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Correlation between Single nucleotide polymorphism (rs20541 A>G) and Serum Interleukin -13 In Asthma

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Abstract:
Asthma is a complex disease with factors owing to heredity and exposures that are environmental resulting in chronic airway inflammation. Differences in immune related genes may lead to susceptibility to disease and alterations of cytokines. The present case–control study aimed to assess the relationship of IL13 single nucleotide polymorphism (SNP) rs20541 (A>G) to asthma risk, and its role in serum IL-13 levels according to various genotypes. A total of 118 subjects were recruited for the study: 60 with asthma and 58 healthy controls. The genotyping of the IL13 rs20541 (A>G) polymorphism was conducted by Sanger sequencing, and serum IL-13 levels were determined by enzyme-linked immunosorbent assay (ELISA). The genotype and allele frequencies of IL13 rs20541 were not significantly associated with asthma susceptibility (P = 0.120). G allele frequency was 33.33% for asthma patients and 24.14% for the control group, respectively, but this difference did not reach statistical significance (P=0.28). Furthermore, serum IL-13 levels did not significantly vary between all of the genotypes (P = 1.543). The results of the present study demonstrate that the IL13 rs20541 polymorphism is not a risk factor for asthma, or controls serum levels of IL-13, in this population from Iraq. Additional large-scale studies are necessary to validate these findings for GWAS and extensive genetic markers associated with personalized asthma treatments.

Keywords: Asthma, Interleukin-13, Single Nucleotide Polymorphism, rs2243250.

الارتباط بين تعدد الأشكال أحادي النوكليوتيد (rs20541 A>G) ومستويات الإنترلوكين-13 في مصل الدم لدى مرضى الربو

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الملخص
يُعدّ الربو مرضًا معقّدًا ينجم عن تفاعل العوامل الوراثية مع التعرضات البيئية، مما يؤدي إلى التهاب مزمن في الممرات الهوائية. وقد تسهم الاختلافات في الجينات المرتبطة بالمناعة في زيادة القابلية للإصابة بالمرض وإحداث تغيّرات في مستويات السيتوكينات. هدفت هذه الدراسة من نوع الحالات والشواهد إلى تقييم العلاقة بين تعدد الأشكال أحادي النوكليوتيد لجين IL13 (rs20541 A>G) وخطر الإصابة بالربو، ودوره في تنظيم مستويات الإنترلوكين-13 في مصل الدم وفقًا للأنماط الجينية المختلفة. شملت الدراسة ما مجموعه 118 مشاركًا، منهم 60 مريض ربو و58 من الأصحاء كمجموعة ضابطة. أُجري تحديد النمط الجيني لتعدد الأشكال IL13 rs20541 (A>G) باستخدام تقنية تسلسل سانغر، كما قيس مستوى الإنترلوكين-13 في المصل بواسطة اختبار الامتصاص المناعي المرتبط بالإنزيم (ELISA). لم تُظهر تكرارات الأنماط الجينية والأليلات لتعدد الأشكال IL13 rs20541 ارتباطًا ذا دلالة إحصائية بقابلية الإصابة بالربو (P = 0.120). بلغت نسبة تكرار الأليل G 33.33% لدى مرضى الربو و24.14% لدى المجموعة الضابطة، إلا أن هذا الفرق لم يصل إلى مستوى الدلالة الإحصائية (P = 0.28). كذلك، لم تختلف مستويات الإنترلوكين-13 في المصل اختلافًا معنويًا بين جميع الأنماط الجينية (P = 1.543). تشير نتائج هذه الدراسة إلى أن تعدد الأشكال IL13 rs20541 لا يُعد عامل خطورة

للإصابة بالربو ولا يؤثر في مستويات الإنترلوكين-13 في المصل ضمن هذه العينة من السكان العراقيين. وتُوصى بإجراء دراسات واسعة النطاق مستقبلًا للتحقق من هذه النتائج ضمن دراسات الارتباط على مستوى الجينوم (GWAS) واستقصاء واسمات وراثية أوسع تدعم العلاجات الشخصية للربو.

الكلمات المفتاحية: الربو، الإنترلوكين-13، تعدد الأشكال أحادي النوكليوتيد، rs2243250.

Introduction

Asthma is a chronic inflammatory disease of the airways which presents with bronchial hyper reactivity, airway remodelling and recurrent wheezing, breathlessness and cough. This disease results from intricate interactions between environmental and host genetic forces that affect responses to the airway, particularly those induced by T helper type 2 (Th2) cytokines. Of the multiple cytokines in this cascade, Interleukin 13 (IL-13) is critical to allergic inflammation and pathogenetic pathways since it stimulates IgE production, select mucus growth, recruiting eosinophils as well as airway hyper-reactivity; all known asthmatic elements. High IL-13 levels in asthmatics correlate with severity of disease and poor clinical outcome [1].

IL-13 is encoded by the IL13 gene on chromosome 5q31–33, which has been linked to asthma susceptibility. Genetic polymorphisms, predominantly single nucleotide (SNPs), in IL13 can influence cytokine expression and function which could have a direct impact on individual risk of and presentation with disease. Among SNPs, rs20541 (A>G), known as the Arg130Gln substitution in exon 4 is one of the most investigated variants. This coding SNP results in an altered amino acid sequence of the IL-13 protein, potentially affecting receptor binding and downstream signaling to regulate cytokine activity [2].

However, the role of rs20541 in susceptibility to asthma and its association with serum IL-13 levels have not been investigated. For instance, a study in asthmatic patients from Iraq found that rs20541 was significantly associated with high concentration of IL-13 and IgE, indicating that the A allele might contribute to asthma development as well as inflammatory markers [1,3]. Similarly, the studies in Egyptian children revealed that rs20541 polymorphism is associated with higher serum IL-13 levels and preferentially discriminates asthma severity, which suggest a functional effect of this variant on disease development [2,4]. Further studies in different populations are still exploring the role of rs20541 on asthma susceptibility and immune phenotypes, but results differ from one ethnic group to another [5, 6].

Although growing evidence has implicated IL-13 rs20541 (A>G) with serum IL-13 levels and asthma, the association between them is not fully elucidated yet which warrant more investigations to confirm a genetic pleomorphism effect on disease susceptibility and cytokine network. Further explicit knowledge of germline-determined molecular features might help move the outcomes from GWAS to identify candidate biomarker(s) for asthma diagnosis and severity assessment with a personalized therapeutic approach.

Methods

Study design and setting

Study Design and Population

The purpose of this case–control study was to explore the potential association of IL13 gene rs20541 polymorphism (A>G) with asthma susceptibility and whether it affected serum IL-13 levels. A total of 60 asthmatic patients and 58 healthy volunteers were enrolled in the study. Asthma participants were recruited from the outpatient clinics and diagnosed based on internationally accepted clinical and spirometric criteria. The control population were age and sex matched patients with no personal or family history of asthma, allergic disease or chronic inflammation.

Sample Collection

Aseptic venipuncture (5 mL) was performed on the participants. Plasma was split into two aliquots:

- One sample was drawn in EDTA vaccutainer tubes for genomic DNA isolation.
- The second component was harvested in no additive tube, allowed to clot and centrifuged in order to decant serum which was kept frozen at −20°C until cytokine analysis.
-

Genomic DNA Extraction

Genomic DNA was prepared from peripheral blood leukocytes as described by the manufacturer. DNA was quantified and purity checked by spectrophotometric method, before storing specimens were at −20°C.

Genotyping of IL13 rs20541 Polymorphism

The genotyping for the IL13 rs20541, A>G SNP was conducted by Sanger sequencing. Portion of the target region that contains SNP was amplified using polymerase chain reaction (PCR) with specific primers (Table-1). PCR products were elected on agarose gel and purified. The reaction sequences were carried out in automatic sequencer for DNA and sequence data processed by appropriate bioinformatics software to test genotypes.

Table 1: Sequence of primers used in the study

Gene	SNPs	Primer sequence 5′ to 3′	Amplicon (bp)
IL-13	rs20541	**Forward Primer:** CAGCAGTTTTCCAGCTTGCA **Reverse Primer:** CCCAAGACATTTTTGACATCAGA	201 bp

Measurement of Serum IL-13 Levels

The levels of IL-13 in serum samples were detected by commercially available ELISA kits according to the manufacturer's instructions. Duplicate samples were included for each assay (to confirm the accuracy), and absorbance was determined with a microplate reader. Cytokine levels were determined by standard curve that was prepared from known concentrations of IL-13.

Statistical Analysis

Genotype and allele frequencies were obtained by direct counting and checked for Hardy–Weinberg equilibrium. Chi square test was used to compare genotype and allele distributions between cases and controls. The comparison of serum IL-13 concentrations between different genotypes was carried out by one-way ANOVA or non-parametric tests when necessary. A p value of < 0.05 was considered significant.

Results

In order to investigate whether the IL-13 rs20541 (A>G) polymorphism might be associated with susceptibility to asthma, we compared genotype and allele frequencies of this nonsynonymous SNP between subjects with bronchial asthma and healthy controls. As shown in Table 2, no significant differences in the distribution of genotypes or alleles between the two groups were observed.

The percentages of AA, AG and GG genotypes in the control group were 60.4%, 31% and 8.6%, respectively, while in asthma patients these were found to be 50%, 33.3% and 16.7%, respectively. A higher tendency of the G allele was also found in asthma cases (33.33%) as compared with controls (24.14%), although not statistically significant and an odds ratio of 1.57, P = 0.120). On the other hand, healthy individuals had higher frequency of A allele (75.86%) than asthma patients (66.67%), and thus it was chosen as the reference allele.

In general, both asthma and sc cases presented greater frequency of the G allele at this SNP, but the data for IL-13 rs20541 seem not be important in susceptibility to asthma in our population.

Table 2: Distribution of IL-13 (rs20541, A>G) genotypes and alleles in relation to asthma risk

SNPs	Genotype	Patients (n=60)	Controls (n=58)	OR (95%CI)	P-value
rs20541 A > G	AA	30 (50%)	35 (60.4%)	Reference	
	AG	20 (33.3%)	18 (31%)	2.33(0.71-7.58)	0.159
	GG	10 (16.7%)	5(8.6%)	1.29(0.58 - 2.89)	0.526
Hw-P		0.0528	0.245		
Allele frequency	A	80 (66.67%)	88(75.86%)	Reference	
	G	40 (33.33%)	28(24. 14%)	1.57(0.88 - 2.77)	0.120

The relationship of IL-13 rs20541 (A>G) and serum IL-13 levels is shown in Figure 1. There were no statistically significant differences in the serum IL-13 levels by genotypes (P = 1.543) and no association between rs20541 and circulating level of IL-13 was found (Table 3).

Table 3: Serum IL-13 levels according to IL-13 rs20541 (A>G) genotypes in asthma patients**.**

IL-13 (pg/mL)			
Genotype	Mean± SD	Test	P-value
AA	77.92±12.27	1.543**	0.225NS
AG	85.31±12.24		
GG	81.85±10.36		

****:** One way Anova test

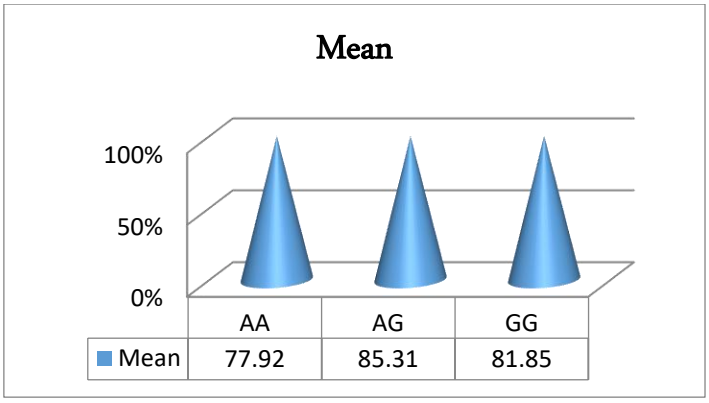

Figure 1: Mean serum IL-13 levels in asthma patients by genotype.

Discussion

A case–control study design was used to investigate the association of IL-13 rs20541 single nucleotide polymorphisms (SNP) with bronchial asthma, as well as its influence on serum IL-13 levels in allergic asthmatics.

The outcomes indicated that the distribution of IL-13 rs20541 (A>G) genotypes and allele frequencies showed no statistically significant difference between asthmatic patients and healthy control group. These results are in accordance with those reported by Abdulla and Mahmood [7] who found similar frequencies of this polymorphism between asthmatic Iraqi patients and controls. Similarly, Sharifi et al. [8] found there was no significant association between the rs20541 G/A polymorphism and asthma in an Iranian population ($p = 0.319$). Moreover, Shirkani et al. [9] reported that the rs20541 variant in the IL-13 gene exon 4 was not associated with a risk of AR.

While there was no significant association between IL-13 rs20541 and the susceptibility to bronchial asthma observed in the current work, other studies have previously shown that this polymorphism is associated with increased plasma IgE levels as well as asthma risk [10,11]. The dissimilar result between the two populations could be due to ethnic diversity and environmental differences that influence patterns of genetic susceptibility. Such an explanation is consistent with a meta-analysis which shows that the failure to find consistent associations between individual SNPs and allergic diseases may be due, in part, to genetic heterogeneity within the population studied as well as environmental exposures [12].

No association were observed in serum IL-13 concentration among the rs20541 genotypes either in normal or OA cases, These data are partly consistent with that reported by Ambrocio-Ortiz et al. [13]. Discrepancies between previous studies could be due to discrepancies in study design, sample size, geographic location and ethnicity.

Conclusion

The genetic polymorphism rs20541 in IL-13 gene did not correlate with asthma susceptibility or serum cytokine levels in the Iraqi patients tested. But it still needed additional studies with large sample and high quality to verify its credibility.

Disclaimer

The paper has never been offered for presentation or publication, including during the submission for some other conference.

Conflict of Interest

No financial, personal or professional interests' conflict of interest to declare.

References

1. Abdulla, A. A., & Shaheed Mahmood, N. (2022). Correlation between IL-13 rs20541 (A>G) gene polymorphism and bronchial asthma among Iraqi patients. Reports of Biochemistry & Molecular Biology, 11(2), 344–349. https://doi.org/10.52547/rbmb.11.2.344.
2. Khattab, M. A., Hussein, M. M., & Khater, W. S. (2023). IL-13 rs20541 single nucleotide polymorphism and serum IL-13 levels in children with bronchial asthma. The Egyptian Journal of Pediatric Allergy and Immunology, 21(1), 27–33. https://doi.org/10.21608/ejpa.2023.170190.1045.
3. Hassan, A. R., Abdallah, A. G., Ismail, N. A., & Fahmy, Y. A. (2024). Association of IL-13 rs20541 and FOXP3 rs3761548 gene polymorphisms and serum IL-13 levels with allergic asthma in Egyptian patients. Egyptian Journal of Immunology, 31(3), 15–27. https://doi.org/10.55133/eji.310302.

4. Letchumanan, G., & Say, Y. H. (2025). Association of IL-13 gene polymorphism (rs20541) with chronic inflammatory diseases: A systematic review and meta-analysis. International Journal of Immunogenetics, 52(6), 313–334. https://doi.org/10.1111/iji.70018.
5. Abdalla, M. A., & Hamdan, H. Z. (2025). IL13 gene polymorphisms among Sudanese patients with bronchial asthma: A case–control study. Molecular Biology Research Communications, 14(1), 27–30. https://doi.org/10.22099/mbrc.2024.50865.2017.
6. Hafez, R. A., Hassan, M. E., Haggag, M. G., Atef, N., Abdallah, A. L., & Gerges, M. A. (2022). Association of interleukin-13 rs20541 gene polymorphism and serum periostin with asthma and allergic conjunctivitis among Egyptian patients. Journal of Asthma and Allergy, 15, 971–982. https://doi.org/10.2147/JAA.S373098.
7. Abdulla, A. A., & Mahmood, N. S. (2022). Correlation between IL-13 rs20541 (A>G) gene polymorphism and bronchial asthma among Iraqi patients. Reports of Biochemistry & Molecular Biology, 11(2), 344. https://doi.org/10.52547/rbmb.11.2.344.
8. Sharifi, A., Ghadiri, A., Salimi, A., Ghandil, P., & Esmaeili, S. A. (2021). Evaluating the distribution of (+2044 G/A, R130Q) rs20541 and (−1112 C/T) rs1800925 polymorphisms in the IL-13 gene: An association-based study with asthma in Ahvaz, Iran. International Journal of Medical Laboratory, 8(1). https://doi.org/10.18502/ijml.v8i1.5675.
9. Shirkani, A., Mansouri, A., Hosseini, R. F., Azad, F. J., Mahmoudian, R. A., Montazer, M., Samimi, A., Momtazi-Borojeni, A. A., Abbaszadegan, M. R., & Gholamin, M. (2019). The role of interleukin-4 and interleukin-13 gene polymorphisms in allergic rhinitis: A case–control study. Reports of Biochemistry & Molecular Biology, 8(2), 111–118.
10. Cui, L., Jia, J., Ma, C. F., Li, S. Y., Wang, Y. P., Guo, X. M., Li, Q., Yu, H. B., Liu, W. H., & Gao, L. B. (2012). IL-13 polymorphisms contribute to the risk of asthma: A meta-analysis. Clinical Biochemistry, 45(4–5), 285–288. https://doi.org/10.1016/j.clinbiochem.2011.12.012.
11. Imraish, A., Abu-Thiab, T., & Zihlif, M. (2021). IL-13 and FOXO3 gene polymorphisms regulate IgE levels in asthmatic patients. Biomedical Reports, 14(6), Article 55. https://doi.org/10.3892/br.2021.1431.
12. Ying, X. J., Zhao, S. W., Wang, G. L., Xie, J., Xu, H. M., & Dong, P. (2013). Association of interleukin-13 SNP rs20541 with allergic rhinitis risk: A meta-analysis. Gene, 521(2), 222–226. https://doi.org/10.1016/j.gene.2013.03.088.
13. Ambrocio-Ortiz, E., Galicia-Negrete, G., Pérez-Rubio, G., Escobar-Morales, A. J., Abarca-Rojano, E., Del Angel-Pablo, A. D., Castillejos-López, M. D., & Falfán-Valencia, R. (2020). Single nucleotide and copy-number variants in IL4 and IL13 are not associated with asthma susceptibility or inflammatory markers: A case–control study in a Mexican-Mestizo population. Diagnostics, 10(5), Article 273. https://doi.org/10.3390/diagnostics10050273.