The Egyptian Journal of Hospital Medicine (January 2024) Vol. 94, Page 61- 67

Study of Interleukin 21 Gene Polymorphism in Egyptian Patients with Allergic Rhinitis and Sinonasal Polyps

Kassem Mohamed Kassem[1], Awad Mohamed El Abd[2], Samer Badie Kamel[1], Dalia Mamdouh Elsayed Salem*[1], Inas A. Ahmed[2], Alaa Mohamed Abdelsamie[1]

Departments of [1]Otorhinolaryngology, and [2]Medical Biochemistry and Molecular Biology, Central Laboratory for Research, Faculty of Medicine, Benha University, Egypt

***Corresponding author:** Dalia Mamdouh Elsayed Salem, **Mobile:** (+20)01015730321, **Email:** daliamamdouh908@gmail.com

ABSTRACT

Background: Sinonasal polyps and allergic rhinitis are prevalent disorders that significantly diminish the standard of living for those who suffer from them. Variants in the interleukin 21 (IL-21) gene have been linked to an increased risk of developing several immune-related diseases. **Objective:** To examine the correlation between IL-21 gene polymorphisms and the likelihood of developing allergic rhinitis and sinonasal polyps.

Patients and Methods: Patients with sinonasal polyps and allergic rhinitis, in addition to a control group, participated in this cross-sectional study. Controls (Group A), patients with AR (Group B), and patients with SNP (Group C) comprised the patient population. TaqMan SNP Genotyping assays were utilised to genotype the IL-21 gene SNP (rs6822844). To identify associations, clinical and genetic data were evaluated.

Results: A notable disparity in the distribution of genotypes was identified in the case-control comparison ($p < 0.006$). In particular, individuals diagnosed with AR had 32 %GG genotypes, 56 %GT genotypes, and 12% TT genotypes. In contrast, the control group demonstrated a mere 4% TT genotypes, 12% GG genotypes, and 84% GT genotypes. The genotype distribution of the SNP patients differed significantly ($p < 0.002$), with 16% carrying the GG genotype, 60% carrying the GT genotype, and 24% carrying the TT genotype. The distribution of genotypes in the control group comprised 4% TT genotypes, 84% GT genotypes, and 12% GG genotypes.

Conclusion: It is possible that Egyptians with certain IL-21 gene variants are more likely to suffer from allergic rhinitis and sinonasal polyps.

Keywords: Interleukin 21, Gene polymorphism, Allergic rhinitis, Sinonasal polyps, Susceptibility.

INTRODUCTION

In the family of type I cytokines, interleukin (IL)-21 is one that CD4+ T cells, such as T follicular helper (Tfh) cells, Th17 cells, and natural killer (NK) T cells, are able to secrete. In addition to regulating CD4+ T cell activation, proliferation, and survival, IL-21 also increases CD8+ T cell and NK cell cytotoxic activity and helps differentiate Tfh and Th17 cells [1].

An IL-21 receptor, a member of the class I cytokine receptor family, binds to a cell surface heterodimeric receptor comprised of a common-chain component (CD134) and a unique receptor (IL-21R). This receptor is shared with receptors for IL-2, IL-4, IL-7, IL-9, IL-13, and IL-15 [2]. Studies have demonstrated that inflammatory diseases, such as cancer, allergies, and autoimmune disorders, are influenced by the binding of IL-21 to its receptor. In addition, IL-21 has the ability to positively influence B cells and enhance their immunity. Plasma cells are generated in humans by memory B cells that are stimulated with IL-21. This process also induces class switch recombination and transforms insensitive naive cord blood B cells into plasma cells that secrete IgG [3].

The persistence and function of germinal centers (GCs), which impact their proliferation, transition into memory B cells, and affinity maturation, are severely impacted by the absence of IL-21 signaling [4]. Other cytokines might play a role in chronic rhinosinusitis (CRS) as well. IL-21 may influence the immune system's innate and adaptive mechanisms. It is a cytokine that performs multiple roles in diverse immune cells, including lymphocytes, natural killer cells, dendritic cells, macrophages, and epithelial cells. It is produced by activated cluster of differentiation (CD)4+ Th cells and natural killer cells [5].

Some research has also found that polyp tissues are rich in B cells and immunoglobulins (Ig), suggesting that local B cell immunity might be a major factor in keeping the nasal inflammatory response going in CRSwNP patients. The results of this study provide more evidence that interleukin-21 (IL-21) plays a harmful function in a variety of inflammatory disorders that affect humans [6].

Airway remodeling, hyperresponsiveness, and strong eosinophil infiltration are hallmarks of allergic disorders, such as allergic rhinitis (AR). Augmented reality's global ubiquity has been on the rise[7,8].

The connection between IL-21 gene polymorphisms and susceptibility to allergic rhinitis and sinonasal polyps was the objective of this investigation.

PATIENTS AND METHODS

Patients:

Patients with allergic rhinitis and sinonasal polyps are the subjects of this case-control study that examines IL-21. From June 2022 to June 2023, participants were selected from the outpatient clinic at Benha University Hospital.

Inclusion criteria were 1) Age ranging from 15 to 40 years 2) Individuals identified as having allergic rhinitis. 3) Individuals who have been identified as having sinonasal polyps.

Received: 01/10/2023
Accepted: 01/12/2023

Exclusion criteria were 1) Those who are under the age of 15 or older than 40 years. 2) Individuals exhibiting craniofacial deficiencies. 3) People suffering from systemic disorders. 4) Individuals with an asthma diagnosis. 5) People who have an adverse reaction to aspirin. 6) Individuals who have had systemic medicinal intervention within the last month, including antihistamines, corticosteroids, and immunotherapy.

Patients were randomly assigned to three groups: Group A included 50 healthy subjects serving as controls, without allergic rhinitis or systemic disease, who volunteered from the hospital. **Group B** comprised 50 subjects experiencing symptoms of rhinorrhea, nasal obstruction, and repeated sneezing, with E.N.T. examinations revealing swollen, moist, and pale bluish nasal mucosa without polyps. **Group C** consisted of 50 subjects with similar symptoms, with E.N.T. examinations indicating swollen, moist, pale bluish nasal mucosa and the presence of non-bleeding, pale pinkish polyps.

Methods:

All studied cases were subjected to the following:

History Taking: Personal History: Data were gathered including the individual's name, age, gender, occupation, place of living, any significant medical habits, and marital status. Current Medical Background: Information regarding the initiation, progression, and persistence of nasal symptoms such as nasal discharge, obstruction, and sneezing. Family History: Any instances of allergic rhinitis in the patient's immediate family were documented. Medication and General Health History: Detailed documentation pertaining to the patient's medications, surgical procedures, and general ailments.

Clinical Examination: A comprehensive general examination was conducted in order to exclude the presence of further inflammatory and systemic conditions. A comprehensive rhinological examination was performed, encompassing palpation, inspection, anterior and posterior rhinoscopy, as well as nasal endoscopy.

Laboratory Investigations: Molecular Estimation: DNA Concentration Estimation: The collection of samples: Under aseptic circumstances, three millilitres of peripheral blood were drawn from each patient and stored at -20°C.

DNA Extraction: The Gene JET Whole Blood Genomic DNA purification Mini Kit (USA) was used to extract DNA from the blood samples in accordance with the manufacturer's procedure. Several procedures were involved in this, such as adding Proteinase K Solution and Lysis Solution, incubating the mixture, and centrifuging it.

Measurement of DNA Concentration: The NanoDrop assay was used to determine the DNA concentration in the samples. Spectrophotometer was equipped with a single sample (1 ul).

Single Nucleotide Polymorphism (SNP) Detection (Genotyping):

The TBX2 receptor antagonist gene SNP (rs6822844) was genotyped utilizing the Step One Plus Real Time PCR apparatus and TaqMan SNP Genotyping kits.

Principle: Using sequence-specific primers, the target DNA was amplified in the TaqMan SNP Genotyping Assay. Then, the fluorescence signal for each allele was detected using TaqMan MGB probes. Alleles in each sample were determined by analysing the fluorescence signals.

Steps: Each PCR tube was supplemented with 1 ug of DNA sample. To each PCR tube, add 11.25 ul of nuclease-free water. The instructions were followed to a then added the reaction mixture to the PCR tubes. Applied a predetermined procedure to the Step One qPCR device by loading the PCR tubes. We automatically identified Allele G (homozygous G/G), Allele T (homozygous T/T), and heterozygous (G/T) by analysing PCR results using Step One Plus software for allelic discrimination.

Ethical approval:

All the patients were asked to sign a document indicating their fully informed permission. Along with a secret code number, each patient was informed of the study's goal. After receiving approval from Benha University's Research Ethics Committee, the Faculty of Medicine conducted the study (Approval code:Ms13-3-2022). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

For the statistical analysis, we used the Windows versions of MedCalc (MedCalc Software, Mariakerke, Belgium) and SPSS (IBM, Armonk, NY, USA, version 22.0). Qualitative data were presented as number and percentage and were compared by Chi-square (χ^2) test. Mean ± standard deviation was used to evaluate quantitative data, which were compared by the Student "t" test, or Mann Whitney U test. A significance level of $P < 0.05$ was used to assess the results.

RESULTS

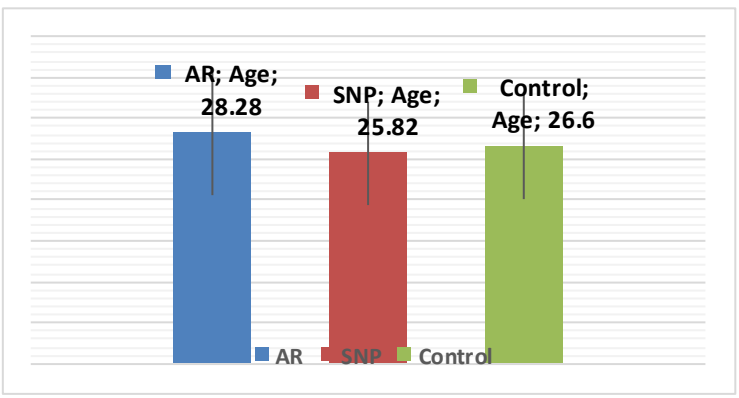
The age difference between the case and control groups was not statistically significant. There was no discernible gender disparity seen among the research groups. The genotype distribution between the study groups was found to be significantly different (**Table 1)**.

Table (1): Age, gender and genotype distribution of the study groups.

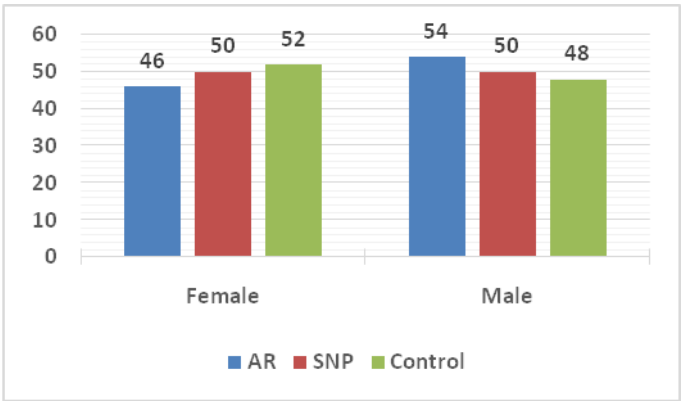
	Cases (n= 100)	Control group (n= 50)	T	p value
Age (Mean ±SD)	27.1±7.1	26.6±6.5	0.4	0.7
Female	48 (48%)	26 (52%)	0.2	0.6
Male	52 (52%)	24 (48%)		
Genotype distribution			10.1	0.006*
GG	20 (26.7%)	6 (12%)		
GT	43 (57.3%)	42 (84%)		
TT	12 (16%)	2 (4%)		

***: Significant**

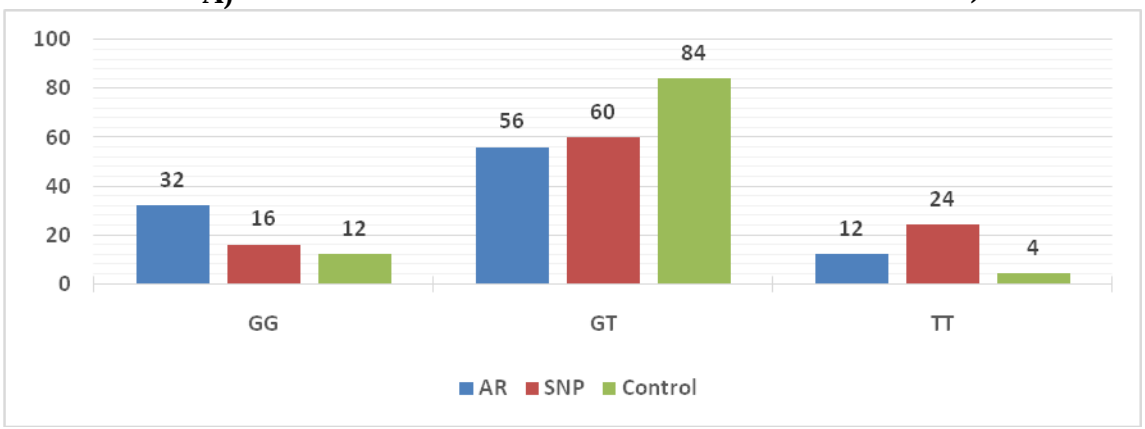
The age distribution among the 3 groups was not significantly different (**Figure 1A)**. No significant gender distribution difference was found among the studied groups **(Figure 1B)**. The genotype distribution varied significantly among the research groups (p < 0.002) **(Figure 1C)**.



A)



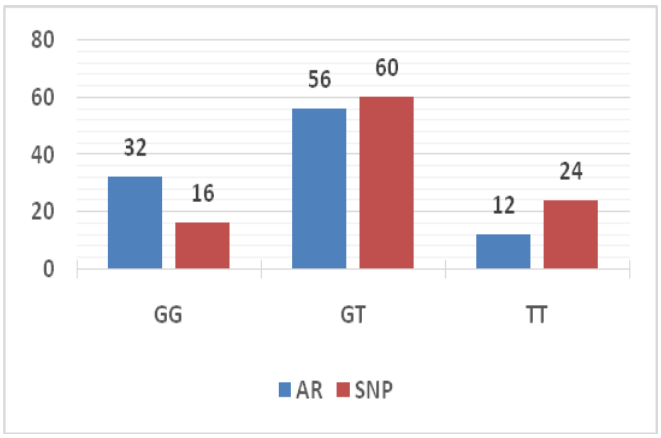
B)



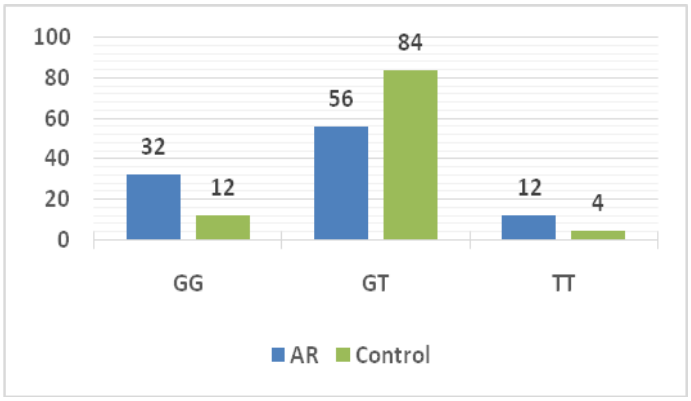
C)

Figure (1): A) Age, B) Gender, and C) genotype distribution of the studies subgroups.

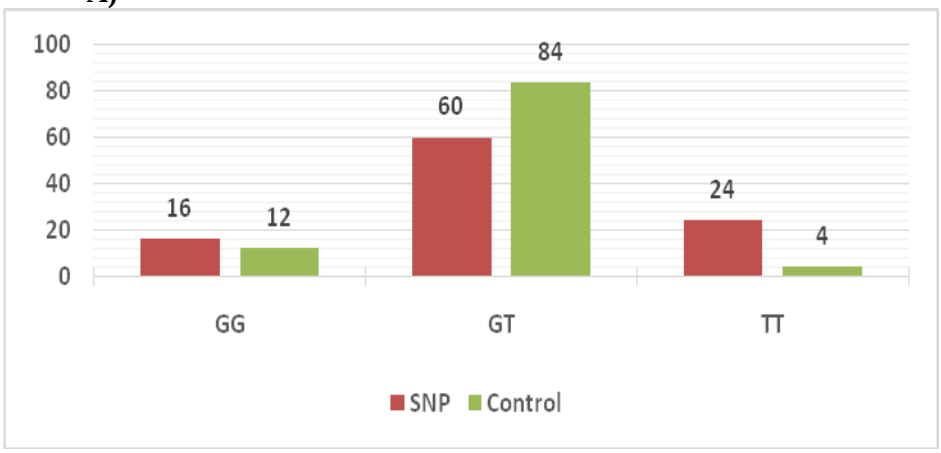
Genotype distribution between AR group and SNP was not significant **(Figure 2A)**. The distribution of genotypes varied significantly (P.0.03) between the AR group and the control group **(Figure 2B)**. The genotype distribution was also significantly (P.0.002) different between the SNP and the control group (**Figure 3C)**.



A)



B)



C)

Figure (2): A) Genotype distribution between AR and SNP, B) Genotype distribution between AR and Control group and C) Genotype distribution between SNP and Control group

The X^2 test did not find a statistically significant difference in the frequencies of rs6822844 of the interleukin 21 gene, minor allele G. On the other hand, compared to the AR patients minor allele T, the SNP patients' levels were significantly higher (P <0.05) **(Table 2)**.

Table (2): Study groups regarding allele distribution.

	AR		SNP		Control group		X^2	p-value
	No.	%	No.	%	No.	%		
G Allele	60	60	46	46	54	54	3.9	0.1
T Allele	40	40	54	54	46	46		

There was a significant difference between the AR group and the SNP group, regarding course and duration of the disease as well as the family history **(Table 3)**.

Table (3): Clinical Data of the studied patients.

Variable		AR (n= 50)		SNP (n= 50)		X^2	p-value
		No.	%	No.	%		
Onset	Gradual	36	72.0%	32	64.0%	0.7	0.4
	Sudden	14	28.0%	18	36.0%		
Course	Progressive	43	86.0%	34	68.0%	4.6	0.03*
	Stationary	7	14.0%	16	32.0%		
Duration (years) Mean ± SD		3.1±1.6		4.12±1.81		2.9	0.004*
History of previous treatment	Negative	7	14.0%	10	20.0%	0.6	0.4
	Positive	43	86.0%	40	80.0%		
Family history	Positive	43	86.0%	18	36.0%	24.3	<0.001*
	Negative	7	14.0%	32	64.0%		

These results were obtained from the AR group's genotype-based clinical evaluation: There was significant difference in all the clinical parameters among the subgroups **(Table 4).**

Table (4): Clinical Data of AR group regarding genotype.

Variable		GG		GT		TT		X^2	p-value
		No.	%	No.	%	No.	%		
Onset	Gradual	12	75.0%	21	75.0%	3	50.0%	1.6	0.4
	Sudden	4	25.0%	7	25.0%	3	50.0%		
Course	Progressive	13	81.3%	25	89.3%	5	83.3%	0.6	0.7
	Stationary	3	18.8%	3	10.7%	1	16.7%		
Duration (years) Mean ± SD		3.13±1.67		3.00±1.56		3.50±1.76		0.2	0.8
History of previous treatment	Negative	2	12.5%	4	14.3%	1	16.7%	0.1	0.9
	Positive	14	87.5%	24	85.7%	5	83.3%		
Family history	Positive	14	87.5%	24	85.7%	5	83.3%	0.1	0.9
	Negative	2	12.5%	4	14.3%	1	16.7%		

The results of the genotype-based clinical evaluation in the SNP group showed a significant difference regarding history of previous treatment among the 3 subgroups **(Table 5)**.

Table (5): Clinical Data of SNP group regarding genotype.

Variable		**GG**		**GT**		**TT**		X^2	p-value
		No.	%	No.	%	No.	%		
Onset	Gradual	6	75.0%	20	66.7%	6	50.0%	1.5	0.5
	Sudden	2	25.0%	10	33.3%	6	50.0%		
Course	Progressive	6	75.0%	22	73.3%	6	50.0%	2.4	0.3
	Stationary	2	25.0%	8	26.7%	6	50.0%		
Duration (years) Mean ± SD		3.25±1.58		4.33±1.77		4.17±2.04		1.1	0.3
History of previous treatment	Negative	2	25.0%	2	6.7%	6	50.0%	10.2	0.006*
	Positive	6	75.0%	28	93.3%	6	50.0%		
Family history	Positive	2	25.0%	12	40.0%	4	33.3%	0.7	0.7
	Negative	6	75.0%	18	60.0%	8	66.7%		

DISCUSSION

Allergic rhinitis (AR) is the most common allergic disease in the world. It is marked by a persistent inflammation of the upper airways caused by immunoglobulin E (IgE), which causes symptoms such as stuffy nose, discharge, discomfort, and sneezing. The immune system, genetics, and environmental stimuli interact to cause AR. A wide variety of cells, cytokines, and chemokines are involved in allergic inflammation. Ten percent to thirty percent of adults are impacted, according to studies [9]. According to ARIA standards, there are chronic and seasonal forms of allergic rhinitis, which is also called nasal allergies or pollinosis. Seasonal pollinosis is worsened by allergic conjunctivitis. In many cases of chronic rhinosinusitis, particularly cases involving nasal polyposis, benign growths of sinonasal mucosa called nasal polyps can be observed (CRSwNP). Some diseases and disorders that may be associated with them include cystic fibrosis, aspirin-exacerbated respiratory disease (AERD), systemic vasculitis, and others. Recurrence of nasal polyps can be prevented with effective treatment that targets the underlying allergic cause [10].

Nasal polyps' etiology and pathology are still hotly contested topics, with theories ranging from aberrant immune-inflammatory responses to bacteria, viruses, and fungi. Increased IL-21 levels in polyp tissues and peripheral blood have been seen in chronic rhinosinusitis (CRS) studies. These findings may be associated with Th17 cells in CRSwNP patients, which produce IL-21 [11]. Reminiscent to observations in allergic rhinitis, patients with atopic CRSwNP showed a decrease in the number of Th17 cells that produce the IL-21 cytokine. House dust mites can cause airway hyperresponsiveness, which is mediated by increased production of Th2 cytokines, and this response may be influenced by signaling through the IL-21 receptor [12]. Increased Th17 cells generating diverse cytokines in CRSwNP patients suggest altered activation both locally and systemically; further investigations are needed to understand the role of Th17 cytokine IL-21 in airway remodeling in CRS. Reduction of Th17-derived IL-21 in polyps from atopic CRSwNP patients is noteworthy and may have a protective function in allergic inflammation [8].

Cytokine interleukin-21 (IL-21) is mostly produced by CD4+ T cells and natural killer T cells (NKT). It influences the survival, activation, and proliferation of T and B lymphocytes and has impacts on the B cell development and immunoglobulin production in vitro. Dendritic cells, CD8+ T cells, B cells, macrophages, monocytes, and IL-21 all contribute to the regulation of the immune system's innate and adaptive components [13]. Th17 cell differentiation, B cell activation, and immunoglobulin synthesis are all impacted by IL-21. Research has shown that rheumatoid arthritis (RA) patients have elevated levels of IL-21 in their synovial tissue. Additionally, experimental arthritis models have shown that inhibiting the IL-21/IL-21 receptor pathways has decreased disease severity [14]. An assortment of autoimmune disorders, including type 1 diabetes, ulcerative colitis, Crohn's disease, psoriatic arthritis, juvenile idiopathic arthritis, and systemic lupus erythematosus, have been linked to the IL2-IL21 area on chromosome 4q27 according to genetic studies [15].

Several autoimmune illnesses have been found to be significantly associated with certain single-nucleotide polymorphisms (SNPs) within this region. These include rs13151961, rs13119723, rs6840978, and rs6822844. The intergenic region between the IL21 and IL2 genes is home to rs6822844, the variant most strongly linked to these illnesses [16]. The molecular function of rs6822844 has not been determined yet; it is thought to be a noncoding polymorphism located between IL21 and IL2. But, it might affect autoimmunity through regulating these two genes' expression or through linkage disequilibrium with a mutated gene that causes it. Mature microRNAs, which bind to complementary regions in the 3 UTR of target mRNAs to silence genes, share a high degree of homology with the sequences surrounding rs6822844[3, 17].

Several autoimmune illnesses have been linked to rs6822844 in populations outside of Europe. In both European-derived and non-European populations, meta-analysis results firmly support this substantial link across many autoimmune disorders, such as Crohn's disease, rheumatoid arthritis, Behcet's disease, and systemic lupus erythematosus [18].

In addition, the autoantibody status of RA patients does not seem to influence the robust connection that has been found between the IL2/IL21 rs6822844 variation and RA susceptibility in the Algerian population [18, 19].

In our study, genotype distribution between AR group and SNP was not significant. The distribution of genotypes varied significantly (P.0.03) between the AR group and the control group. The genotype distribution was also significantly (P.0.002) different between the SNP and the control group.

In our study, there was a significant difference between the AR group and the SNP group, regarding course and duration of the disease as well as the family history.

In our study, the X^2 test did not find a statistically significant difference in the frequencies of rs6822844 of the interleukin 21 gene, minor allele G. On the other hand, compared to the AR patients minor allele T, the SNP patients' levels were significantly higher ($P < 0.05$).

CONCLUSION

Finally, our research has shown that sinonasal polyps and allergic rhinitis are both associated with the IL21 genetic polymorphism (rs6822844).

- **Sources of funding:** There were no grants awarded to this research by governmental, private, or non-profit funding bodies.
- **Author contribution:** Authors contributed equally to the study**.**

Financial support and sponsorship: Nil

Conflict of Interest: Nil

REFERENCES

1. **Mesas-Fernández A, Bodner E, Hilke F *et al.* (2023):** Interleukin-21 in autoimmune and inflammatory skin diseases. Eur J Immun., 53:e2250075. doi: 10.1002/eji.202250075.
2. **Mehta D, Wurster A, Grusby M (2004):** Biology of IL-21 and the IL-21 receptor. Immunol Rev., 202: 84-95.
3. **Ren H, Lukacher A, Rahman Z *et al.* (2021):** New developments implicating IL-21 in autoimmune disease. Journal of Autoimmunity, 122: 102689.
4. **Zotos D, Coquet J, Zhang Y *et al.* (2010):** IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med., 207:365-78.
5. **Cosenza M, Sacchi S, Pozzi S (2021):** Cytokine release syndrome associated with T-cell-based therapies for hematological malignancies: pathophysiology, clinical presentation, and treatment. Int J Mol Sci., 22: 7652. doi: 10.3390/ijms22147652.
6. **Buchheit K, Hulse K (2021):** Local immunoglobulin production in nasal tissues: A key to pathogenesis in chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol., 126: 127-34.
7. **Liu Y, Sha J, Meng C *et al.* (2022):** Mechanism of lower airway hyperresponsiveness induced by allergic rhinitis. J Immunol Res., 22:4351345. doi: 10.1155/2022/4351345.
8. **Kim S, Cho K (2023):** Treatment strategy of uncontrolled chronic rhinosinusitis with nasal polyps: A review of recent evidence. International Journal of Molecular Sciences, 24:5015. doi: 10.3390/ijms 24055015.
9. **Iordache A, Balica N, Horhat I *et al.* (2022):** Allergic rhinitis associated with nasal polyps and rhinosinusitis - histopathological and immunohistochemical study. Rom J Morphol Embryol., 63: 413-9.
10. **Small P, Keith P, Kim H (2018):** Allergic rhinitis. Allergy Asthma Clin Immunol., 14:51. doi: 10.1186/s13223-018-0280-7.
11. **Hulse K, Stevens W, Tan B *et al.* (2015):** Pathogenesis of nasal polyposis. Clin Exp Allergy, 45: 328-46.
12. **Huang J, Xu Y (2023):** Autoimmunity: A new focus on nasal polyps. International Journal of Molecular Sciences, 24: 8444. doi: 10.3390/ijms24098444
13. **Czajka-Francuz P, Cisoń-Jurek S, Czajka A *et al.* (2022):** Systemic interleukins & rsquo; profile in early and advanced colorectal cancer. International Journal of Molecular Sciences, 23:124. doi: 10.3390/ ijms23010124
14. **Long D, Chen Y, Wu H *et al.* (2019):** Clinical significance and immunobiology of IL-21 in autoimmunity. Journal of Autoimmunity, 99:1-14.
15. **Maiti A, Kim-Howard X, Viswanathan P *et al.* (2010):** Confirmation of an association between rs6822844 at the Il2-Il21 region and multiple autoimmune diseases: evidence of a general susceptibility locus. Arthritis Rheum., 62:323-9.
16. **Thompson S, Sudman M, Ramos P *et al.* (2010):** The susceptibility loci juvenile idiopathic arthritis shares with other autoimmune diseases extend to PTPN2, COG6, and ANGPT1. Arthritis Rheum., 62: 3265-76.
17. **Ren K, Tang J, Nong L *et al.* (2019):** Association between interleukin-21 gene rs6822844 polymorphism and rheumatoid arthritis susceptibility. Biosci Rep., 39: BSR20190110. doi: 10.1042/BSR20190110
18. **Louahchi S, Allam I, Raaf N *et al.* (2016):** Association of rs6822844 within the KIAA1109/TENR/IL2/IL21 locus with rheumatoid arthritis in the Algerian population. HLA Immune Response Genetics, 87: 160-64.
19. **Yu M, Hou J, Zheng M *et al.* (2020):** IL-21 gene rs6822844 polymorphism and rheumatoid arthritis susceptibility. Biosci Rep., 40: BSR20191449. doi: 10.1042/BSR20191449.