44.28 ± 42.65% respectively. A similar finding was also observed among the control group with a mean of 28.03 ± 9.12% and 11.77 ± 5.00% respectively. Finally a significantly higher AEC was observed in controls with GA as compared to GG genotype with a mean of 250.00 ± 51.96% versus 52.94 ± 36.87% respectively.*

Conclusion: *The common variant IL-13 gene polymorphism R130Q is frequently associated with pediatric asthma. This variant is more active than the wild type in inducing allergic inflammation as reflected by the higher serum total IgE and AEC. Hence, IL-13R130Q may be candidate for future gene therapy targeted at reducing the ill-effects of this polymorphism.*

Keywords: IL-13R130Q – bronchial asthma - pediatrics.

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INTRODUCTION

Asthma and its associated intermediate phenotypes are under a substantial degree of genetic control. Identifying the genes underlying asthma offers a mean of better understanding its pathogenesis[1]. The importance of genetic factors in influencing the risk of developing allergic inflammation is well established[2]. Linkage association studies and genome-wide screening suggest that multiple genes are involved in the pathogenesis of asthma. However, many segregation studies suggest that a major gene could be involved in asthma, but until now different genetic models have been obtained[3].

IL-13 is an immunoregulatory cytokine produced primarily by activated Th2 cells, mast cells and natural killer cells (NK) cells. IL-13 is a 17-kD glycoprotein[4]. The gene encoding IL-13 is located only 25 kilobases upstream of the gene for IL-4 and in the same orientation, leading to the speculation that these genes arose as a duplication event during evolution. In addition to their structural similarities they share considerable functional similarities. They are both known to have a number of actions relevant to the asthmatic

diathesis such as the regulation of isotype class switching in B cells to IgE synthesis, induction of the expression of MHC II and CD23, the induction of adhesion molecule expression on endothelial cells e.g. vascular cell adhesion molecule-1 (VCAM-1), chemokine production (eotaxin), the activation of mast cells and eosinophils, and the inhibition of proinflammatory gene expression (IL-1, TNF and IL-6). This overlap in function is due to the sharing of a receptor chain in their individual multimeric receptor complexes IL-4R[5].

The central role of IL-13 in asthma and allergy is also highlighted by studies that demonstrated that the biologic effects of IL-9 and IL-25 are mediated via IL-13, and that histamine can induce IL-13 production in some experimental circumstances[6].

IL-13 induces airway hyperreactivity (AHR) as it is known to regulate the production of a number of secreted molecules that alter the contraction or relaxation of airway smooth muscle cells and it inhibits the activity of inducible nitric oxide synthase, which would result in a decrease in production of the bronchodilator nitric oxide[7].

Genetic polymorphism arises from mutation. Different classes of polymorphism are generally named on the basis of the type of mutation from which they result. The simplest class of polymorphism derives from a single base mutation that substitutes one nucleotide for another. Such polymorphism has been called a single nucleotide polymorphism, or SNP.

Common single-nucleotide polymorphisms in IL-13 are associated with allergic phenotypes in several ethnically diverse populations. In particular, IL13+2044G→A is expected to result in the nonconservative replacement of arginine 130 (R130) with glutamine (Q)[8]. The impact of IL13+2044G→A on the functional properties of IL-13 was examined by comparing the activity of wild type (WT) IL-13 and IL-13 R130Q on primary human cells involved in the effector mechanisms of allergic inflammation. Notably, IL-13 R130Q was neutralized less effectively than WT IL-13 by an IL-13Rα2 decoy. Decreased neutralization of the minor variant could contribute to its enhanced in vivo activity. Neither of the IL-13 variants was able to engage T cells, which suggests that increased allergic inflammation in carriers of IL13+2044A depends on enhanced IL-13–mediated Th2 effector functions rather than increased Th2 differentiation. Collectively, data indicate that natural variation in the coding region of IL-13 may be an important genetic determinant of susceptibility to allergy[9].

IL-13 R130Q is more active than WT IL-13 in up regulating CD23 expression in primary monocytes[10]. IL-13 R130Q is more active than WT IL-13 in inducing hydrocortisone-dependent, but not CD40-dependent, IgE synthesis. Together with IL-4, IL-13 is the only cytokine which has been shown to induce IgE class switching. This effect requires synergistic interactions with a second signal typically provided by CD40 engagement[11].

With this as a background, we were stimulated to study the association of the IL-13 variant R130Q with childhood bronchial asthma and its relation to various clinical and laboratory phenotypes of the disease.

METHODS

Study Population

This case-control pilot study comprised 20 asthmatic and 20 healthy children as a stratified non-randomized sample. The asthmatic children were enrolled consecutively from the Pediatric Allergy and Immunology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt. The diagnosis of asthma in the studied patients was made according to the criteria of the American Thoracic Society[12]. They were 10 males and 10 females. Their ages ranged from 5 to 13 years with a mean age of 8.75±2.88 years. Their duration of illness ranged from 1 year to 12 years, with a mean of 6.43 ±2.78 years. According to the GINA[13] classification, the selected patients were classified into 7 patients with severe persistent asthma and 13 patients with moderate persistent asthma. The patients were studied during acute asthma exacerbation, and graded into 7 with mild, 7 with moderate and 6 with severe exacerbation. Weight and height measurements were recorded and plotted against the normal percentiles for age[14]. Children with data suggestive of chest infection, parasitic or concomitant diseases were excluded from the study. The control group comprised 20 clinically healthy children (10 males and 10 females). Their ages ranged from 5 to 13 years with a mean value of 8.55±2.84 years. They had no personal or family history of atopy.

An informed consent was obtained from the parents or care-givers of all children before enrollment. The study protocol was approved by the ethics committee of the Department of Pediatrics, Ain Shams University.

Study Measurements

Blood sample collection and processing

Six ml venous blood sample was drawn aseptically from every subject in vacutainer cell preparation tube (CPT) with sodium citrate for blood cell counting, total IgE assay and detection of IL-13

gene polymorphism R130Q. The tube was stored upright at room temperature and centrifuged within two hours at 2760 rpm for 20 minutes at 22˚C. The whitish mononuclear cell layer was collected with a Pasteur pipette and transferred to 25 ml size conical centrifuge tube. Washing with PBS with centrifugation at 560 rpm for 15 minutes and discarding of the supernatant was done twice then the cell pellet was resuspended and subsequently used for DNA extraction.

Detection of IL-13 gene polymorphism (R130Q)

DNA extraction from mononuclear cells using (Purescript total DNA Isolation kit, Gentra, Cat. No.5500A, USA) in accordance with the manufacturer's instructions, then DNA was amplified using standard procedures[15]. The primer pairs used were forward (GGCGTTCTACTCACGTGCTGACC) and reverse primers (GCTAAGGAATTTTACCCCTCCC), PCR amplification was carried out in 20 µL containing 0.1 µM of each primer, 1.5 mM MgCl2, 200 µM dNTPs, 50 ng of DNA template, 1 U of Taq DNA polymerase, and 1X PCR buffer in a Gene Amp PCR system (PerkinElmer 9600 Thermocycler, Waltham, Massachusetts, USA).

Amplification products were double strands sequenced using (ALF express autoread sequencing kit, Amersham pharmacia biotech, USA). DNA sequence analysis of the amplified products was done in four steps namely denaturation reaction, annealing reaction, labeling reaction and terminal reaction. Then 8µL of each sequencing reaction product were loaded into wells of sequencing gel, this was followed by electrophoresis of the gel, exposing and developing the film then the DNA sequence was read from the film using ALF express sequencer and the resultant chromatogram was analyzed by consed 30 software, and a blast at (http:www.ncbi.nucleotide.gov) search.

Serum total IgE

Serum total IgE was measured by ELISA technique (Genzyme Diagnostics, Medix Biotech, San Carlos, Calif). The value of IgE used for data analysis was calculated as a percentage of the highest normal for age[16].

Complete blood counting

Complete blood counting (CBC) was done using Coulter Counter (Coulter Microdiff 18, Fullerton, CA, USA) and the differential counts were carried out manually. Blood sampling of all subjects was performed at the same time daily (10 am) to avoid diurnal variations in eosinophil counts. The normal value of absolute eosinophilic count for age group 5-13 is 0.1- 1 $x10^3/\mu l$.

Statistical analysis

Data were analyzed by computer using the statistical program SPSS for Windows version 13. The mean, SD, median and IQ range were presented for the descriptive analysis of the groups. Groups were compared using the Student's t-test or the Mann-Whitney test (in case of non-parametric data). Fisher's Exact test was used for comparison of categorical data. In all tests p values less than 0.05 were considered statistically significant.

RESULTS

In our study, 50% of the asthmatic children had the common variant of IL-13, IL-13R130Q (GA), while the other 50% had the wild type (WT) IL-13 (GG). On the other hand only 3/10 (15%) of the healthy controls were positive for IL-13R130Q, and the remainder were presenting the wild GG genotype (fig. 1). The difference between the frequencies of patients and controls was statistically significant.

Comparison of serum total IgE levels as percent of normal for age in asthmatic children with GA and GG genotypes revealed that the levels were significantly higher in patients with GA compared to those with GG genotype with a mean of 373.25 ± 238.11% and 44.28 ± 42.65% respectively. Also the frequency of elevated total IgE was 90% in patients with the GA genotype compared to only 10% in those with the GG genotype ($p<0.001$). Control children with GA genotype also had significantly higher serum total IgE as percent of normal for age (mean of 28.03 ± 9.12%) as compared to those with GG genotype (mean of 11.70 ± 5.00%).

The AEC, as percent of normal, was slightly higher in GA patients compared to GG patients (mean values: 94.50 ± 83.80 and 67.20 ± 1.46 respectively $p>0.05$). However the respective values in controls were significantly different [mean values 250.00 ± 51.96 and 52.94 ± 36.87 respectively]. The frequency of elevated AEC was higher in GA patients compared to those with GG genotype (40% versus 10% respectively). On the other hand, all GA controls had an elevated AEC above normal in comparison to 17.64% only in controls with the GG genotype.

The present study failed to demonstrate a relation of the genetic profile of IL-13 to history of upper respiratory tract infections or food allergy as triggering factors for bronchial asthma exacerbations.

The impact of the presence of polymorphism of the gene of IL-13 on the severity of asthma was explored. No significant differences were observed between asthmatic patients harboring the gene polymorphism R130Q (GA) and those with the wild type (GG genotype) as regards the grading of asthma or the severity of the acute exacerbation. In this context, 60% of GA genotype harborors had moderate persistent asthma and 40% had severe persistent asthma in comparison to 70% and 30% respectively in the GG genotype harborors (p>0.05).

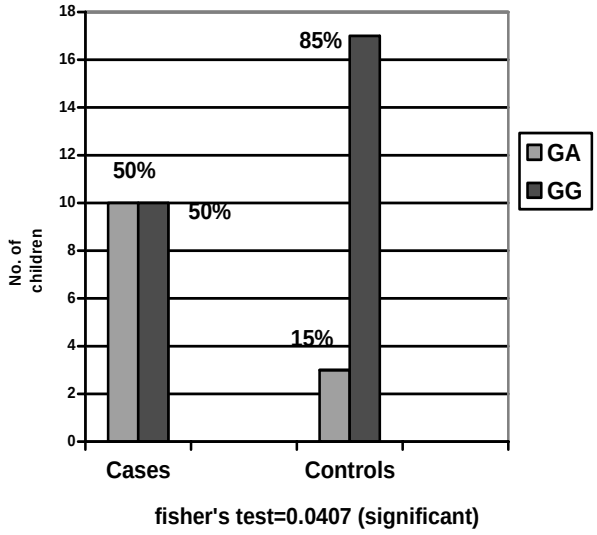


Figure 1. Interleukin 13 gene polymorphism R130Q (GA) in patients and controls.

Table 1. Demographic differences among asthmatic patients with GA and those with GG genotypes

	GA (n=10)	GG (n=10)	Test (value)	P-value
Age	10.10 ±2.92	7.40 ±2.22	t (2.33)	0.032*
Sex				
Males	6 (60%)	4 (40%)	Fisher's Exact Test	
Females	4 (40%)	6 (60%)	(0.656)	
Onset	3.05 ±1.83	1.58 ±0.76	z (1.81)	0.06
Duration	6.85 ±3.25	6.00 ±2.32	z (0.76)	0.45
Ht centile	52.50 ±14.19	47.50 ±7.91	z (0.68)	0.49
FH			Fisher's Exact	
+ve	7 (70%)	7 (70%)	Test	
- ve	3 (30%)	3 (30%)	(1.000)	
URTI				
+ve	4 (40%)	7 (70%)		
-ve	6 (60%)	3 (30%)	(0.369)	
History of food allergy				
+ve	4 (40%)	6 (60%)		
-ve	6 (60%)	4 (40%)	(0.656)	

FH: family history; GA: IL- I3R130Q; GG: normal IL-13, Ht= height; n=number, ns= non significant, s= significant; t=t value of student-t test, URTI: upper respiratory tract infection. ht= weight, z=z value of Mann-Whitney test.

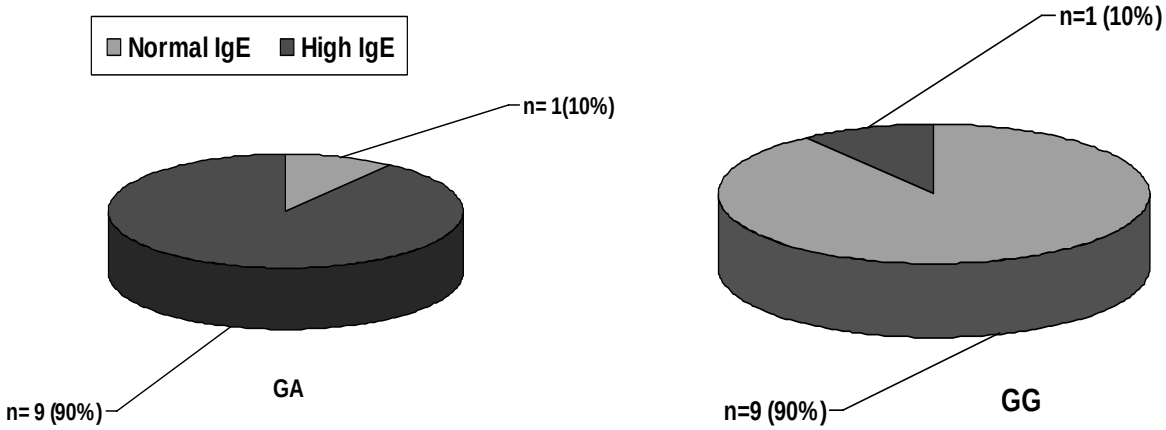
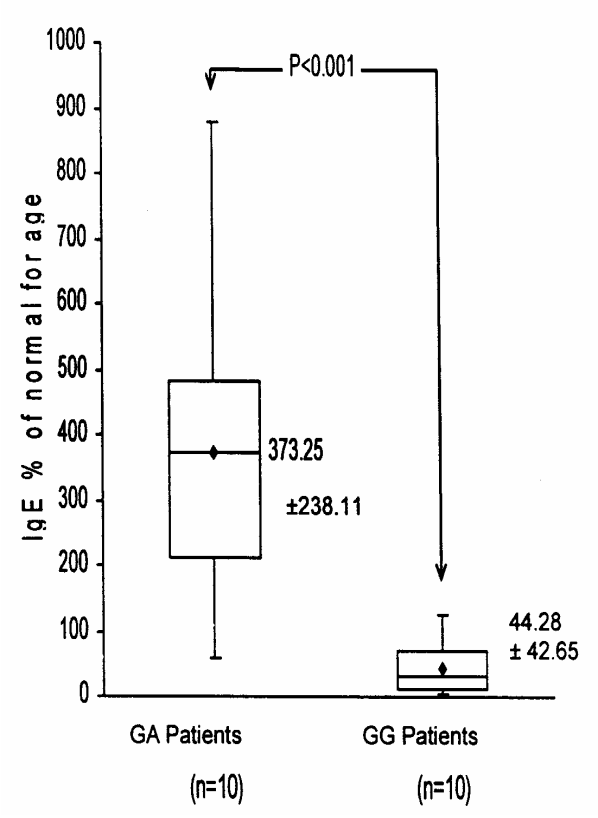
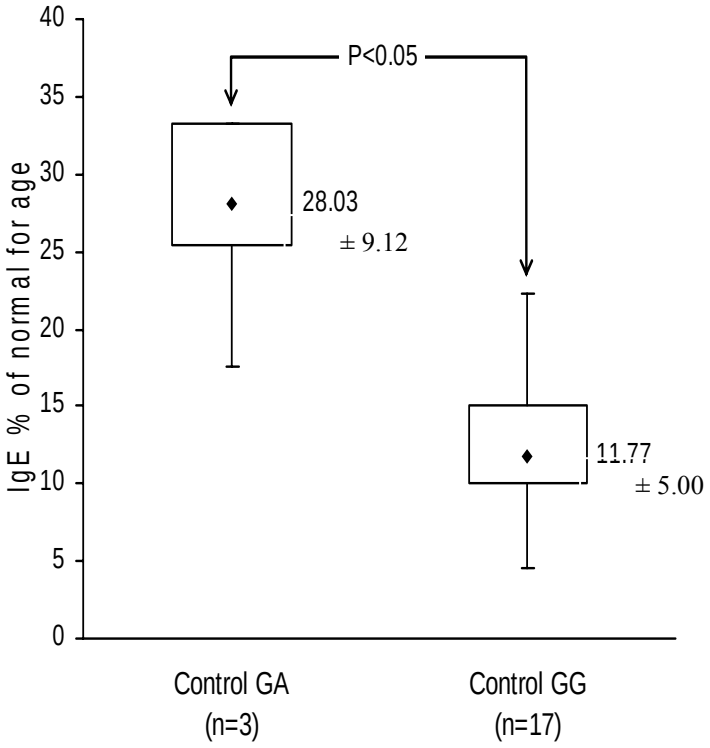


Figure 2. Distribution of asthmatic children with high IgE level.



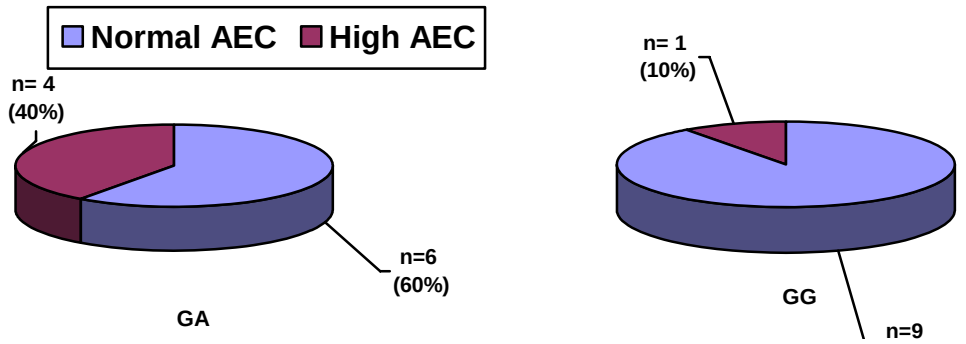
z = 3.48

Figure 3. Serum total IgE as percent of normal for age of asthmatic patients with GA and GG genotypes.



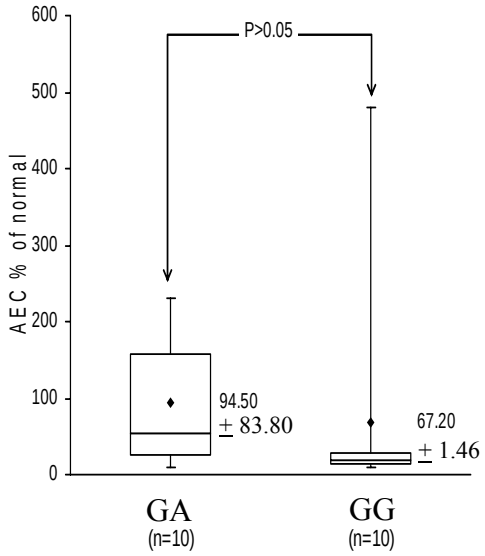
z = 2.49

Figure 4. Serum total IgE as percent of normal for age of controls with GA and GG genotype.



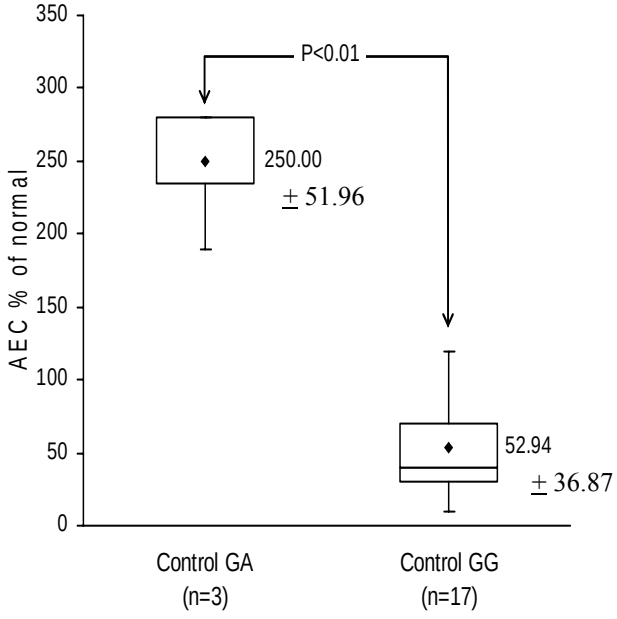
z = 1.66 **p > 0.05**

Figure 5. Distribution of asthmatic children with high absolute eosinophilic count (AEC). level.



z = 1.66

Figure 6. Absolute eosinophilic count as percent of normal for age of GA and GG patients.



z = 2.70

Figure 7. Absolute eosinophilic count as percent of normal for age of controls with GA and GG genotypes.

DISCUSSION

Polymorphisms that may dysregulate the function and/or the expression of IL-13 occur frequently in the population and have been consistently associated with allergic phenotypes[17]. Arima and associates[18] have recently provided evidence that the Arg110Gln may be a functional variant. Utilizing a mutant recombinant IL-13, they showed that recombinant IL-13 containing the Gln110 variant bound the IL-13 receptor α2 chain with lower affinity than the wild-type IL-13, resulting in a lower clearance rate of the cytokine. Furthermore the asthmatic patients homozygous for the Gln110 variant had higher serum levels of IL-13 than those without the variant. They postulated that impaired binding of IL-13 by the soluble IL-13Rα2 chain leads to higher serum levels and prolonged activity of IL-13 in vivo.

The present study revealed a significantly more frequent occurrence of the IL-13 polymorphism R130Q (GA) in asthmatic children than in healthy controls (50% versus15% respectively). This indicates that natural variation of the coding region of IL-13 may be an important genetic determinant of the susceptibility to asthma, based on the fact that IL-13R130Q is significantly more active than WT IL-13 in enhancing essential effector pathways of allergic inflammation in primary human cells[10]. This can be simply explained by the hypothesis that R130 is important for IL-13 binding to IL-13 Rα2 which is a key negative regulator of IL-13 responses in vivo. Hence, this IL-13 variant might to some extent escape the damping mechanisms that normally restrain the activity of WT IL-13 in vivo.

This comes in accordance with a case- control study on British and Japanese panels[8] in which it was found that IL-13 R130Q was significantly more common among asthmatics than controls in both populations and that this IL-13 variant seemed to have longer plasma half- life. In addition, Homšak et al[19] suggested that IL-13 polymorphism R130Q is highly connected with both types of asthma atopic and non atopic. However, other researchers[20] found a weak evidence of association between IL-13R130Q allele and increased risk of atopy and atopic dermatitis, most strongly in white children.

Chen and associates[21] indicated that SNP in IL-13 namely R130Q resulted in a significant biological difference as evidenced by the fact that R130Q induced airway hyperresponsiveness with doses of recombinant IL-13 R130Q significantly lower than that of the WT IL-13.

In contrast, other studies suggested that the R130Q polymorphism of IL-13 gene was not associated with asthma in Chinese children and it did not correlate with subjective or objective indicators of asthma severity[22]. Also they found that R130Q did not increase in frequency with airway hyperresponsiveness, asthma diagnosis or severity but was correlated with overall allergy phenotype, rather than an isolated association with one specific component of allergy such as elevated serum IgE[22,23]. Moreover, no association or linkage of the R130Q variant and lung functions in COPD patients was found in some studies; a finding which was explained by the hypothesis that IL-13 may only contribute to COPD in those individuals who have a concurrent asthma and/or airway hyper-responsiveness[24]. As obvious from these data, the results of studies on the polymorphism of IL-13 gene vary widely especially from a population to another: IL-13R130Q SNPs being prominent among British, Japanese as well as Egyptian asthmatics whereas this was not the case with the Chinese.

Previous reports have shown that genetic variants of the IL-13 promoter and coding regions were involved in the pathogenesis of asthma[25,26]. Hence, the impact of IL13 R130Q polymorphism on IgE levels was explored. Comparison of serum total IgE as percent of normal for age in asthmatic children with GA and GG genotypes revealed that IgE% was significantly higher in patients with the GA genotype with a mean of 373.25% and 44.28% respectively. Also the total IgE was elevated in 90% of patients with GA genotype compared to only 10% in those with GG genotype. This finding can be explained by the ability of IL-13R130Q to over express CD23 which contributes to the increase in the IgE- dependant inflammation[10].

The IgE levels as percent of normal for age was significantly higher in our asthmatic patients than in healthy controls with a mean of 208.77% and 14.21% respectively. IgE molecules play a crucial role in allergic respiratory diseases and may cause chronic airway inflammation in asthma through activation of effector cells via high affinity FcЄRI or low affinity FcЄRII IgE receptors[27]. Allergen-specific IgE synthesized in response to allergens in the environment becomes fixed to FcЄRI on the membranes of mast cells and basophils. Aggregation of receptor bound IgE molecules on re-exposure to specific allergen results in the production of mediators that produce the allergic response[28].

Strange enough, control children with GA genotype also had significantly higher serum total IgE as percent of normal for age as compared to those with GG genotype. This occurred in spite of the fact that both subgroups had within normal

values of IgE. This may reflect a susceptibility to later develop an atopic phenotype among at-present clinically normal children. Several studies found that R130Q polymorphism of IL-13 gene is associated with elevated serum total and allergen-specific IgE and stated that this variant is useful in the detection of subjects at risk of having elevated serum IgE or atopy[22,23]. On the contrary, Homšak and associates[19] found no association or linkage of the R130Q variant to IgE level.

Zitnik et al[29] analyzed the effect of two functional IL-13 variants (R130Q and c.1-1111 C>T) in a large population of white children with atopic dermatitis and a positive family history of asthma and found that IL-13 R130Q variant showed statistically significant effects on total serum IgE levels with highest values in homozygous subjects up to the age of four years. Allele frequencies for both IL-13 variants were similar to previous reports in white populations[30].

Previously believed to have an exclusive role in the release of cytotoxic mediators in the defense against helminthic infections, eosinophils are now considered key players in inflammatory reaction and regulation of immune response. Through activation of a large variety of membrane receptors and production of various pharmacologically active mediators, eosinophils may exert detrimental role in tissues in which they have been recruited[31]. Hypereosinophilia in the blood and tissues is one of the main characteristics of the pathophysiology of allergic diseases. Recent human experiments indicated that eosinophils may control the bronchial remodeling that occurs in asthma. Furthermore, eosinophils are a rich source of fibrogenic factors, particularly TGF-B[32].

Whether or not the studied polymorphism affects the AEC was subject to exploration. At first, the AEC of asthmatic and normal children were found comparable; a result that could be attributed to corticosteroids' intake by asthmatic patients causing a reduction in their AEC. A search for heterogeneity of AEC between the GA and GG genotypes revealed that although the former was associated with higher AEC, yet the difference among the two groups was statistically insignificant. This was not the case with the control group where the GA genotype was associated with significantly higher mean AEC with 100% of GA controls exhibiting high AEC compared to 17.64% only in controls with the GG genotype. Demo et al[23] found that R130Q was correlated with the overall allergy (combining eosinophil count and total serum IgE) as well as with eosinophil count alone.

Indeed, IL-13 has a major role in allergic inflammation through regulation of recruitment and activation of inflammatory cells. It has several actions relevant to recruitment of T cells and eosinophils into the lung. Among these actions are its role in up- regulation of vascular cell adhesion molecule 1 expression, its ability to prolong eosinophil survival, and its activity as an eosinophil activation and chemotactic agent. IL-13-induced eosinophilia is dependent on IL-5 and eotaxin, suggesting that IL-13 induces the production of these two eosinophil-active mediators[33].

Environmental triggers of asthma exacerbations, namely, upper respiratory tract infections and food allergy proved to be irrelevant to the genotype of IL-13, a finding which can be attributed to the small number of the patients examined. However it might indicate that the aberrant response of asthmatic individuals to environmental triggers such as infections and food allergens, is perhaps dictated by genetic polymorphism other than that of IL-13. Wider scale studies will help decide the exact nature of this relationship. Leung and associates[22] suggested that R130Q is linked to serum concentrations of specific IgE to dogs and cockroaches. Zitnik et al[29] reported a strong association between IL-13 SNPs R130Q and c.1-1111 C>T and food allergens particularly hen's egg .

The studied polymorphism of the gene of IL-13 had no impact on the severity of asthma or the severity of the acute exacerbations. This finding was supported by DeMeo et al[23] who also found no correlation between IL-13R130Q and asthma severity. Mijin Kang et al[33] reported that the +2044G/A polymorphism located in the coding region of IL-13 gene was associated with high total IgE and maximum percent fall in FEV1 (%) in children with asthma and exercise induced hyperresponsiveness suggesting that IL-13 polymorphism may modulate the severity of exercise induced hyperresponsiveness in Korean children with asthma.

The results of the present study provide preliminary evidence for the association of the common variant IL-13 gene polymorphism namely IL-13R130Q with the development of pediatric asthma. This variant is more active than the wild type IL-13 in inducing allergic inflammation as reflected by the higher serum total IgE and to some extent, AEC. IL-13R130Q might be an important genetic determinant of susceptibility to asthma in clinically normal children as it is associated with higher total IgE and AEC. The decision concerning

its legibility for future gene therapy targeted at reducing its ill- effects awaits larger scale studies.

In view of the varying results of genetic studies among different populations in different locations of the world, it becomes a necessity to establish the exact genetic profile unique for each population of asthmatic children. This will help direct future research in the field of gene therapy of asthma to suit each genotype.

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