Original article

Interaction of Two Variants of IL4 Receptor-A Gene with Serum IgE Level and Some Risk Factors for Childhood Asthma in Karbala Governorate

Ali Mansoor Al-Ameri[1*], Jaafar Kadhim Naama[2] and Munther Hussain Al-Kadhimy[3]

[1]Medical Microbiology Dept., College of Medicine, Karbala University, kerbala, iraq
[2] Medical Microbiology Dept., College of Medicine, Kufa University, najaf, iraq
[3] Institute Of Liver Studies, King's College Hospital/ London.

Abstract

Background: Asthma and related allergic diseases are complex genetic diseases with major environmental influences that occur in a developmental context. Susceptibility to asthma is influenced by genes and environment; implicated genes may indicate pathways for therapeutic intervention.
Aim: The present study aims to test the linkage association of *IL-4*Ra gene polymorphisms, E375A and C406R, determined by PCR/RFLP assay, with asthma development from 100 asthmatic children. In addition to their association with elevated serum IgE level in asthmatic children and possible interaction with other contributing factors including high BMI, history of prematurity, neonatal jaundice or vitamin D deficiency, exposure to bottle feeding and family history of atopy and/or asthma.
Patients and methods: This is a cross sectional survey study done in Kerbala Pediatric teaching Hospital during April, 2011 through February, 2013.
Results & Discussion: Data of the current study suggested a significant linkage association between IL4RA single nucleotide polymorphisms, SNPs, (E375A and C406R) and development of childhood asthma in the recruited participants, r= 0.82 and 0.67, respectively. Interestingly, the latter effect is synergistically increased upon gene-environment interaction with any of the studied risk factors tested in this study, except past history of neonatal jaundice.
The presence of E375A and C406R SNPs of IL4RA gene have potential effect on development of childhood asthma. Secondly, this effect is synergistically reinforced via gene-environment interaction of these SNPs with other asthma contributors including high BMI, history of prematurity, formula feeding, vitamin D deficiency and positive family history of asthma.
Conclusion: Our study reinforced the theory that asthma is a multifactorial disease suggesting a noticeable interaction between genetic and environmental factors in the development of this disease.
Key words: IL4 Receptor-alpha Gene, IgE , Childhood Asthma

Introduction

Asthma phenotypes in children could be summarized as; transient wheeze, persistent wheeze and late-onset wheeze with varying characteristics[(1)]. The etiology of asthma is likely multifactorial. Genetic factors may control individual predispositions to asthma[(2)]. In fact, developing approaches capable of identifying functional gene variants and the relevant mechanisms is undoubtedly a priority, particularly if

*For Correspondence: Email to alimansoor699@gmail.com

these variants are to become therapeutic targets. Thus, the ultimate promise of studying asthma genetics is not only to help unravel disease pathogenesis, but also, and perhaps more importantly, to lead to more effective prevention and cure[3]. Many asthma-related genes were implicated in asthma susceptibility of which, interleukin (IL)-4 receptor alpha (IL4RA) gene polymorphisms namely (E375A and C406R) were taken in consideration in this study. The literature contains conflicting reports on the association of common variants of the IL4RA gene with asthma[4]. It is well known that IgE plays a pivotal role in immunopathogenesis of asthma. Cross-linking of IgE-allergen complex with Fc εRI is the key step in immediate-type and inflammatory allergic reactions including asthma via mediating activation of the targeted mast cell and subsequent degranulation and mediators release. Additionally, determination of IgE antibodies is currently the most widely used test system in clinical routine for diagnostic purposes[5]. An association between extreme preterm birth and chronic respiratory morbidity, including asthma, has been established [6]. However, compared to those studies linking short term respiratory morbidities with prematurity, few studies have focused on the impact of prematurity on longterm respiratory outcomes, such as the development of asthma [7,8]**.** Hence, a link between history of prematurity, polymorphism in interleukin (IL)-4 receptor alpha (IL4RA) gene and asthma susceptibility was examined in the current study. A number of observational studies suggest associations between serum vitamin D level and childhood asthma [9-14]. A protective role was suggested by most recent studies; and this was explained by the effect of vitamin D in promoting IL-10 production in human B cells thus favoring rerulatory T-helper arm of the immune response[15]. Herein, we try to fix this association and to further test for any additional role of IL4RA gene SNPs with history of vit. D deficiency and asthma. Data from a recent metaanalysis study[16] revealed that an association between neonatal jaundice as a newly introduced etiologic factor in asthma in children was strongly suggested. Studies suggested many possible explanations of the causal association between neonatal jaundice and childhood asthma susceptibility[17-19]. In this study, an interaction of this factor with genetic variants of the IL4RA gene and their role in asthma development was investigated. Various studies of patients with asthma have reported an association between formula feeding and diagnosed asthma[20-25]. Plausible mechanisms to explain this association still controversial.[26] However, in the current study a possible interaction with genetic polymorphism of IL4RA gene was tested to clarify the gene-environment linkage association with childhood asthma. The link between asthma and obesity in childhood has been examined in many epidemiological studies[27,28]. A recent meta-analysis showed that children with high body weight were at increased risk of developing asthma with variable explanations[29-31]. Further link with genetic polymer-phism of IL4RA gene was estimated here to fix such causal association. The objectives of the present study are to determine the predictive value of serum IgE level in childhood asthma, to investigate the linkage association between childhood asthma development, elevated IgE and IL4RA SNPs (E375A and C406R), and to mount a gene-environment interaction study by considering a number of environmental and biological risk

factors (high BMI, formula feeding, history of prematurity, neonatal jaundice and vit. D deficiency, family history of atopy and/or asthma).

Patients and Methods

This is a cross sectional survey conferring a linkage association and a gene-environment interaction study regarding childhood asthma susceptibility. From January, 2011 through Febreuary, 2013, a total of 100 doctor-diagnosed asthmatic children (69 boys and 31 girls; mean age was 6 years, range 2–15 years) and 30 healthy control, were recruited from Kerbala Pediatric Teaching Hospital. Inclusion criteria for patients include those children aged 2-15 years with doctor-diagnosed moderate to severe asthma. Exclusion criteria involve children with cardiac diseases or respiratory illness due to other causes a foreign body inhalation, cystic fibrosis[1] or those who have recently received a full course of steroid, montilukast or other immunomodulator (s). Informed consent was obtained for all the participants. All subjects were tested for total serum IgE level and respond to a pre-formed questionnaire to mount an idea about exposure to the above mentioned asthma-associated risk factors. Clinical asthma, characterized by increased airway responsiveness, reversible airway obstruction, and airway inflammation, was defined according to pediatrician's diagnosis. Later on, serum IgE was demonstrated by enzyme-linked immunosorbant assay (ELISA) in serum samples from 100 asthmatic children and 30 controls. For asthmatic children and healthy control group, two IL4R α gene SNPs, (E375A and C406R) were investigated by PCR/RFLP assay; and a linkage association with elevated serum IgE level was tested. In addition, a gene-environment interaction between these two SNPs and some risk factors including high BMI, history of doctor diagnosed vitamin D deficiency, prematurity or neonatal jaundice, type of feeding and family history of atopy and/or asthma. Exposure to the mentioned risk factors was estimated according to history-based formula including the response to a pre-formed questionnaire form. Buffy coat samples were taken from venous blood and were genotyped for the presence of E375A or C406R SNPs from IL4RA gene by performing polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The E375A and C406R polymerphisms were contained within the same 334-bp PCR product and were digested with the restriction enzymes *Cac*8I (for E375A) or *Tsp*45I (for C406R), to distinguish the alleles (and the alleles were resolved by electrophoresis on a 2 % agarose gel) [33]. By using a thermocycler (Cleaver Scientific), the following reaction components were taken according to the kit instructions with some modifications: a final volume of 50 µl solution containing 25 µl of the PCR Direct Red Dye Premix mastermix and 0.2 µM of the forward primer:

5'-CAGCATGGTGCCCAGTGGAG-3'

And the reverse primer:

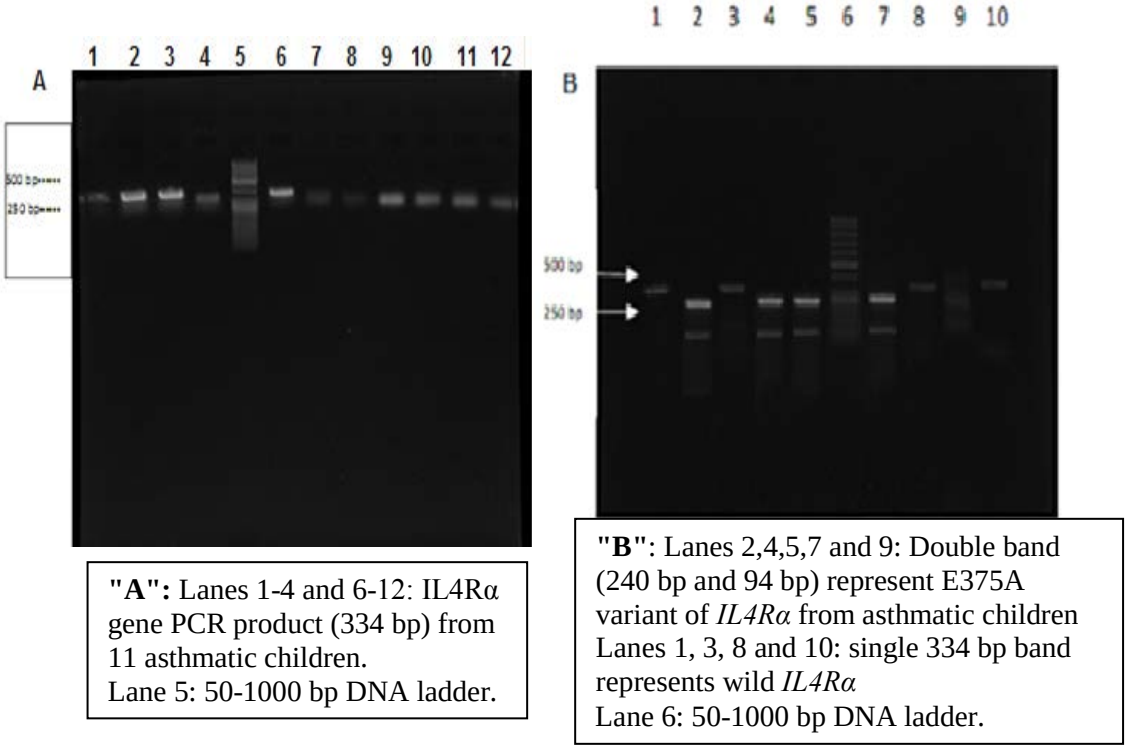
5'-CTTGGGAACTCATCCCAGGGC-3'[33].

Together with 5 µl of blood buffy coat, the volume was completed to 50µl by deionized water enclosed with the kit. Cycling conditions included an initial denaturation at 98^{o}C for 2 min, followed by 35 cycles at 96^{o}C for 30s, annealing temperature of each polymorphism (61^{0}C)[33] for 30 s, 72^{o}C for 40 s, and a final extension at 72^{o}C for 10 min according to kit leaflet with some modifications. Complete cleavage of the 334-bp PCR-amplificated DNA segment of IL4RA

gene by a restriction endonuclease was done. Upon analysis by gel electrophoresis, the cleavage products visualized as fragmented bands with varying MW as follows[33]: Double band representing E375A SNP digested with *Cac*8I enzyme, Double band representing C406R SNP digested with *Tsp* 45I enzyme; and Single 334-bp band representing wild IL4RA "can't be digested by any of the two enzymes". The current study also included measurement of body mass index (BMI). The equation child's weight (in Kg) divided by the square height (in meter^2) was employed to asses BMI at the initial visit. Overweight is defined as a BMI above the 91st centile (i.e.) results of BMI greater than 19 were regarded abnormally high [34]. Further assessment involved a response to questionnaire regarding history of previous doctor diagnosed vitamin D deficiency, prematurity or neonatal jaundice, type of feeding, family history of atopy and/or asthma; according to prepared questionnaire form. Genotypic finding were compared with those from the 30 healthy control group. Statistical analyses regarding allele frequencies between asthmatic and control groups were also analyzed using the $\chi 2$ test or the Fisher exact test. Pearson coefficient and odds ratios (ORs) for elevated serum IgE and the risk of asthma and their 95% confidence intervals (CIs) were calculated using logistic regression analysis. All the above statistical analyses were performed by Microsoft axcel and the online Graph Pad software "Prism".

Results

Our data from the PCR/RFLP assay revealed a significantly increased frequency of IL4RA gene SNPs (E375A and C406R) genotyped from the 100 asthmatic children compared to the control group, (p value < 0.05), as shown in Figures 1 and 2.



"A": Lanes 1-4 and 6-12: IL4Rα gene PCR product (334 bp) from 11 asthmatic children.
Lane 5: 50-1000 bp DNA ladder.

"B": Lanes 2,4,5,7 and 9: Double band (240 bp and 94 bp) represent E375A variant of *IL4Rα* from asthmatic children
Lanes 1, 3, 8 and 10: single 334 bp band represents wild *IL4Rα*
Lane 6: 50-1000 bp DNA ladder.

Figure 1 Agarose gel elecrophpresis of IL4Rα gene, "A" before digestion, "B" after digestion with *CAC* 8I enzyme.

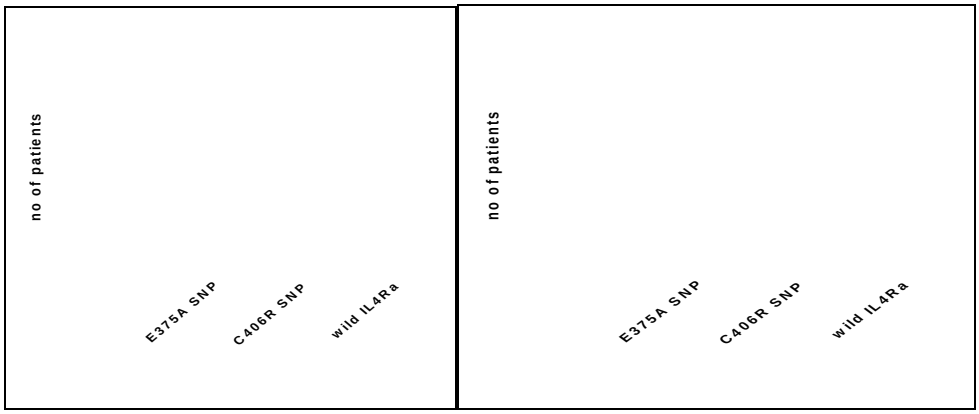


Figure 2: Frequency distribution of IL4Rα SNPs in "A" 100 asthmatic children compared to "B" control group.

Data from the current study has shown a significantly elevated serum IgE level in association with E375A and C406R SNPs compared to data from asthmatic patients with wild type non mutated IL4RA gene (Table 1). Gene-Gene Interaction Analysis revealed direct correlation between positive family history of atopy and/or asthma and occurrence of childhood asthma. In addition, results of frequency distribution of IL4Ra SNPs "E375A and C406R" suggested that children carrying one of these asthma candidate genes were more susceptible to asthma development in case of positive family history of atopy and/or asthma than those with wild type *IL4Ra* indicating a synergistic interaction between multiple asthma-contributing genes (Figure 3).

Table 1: Serum IgE levels in relation to IL4Rα SNPs "E375A and C406R" from 100 asthmatic children and 30 control group. (** significant association with high IgE level*)

participants		Type of *IL4Rα* SNPs			Total number
		E375A	C406R	Wild *IL4Ra*	
Mean serum	Asthmatic	114.67*	108.28*	67.98	100
	Control group	46.23	38.09	35.84	30
P value		<0.05	<0.05	>0.05	

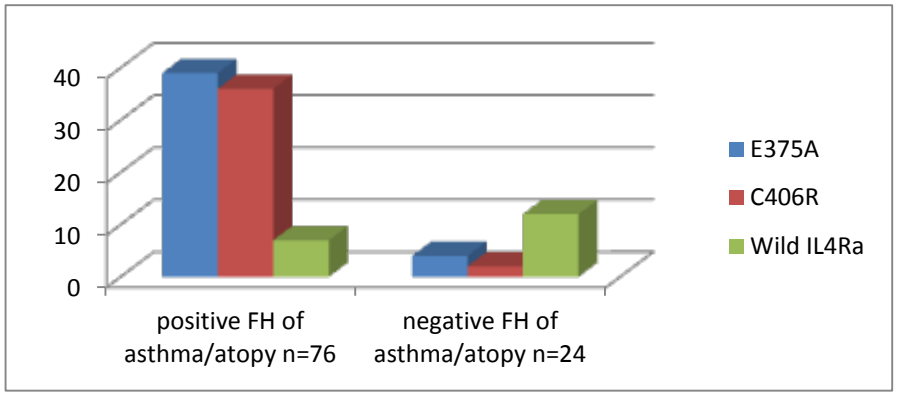


Figure 4: Association of IL4RA SNPs with asthma and interaction with family history of atopy or asthma.

Gene-environment interaction analysis has shown a significant impact of high BMI on childhood asthma development, p value <0.05. (Figure 4)

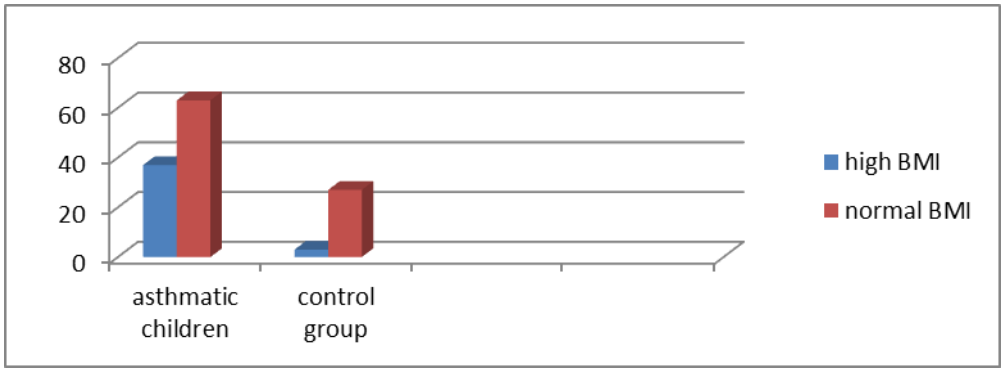


Figure 4: Association of asthma development and high body mass index in 100 asthmatic children compared to the healthy control group.

More importantly, children carrying asthma candidate IL4Ra SNP, E375A or C406R, were significantly more susceptible to adverse effects of high BMI, compared to those with wild *IL4RA* gene. Regarding role of early insertion of formula feeding, results have shown a significant association with childhood asthma development when compared to the control group (figure 5).

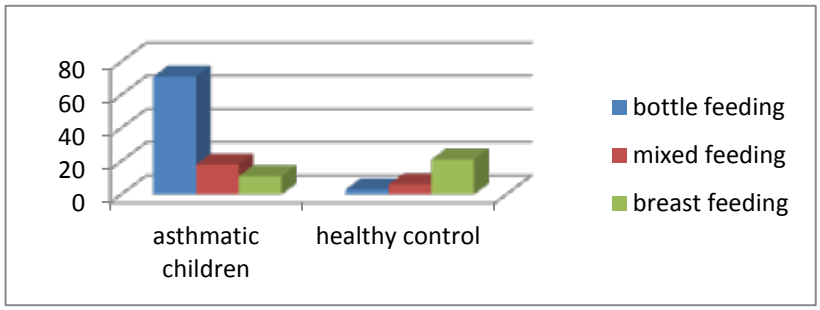


Figure 5: Association of asthma development and insertion of bottle feeding in 100 asthmatic children compared to the healthy control group.

Interestingly, a synergistic gene-environment interaction between bottle feeding and IL4Ra SNP, E375A or C406R. Thus children with mutant *IL4RA* gene were significantly more susceptible to adverse effects of bottle feeding, compared to those with wild gene p value <0.05. Results concerning history of vitamin D deficiency revealed a significant association with development of childhood asthma when compared to the control group, p value <0.05 (Figure 6).

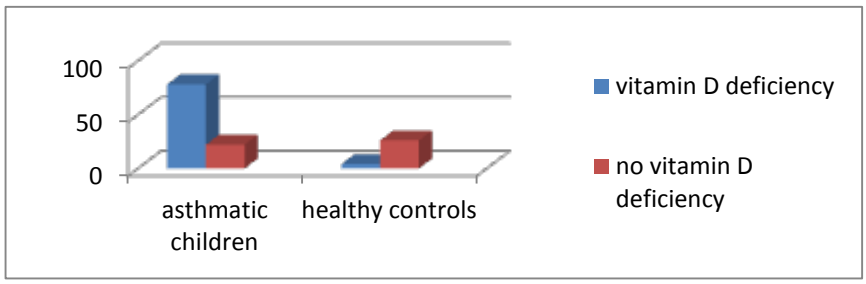


Figure 6: Relation between history of vitamin D deficiency and asthma development in 100 asthmatic children compared to the control group.

Again, a synergistic interaction between history of vitamin D deficiency and the presence of one of the IL4Ra SNPs, E375A or C406R which was found to potentiate the adverse effect of that asthma-related

risk factor, p value <0.05. Regarding past history of prematurity, our results revealed significant association with childhood asthma development and a synergistic gene-environment interaction with IL4Ra SNPs in this aspect, p value <0.05. Results concerning past history of neonatal jaundice revealed neither significant association with asthma nor synergistic gene-environment interaction with IL4Ra SNPs, p value > 0.05.

Discussion

Data of the current study suggested a significant linkage association between IL4RA single nucleotide polymorphisms, SNPs, (E375A and C406R) and development of childhood asthma in the recruited participants. A finding which is consistant with some previous genetic studies, while disagrees with others (see below). In addition, this finding represents a mere causal association of these two mutations in asthma development apart from the synergistic interaction with other asthma risk factors taken in consideration in this study. Such gene-environment interactions are to be shown in the following sections. Interlukin-4 is an important cytokine in allergic inflammation and in IgE isotype switching, via its binding to IL4RA a linked region in many asthma populations[35]. It was shown that many genes such as Interleukin (IL)-13, IL-4 , interleukin 4 receptor (IL-4Ra) , signal transducer and activator of transcription 6 (STAT6) and tumor necrosis factor-alpha (TNF a) genes are key inflammatory genes in the development of allergic diseases such as asthma and the list is expanding[36]. Several authors have recently analyzed the role of those genetic factors in asthma, although the results are contradictory[37-42]. In addition, no one gene was said to be an ‘asthma' gene in all populations. This likely reflects the complex etiology of this condition, the modest effects of these genes on risk and the important roles of gene–gene and gene–environment interactions in determining asthma susceptibility [43]. In view of the diversity of those candidate genes, we selected two SNPs from IL4Ra gene, a common asthma susceptibility gene, which are (E375A and C406R), and investigated their associations with asthma in our study. A significant positive linkage association was found in the current study between the two chosen mutations in IL4RA gene (E375A and C406R) and occurrence of childhood asthma. Although the results of this study indicate that the IL-4 receptor α gene does not appear to be directly involved in atopy or asthma, it may nevertheless interact with other factors which contribute to the pathogenesis of the disease. A finding in agreement with data from previous genetic studies in this field[35,44] and conflicting with others[40]. A gene–gene interaction of IL4RA with IL-13 was recently described in a Dutch population with asthma[33] However, there is so much controversy and complexity regarding the real causal association of even the most reproducible genes such as IL13, IL4, IL4RA, CD14, ADRB2, FCER1B, TNF and ADAM33 as extensively discussed elsewhere[45], inconsistent associations may reflect flaws in study design, size or analysis. Hence, a role for environmental factors and processes rooted in the biology of complex diseases in general, and asthma and allergy in particular, should also be considered.[3] On the other hand, it was suggested that the IL4RA gene does not exert a substantial effect on the inheritance of atopy or asthma in the population tested. These data are in agreement with the majority of studies in which no common variant of the gene was

found to be associated with asthma-related phenotypes across different ethnic groups, and in particular with a study in German and Swedish families in which the same polymorphisms were typed[46]. It was reported that negative results for linkage and association of five SNPs in the IL4RA gene with allergic asthma or intermediate phenotypes in a sample of allergic asthmatic families from north-east Italy[4]. Based on a considerable number of polymorphisms, Ober *et al*. (2000) suggested a likely at-risk haplotype in the Hutterites, but failed to find individual SNPs or a combination of SNPs associated with atopy or asthma in the samples of the population studied[47]. Owing to these data, many gene and environmental factors are associated with asthma, but the effect of each of these factors is mild. It was known that this disease has complex etiologies including the dependence of genotypic effects on environmental factors (i.e., gene-environment interactions) and genotypes at other loci (i.e., gene-gene interactions) [36]. Recently, there has been increased interest in gene-gene and gene-environment interactions, which may affect asthma pathophysiology. Asthma candidate genes are thought to contribute only 40–60% overall risk [40]. Gene-gene and gene-environmental interactions could explain the residual influence for asthma etiology when a single candidate gene is considered [36]. In fact, several genetic mechanisms are suggested to work together in order to mount susceptibility to such a complex disease as asthma. This may explain in part the data conflict regarding involvement of particular gene in development of asthma. This phenomenon necessates the need for studying possible synergistic interaction between the implicated gene varients[36]. In addition, environmental factors are clearly important in the pathogenesis of asthma; and, unfortunately, gene-environment interactions are infrequently included in genetic studies[48]. Analysis of serum IgE levels in association with genotypes indicated that patients with the E375A/C406R genotype have significantly higher levels of IgE. Moreover, the current study has shown a significant linkage association between IL4RA SNPs (E375A and C406R) with increases serum IgE level and occurrence of asthma. These data are consistent with other previous study that found a significant association between E375A, C406R, and S478P and total serum IgE levels was observed[49]. Hence, it was suggested that the polymorphism associated with asthma and atopic phenotypes is either E375A, C406R, S478P, a combination of these loci, or an additional, as yet unknown variant in LD with these polymorphisms (as also concluded in other populations)[47]. However, our results are inconsistent with a previous study reporting association of the IL4RA SNP (P478 and R551 haplotype) with lower IgE levels[50]. The similar validated results were also found in Japanese and British populations, but not in a Costa Rican population[51]. In agreement with our findings, two recent high resolution studies of the 5q31–33 region identify regions of linkage and haplotypes associated with atopy and high total IgE phenotypes distal to the IL-4–IL13 locus[52,53]. Another study suggested an association between the IL-4Ra promoter polymorphisms at positions –1098, –590, and –33 and IL-4 RA at position +1902 and asthma, and between the haplotypes of these polymorphisms and total IgE in asthmatic patients. However, the author did not find any association between these

polymorphisms and asthma severity [35]. Owing to these data, both our selected SNPs have previously been reported to be highly linked to childhood asthma in the recruited patients, and were found to be significantly associated with the risk of elevated serum IgE levels. This is in contrast with previous reports in which no associations were found between the IL4 polymorphisms and any measure of atopy, such as total or specific IgE levels, suggesting that polymorphisms of the IL-4 promoter may be more related to asthma per se than to atopy or atopic asthma[32]. However, another study found a risk haplotype composed by V75 and R576 in the IL-4Rα gene was associated with susceptibility to asthma and resulted in the expression of an IL-4Rα with enhanced sensitivity to IL-4[54]. Data on the correlation between T–590C on IL4RA gene and bronchial asthma are controversial[55]. Data suggested a synergistic interaction of the studied IL4RA SNPs (E375A and C406R) with high BMI in asthmatic children recruited to the study. This observation is in agreement with an idea stating that obesity is an important risk factor in asthma[29,56,57], and that patients with allergic asthma showed significantly higher body mass index (BMI) and insulin resistance than non-asthmatic controls[26]. Our results revealed a significant positive correlation between occurance of childhood asthma and history of early introduction of formula feeding during the first 6 months of age. In addition, this association is synergistically enhanced in the presence of one of the two studied IL4RA SNPs. In fact, the gene-environment interaction studies in this regard are lacking and data nearly restricted to causal association testing. In 2001, a meta -analysis was carried out concerning breastfeeding and it was found that although some protective effect against atopic dermatitis and/or wheezing existed in studies lasting <4 years, the benefits were less pronounced in studies where participants were followed for a longer period of time. More recent studies [58] and meta-analyses have confirmed the results of the first one[59]. On the other hand, the risk of asthma was found to be enhanced in breastfed children after the age of 6 years in some[60] but not all prospective studies[61]. Results from a developing country suggest a protective effect of prolonged breastfeeding on the development of allergic disease, particularly hay fever, in children born to nonallergic parents. This protective effect was not found in children with an allergic predisposition[62]. Overall, breast-feeding is therefore highly recommended for all infants, irrespective of atopic heredity, because its preventive effect on atopy is not demonstrated[63]. Reasons for these controversies include methodological differences and flaws in the studies performed to date, the immunologic complexity of breast milk itself and, possibly, genetic differences among patients that would affect whether breastfeeding is protective against the development of allergies or is in fact sensitizing[64]. Thus, the relationship between breastfeeding and allergic disease risk has been controversial. Studies show a paradoxical effect of breastfeeding on the prevention of asthma, with an apparent protective effect against early wheezing illness in the first years of life yet an increased risk of asthma in later life; however, these findings must be interpreted with caution[65]. Existing studies fail to adequately adjust for confounders, including the critical issues of protection against early life respiratory illnesses and reverse causation. Therefore, it is possible that the effect of breastfeeding on early wheezing

illness reflects protection against respiratory infection, the predominant trigger of wheezing in early childhood, rather than a true reduction in risk of asthma [65]. Indeed, it was reported that families at high risk of allergic disease are more likely to select breastfeeding. Therefore the association between breastfeeding for more than 6 months and an increased risk of allergic disease observed in their cohort of 620 high-risk infants can potentially be explained by the fact that high-risk families choose to breast feed longer rather than by the fact that breast feeding increases the risk of allergic disease [64]. The current study revealed a significant genetic interaction between positive family history of asthma/atopy, presence of E375A or C406R SNPs and the susceptibility to childhood asthma. This may be a confirmatory evidence about complimentary effect of multiple genetic susceptibility (gene-gene interaction) idea in asthma development[36]. Various genetic reports confirm such gene-gene interaction effect on susceptibility to asthma[66]. Many are included in recent metaanalysis studies. For instance, in a recent meta-analysis of 20 genome-wide linkage studies there were only two chromosomal regions (2p21–p14 and 6p21) that showed significant evidence for linkage in European families, after adjustment for multiple comparisons[48]. Linkage studies have good power to detect rare high risk disease-causing alleles but are less effective at detecting more common risk alleles with modest effect sizes. This may explain at least some of the lack of reproducibility observed in these asthma linkage studies [35,48]. In a white Dutch population, Howard et al. found a gene-gene interaction between S478P of the IL-4Rα gene and −C1112T of the IL-13 gene markedly increased an individual's susceptibility to asthma[44]. Recently, Chan et al. reported IL-13 R130Q together with IL-4Rα I50V might result in an increased risk for asthma in a Hong Kong population of China[67]. Other investigators have chosen the six-locus model as the best one for determining the susceptibility of gene-gene interactions to asthma in a Chinese population. Data based on its maximum testing accuracy and cross-validation consistency. The best six-locus model consisted of IL-4 −C33T, IL-13 R130Q, IL-4Rα I75V, IL-4Rα Q576R, STAT6 C2892T, and CD14 −C159T polymorphisms[68]. Among these SNPs, I75V and Q576R polymorphisms in the IL-4Rα gene were significantly associated with asthma in haplotype analysis and MDR analysis, suggesting a combined effect for IL-4Rα on asthma development. Compared to the single SNP effect of IL-13 R130Q and the haplotype combined effect of IL-4Rα,a greater odds ratio for the best model with six-locus indicated that a synergistic interaction among the six polymorphisms was more strongly associated with asthma development [68]. These results suggest that some polymorphisms are linked and that certain haplotypes may have a stronger impact on diseases compared with single-nucleotide polymorphisms alone, which is also supported by Ober et al. Based on a considerable number of polymorphisms in the IL-4Rα gene, the investigator reported a six-locus risk haplotype in the Hutterites and several outbred populations, but failed to find individual SNPs associated with atopy or asthma in the samples of the population studied[51]. Data of the current study revealed that history of prematurity strongly interact with E375A and C406R SNPs in asthma development in the recruited children. This finding is consistent with several studies in this aspect most of which

focused on the impact of gestational age for very preterm infants (≤32 weeks) or all preterm infants (< 37 weeks), and the magnitude of the association with asthma varied substantially between studies[6,8,69].The few studies of late prematurity and the development of asthma showed mixed results. Using survey data from a nationally representative sample, Abe et al did not find an association with late-preterm birth when asthma was assessed on the basis of caregiver reports[8]. Escobar et al, using a large cohort, did find a statistically significant association between late prematurity and recurrent wheeze in children up to 3 years of age[69]. Mechanistically, it has been proposed that chorioamnionitis, because of the associated proinflammatory state and heightened immune response, might play a role in asthma development for infants born preterm[70,71]. The current study revealed a significant interaction between the past history of Vitamin D deficiency, presence of E375A and C406R SNPs and the occurrence of asthma in the recruited children. Our finding are in agreement with data from previous works in this regard. Many retrospective cohort studies have shown remarkable effect of positive history of Vit D deficiency on childhood asthma development with variable explanations to this phenomenon[72-77]. Nutrients such as vitamin D have notable effects on the immune system, with a vitamin D-deficient diet being associated with decreased regulatory responses to allergen[78,79]. Importantly, these effects occur even prenatally[80]. The current study revealed non significant association of past history of neonatal jaundice and development of asthma. Similarly, no synergistic interaction between it and the presence of E375A and C406R SNPs in the development of asthma in the recruited participants was observed. Our finding disagrees with the idea of positive causal association between history of neonatal jaundice and development of asthma irrespective of the IL4RA gene SNPs. Our data disagree with that from a recent metaanalysis study which revealed an association between neonatal jaundice as a newly introduced etiologic factor in asthma in children was strongly suggested, and the influence in females is stronger[16]. Despite different explanation for this causal relation**,** researches considering interaction with another genetic variant are lacking[18,19,81,82].

Conclusion

Genetic analysis of the polymorphisms of the IL-4RA gene SNPs (E375A and C406R) revealed their potential linkage association with development of childhood asthma. In addition, E375A and C406R SNPs of IL4RA gene have a conciderable effect potentiating the adverse effect of some susceptibility risk factors to childhood asthma including high BMI, history of prematurity, bottle feeding, vitamin D deficiency, and positive family history of atopy and/or asthma. A statistically significantt association between the above mentioned SNPs and serum total IgE was also found. Our results highlight the value of allele frequency and of the association between these polymorphisms and their phenotypic manifestations, together with their gene-environment interaction effect which is highly variable according to population.

Recommendations

Owing to the data from the present study, it is recommended that:

- Studies analyzing additional asthma phenotypes (eg, severe persistent asthma) in larger scale would

enable us to test possible associations between the genetic polymorphisms for *IL-4RA* and that asthma phenotype.

- Further studies with more gene tagging or functional SNPs using DNA sequencer to discriminate between homo- and heterozygous genotypes and with larger sample size are required to elucidate the role of multiple genetic factors in the development of childhood asthma.
- Given the proven ability of vitamin D to modulate T-cell responses, further cohort research, particularly, early life intervention studies need to be carried out to establish whether early life dietary intervention can be used as a public health measure to reduce the prevalence of childhood asthma.
- Future research that takes into account the potential contribution of confounding factors and effect modifiers is needed to clarify the role of breastfeeding in development of allergic disease and to inform current clinical guidelines on the prevention of allergic diseases including childhood asthma.

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