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Influence of Single Nucleotide Polymorphisms on CRS Outcomes: A Preliminary Observational Study

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Objective(s): To conduct a preliminary investigation into the relationship between specific SNP variants, type II inflammation, and the effectiveness of dupilumab therapy and surgery in patients with CRS.

Methods: In this prospective study, 48 subjects were enrolled, comprising 32 CRS patients and 16 healthy controls. The CRS patients were subjected to either dupilumab therapy or endoscopic surgery according to EPOS guidelines. SNP variants were identified using the TaqMan SNP genotyping technique. The identified SNP profiles were compared between the control group and CRS patients, and their potential influence on treatment outcomes was evaluated. Treatment responses were assessed based on symptom scores, such as SS-I, SNOT-22, disease progression using the NPS findings, and SNP profiles at a 6-month follow-up. The primary measures included the Nasal Polyp Score, Smell Identification Test (SIT) score, and SNOT-22 outcomes.

Results: Dupilumab therapy and surgery significantly decreased NPS, with the last showing superior results. However, dupilumab therapy resulted in a significantly improved SIT score. Significant differences were observed in SNP profiles, particularly with rs1800629 (TNFA), rs2856838 (IL1a), rs17561 (IL1a), and rs1805011 (IL4R). In particular, the expression of rs2856838 and rs1805011 variants in the dupilumab group was associated with significantly better SIT and SNOT-22 outcomes than non-expressors. Also, the surgery group patients expressing the rs2856838 variant reported significant improvements in SNOT-22 scores.

Conclusion: These preliminary findings suggest that SNP genotypes may guide personalized treatment strategies for CRS. Further larger prospective studies are required to confirm these initial observations.

Key Words: CRS, dupilumab, genetic polymorphisms, SNP.

Level of Evidence: 2

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a widespread inflammatory condition causing significant morbidity and impairing quality of life.[1] Despite extensive research, the pathogenesis of CRS remains complex and multifactorial. Increasingly, genetic factors are being recognized as critical to both disease susceptibility and its progression.[2–4] Single nucleotide polymorphisms (SNPs), which are the most common type of genetic variation among people, have attracted interest due to their potential association with CRS.[5,6] A recent systematic review unveiled the primary SNPs significantly associated with chronic rhinosinusitis and the specific pathways they affect.[7] However, due to the variability in extraction methods and sequence sampling, further research involving larger cohorts is required to more definitively identify significant SNPs.

This preliminary study aimed to explore the relationship between CRS and five specific SNPs identified from a prior literature research,[7] with a particular focus on how these genetic variations affect the incidence and progression of CRS. By shedding light on these genetic aspects of CRS, we hope to elucidate potential predictive factors, contribute to more comprehensive understanding

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of disease pathogenesis, and pave the way for personalized therapeutic approaches.

MATERIALS AND METHODS

Study Design, Settings, and Patient Participants

This preliminary study was designed as a prospective, open-label, parallel-group observational study, adhering to the EQUATOR guidelines (https://www.equator-network.org/) and in compliance with STrengthening the REporting of Genetic Association Studies (STREGA) checklists.[8] The study received approval from the Human Medical Research and Ethics Committee of the University of Catania and was conducted in accordance with the Declaration of Helsinki (code 24121–21/05/2021). The study participants were all adults (aged 18 years and above) recruited from our tertiary otolaryngological center between January 2021 and July 2023. All participants were screened using the latest EPOS (European Position Paper on Rhinosinusitis and Nasal Polyps) guidelines, and EUFOREA criteria to identify severe, uncontrolled Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) and Th2 biomarkers present in the blood.[9] All the participants enrolled in the study had undergone at least one surgery previously for severe CRSwNP, during which tissue was harvested for genetic examination. Exclusion criteria applied to all groups were autoimmune diseases, genetic or congenital diseases affecting the respiratory tract, current pregnancy or breastfeeding, acquired immunodeficiencies, active neoplasms, prior chemoradiation therapies, known previous or existing non-CRS-related olfactory disorders, and other ongoing biologic therapies. Treatment selection for eligible subjects was based on their inflammatory endotype (Figure 1). Patients exhibiting Th2 inflammation[9] were assigned to a biological treatment. Conversely, patients demonstrating non-Th2 profiles were assigned to surgical treatment. A third group comprising individuals without CRS or related comorbidities was included to investigate the assay the SNPs in a healthy population. This distinction in treatment adopted was based on inflammatory profiles was made in line with current clinical practice, allowing us to study the interaction between genetic variations and specific treatment modalities in well-defined subgroups of patients.[9] To ensure a homogeneous population for variables potentially influencing SNPs expression, all study participants (healthy controls and patients with CRS) shared the same environment (born and raised in the same city), nutritional habits (Mediterranean diet), and ethnicity (Caucasians). To minimize potential selection bias, the geneticist evaluating the nasal and blood samples was blinded to the included assignments.

Patient Assessment and Outcomes

Participants were evaluated at baseline, with post-treatment (surgery or dupilumab) follow-up visits scheduled at interval of 1, 3, and 6 months. Each participant underwent a lung examination, and asthma diagnoses were made following the most recent guidelines.[10] Symptoms were assessed using a Visual Analog Scale (VAS), which ranged from 0 (absence of symptoms) to 10 (the most severe symptoms), for evaluating nasal obstruction, headache, and rhinorrhea. Type 2 inflammation was assessed at baseline in accordance with the EPOS 2020 guidelines.[9] Nasal polyp size was determined through nasal endoscopy with a 2.7-mm flexible endoscope (Olympus, Germany) and assessed through the nasal polyp score (NPS).[11] The aggregate scores for both nostrils ranged from 0 to 8, with higher scores indicating more severe conditions. The sense of smell was evaluated using the identification subset of the Sniffin Sticks-16 items (SS-I) (Burghart, Wedel, Germany).[12] The minimal clinically important difference (MCID) for olfactory recovery was defined as an increase of at least 3 points, as reported by Gudziol et al.[13] The hyposmia cut-off was adjusted according to the age-related values of the SITdomain, as described by Oleszkiewicz et al.[14] The impact of CRSwNP on Health-Related Quality of Life (HRQoL) was assessed using the Sinonasal Outcome Test (SNOT-22).[15] Our primary endpoints were the correlations between SNPs profiles, symptoms (NPS and the SNOT-22), and olfactory outcomes (SIT score). For secondary endpoints, we conducted treatment subgroup analyses considering specific SNP profiles.

DNA Extraction

We collected nasal tissue (in the dupilumab and surgical groups) and blood samples (in healthy group) for DNA isolation during endoscopic sinus surgery. Using this method, we collected relevant samples in both cases, as demonstrated by studies on concordance between SNP profiles collected from various tissue types within the same individual,[16] without subjecting healthy participants to unnecessary invasive procedures.

Nasal polyp specimens were endoscopically harvested from the anterior ethmoid area and from adjacent healthy nasal mucosa. The DNA from tissue samples was extracted following the protocol given in the QIAamp DNA Mini Kit (Ref. 51304, QIAGEN GmbH, Qiagen Strasse 1, 40724 Hilden, Germany), while for the blood samples, the same kit was used but following the DNA Purification from Blood or Body Fluids (Spin Protocol) protocol reported in the QIAamp® DNA Mini and Blood Mini Handbook. Quantification of samples was carried out using Eppendorf BioPhotometer® D30 (Fisher Scientific Italia, Strada Rivoltana Km 4, 20053 Rodano (MI), Italy).

TaqMan SNP Genotyping

The selection of SNPs for analysis was based on previous systematic literature research to identify potential association with CRSwNP. If no such SNPs had been reported in the literature, genome-wide association studies (GWAS) were used to identify potential SNPs of interest. Genotyping of the five genetic variants identified was performed using five TaqMan SNP Probe (Table S1) designed and synthesized by ThermoFisher (Thermo Fisher Scientific, 168 Third Avenue, 02451 Waltham, Massachusetts, USA), the QuantiNova® Probe PCR Kit Mastermix (Ref. 208252, QIAGEN GmbH, Qiagen Strasse 1, 40724 Hilden, Germany), and the LightCycler®480 Roche Molecular Systems (Rotkreuz, Switzerland Forrenstrasse 2, 6343 Rotkreuz, Switzerland). The most frequent allele was detected on the VIC/HEX Channel (535–580 nm), while the minor frequent allele on the FAM channel (495–517 nm). The final reaction volume for PCR was 10 μL, which contained 4 μL of 10 ng/μL genomic DNA, 5 μL of QuantiNova® MasterMix, and 1 μL of the 1:10 Diluted TaqMan SNP Probe. PCR amplification was carried out in 96-well plates containing unknown genotype samples and no-template controls. Thermal cycle conditions were as follows: a PCR initial activation step of 95°C for 2 min and 40 cycles of a two-step cycling of 95°C for 5 s (denaturation) and 60°C for 30 s (combined annealing/extension). PCR efficiencies, melting curve analysis, and expression rate were calculated using the LightCycler® 480 software (Roche, Monza, Italy). SNP genotyping consulting was performed using suitable platforms like microarray-based genotyping or next-generation sequencing, depending on the number of SNPs to be analyzed and available

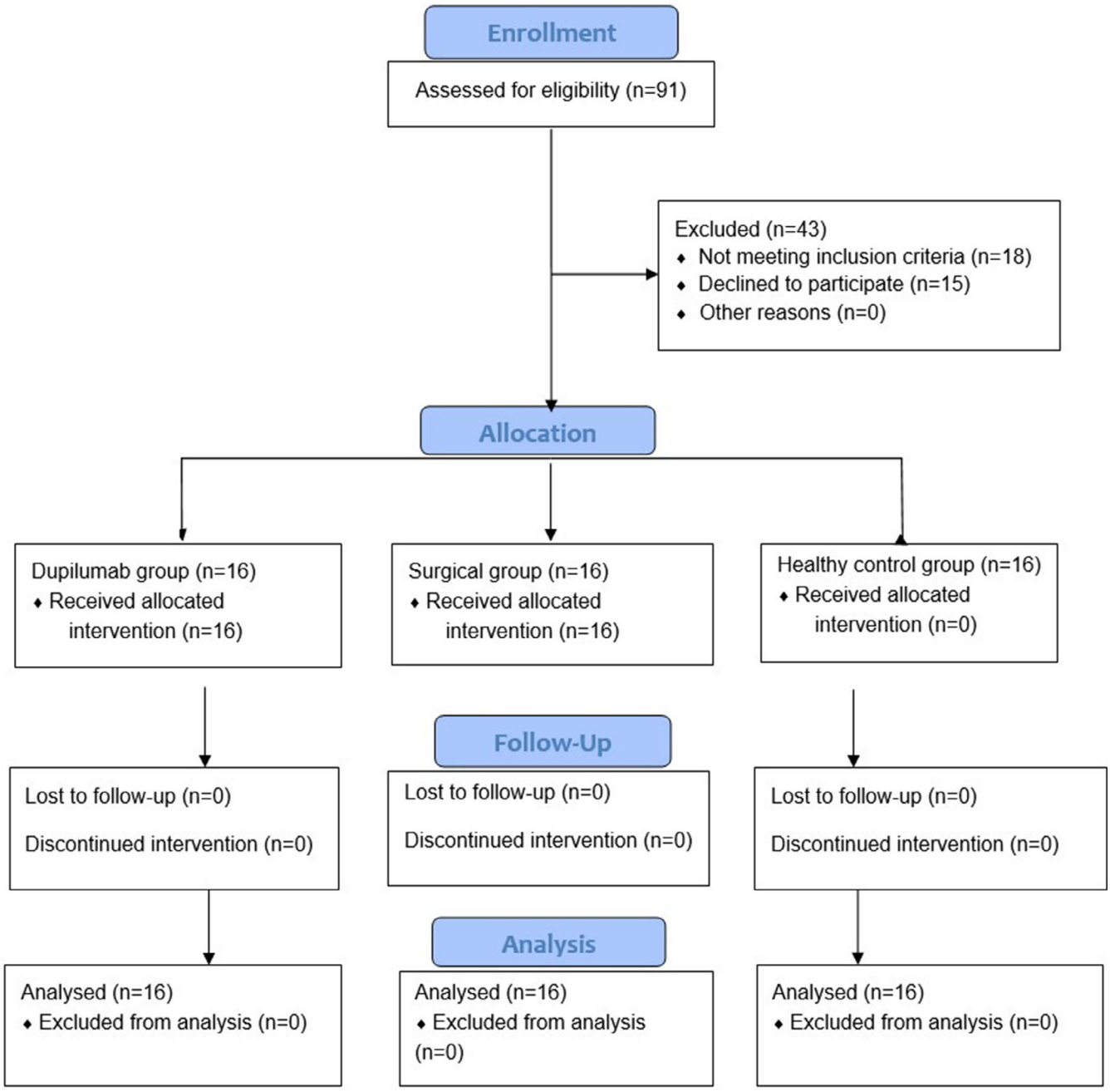

Fig. 1. Flow diagram for the observational study protocol. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

resources. These high-throughput techniques allowed for the analysis of multiple SNPs simultaneously, providing a comprehensive genetic profile.

Treatment

All patients recruited had not experienced improvement after receiving medical therapy, comprising intranasal corticosteroid sprays (INCS) and short courses of oral corticosteroids (OCS), in line with the latest guidelines for CRS management.[9] Patients in the dupilumab group received biological therapy complying with the EPOS guidelines. The dupilumab was administered subcutaneously at a dosage of 300 mg every 2 weeks (using a safety syringe) over a period of 6 months. Conversely, the surgical group underwent functional endoscopic sinus surgery (FESS), following the Messerklinger method which emphasizes preservation of the middle turbinate. The operation was performed under general anesthesia by two experienced rhinology surgeons, with the extent of the surgery determined based on the CT scan results.

Post-surgery, expandable sponges (Merocel, Medtronic-XOMED, Jacksonville, FL) were placed in the surgical area and kept there for 24 to 48 h before removal. Thereafter, a normal saline lavage was administered for a period of 2 to 3 months.

Statistical Analysis

The distribution of each variable of interest was explored using the Kolmogorov–Smirnov test. Continuous variables were summarized as means with standard deviation (SD) or as medians with interquartile range (IQR) in the case of Gaussian or non-Gaussian distribution, respectively. Between-group comparisons were made using the analysis of variance, Kruskal–Wallis test, Student's *t*-test, or Wilcoxon test, as appropriate. Categorical variables were reported as frequencies and percentages and were compared using the Pearson chi-square or Fisher's exact tests, as appropriate. Box plots were then generated based on SS-I, NPS, and SNOT-22 scores at the initial and follow-up stages, divided according to the identified SNP profiles. All tests were conducted at a 2-sided 5% significance level and were performed with R, version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS Statistics Software for Windows (IBM Corp., Released 2017, Version 29.0, Armonk, NY: IBM Corp.).

RESULTS

All 48 participants completed the study and were included in the preliminary analysis. Table I provides a comparison of the two treatment groups at baseline in terms of clinical symptoms, demographic features, and comorbidities (Table I). At the 6-month follow-up, both dupilumab and surgery treatments resulted in a significant reduction in NPS ($p < 0.001$ for both). However, the NPS improvements were more pronounced in the Surgery group ($p < 0.001$) (see Table II). It is noteworthy that both treatments significantly improved the SIT score ($p < 0.001$), with a significantly greater improvement observed in the dupilumab group during intergroup analysis ($p < 0.001$). Conversely, at intergroup analysis for VAS scores, the dupilumab group was greater but not statistically significant ($p > 0.05$).

SNP Profiles

Our study found significant differences in the SNP profiles among the three included groups, specifically in rs1800629 (TNFA), rs2856838 (IL1a), rs17561 (IL1a), and rs1805011 (IL4R). The SNP data of the participants are summarized in Table III. Only one patient treated with dupilumab (6.25%) exhibited expression of the rs1800629 variant (TNFA), while it was absent in all individuals in the surgical and healthy control groups ($p = 0.360$). The rs2856838 SNP (IL1a intronic variant) showed more variability in the dupilumab group, expressing a variant in 14/16 (87.5%) subjects. Interestingly, statistical significance was found between dupilumab and Surgery group rates ($p < 0.001$). Regarding the SNPs of IL4R, different results were identified between intronic variant and rs1805011 (nonsynonymous coding variant). Notably, none of the individuals tested across the three groups exhibited the genetic variant of rs3024608 (IL4R intronic variant). By contrast, the variant rs1805011 SNP (IL4R coding nonsynonymous variant) was expressed in 37.5% of the dupilumab group, while the surgical and healthy control groups had lower rates (6.25% and 19.75%, respectively; $p = 0.090$). The difference reached statistical significance when comparing between dupilumab and surgical groups ($p < 0.001$). After a 6-month follow-up, noteworthy associations were identified between clinical outcomes and two key SNPs, rs2856838 and rs1805011, across all samples (Table IV). In the dupilumab group, patients with the rs1805011 expressed genotype showed substantially greater IgE levels ($p = 0.041$) and lower SIT scores ($p = 0.001$) in comparison with the non-expressed group. Conversely, there were no discernible variations between the expressed and non-expressed groups in terms of eosinophil counts, nasal polyp scores (NPS), SNOT-22, or VAS ratings. On the contrary, patients in the Surgery group with the expressed genotype of rs2856838, significantly reduced their SNOT-22 scores ($p = 0.007$) as compared to the non-expressed group (Fig. 2). Conversely, IgE levels, eosinophil counts, SIT scores, NPS, and VAS scores did not significantly differ between the rs2856838 expressed and non-expressed groups. The Kruskal–Wallis test, used for intergroup analysis, confirmed that some SNP expressions had a

TABLE I.
Demographic Features and Clinical Parameters of Patients Enrolled in this Study.

Variable	Surgery ($n = 16$)	Dupilumab ($n = 16$)	Overall ($n = 32$)	*p*
Age - years, median (IQR)	54.00 [49.75, 58.00]	57.00 [50.00, 59.00]	54.50 [50.00, 59.00]	0.571
Male sex, *n* (%)	12 (75.0)	10 (62.5)	22 (68.8)	0.703
BMI - Kg/m^2, median (IQR)	29.00 [26.75, 30.00]	26.50 [25.00, 29.00]	27.50 [26.00, 29.25]	0.031
Comorbidities				
Atopy, *n* (%)	9 (56.2)	10 (62.5)	19 (59.4)	1.000
Asthma, *n* (%)	4 (25.0)	6 (37.5)	10 (31.2)	0.703
Aspirin intolerance, *n* (%)	1 (6.2)	3 (18.8)	4 (12.5)	0.593
N-ERD, *n* (%)	1 (6.2)	2 (12.5)	3 (9.4)	1.000
IgE total - kU/L, median (IQR)	71.00 [54.25, 80.00]	421.00 [286.00, 450.00]	160.50 [72.50, 420.50]	<0.001
Blood eosinophil count - 10^3/μL, median (IQR)	180.00 [167.50, 190.00]	520.00 [490.00, 582.50]	270.00 [180.00, 520.00]	<0.001

*p-value <0.05, **p-value <0.01.
BMI = body mass index; N-ERD = NSAIDs exacerbated respiratory disease.

TABLE II.
Outcomes Comparison Between Study Groups.

Outcome	Baseline	6-month follow-up	*p*
Surgery			
IgE total - kU/L, median (IQR)	71.00 [54.25, 80.00]	68.50 [44.25, 76.75]	0.777
Eosinophil blood count, median (IQR)	180.00 [167.50, 190.00]	160.00 [147.50, 182.50]	0.149
SIT Score, median (IQR)	2.00 [1.75, 5.00]	6.00 [5.75, 8.00]	<0.001
NPS, median (IQR)	5.00 [4.75, 6.00]	0.00 [0.00, 1.00]	<0.001
SNOT-22, median (IQR)	51.50 [46.00, 56.00]	17.50 [15.00, 23.00]	<0.001
VAS obstruction, median (IQR)	7.00 [6.00, 8.00]	2.00 [1.00, 2.00]	<0.001
VAS rhinorrhea, median (IQR)	7.00 [6.00, 8.00]	1.00 [0.75, 2.00]	<0.001
VAS headache, median (IQR)	5.00 [4.00, 6.00]	1.00 [1.00, 1.25]	<0.001
Dupilumab			
IgE total - kU/L, median (IQR)	421.00 [286.00, 450.00]	139.00 [74.75, 247.75]	<0.001
Eosinophil blood count, median (IQR)	520.00 [490.00, 582.50]	525.00 [485.00, 597.50]	0.691
SIT Score, median (IQR)	3.00 [1.75, 5.00]	11.00 [9.00, 14.25]	<0.001
NPS, median (IQR)	5.50 [5.00, 6.00]	2.00 [1.00, 2.25]	<0.001
SNOT-22, median (IQR)	48.00 [39.25, 70.25]	13.50 [7.00, 22.00]	<0.001
VAS obstruction, median (IQR)	8.00 [7.00, 9.00]	2.00 [1.00, 2.25]	<0.001
VAS rhinorrhea, median (IQR)	8.00 [7.00, 8.00]	1.00 [1.00, 2.00]	<0.001
VAS headache, median (IQR)	5.00 [5.00, 6.00]	2.00 [1.00, 2.00]	<0.001

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
NCS = nasal congestion score; NPS = nasal polyp score; SIT = Sniffin sticks identification test; SNOT-22 = Sinonasal outcome test; VAS = visual analogue scale.

TABLE III.
Subgroup SNP Variants Expression.

	Dupilumab ($n = 16$)		Surgery ($n = 16$)		Healthy ($n = 16$)		
	n	%	*n*	%	*n*	%	*p*-value
rs1800629 (TNFA)							
Nex group	1	6.25	0	0	0	0	0.360
Ex group	15	93.75	16	100	16	100	
rs2856838 (IL1a)							
Nex group	2	12.5	7	43.75	5	29.8	0.147
Ex group	14	87.5	9	56.25	11	70.2	
rs17561 (IL1a)							
Nex group	0	0	2	12.5	2	12.5	0.335
Ex group	16	100	14	87.5	14	87.5	
rs3024608 (IL4R)							
Nex group	16	100	16	100	16	100	-
Ex group	0	0	0	0	0	0	
rs1805011 (IL4R)							
Nex group	10	62.5	15	93.75	13	81.25	0.090
Ex group	6	37.5	1	6.25	3	19.75	

Ex = expressed; Nex = non-expressed.

substantial impact on treatment efficacy. A highly significant difference ($p < 0.001$) was found in SIT and NPS scores among the identified SNP profiles (Fig. 3A,B). In addition, a genotype-dependent difference was demonstrated for IgE levels ($H = 77.60$, $p < 0.001$) and eosinophil counts ($H = 100.2$, $p < 0.001$) (Fig. 4A,B).

DISCUSSION

Our study represents the first evidence in literature evaluating the correlation between dupilumab and surgical treatments in managing CRS clinical symptoms, in relation to the SNPs expression. As recently hypothesized, the genotype-dependent effects may have potential

TABLE IV.
Outcomes Comparison According to rs1805011 and rs2856838 Expression in Dupilumab and Surgical Group.

Dupilumab				Surgery		
rs1805011				rs2856838		
Outcome	ex	nex	*p*-value	ex	nex	*p*-value
IgE total - kU/L, median (IQR)	200.5 [89.25, 331.75]	82.5 [39.5, 159.0]	0.041	74.00 [23.00, 76.00]	65.00 [43.50, 91.00]	0.918
Eosinophil blood count, median (IQR)	585 [480, 620]	505 [462.5, 557.5]	0.514	170 [140, 190]	150 [140, 190]	0.831
SIT score, median (IQR)	9 [6.75, 10.5]	15 [14, 15.25]	0.001	7 [3, 8]	6 [5.5, 7]	0.741
NPS, median (IQR)	2 [2, 3.5]	1 [0, 2.25]	0.087	1 [0, 1]	0 [0, 1]	0.546
SNOT-22, median (IQR)	13.5 [7, 22.75]	11 [2.75, 16.75]	0.383	15 [15, 15]	22 [20, 30]	0.007
VAS obstruction, median (IQR)	2 [1, 4]	1 [0, 2.25]	0.103	2 [1, 2]	2 [1, 2]	0.539
VAS rhinorrhea, median (IQR)	1 [1, 2]	1.5 [0.75, 2]	0.953	2 [0, 2]	1 [0.5, 1]	0.30
VAS headache, median (IQR)	1.5 [1, 2]	2 [0.75, 2]	0.906	1 [1, 2]	1 [0.5, 1]	0.347

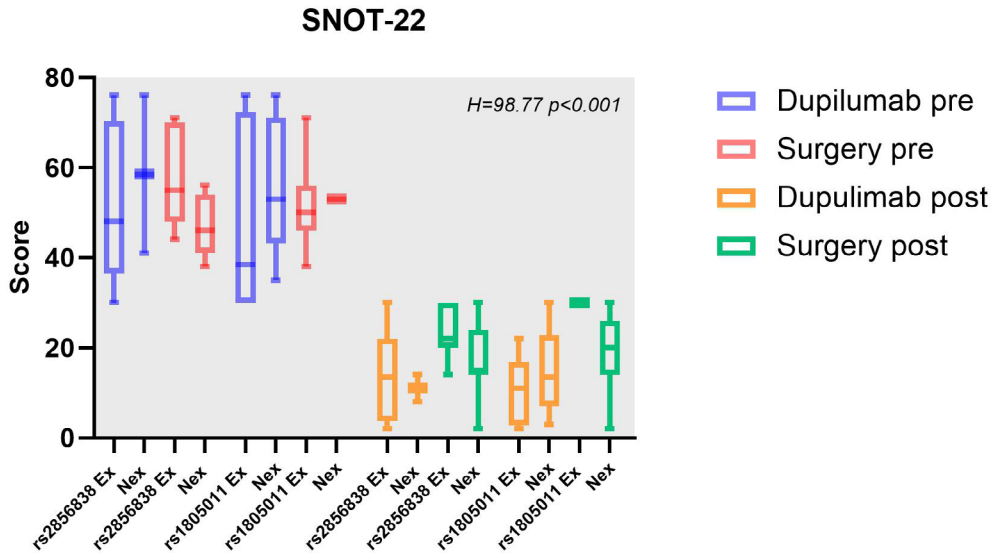

Fig. 2. Baseline versus 6-month outcomes comparison represented by box plot. The Kruskal–Wallis for SNOT-22 scores was statistically significant among all different treatment outcomes. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

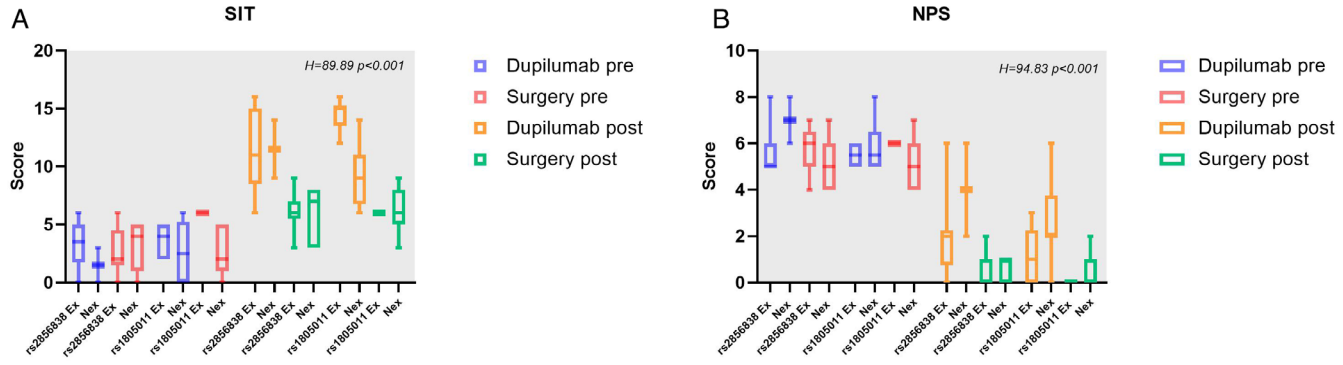

Fig. 3. Box plots for (A) SIT scores; (B) NPS scores; the Kruskal–Wallis tests indicated statistical significance among all different treatment outcomes. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

implications in various areas as personalized medicine.[17–19] A recent in-depth review identified major genetic variations linked to CRS, treatment response, and associated comorbidities.[7] Genetic alterations have been observed to disrupt downstream signal transmission, resulting in the activation of specific altered

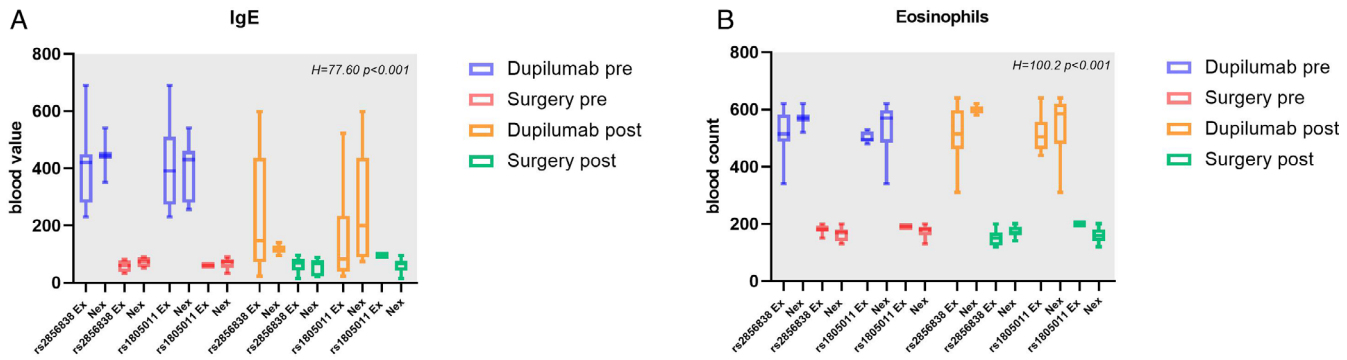

Fig. 4. Box plots for (A) IgE blood levels, (B) eosinophil blood count. The Kruskal–Wallis tests indicated statistical significance among all different treatment outcomes. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

pathways. These SNPs contribute to CRS through various mechanisms, including barrier deficits, alterations in ion channels, and changes in genes involved in the TH2 inflammation, affecting how the disease appears and the effectiveness of treatments. In our study, we observed distinct expression patterns of SNPs among the three groups included, specifically in the rs1800629 (TNFA), rs2856838 (IL1a), rs17561 (IL1a), and rs1805011 (IL4R) SNPs. The TNF-α (rs1800629) polymorphism has been reported to influence the risk of different inflammatory disorders, such as chronic periodontitis and type 2 diabetes mellitus.[20] However, in our preliminary findings, we observed that the rs1800629 allele was present only in the dupilumab group, which was specifically selected for type II inflammation. In the study conducted by Mfuna Endam et al., an association between CRS with nasal polyposis and the SNPs rs2856838 was identified.[6] The authors reported a protective effect of this SNP expression (OR, 0.63; $p = 0.003$), which was further enhanced in individuals homozygous for the minor allele (OR, 0.38; $p = 0.002$). In our study, the rs2856838 SNP showed more variability among the three groups included, with a higher rate of expression in the dupilumab group. Notably, subjects expressing the rs2856838 variant in the dupilumab group had greater SIT outcomes. On the contrary, worse rs2856838-related SNOT-22 scores were observed than non-expressors. Conversely, the expression of the rs2856838 variant in surgery patients was associated with improved SNOT-22 outcomes than non-expressors. The same genotype-dependent effects were not observed in the NPS scores following surgery. The close association between type II inflammation and asthma is also well-documented in the literature.[21–23] In a cohort study of 400 patients, Sun et al. found that the IL-4R SNP variation was associated with the development of asthma and distinctive palmar pattern (rs3024608, $p = 0.029$).[24] Conversely, in our study, no significant correlation was found for the rs3024608 SNP (IL4R intronic variant) in any of the individuals tested across the three groups. Interestingly, our study yielded significant findings related to a different IL4R SNP that expresses a nonsynonymous coding variant. The rs1805011 SNP frequence was significantly higher in the dupilumab group than the surgical group ($p < 0.001$).

While not statistically significant at three groups comparison, the higher expression rate in the dupilumab group (37.5%) compared with the surgery group (6.25%) and healthy controls (19.75%) could potentially indicate a relationship between this SNP and treatment response or disease severity, rather than disease presence alone.

Interestingly, the expression of the rs1805011 variant resulted in improved SIT and SNOT-22 outcomes in the dupilumab group. It appears that dupilumab may also have a genotype-dependent effect on reducing IgE levels in patients with rs1805011 variant. Indeed, the dupilumab group selected for higher IgE levels showed consistency in distinct IL4R SNP patterns. These results may suggest potential for personalized treatment strategies for CRS, in which genetic profiling could identify patients more likely to respond to biologics targeting the IL-4/IL-13 pathway. Despite these promising findings, no significant differences were observed in eosinophil blood count between the expressor and non-expressors subjects for both rs2856838 and rs1805011 variants, regardless of whether they were treated with dupilumab or underwent surgery. Our findings may contribute to the field of pharmacogenomics, potentially saving health care resources by avoiding less effective treatments for certain genotypes.[25] Although these implications offer exciting possibilities, it is important to acknowledge the complexities involved. The relationship between genotype-treatment response might not be straightforward and could be influenced by multiple factors, including other genetic variants, epigenetic changes, environmental factors, and lifestyle choices.[26] In our preliminary findings, SNP variants were expressed in CRS subjects regardless of the presence of type II inflammation and were also variably identified in the general population. This suggests that specific genetic variations identified through SNP analysis may play a role in the development of CRS. Moreover, our study revealed the possible role of multifactorial pathogenesis of the CRS, with potential contribution of other factors, such as environmental influences, to the development of the disease from healthy subjects. For the first time, our research also revealed intriguing associations between SNP expression and the clinical results of several CRS treatments. The rs1805011 expression in the IL4R gene was linked to worse olfactory results and

greater IgE levels. On the contrary, no appreciable differences were observed in eosinophil counts, nasal polyp scores (NPS), SNOT-22, or VAS ratings between the expressed and non-expressed groups. Conversely, participants in the Surgery group who expressed the rs2856838 variation in the IL1A gene were linked to improved quality of life after surgery for CRS. It is interesting to note that in the Surgery cohort, there was no significant difference seen between the expressed and non-expressed groups for IgE levels, eosinophil counts, SIT scores, NPS, or VAS scores.

Study Limitations

Although the variance in SNP distributions among our study groups was intriguing, especially for rs1805011, the limited participant pool derived from the monocentric study restricted the statistical strength needed to identify significant differences between the treatment groups. Further complexity arises from unregulated or unmeasured factors that could have influenced the SNP phenotype expression, as lifestyle habits, administration of other drugs, or variations in post-treatment care, could all have shaped the outcomes we observed.[27–31] One of the most significant challenges in this study is the inter-patient variability of the SNP profiles, leading to substantial differences among individuals. In addition, the dupilumab group had higher IgE levels than the surgical group, as a consequence of our study design, probably due to the fact that we assigned biologic treatment to patients with Th2 inflammation according to their inflammatory endotype. These basal variations in IgE levels between treatment groups may explain some of the variety of SNPs in IL4R, particularly the rs1805011 variant. Larger randomized trials are needed to clarify the relevance of SNPs in CRS treatment outcomes, as this possible confounding factor underscores how difficult it is to assess genetic relationships in diverse patient populations.

CONCLUSIONS

This preliminary study suggested that SNP genotypes may play a role in personalizing treatment strategies for CRS. The outcomes of each CRS treatment may vary among patients, potentially influenced by their unique SNP profiles. Intriguingly, patients with type II inflammation treated with dupilumab could experience different outcomes depending on SNP expression. These observations underscore the potential for developing personalized treatment plans that take into account the individual SNP genotypes of patients. Despite this promising lead, there is a need for larger, prospective studies to confirm the impact of SNP genotypes on long-term clinical efficacy and disease progression.

BIBLIOGRAPHY

1. Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on Rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;58(1):1-464. https://doi.org/10.4193/Rhin19.248.
2. Ramakrishnan VR, Hauser LJ, Feazel LM, Ir D, Robertson CE, Frank DN. Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. *J Allergy Clin Immunol*. 2015;136(2):334-342.e1. https://doi.org/10.1016/j.jaci.2015.03.018.
3. Dżaman K, Czerwaty K. Roles of exosomes in chronic rhinosinusitis: a systematic review. *Int J Mol Sci*. 2022;23(19):11284. https://doi.org/10.3390/ijms231911284.
4. Zhong B, Seah JJ, Liu F, Ba L, du J, Wang DY. The role of hypoxia in the pathophysiology of chronic rhinosinusitis. *Allergy*. 2022;77(11):3217-3232. https://doi.org/10.1111/all.15384.
5. Maniaci A, Rosolia G, Musso N, et al. Single-nucleotide polymorphism and downstream signaling associated with chronic rhinosinusitis, asthma, allergy and ASA intolerance: a narrative review. *Otorhinolaryngology*. 2022;72:198-203. https://doi.org/10.23736/S2724-6302.22.02453.
6. Mfuna Endam L, Cormier C, Bossé Y, Filali-Mouhim A, Desrosiers M. Association of IL1A, IL1B, and TNF gene polymorphisms with chronic rhinosinusitis with and without nasal polyposis: a replication study. *Arch Otolaryngol Head Neck Surg*. 2010;136(2):187-192. https://doi.org/10.1001/archoto.2009.219.
7. Antonino M, Nicolò M, Jerome Renee L, et al. Single-nucleotide polymorphism in chronic rhinosinusitis: a systematic review. *Clin Otolaryngol*. 2022;47(1):14-23. https://doi.org/10.1111/coa.13870.
8. Little J, Higgins JP, Ioannidis JP, et al. STrengthening the REporting of genetic association studies (STREGA)—an extension of the STROBE statement. *Genet Epidemiol*. 2009;33(7):581-598. https://doi.org/10.1002/gepi.20410.
9. Fokkens WJ, Viskens AS, Backer V, et al. EPOS/EUFOREA update on indication and evaluation of biologics in chronic Rhinosinusitis with nasal polyps 2023. *Rhinology*. 2023;61(3):194-202. https://doi.org/10.4193/Rhin22.489.
10. Reddel HK, Bacharier LB, Bateman ED, et al. Global initiative for asthma strategy 2021: executive summary and rationale for key changes. *Eur Respir J*. 2022;59(1):2102730. https://doi.org/10.1183/13993003.02730-2021.
11. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis initiative. Rhinosinusitis: developing guidance for clinical trials. *J Allergy Clin Immunol*. 2006;118(4 Suppl):S17-S61. https://doi.org/10.1016/j.jaci.2006.07.002.
12. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem Senses*. 1997;22(1):39-52. https://doi.org/10.1093/chemse/22.1.39.
13. Gudziol V, Lötsch J, Hähner A, Zahnert T, Hummel T. Clinical significance of results from olfactory testing. *Laryngoscope*. 2006;116(10):1858-1863. https://doi.org/10.1097/01.mlg.0000234915.51189.cb.
14. Oleszkiewicz A, Schriever VA, Croy I, Hähner A, Hummel T. Updated Sniffin' sticks normative data based on an extended sample of 9139 subjects. *Eur Arch Otorhinolaryngol*. 2019;276(3):719-728. https://doi.org/10.1007/s00405-018-5248-1.
15. Kennedy JL, Hubbard MA, Huyett P, Patrie JT, Borish L, Payne SC. Sinonasal outcome test (SNOT-22): a predictor of postsurgical improvement in patients with chronic sinusitis. *Ann Allergy Asthma Immunol*. 2013;111(4):246-251.e2. https://doi.org/10.1016/j.anai.2013.06.033.
16. Shao W, Ge Y, Ma G, et al. Evaluation of genome-wide genotyping concordance between tumor tissues and peripheral blood. *Genomics*. 2017;109(2):108-112. https://doi.org/10.1016/j.ygeno.2017.01.003.
17. La Mantia I, Grigaliute E, Ragusa M, et al. Effectiveness and rapidity on olfatory fuction recovery in CRS patients treated with Dupilumab: a real life prospective controlled study. *Eur Arch Otorhinolaryngol*. 2024;281(1):219-226. https://doi.org/10.1007/s00405-023-08184-6.
18. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *Lancet*. 2019;394(10209):1638-1650. https://doi.org/10.1016/S0140-6736(19)31881-1.
19. La Mantia I, Grigaliute E, Ragusa M, et al. Effectiveness and rapidity on olfactory function recovery in CRS patients treated with dupilumab: a real life prospective controlled study. *Eur Arch Otorhinolaryngol*. 2024;281(1):219-226. https://doi.org/10.1007/s00405-023-08184-6.
20. Shi LX, Zhang L, Zhang DL, et al. Association between TNF-α G-308A (rs1800629) polymorphism and susceptibility to chronic periodontitis and type 2 diabetes mellitus: a meta-analysis. *J Periodontal Res*. 2021;56(2):226-235. https://doi.org/10.1111/jre.12820.
21. Madore AM, Vaillancourt VT, Bouzigon E, et al. Genes involved in interleukin-1 receptor type II activities are associated with asthmatic phenotypes. *Allergy Asthma Immunol Res*. 2016;8(5):466-470. https://doi.org/10.4168/aair.2016.8.5.466.
22. Lando V, Calciano L, Minelli C, et al. IL18 gene polymorphism is associated with total IgE in adult subjects with asthma. *J Clin Med*. 2023;12(12):3963. https://doi.org/10.3390/jcm12123963.
23. Gaurav R, Varasteh JT, Weaver MR, et al. The R213G polymorphism in SOD3 protects against allergic airway inflammation. *JCI Insight*. 2017;2(17):e95072. https://doi.org/10.1172/jci.insight.95072.
24. Sun L, Xue W, Li J, Zhou Z, Han W. Palm dermatoglyphs and interleukin-4 receptor polymorphisms in asthma. *Biomed Rep*. 2017;6(1):21-26. https://doi.org/10.3892/br.2016.803.
25. Matera MG, Rinaldi B, Calzetta L, Cazzola M. Pharmacogenetic and pharmacogenomic considerations of asthma treatment. *Expert Opin Drug Metab Toxicol*. 2017;13(11):1159-1167. https://doi.org/10.1080/17425255.2017.1391215.

26. Oblak L, van der Zaag J, Higgins-Chen AT, Levine ME, Boks MP. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing Res Rev*. 2021;69:101348. https://doi.org/10.1016/j.arr.2021.101348.
27. Denham J, O'Brien BJ, Harvey JT, Charchar FJ. Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. *Epigenomics*. 2015;7(5):717-731. https://doi.org/10.2217/epi.15.29.
28. Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. *Mol Aspects Med*. 2013;34(4):753-764. https://doi.org/10.1016/j.mam.2012.07.018.
29. Bar-El Dadon S, Reifen R. Vitamin a and the epigenome. *Crit Rev Food Sci Nutr*. 2017;57(11):2404-2411. https://doi.org/10.1080/10408398.2015.1060940.
30. Ararat-Sarria M, Patarroyo MA, Curtidor H. Parasite-related genetic and epigenetic aspects and host factors influencing plasmodium falciparum invasion of erythrocytes. *Front Cell Infect Microbiol*. 2019;8:454. https://doi.org/10.3389/fcimb.2018.00454.
31. McClay JL. Epigenetic regulation of drug metabolism in aging. *Aging (Albany NY)*. 2021;13(13):16898-16899. https://doi.org/10.18632/aging.203312.