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THE GENETIC AND MICROBIAL LANDSCAPE OF CHRONIC RHINOSINUSITIS: INVESTIGATING THE IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS AND MICROBIOME DIVERSITY ON DISEASE PROGNOSIS

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INTRODUCTION

1. Background

Chronic rhinosinusitis (CRS), defined as a persistent inflammation of the paranasal sinuses and nose that lasts for at least twelve weeks, is one of the most common chronic diseases globally[1]. This pathology, which affects a large portion of the world's population regardless of age, sex and geographical origin, has a significant impact on the quality of life of patients. Manifesting with symptoms such as nasal congestion, post-nasal rhinorrhea, decreased sense of smell and facial pain, CRS can greatly limit daily activities, affect sleep and cause significant emotional distress. CRS presents mainly in two distinct forms: with polyposis (CRSwNP) and without polyposis (CRSsNP)[2-5]. Both are united by a series of clinical symptoms, which however can vary in intensity and severity. However, despite the symptomatic similarities, CRSwNP and CRSsNP are characterized by fundamental differences in pathophysiology, response to treatment and long-term prognosis [3]. These differences indicate that the two types of CRS are likely the result of different pathogenetic mechanisms. Understanding and detailing the similarities and differences between CRSwNP and CRSsNP is a crucial aspect of clinical research[5-8]. This distinction can lead to the development of more effective and personalized treatments, aimed at meeting the specific needs of patients. However, the complexity and heterogeneity of the clinical and pathological picture of these diseases represent a considerable challenge [9-12]. Despite significant advances in research and clinics, many questions still remain unanswered. These open questions include fundamental aspects such as the molecular and cellular mechanisms underlying the pathogenesis of CRSwNP and CRSsNP, factors contributing to treatment response, and reasons for differences in long-term prognosis[13-16]. Furthermore, research is still active in the attempt to identify reliable and non-invasive biomarkers for the diagnosis and monitoring of the disease, and for the development of new therapeutic strategies[18-20]. All this emphasizes the importance of continuing to investigate these aspects in depth, in order to improve the clinical management of this pathology and the quality of life of patients[21]. Chronic rhinosinusitis (CRS) is a condition that can present with a variety of symptoms, many of which are common in both the polyposis (CRSwNP) and non-polyposis (CRSsNP) forms. However, there are also some differences in symptoms between these two variants. The most common symptoms of CRS include:

- Nasal congestion or obstruction

- Post-nasal rhinorrhea (flow of mucus from the nose into the throat)

- Reduction or loss of smell

- Pain or pressure in the face

Additionally, patients may experience other symptoms such as headache, cough, fatigue, bad breath, fever, and difficulty concentrating.In CRS with polyposis (CRSwNP), patients often experience a more marked loss of smell than those with CRS without polyposis[22-26]. Nasal polyps can also cause a feeling of "fullness" in the nose and face, and in some cases they may be visible inside the nose. For CRS without polyposis (CRSsNP), patients tend to report symptoms of facial pain or pressure more often than those with CRSwNP. Additionally, post-nasal rhinorrhea and cough can be particularly problematic in this form of CRS. It is important to note that the symptoms of CRS can vary greatly between individuals, and the severity of symptoms does not always correspond to the degree of inflammation observed during the medical examination. Therefore, a comprehensive clinical evaluation is critical to the diagnosis and management of this condition. Treatment of chronic rhinosinusitis (CRS) focuses on controlling symptoms, improving quality of life, and preventing flare-ups [27–30]. In the forms with polyposis (CRSwNP) and without polyposis (CRSsNP), treatments may include a combination of medical therapies and, in some cases, surgery. Nasal corticosteroids, which reduce inflammation, are often the first line of treatment for CRS. These medications can help reduce symptoms such as nasal congestion and runny nose. In some cases of CRS, symptoms may be caused or aggravated by a bacterial infection, and in these cases, a course of antibiotics may be helpful [31-33]. Saline nasal washes can help remove excess mucus and keep your nostrils hydrated, reducing symptoms such as congestion and runny nose. In some cases, especially in the presence of nasal polyps, oral corticosteroids may be prescribed. However, due to possible side effects, these drugs are generally reserved for more severe cases or as a short-term treatment. When medical treatment is not sufficient or symptoms are particularly severe, surgery may be necessary[34-37]. Sinus surgery can range from minor procedures, such as the removal of nasal polyps, to more complex procedures such as endoscopic sinus surgery. The goal of surgery is to improve sinus drainage and remove areas of chronic inflammation. In recent years, new biological treatments have been developed for CRSwNP. These drugs, such as dupilumab and omalizumab, work by modulating specific immune responses that contribute to chronic inflammation in CRSwNP.Currently, these drugs are reserved for patients with severe disease that does not respond to standard treatments. The best treatment for CRS varies between individuals, and should be personalized based on the severity of the disease, specific symptoms, the presence of other medical conditions, and the response to previous treatments. The management of CRS therefore requires a multidisciplinary approach involving doctors from different specialties, such as otolaryngologists, allergists and immunologists. Oral corticosteroids are powerful medications that can be very effective at reducing inflammation and relieving symptoms in a number of conditions, including severe forms of chronic rhinosinusitis. However, long-term or high-dose use of oral corticosteroids can lead to a number of side effects, which include[38-40]:

- Weight gain and redistribution of body fat

- This can lead to swelling of the face (sometimes called "moon face"), accumulation of fat on the back and abdomen, and thinning of the arms and legs.

- Osteoporosis: Long-term use of corticosteroids can lead to bone loss, increasing the risk of fractures.

- Hypertension (high blood pressure): Corticosteroids can increase blood pressure.

- Diabetes: The use of corticosteroids can increase blood sugar levels, sometimes leading to diabetes.

- Mood and sleep problems: The use of corticosteroids can lead to mood changes, including anxiety, irritability, and depression. Some people may also have sleep problems.

- Eye problems: Long-term use of corticosteroids may increase the risk of cataracts and glaucoma.

- Weakened immune system: Corticosteroids can reduce the body's ability to fight infections.

- Cushing's syndrome: This is a rare but serious condition that can develop following long-term use of high-dose corticosteroids. Symptoms may include weight gain, tiredness, high blood pressure, high blood sugar levels, and fragile skin.

- Gastric problems: The use of corticosteroids may increase the risk of ulcers and bleeding in the stomach or intestines.

It is important to note that not all people who take oral corticosteroids experience these side effects, and the risk varies depending on the dose and duration of treatment. If you are taking oral corticosteroids and are concerned about side effects, talk to your doctor. You should never stop taking corticosteroids without consulting a doctor, as this can lead to severe corticosteroid withdrawal syndrome.Biological therapies, or biologics, are treatments made with natural substances derived from living organisms [41-45]. They can be proteins, cells, genes or complexes of these substances. They work by targeting specific parts of the immune system that contribute to inflammation and disease. Here are some additional points to better understand biologics:

- Precision medicine: Biologics are often part of an approach to therapy known as precision medicine, in which treatments are customized to a patient's individual characteristics, needs and preferences. That's because biologics are often designed to target specific proteins or cells involved in the immune response, which can vary from person to person.

- Administration: Biologics are typically administered via injection or infusion because they are composed of large, complex molecules that cannot be effectively absorbed when taken orally.

- Cost: Biologics are generally more expensive than conventional drug therapies. This is due to the complexity of manufacturing these drugs, which often involves sophisticated manufacturing and purification processes.

- Side effects: Like all drugs, biologics can have side effects. These may vary depending on the specific drug and the person taking it. Some common side effects may include injection site reactions, infections, fatigue, and headaches. Biologics can sometimes alter the body's immune response, making a person more susceptible to infections or autoimmune diseases.

- Monitoring: Patients taking biologics may require regular monitoring to ensure the medication works as intended and to monitor for any side effects. This monitoring may include blood tests, pulmonary function tests, or other diagnostic tests.

- Access: Not all patients can access biologics due to cost, insurance coverage policies, and drug availability. Doctors and patients must work together to find the most effective and accessible treatment for their specific situation.

Biological treatments represent a new frontier in managing many chronic diseases, including chronic rhinosinusitis with nasal polyposis (CRSwNP). These drugs work by modulating specific immune responses that contribute to chronic inflammation. Some examples of biological treatments currently used or being tested for CRSwNP[45-48]:

- Dupilumab (Dupixent): Dupilumab is a monoclonal antibody that inhibits the interleukins IL-4 and IL-13, which play a key role in allergic inflammation and have been identified as important in the pathogenesis of CRSwNP. It is approved for use in CRSwNP in many countries.

- Omalizumab (Xolair): Omalizumab is a monoclonal antibody that inhibits immunoglobulin E (IgE), a key molecule in allergy and inflammation. It is currently approved for use in asthma and chronic urticaria, and studies suggest it may also be useful in CRSwNP.

- Mepolizumab (Nucala) and Reslizumab (Cinqair): These are monoclonal antibodies that inhibit interleukin 5 (IL-5), an important promoter of eosinophilic inflammation, a type of inflammation often found in CRSwNP.

- Benralizumab (Fasenra): It is another monoclonal antibody that inhibits IL-5, but it also directly removes eosinophils, a type of immune cell involved in CRSwNP, from the body.These drugs represent promising treatment options for patients with CRSwNP, especially for those who do not respond to corticosteroid treatment or who have significant side effects from these drugs. However, the use of these drugs should be guided by an experienced doctor as they may have side effects and are not suitable for all patients. Other biologic therapies are currently in development and testing. Surgery for Chronic Rhinosinusitis (CRS) is a treatment option that may be considered when medical treatments are not effective in controlling symptoms or when complications of the disease occur[49-51]. The main goal of surgery is to improve sinus drainage and remove areas of chronic inflammation. Surgical techniques for CRS include endoscopic sinus surgery (FESS, Functional Endoscopic Sinus Surgery), balloon sinusotomy, and open sinus surgery. Endoscopic sinus surgery is the most common type of surgery for CRS. This procedure uses an endoscope, a thin, flexible tube with a light and camera, to view the breasts. The surgeon then removes polyps, scar tissue, and other areas of inflammation. Balloon sinusotomy is a minimally invasive procedure that uses a small balloon that is inflated to widen the sinus passages and improve drainage. Open sinus surgery may be necessary in more severe cases, such as when there is widespread nasal polyposis or if there are complications such as abscesses or invasive fungal infections. This procedure is more invasive and may result in hospitalization and a longer recovery time. Like any surgical procedure, there are risks associated with sinus surgery, including the risk of infection, bleeding, and damage to other structures in the nose and sinuses. Furthermore, in some patients, CRS may recur after surgery, and additional treatments may be necessary. The decision to undergo surgery for CRS should be made by considering various factors, including the severity of symptoms, response to medical treatments, presence of complications, the patient's overall health condition, and individual preferences. This decision should be made collaboratively between the patient and his or her doctor. The decision to undergo surgery for Chronic Rhinosinusitis (CRS) is a complex process that involves the analysis of clinical and personal variables. The effectiveness of surgery for CRS has been extensively studied, however, the indication for surgery must be individualized for each individual. Primarily, the severity of the symptoms and the impact on the individual's quality of life must be taken into consideration. If the symptoms are severe e significantly affect the quality of life despite the use of medical therapies, surgery may become a reasonable therapeutic option. Second, response to medical treatments is critical. A patient who has not responded to a full range of medical therapies, including antibiotics, nasal corticosteroids, and nasal washes, may be a candidate for surgery. The presence of complications is another determining factor. Complications such as nasal polyps, abscesses or invasive fungal infections may often require surgery for management. The patient's general health condition is an additional element to consider. Patients with comorbidities that may increase the risk of surgical complications may not be ideal candidates for surgery[52-55]. Finally, patient preferences are of paramount importance. Patients should be fully informed about the potential

benefits and risks of surgery. The final decision must be made jointly between the patient and the doctor, carefully evaluating the specific circumstances of the case. Therefore, the decision to undergo surgery for CRS must be the result of a thorough evaluation of symptoms, response to medical treatments, presence of complications, the patient's overall health, and personal preferences. Surgery for Chronic Rhinosinusitis (CRS) is a procedure that, like any surgical procedure, involves a number of risks and potential complications. Their degree and probability of occurrence can vary depending on several factors, including the patient's general health status, the extent of the disease and the specific type of surgical procedure [56-58]. One of the risks that can occur is infection. This risk is present in any type of surgery. Infections can be localized, i.e. limited to the area of surgery, or systemic, when they spread throughout the body. Infections may require additional treatments, such as antibiotics, and in some cases can lead to more serious complications. Bleeding is also a common risk in all surgical procedures. Although severe bleeding is rare in sinus surgery, it is always a possibility. In rare cases, if the bleeding cannot be controlled, a blood transfusion or further surgery may be necessary. Another risk is damage to surrounding structures. During the surgery, the structures inside the nose and sinuses are at risk. This can include damage to the walls of the sinuses, the bones of the face, and in very rare cases, the brain or eyes. If such damage occurs, additional surgeries may be necessary to repair the damaged structures. The formation of adhesions, abnormal connections between tissues, is a potential post-operative complication. Adhesions can cause symptoms such as nasal obstruction and may require additional surgery to remove. Recurrence of the disease is another possible complication. Although surgery can remove inflamed tissue and improve symptoms, CRS can return. This may require further treatment, which may include both medical therapies and surgery. Some patients may experience changes in their sense of smell after surgery. These changes can range from a temporary reduction in smell to a permanent loss, e.g can affect the patient's quality of life. Finally, as with any surgery, it is normal to experience post-operative pain and swelling. These symptoms usually improve with time and can be managed with pain medications and anti-inflammatory therapies. Before making the decision to undergo surgery for CRS, it is critical that you discuss these risks and complications with your doctor. They are able to provide you with detailed and personalized information based on your specific condition, your general health and the type of surgical procedure proposed. This will help you make an informed and informed decision. During surgery for Chronic Rhinosinusitis (CRS), there are several anatomical structures within the nose, sinuses, or surrounding areas that can potentially be damaged. These include the walls of the sinuses, bones of the face, brain and eyes. The walls of the nasal sinuses, which are empty cavities located within the bones of the face, can be subject to injury during the surgical procedure. These lesions can lead to several complications, including bleeding and adhesion formation. Adhesions are abnormal connections between tissues that can cause further problems, such as nasal obstruction, and may require further surgery to remove. The bones of the face, which form the surrounding structure of the nose and sinuses, can also be damaged during surgery. This

type of damage is rarer, but if it occurs, it can cause pain, swelling, or cosmetic changes. In some cases, additional surgery may be needed to repair damaged bones. The brain is another structure that, although very rarely, can be damaged during sinus surgery. This could happen especially if the disease has spread to the frontal and sphenoid sinuses, which are located close to the brain, or if there are anatomical abnormalities. Although the risk is minimal, if such damage were to occur, serious complications could result. Finally, the ethmoid sinuses, located near the eyes, can be damaged during the operation. This is a rare event, but if it were to occur, it could lead to vision problems, ranging from mild changes to more serious problems. It is important to remember that the risk of damage to these structures is typically low, especially when the surgery is performed by a surgeon with experience in these types of procedures. Before the surgery, the doctor will discuss all these potential risks with the patient and will take all necessary measures to minimize them. In this way, the patient can make an informed and conscious choice regarding his treatment.

1.1 SNP and CRS

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, with genetic factors being increasingly recognized as critical in disease susceptibility and progression[2-4]. Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people, and their potential association with CRS has been investigated in the literature [59,60]. A recent systematic review unveiled the primary single-nucleotide polymorphisms (SNPs) significantly associated with chronic rhinosinusitis, along with the specific pathways they affect [6]. However, due to the variability in extraction methods and sequence sampling, additional research involving larger cohorts is required to more definitively identify significant SNPs.

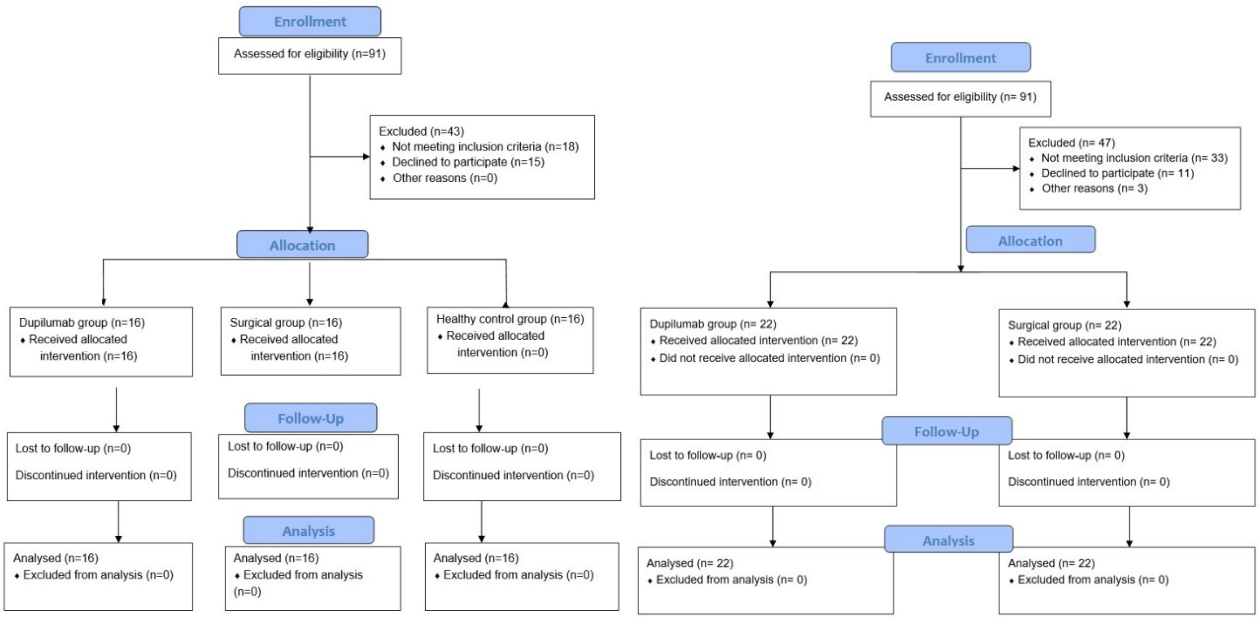
1.2 Microbiome and CRS

The human nasal microbiota plays a crucial role not only in maintaining health but also in disease state, serving as the first line of defense in the upper respiratory tract. In recent years, the role of the nasal microbial composition in CRS has received increasing attention [61-63]. In fact, the imbalance of the microbiota, defined as dysbiosis, can perpetuate inflammation and contribute to the persistence of CRS symptoms[64-66]. However, with a better understanding of the nasal microbiota's role in CRS, there is now a growing interest in novel treatment strategies aimed at restoring healthy microbiota[67-69]. Thus, given the potential impact of treatments on the nasal microbiota, it is critical to understand how different treatment modalities can affect nasal repopulation. Further knowledge on this topic may significantly impact the course of CRS, its outcomes, and the effectiveness of these treatments[70-73]. Dupilumab, an interleukin-4 receptor alpha antagonist, has emerged as a promising option11, inhibiting the signaling of type II inflammation. Type II inflammation itself has been shown to be associated with the colonization of

different pathogens such as Staphylococcus Aureus or Pseudomonas Aeruginosa. In this prospective observational study, we aimed to investigate and compare the changes in nasal repopulation following surgical treatment and Dupilumab therapy in treatment-naive patients with severe CRS. Moreover, we aimed to explore the relationship between SNPs and CRS, focusing on the incidence and progression of CRS in relation to specific SNPs. By shedding light on these genetic aspects of CRS, we hope to elucidate potential genetic risk factors, contribute to the understanding of CRS pathogenesis, and pave the way for personalized therapeutic approaches.

2. Materials and methods

This study was designed as a prospective, open-label, parallel-group trial, conducted by adhering to the EQUATOR guidelines (https://www.equator-network.org/) and in compliance with the CONSORT (Consolidated Standards of Reporting Trials) and STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) checklists [74]. The Human Medical Research and Ethics Committee of the University of Catania granted approval for the study, which was carried out in accordance with the Declaration of Helsinki (code 24121-21/05/2021). The study's 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 most recent EPOS (European Position Paper on Rhinosinusitis and Nasal Polyps) guidelines and the EUFOREA criteria to identificate severe uncontrolled Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) and Th2 biomarkers [1]. Moreover, a control group of healthy patients was included. The studies design protocols are illustrated in Figure 1 and 2.



1) 2)

Fig. 1-2 *Consort flow diagram for the microbial study protocol.*

Exclusion criteria were autoimmune diseases; genetic, congenital, systemic diseases affecting the respiratory tract, concurrent pregnancy or breastfeeding or acquired immunodeficiencies; active neoplasms; previous chemoradiation therapies; known previous or existing non-CRS related olfactory disorders; other ongoing biologic therapies. Consequently, eligible subjects were equally distributed into three homogeneous groups: a Dupilumab group (CRSwNP and Th2 inflammation), Surgical group (CRSwNP non Th2), Healthy control group. The nasal SNPs assessment at baseline was performed in each group enrolled. To minimize potential selection bias, the genetist evaluating the nasal and blood samples was blinded to the included group. Treatment outcomes were compared at baseline and follow-up.

2.1 Patient assessment and outcomes

Participants were evaluated at baseline and during follow-up visits scheduled at 1, 3, and 6 months. Each participant underwent a pneumonological examination, and asthma diagnoses were made following the most recent guidelines [9]. Symptoms were assessed using a visual analog scale (VAS), ranging from 0 (absence of symptoms) to 10 (most severe symptoms), for evaluating nasal obstruction, headache, and rhinorrhea. Type 2 inflammation was assessed at baseline via laboratory tests for blood markers, including eosinophil count (EOS) and immunoglobulin E (IgE), following the EPOS 2020 guidelines [1]. Nasal polyp size was determined through nasal endoscopy using a 2.7-mm flexible endoscope (Olympus, Germany), allowing us to define the nasal polyp score (NPS). 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) [11]. The SS-I involves 16 common odors, each accompanied by four verbal descriptors, including three distractors and one target, with a normal score being 12 correct responses. The Minimal Clinically Important Difference (MCID) for olfactory recovery, utilized to ascertain the rate of improvement in olfactory function, was defined as an increase of at least 3 points, as per the findings of Gudziol *et al.* [75]. We accordingly adjusted the hyposmia cut-off according to the age-related values of the SS-I domain as described by Oleszkiewicz *et al.* [76]. The impact of Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) on Health-Related Quality of Life (HRQoL) was assessed using the Sinonasal Outcome Test (SNOT-22), which consists of 22 items divided into 5 domains [77]. The SNOT-22 questionnaire has total scores ranging from 0 to 110, with higher scores indicating a poorer HRQoL. Our primary endpoints were the correlations between SNPs profiles and symptoms (NPS, and the SNOT-22) and olfactory outcomes (SS-I score). For secondary endpoints, we conducted subgroup analyses within each treatment group, considering specific SNPs profiles.

2.2 Sample Collection and SNPs Analysis

During endoscopic sinus surgery at the University Hospital from January 2021 to July 2023, Nasal tissue and blood samples for DNA isolation were gathered using CultureSwab (BD, Franklin Lakes, NJ). The nasal polyps specimens were endoscopically taken towards the anterior ethmoid area and in healthy nasal mucosa ajacent . All participants were subjected to a baseline assessment for SNP analysis.

During endoscopic sinus surgery at the University Hospital, nasal swabs were gathered from January 2021 to July 2023. Specimens for DNA isolation were collected during the procedure using CultureSwab (BD, Franklin Lakes, NJ). The swabs were endoscopically directed towards the ethmoid area, an adjacent sinus, or both when pus was detected[5]; measures were implemented to prevent contamination from the anterior nasal cavity during the probing process. After at least five complete cycles of rotation to complete saturation, the probes were placed in sterile Eppendorf tubes containing 2 ml of saline solution NaCl 0.9%, and delivered to the BIOMETEC Department of the University of Catania for microbiological analysis.

2.3 Microbiota Analysis

Nasal swabs were processed for routine microbiological cultures at the BIOMETEC laboratory of Medical Molecular Microbiology and Antibiotic Resistance (MMAR). The collected sample was plated on Tryptic Soy Agar (Oxoid, Basingstoke, UK) with 5% horse blood (Thermo Scientific, Basingstoke, UK) and cultured overnight at 37°C in 5% CO_2, and also on selective media Mannitol Salt Agar and MacConkey Agar (Oxoid, Basingstoke, UK) that were incubated overnight at 37°C in aerobic conditions. For anaerobic conditions, plates were cultivated using anaerobic bags (Biomeriux, France) at 37°C for 48 hours. Subsequently, each morphologically different colony was taxonomically identified at the species level by amplification and sequencing of the 16S rRNA[17], tuf (TUF-F/TUF-R) for *Staphylococcus* spp.[18] and 68d and DG74 for Gram-negative bacteria for accurate identification[19]. Genomic DNA was extracted using a PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. All PCR products obtained were purified using the QIAquick PCR gel extraction kit (Qiagen) and sequenced. Sequence analyses were performed using Gapped BLAST.

2.4 SNPs analysis

DNA were extracted from the blood samples using a standard extraction kit following the manufacturer's instructions. Extracted DNA was be quantified and qualified before performing SNP analysis. The selection of SNPs for analysis will be based on previous literature indicating potential association with CRSwNP or response to treatments like Dupilumab or surgical intervention. If no such SNPs have been reported in the literature, genome-wide association studies (GWAS) was used to identify potential SNPs of interest. SNP genotyping was performed using suitable platforms like microarray-based genotyping or next-generation sequencing, depending on the number of SNPs to be analyzed and available resources. These high-throughput techniques allow for the analysis of multiple SNPs simultaneously, providing a comprehensive genetic profile. Following genotyping, allele frequencies will be calculated for each SNP, and statistical analysis will be performed to identify any significant differences in allele frequencies between the Dupilumab and Surgical groups. This will involve logistic regression analysis, adjusting for potential confounding factors such as age, sex, and other relevant clinical variables. The results of the SNP analysis were interpreted in the context of CRSwNP pathogenesis and treatment response, potentially identifying genetic factors that could influence disease development and response to treatment.

2.5 Treatment

All patients recruited experienced a lack of improvement after receiving medical therapy, which included Intranasal Corticosteroid Sprays (INCS) and short courses of Oral Corticosteroids (OCS). This treatment was in accordance with the most recent guidelines for Chronic Rhinosinusitis (CRS) management [8]. As per the EPOS guidelines, patients also received a single course of oral corticosteroids, while the intranasal corticosteroid was administered continuously. Patients in the active group received biological therapy with dupilumab, complying with the EPOS 2020 guidelines [8]. The dupilumab was administered subcutaneously at a dosage of 300 mg every two weeks (using a safety syringe) over a period of 6 months. On the other hand, the surgical group underwent Functional Endoscopic Sinus Surgery (FESS) following the Messerklinger method, which emphasizes the preservation of the middle turbinate. This operation was performed under general anesthesia by two proficient rhinology surgeons. The scope of the surgery was determined according to the CT scan results. After surgery, expandable sponges (Merocel, Medtronic-XOMED, Jacksonville, FL) were placed in the surgical area and kept there for 24 to 48 hours before removal. Subsequently, a normal saline lavage was applied for a period of 2 to 3 months.

2.6 Statistical analysis

Standard descriptive statistics were applied in our research, displaying the mean and standard deviation for continuous variables, and percentages for categorical variables. The sample size for the study was computed based on a 95% confidence level, a p-value less than 0.05, a power of 0.8, and a mean difference set at 2.0. This calculation indicated a minimum requirement of 33 patients (11 per group) to achieve a mean difference of 2.0. Moreover, a 30% dropout rate was considered in the sample size estimation, bringing the total to 48 patients. The independent t-test was employed for normally distributed values, whereas the Mann-Whitney U test was applied to non-normally distributed values. The chi-square test was used to evaluate the disparity between the observed and predicted data. 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 SNPs profiles. Subsequently, the Kruskal-Wallis test was conducted to evaluate differences between groups. Violin plots were subsequently produced using SS-I, NPS and SNOT-22 scores at baseline and follow-up, stratified according to identified microbial profiles. Consequently, Kruscall-Wallis test was performed to assess intergroup differences. A p-value less than 0.05 was accepted as statistically significant. All statistical analyses were performed using the IBM SPSS Statistics software for Windows (IBM Corp., Released 2017, Version 29.0, Armonk, NY: IBM Corp).

3. Results

All 48 participants completed the study and were included in the analysis. Table I summarized baseline comparison of the two intervention groups in terms of clinical symptoms, demographic features, and comorbidities (Table I).

	Dupilumab (n=16)	Surgery (n=16)	*p-value*
Age	54.43 ± 6.25	51.06 ± 11.98	0.326
Sex	10M/6F (62.5%)	12M/4F (75%)	0.445
BMI	26.68 ± 2.24	28.43 ± 1.86	0.022
Blood eosinophilia	521.87 ± 74.58	175 ± 20.97	<0.001
IgE total (kU/L)	399.75 ± 120.23	65.25 ± 18.61	<0.001
Aspirin Intolerance	3 (18.75%)	1 (6.25%)	0.285
N-ERD	2 (12.5%)	1 (6.25%)	0.544
Comorbidities			
Atopy	10 (62.5%)	9 (56.25%)	0.718
Asthma	6 (37.5%)	4 (25%)	0.445
SNP phenotypes			
rs1800629, MAF=0.11			
HomoG	1 (6.25%)	0	-
HomoA	12 (75%)	13 (81.25%)	0.668
EteroGA	3 (18.75%)	3 (18.75%)	-
rs2856838, MAF=0.38			
HomoG	2 (12.5%)	7 (43.75%)	0.049
HomoA	4 (25%)	4 (25%)	-
EteroGA	10 (62.5%)	5 (31.25%)	0.076
rs17561, MAF=0.28			
HomoA	0	2 (12.5%)	-
HomoC	11 (68.75%)	11 (68.75%)	-
EteroAC	5 (31.25%)	3 (18.75%)	0.294
rs3024608, MAF=0.07			
HomoC	16 (100%)	16 (100%)	-
HomoG	0	0	-
EteroCG	0	0	-
rs1805011, MAF=0,31			
HomoA	10 (62,5%)	16 (100%)	-
HomoC	0	0	-
EteroAC	6 (37.5%)	0	-

Table I. Demographic features and clinical parameters of patients enrolled in this study. Abbreviations: BMI, body mass index; N-ERD, NSAIDs Exacerbated Respiratory Disease.

All 44 participants completed the study. At baseline, the two groups were comparable in terms of clinical symptoms and demographic features (Table II).

	Dupilumab (n=22)	Control (n=22)	*p-value*
Age	52.09	48.68	0.286
Sex	17M/5F (77.27)	12M/10F (54.54%)	0.469
BMI	27.04 ± 2.1	27.63 ± 1.89	0.321
Blood eosinophilia	521,87 ± 74,58	502,13 ± 62,41	0.346

IgE total (kU/L)	399,75 ± 120,23	378,23 ± 117,67	0.551
Aspirin Intolerance	4 (18.18%)	3 (13.63%)	0.725
N-ERD	3(13.63%)	2 (9.09%)	0.671
Comorbidities			
Atopy	14/22 (63.63%)	16/22 (72.72%)	0.778
Asthma	9 (40.9%)	8/22 (36.36%)	0.836

Table II. Demographic features and clinical parameters of patients enrolled in this study. Abbreviations: BMI, body mass index; N-ERD, NSAIDs Exacerbated Respiratory Disease.

	Total (n=44)			**Dupilumab (n=22)**			**Surgery (n=22)**		
	Baseline	6 months	*p-value*	Baseline	6 months	*p-value*	Baseline	6 months	*p-value*
Blood eosinophilia	512,45 ± 78.42	543.26 ± 84.15	<0.001	521,87 ± 74,58	556.14 ± 66.79	0.042	502,13 ± 62,41	446.31 ± 105.38	0.663
NPS	5.52 ± 0.99	1.23 ± 1.52	<0.001***	5.77 ± 0.97	2 ± 1.79***	<0.001	5.27 ± 0.98	0.45 ± 0.59***	<0.001
SNOT 22	56.16 ± 15.50	16.14 ± 8.41	<0.001***	55.22 ± 16.68	13.36 ± 9.46***	<0.001	57.09 ± 14.93	19.13 ± 6.01***	<0.001
SSIT Score	3.02 ± 1.95	8.41 ± 3.40	<0.001***	2.9 ± 2.09	10.45 ± 3.67***	<0.001	3.13 ± 1.88	6.36 ± 1.39***	<0.001
VAS Obstruction	7.82 ± 1.27	1.61 ± 1.03	<0.001***	7.95 ± 1.25	1.27 ± 0.63***	<0.001	7.68 ± 1.32	1.95 ± 1.25***	<0.001
VAS Rinorrhea	7.07 ± 1.14	1.05 ± 0.82	<0.001***	7.18 ± 1.13	0.77 ± 0.68***	<0.001	6.95 ± 1.17	1.31 ± 0.89***	<0.001
VAS Headache	5.20 ± 0.97	1.27 ± 1.01	<0.001***	5.36 ± 1.09	1.5 ± 1.05***	<0.001	5.04 ± 0.84	1.04 ± 0.95***	<0.001
Species									
S. aureus	19 (43.18%)	25 (56.82%)	0.200	14 (51.85%)	14 (51.85%)	>0.05	5 (22.72%)	11 (50%)	0.097
P. aeruginosa	7 (15.91%)	2 (4.55%)	0.078	6 (22.22%)	0*	-	1 (4.54%)	2 (9,09%)	0.556
S. epidermidis	16 (36.36%)	33 (75%)	<0.001***	10 (37.03%)	16 (59.25%)	0.160	6 (27.27%)	17 (77.27%)	0.007**
Other aerobic species	30 (68.18%)	29 (65.91%)	0.820	13 (59.25%)	20 (74.07%)	0.123	17 (77.27%)	9 (40.90%)	0.061

Table III. Subgroup outcomes comparison. Abbreviations: m, month; NPS, nasal polyp score; SNOT-22, Sino-nasal outcome test; NCS, nasal congestion score; SSIT, Sniffin sticks identification test; VAS, visual analogue scale. * p<0.05, ** p<0.01, *** p<0.001 at intergroup analysis for each follow up.

At 6-months follow-up, both Dupilumab and surgery treatments led to a significant reduction in NPS (p<0.001 for both). However, the improvements were superior in the Surgery group (p<0.001) (Table III). In contrast, the SSIT score was significantly greater in the Dupilumab group (p<0.001). Also in SNPs groups at 6-months follow-up, both Dupilumab and surgery treatments led to a significant reduction in NPS (p<0.001 for both). However, the improvements were superior in the Surgery group (p<0.001) (Table IV).

	Dupilumab (n=16)			**Surgery (n=16)**		
	Baseline	6 months	*p-value*	Baseline	6 months	*p-value*
IgE total (kU/L)	399.75 ± 120.23	176,06 ± 131,56	<0,001	65.25 ± 18.61	61.56 ± 25.77	0,646
Blood eosinophilia	521.87 ± 74.58	529.37 ± 91.39	0,801	175 ± 20.98	163.33 ± 23.09	0,145
SSIT Score	3.06 ± 2.14	11 ± 2.64	<0,001	2.87 ± 1.96	6.12 ± 1.89	<0,001

NPS	5.75 ± 1	1.33 ± 1.15	<0,001	5.37 ± 1.08	0.5 ± 0.63	<0,001
SNOT 22	52.87 ± 17.32	10.66 ± 2.51	<0,001	52.87 ± 10.13	20.5 ± 7.91	<0,001
VAS Obstruction	7.81 ± 1.32	2.33 ± 1.52	<0,001	7.31 ± 1.30	1.69 ± 0.70	<0,001
VAS Rinorrhea	7.68 ± 1.01	1.31 ± 0.57	<0,001	7 ± 0.97	1.06 ± 0.77	<0,001
VAS Headache	5.31 ± 0.94	1.66 ± 0.58	<0,001	5 ± 0.82	1.13 ± 0.81	<0,001

Table IV. Subgroup outcomes comparison. Abbreviations: m, month; NPS, nasal polyp score; SNOT-22, Sino-nasal outcome test; NCS, nasal congestion score; SSIT, Sniffin sticks identification test; VAS, visual analogue scale. * p<0.05, ** p<0.01, *** p<0.001 at intergroup analysis for each follow up.

It's noteworthy that both treatments significantly improved the SSIT score (p<0.001), but the improvement was significantly greater in the Dupilumab group at intergroup analysis (p<0.001). Conversely, at intergroup analysis for VAS scores, the Dupilumab group were greater but not statistically significant (p>0.05).

3.1 SNPs Profiles

Our study found significant differences in the SNP profiles among the 3 included groups, specifically in rs1800629 (TNFA), rs2856838 (IL1a), rs17561 (IL1a), and rs1805011 (IL4R). Only one patient treated with Dupilumab (6.25%) presented expression of the rs1800629 allele (TNFA) while it was absent in all individuals in the surgical and control groups (p=0.360). The rs2856838 (IL1a intronic variant) SNP showed more variability in the Dupilumab group, expressing a variant in 14/16 (87.5%) subjects. However, statistical significance was found only with Surgery group (p=0.049). Regarding the IL4R SNPs, different results were found among rs3024608 (IL4R intronic variant) and rs1805011 (coding non synonymous) SNP. The intronic variant was non-significant in our study at intergroup analysis. The SNP data of the participants were categorized and analyzed as shown in Table V.

	Dupilumab (n=16)		**Surgery (n=16)**		**Healthy (n=16)**		*p-value*
	n	%	n	%	n	%	
rs1800629 (TNFA)							
Nex	1	6.25	0	0	0	0	0.360
Ex	15	93.75	16	100	16	100	
rs2856838 (IL1a)							
Nex	2	12.5	7	43.75	5	29.8	0.147
Ex	14	87.5	9	56.25	11	70.2	
rs17561 (IL1a)							
Nex	0	0	2	12.5	2	12.5	0.335

Ex	16	100	14	87.5	14	87.5	
rs3024608 (IL4R)							
Nex	16	100	16	100	16	100	ns
Ex	0	0	0	0	0	0	
rs1805011 (IL4R)							
Nex	10	62.5	15	93.75	13	81.25	0.090
Ex	6	37.5	1	6.25	3	19.75	

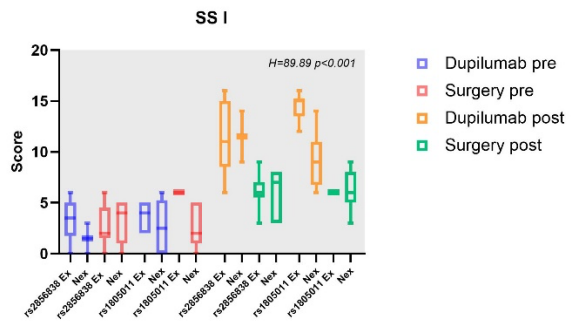
Table V. Subgroup SNPs variants expression. Abbreviations: Ex, Expressed. Nex, non expressed.

Interestingly, none of the individuals tested in the three groups had the genetic marker rs3024608 (IL4R intronic variant). In contrast, the marker rs1805011 (IL4R coding non synonimous variant) was expressed in 37.5 percent of the Dupilumab group, while the surgical and control groups had lower rates (6.25 % and 19.75%, respectively) but not significant (p=0.09). Conversely, at intergroup analysis the expression of rs1805011 SNP variant was significantly higher Dupilumab patients than the Surgery ones (p=0.032). A slight difference was instead reported compared to healthy subjects.

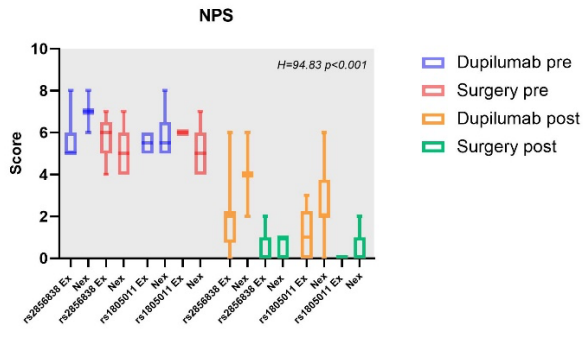
3.2 Clinical and SNPs outcomes

After a 6-month follow-up, valuable associations were identified between clinical outcomes and two key SNPs, rs2856838 and rs1805011 across all samples. Patients expressing rs2856838 variant in the Dupilumab group had higher SSI scores (11.71 ± 3.34 vs. 8±1; p=0.150) but worse SNOT-22 scores (11 ± 3 vs. 13.83 ± 8.87; p=0.333) compared to the non-expressors. Also rs1805011 variant expression exhibited better outcomes in Dupilumab group with higher SSI scores than not expressed (14.83 ± 0.68 vs. 9.1 ± 2.38; p<0.001) and superior improvements in SNOT-22 scores (10.67 ± 2.52 vs. 15.2 ± 8.70; p<0.001). Interestingly, the SNP rs1805011 variant presented significant greater SS-I scores than rs2856838 (p<0.001).

a)



b)



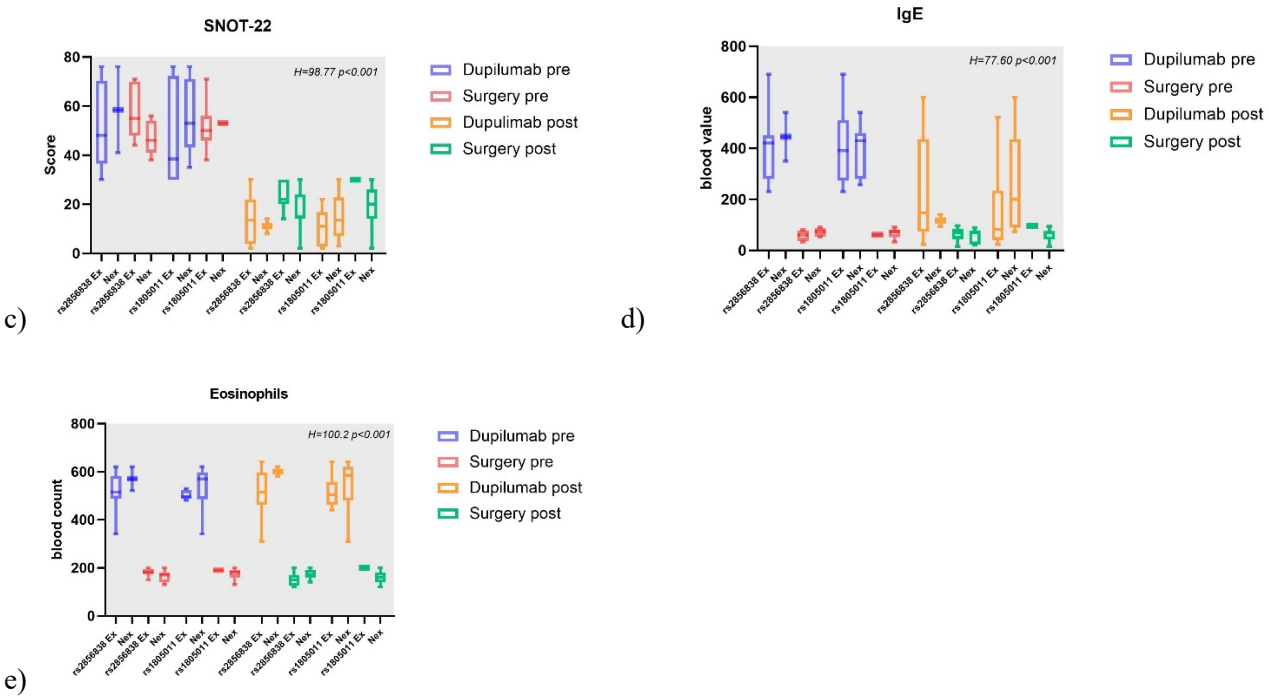


Fig. 3. Baseline vs. 6-months outcomes comparison represented by Violin Plot. a) SS-I scores b) NPS scores ; c) SNOT-22 scores; d) IgE blood level e) Eosinophil blood count. The Kruskal-Wallis tests indicated statistical significance among all different treatment outcomes. Surgical group reported better outcomes for the Nasal Polyp Score (NPS) but there were no significant differences at intragroup analysis.

SNP polymorphisms and clinical outcomes

Dupilumab appears to have a genotype-dependent effect on SSI and IgE levels in patients with rs1805011, while surgery significantly improves SNOT-22 scores in the ex group of rs2856838 (Table VI).

	Dupilumab						**Surgery**		
	rs2856838			**rs1805011**			**rs2856838**		
	ex	*nex*	*p-value*	*ex*	*nex*	*p-value*	*ex*	*nex*	*p-value*
SSI	11.71 ± 3.34	8 ± 1	0.150	14.83 ± 0.68	9.1 ± 2.38	<0.001	6.11 ± 1.59	6.14 ± 2.09	0.975
SNOT-22	11 ± 3	13.83 ± 8.87	0.333	10.67 ± 2.52	15.2 ± 8.70	0.095	23.77 ± 5.28	14.9 ± 0.4	<0.001
NPS	1.85 ± 1	3.5 ± 1.5	0.055	1.16 ± 1.06	2.7 ± 1.79	0.092	0.44 ± 0.68	0.57 ± 0.49	0.662
IgE levels	158.71 ± 112.22	297.5 ± 157.5	0.135	98.83 ± 66.14	222.4 ± 132.71	0.025	63.88 ± 24.42	58.57 ± 25.3	0.678

Eosinophil Blood Count	519.28 ± 89.87	600 ± 20	0.238	515 ± 63.44	538 ± 99.57	0.578	160.11 ± 26.83	162.85 ± 23.73	0.831

Table VI. SNPs-based outcomes of each treatment group.

In the Dupilumab group we found For the SSI Sniffin Stick Test olfaction both rs2856838 (11.71 ± 3.34 vs. 8 ± 1, p=0.150) and rs1805011 ex genotype (14.83 ± 0.68 vs. 9.1 ± 2.38; p<0.001) had a higher mean score than those with the nex genotype. Surgery did not lead to a significant difference in SSI scores between the ex and nex groups of rs2856838 (p=0.975). Instead for the SNOT-22 there was no significant difference between the ex and nex groups of rs2856838 and rs1805011 who were treated with Dupilumab (p=0.333 and p=0.095 respectively). However, surgery significantly improved SNOT-22 scores in the ex group of rs2856838 compared to the nex group (23.77 ± 5.28 vs. 14.9 ± 0.4; p<0.001). NPS was slightly lower in the ex group of both rs2856838 and rs1805011 who were treated with Dupilumab, but these differences were not statistically significant (p=0.055 and p=0.092 respectively). There was also no significant difference in NPS scores after surgery between the ex and nex groups of rs2856838 (p=0.662).
For IgE blood levels, patients with the ex genotype of rs2856838 had lower mean levels when treated with Dupilumab (158.71 ± 112.22) compared to the nex group (297.5 ± 157.5), but this difference was not statistically significant (p=0.135). In contrast, Dupilumab significantly reduced IgE levels in the ex group of rs1805011 compared to the nex group (98.83 ± 66.14 vs. 222.4 ± 132.71; p=0.025). Surgery did not significantly affect IgE levels in either genotype of rs2856838 (p=0.678). Finally, for the eosinophil blood count, there were no significant differences between the ex and nex groups in both rs2856838 and rs1805011 who were treated with Dupilumab (p=0.238 and p=0.578 respectively), or in those who underwent surgery (p=0.831).
Microbiological outcomes

The presence of S. aureus rose modestly from 43.18% at the start of the study to 56.82% at the six-months (p=0.200). Conversely, P. aeruginosa decreased from 15.91% to 4.55% (p=0.078). Most notably, we observed a striking increase in S. epidermidis, which nearly doubled from 36.36% at baseline to 75% after six months (p < 0.001). The prevalence of other bacterial species had only minor fluctuations, shifting from 68.18% initially to 65.91% (p=0.820). At subgroup analysis, the prevalence of S. aureus in the Dupilumab group remained steady at the 6-months follow-up (51.85%). On the other hand, the Surgery group saw an increase from 22.72% at baseline to 50% after 6 months (p=0.097). Interestingly, P. aeruginosa was completely eradicated in the Dupilumab group, down from an initial 22.22%. In contrast, the Surgery group experienced an increase from 4.54% at baseline to 9.09% (p=0.576). The prevalence of S. epidermidis increased significantly in both groups. In the Dupilumab group, it rose from 37.03% to 59.25% (p=0.160), while in the Surgery group, it escalated from 27.27% to 77.27% (p=0.007). As for the presence of Other microbial species, an increase was found in the Dupilumab group, from 59.25% at baseline to 74.07% (p=0.123). Conversely, the

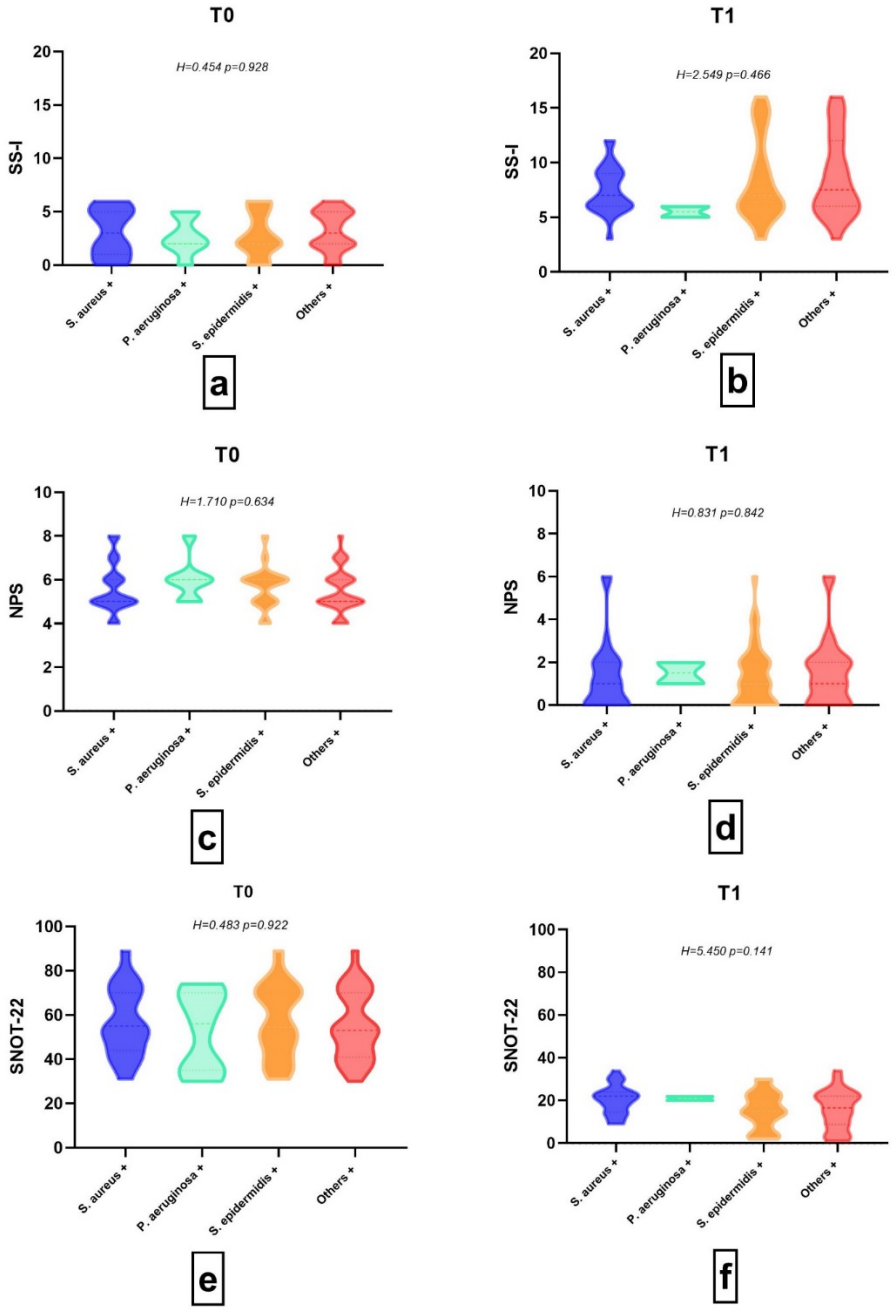
Surgery group experienced a decrease, dropping from 77.27% at baseline to 40.90% after 6 months (p=0.061). Other bacterial species less prevalent than these three populations are reported in Supplementary Table I.

	Total (n=44)	
	Baseline	6-months
Gram positive bacteria		
S. aureus	19 (43.18%)	25 (56.82%)
S. epidermidis	16 (36.36%)	33 (75%)
S. haemolyticus	3 (6.82%)	2 (4.54%)
S. warneri	2 (4.55%)	3 (6.82%)
S. capitis	1 (2.27%)	0
S. hominis	1 (2.27%)	0
S. lugdunensis	1 (2.27%)	1 (2.27%)
S. saprophyticus	1 (2.27%)	0
S. conhii	0	1 (2.27%)
Gram negative bacteria		
P. aeruginosa	7 (15.91%)	2 (4.55%)
C. koseri	3 (6.82%)	2 (4.55%)
E. aerogenes	3 (6.82%)	2 (4.55%)
E. cloacae	3 (6.82%)	1 (2.27%)
E. hormaechei	2 (4.55%)	6 (13.64%)
C. freundii	1 (2.27%)	0
C. violaceum	1 (2.27%)	0
E. coli	1 (2.27%)	1 (2.27%)
H. alveii	1 (2.27%)	0
K. pneumoniae	1 (2.27%)	2 (4.55%)
M. morganii	1 (2.27%)	0
S. liquefaciens	1 (2.27%)	0
C. diversus	0	1 (2.27%)
E. kobei	0	1 (2.27%)
E. ludwigii	0	1 (2.27%)
K. aerogenes	0	1 (2.27%)

Table S1: Percentage of colonized patients for each microbial species at baseline and 6-months follow-up.

3.3 Microbial profiles outcomes

Microbial profiles influenced clinical outcomes. Patients with P. aeruginosa had higher SNOT-22 scores (21.00 ± 1.41) and lower SS-I scores (5.50 ± 0.71) than other species analyzed at follow-up (Table III). Conversely, patients with S. epidermidis demonstrated lower SNOT-22 scores among all groups and greater improvements in olfaction than S. aureus and P. aeruginosa. No significant differences were found in SS-I scores between the ifferent groups at the Kruskal-Wallis test (Fig.4a-Fig.4f).



Additionally, the subgroup analysis for the dupilumab group showed that the S. aureus patients had significantly worse SS-I (8.21 ± 2.52 vs.10.88 ± 3.96; p=0.039, respectively) and SNOT-22 scores outcomes than S. epidermidis ones (18.57 ± 7.79 vs. 11.94 ± 9.07; p=0.041, respectively). Lastly, for the Nasal Polyp Score (NPS), there were no significant differences observed between groups. In the surgical group sub-analysis, only the SNOT-22 scores indicated a significant difference between S. aureus and S. epidermidis patients.

4. Discussion

This study evaluated the efficacy of Dupilumab and surgery treatment in managing clinical symptoms, and the correlation between these treatments and Single Nucleotide Polymorphisms (SNPs) in patients[59,60]. The

findings suggest that both Dupilumab and surgery significantly reduced Nasal Polyp Score (NPS) and improved the Sniffin' Sticks Identification Test (SSIT) score. However, the improvement was notably superior in the surgery group for NPS and in the Dupilumab group for SSIT. The SNP profiles showed significant differences among the groups, particularly in the rs1800629 (TNFA), rs2856838 (IL1a), rs17561 (IL1a), and rs1805011 (IL4R) SNPs. The rs1800629 allele was present only in the Dupilumab group, while the rs2856838 SNP showed more variability in the same group, with statistical significance found only in comparison to the surgery group. Interestingly, the rs3024608 SNP (IL4R intronic variant) was not significant in our study, and none of the individuals tested in the three groups had this genetic marker. On the other hand, the rs1805011 SNP (IL4R coding non synonymous variant) was expressed in 37.5% of the Dupilumab group, with significantly higher expression than in the surgery group. Associations were found between clinical outcomes and two key SNPs, rs2856838 and rs1805011, across all samples. Patients expressing the rs2856838 variant in the Dupilumab group had higher SSIT scores but worse SNOT-22 scores compared to non-expressors, while rs1805011 variant expression resulted in higher SSI scores and improved SNOT-22 scores in the Dupilumab group. Dupilumab seemed to have a genotype-dependent effect on SSI and IgE levels in patients with rs1805011, while surgery significantly improved SNOT-22 scores in the ex group of rs2856838. However, these genotype-dependent effects were not significantly different in the NPS scores after surgery for the ex and nex groups of rs2856838. The study also found that Dupilumab significantly reduced IgE levels in the ex group of rs1805011 compared to the nex group, which signals a possible genotype-dependent effect on IgE levels. Despite these promising outcomes, the study found no significant differences in eosinophil blood count between the ex and nex groups in both rs2856838 and rs1805011 who were treated with Dupilumab or underwent surgery. The genotype-dependent effects of Dupilumab and surgery on treatment outcomes identified in this study have potential implications in various areas. One of the most significant is the field of personalized medicine. If certain genotypes respond better to specific treatments, clinicians could use this genetic information to guide treatment choices and optimize patient outcomes. These findings could also help in the development of predictive models. Such models could estimate the likely success of Dupilumab or surgical interventions based on an individual's SNP profile, assisting in treatment decisions and setting realistic expectations for patients. Understanding the link between specific genetic variants and treatment response could lead to the identification of new therapeutic targets. This could pave the way for the development of novel treatments for conditions associated with these SNPs. The findings also contribute to the field of pharmacogenomics, the study of how genes affect a person's response to drugs. This could result in safer and more effective drug prescriptions, minimizing the risk of adverse drug reactions and maximizing drug efficacy. If genetic profiling can predict treatment response, it could potentially save healthcare resources by avoiding less effective treatments for certain genotypes. This could lead to more cost-effective healthcare delivery. In future clinical studies, genotypes could be considered as stratification criteria or as factors in subgroup analyses. This could provide more granular insights into the efficacy and safety of treatments. While these implications offer exciting possibilities, it's important to note the complexities involved. The genotype-treatment response relationship might not be straightforward and could be influenced by multiple factors,

including other genetic variants, epigenetic changes, environmental factors, and lifestyle choices. Therefore, more comprehensive studies are needed to confirm these findings and further explore their potential implications. This study offers critical advancements in our understanding of the nasal microbiota's relationship with CRS treatments including novel strategies like Dupilumab. Although the role of the nasal microbiome21–24 in CRS is increasingly recognized, the correlation between different microbiological profiles including the role of S. epidermidis, and P. aeruginosa and nasal outcomes have not been analyzed to date. We isolated a high prevalence of bacterial populations of Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa, along with different bacterial species belonging to Staphylococcus spp. and Enterobacterales that were sporadically present with no apparent trend in colonizing the nasal cavity. For anaerobic species, only a small percentage of Gram positive bacteria was found, namely Peptostreptococcus spp. that are usually an important genus for CRS patients, but these isolates were scarce compared to the most prevalent populations. These results might be due to the limitations and bias of the culturing methods for fastidious bacteria. Throughout our study, we observed shifts in specific bacterial species over a six-month period, as we found that both surgical interventions and dupilumab treatments elicit changes in the nasal microbiota. Overall, the colonization of S. aureus moderately increased from 43.18% to 56.82% (p=0.200), S. epidermidis colonization went from 36.36% to 75%, and P. aeruginosa prevalence dropped from 15.91% to 4.55% (p=0.078). Our data, however, represent a significant breakthrough in understanding the link between nasal outcomes and microbial profiles, presenting for the first time the beneficial role of the Staphylococcus epidermidis, a recurrent saprophyte in nasal respiratory skin and mucosa. It is interesting that S. epidermidis colonization increased in the 6-months follow-up groups, especially for surgical group, but there was no statistical different trend at treatments comparison. On the contrary, our data found a variable change in the microbiological profile for other prevalent species. Despite dividing the participants in two randomized groups, at baseline the Dupilumab group showed a S. aureus colonization rate of 51.85% (14 patients) that remained constant at the 6-month follow-up; on the other hand, the Surgery group experienced anincrease in S. aureus colonization (from 22.72% to 50%; $p<0.097$). As previously described22, this increase might be explained by the impact of ESS on microbial populations in the nasal cavity, leading to a shift to certain populations like S. aureus. Similarly, Jain et al. reported changes in bacterial composition and abundance in the middle meatus after endoscopic surgery (ESS), and patients subjected to ESS experienced increased bacterial richness for the Staphylococcus genus ($p = 0.002$) compared to other taxa. S. aureus colonization has also been linked to worse outcomes in patients after ESS21. Our data suggests that surgical intervention might lead to a significant shift towards S. aureus colonization in the bacterial repopulation of the nasal cavity. On the other hand, dupilumab treatment appears to stabilize the balance of these populations. Additionally, the dupilumab treatment showed an important shift in P. aeruginosa colonization from 22.22% (6 patients) to a complete eradication, that might be involved in better outcomes for patients as this species is associated to negative results in patients with CRS after ESS(24). This dynamic response could be shaped by various factors, such as the environmental context and individual characteristics like immune responses11. Probably, dupilumab treatment can trigger a more immediate shift in the nasal microbiota to a healthier

repopulation, acting as a monoclonal antibody and inhibiting IL-4 and IL-13 cytokines by blocking the interleukin-4 receptor alpha. Nevertheless, the precise influence of dupilumab on the microbiota remains unclear and necessitates further exploration. Our results have substantial implications for the treatment of CRS. Both dupilumab and surgery group had better outcomes in SNOT-22, NPS and SS-I scores compared to baseline, confirming the success of these treatments. Nonetheless, although when analysed together no bacterial species had significant impact on NPS and SNOT over the six-month period, different results were found between surgery and dupilumab recolonization. The surgical group showed a significant trend only in the SNOT-22 scores, with worse outcomes for S. aureus than S. epidermidis and Other bacterial species ($p<0.01$ for both). Moreover, in the dupilumab group S. aureus colonization was also linked to worse results in SNOT-22 and SS-I scores compared to other bacterial groups like S. epidermidis ($p=0.039$ and $p=0.041$, respectively) and other bacterial species ($p=0.051$ and $p=0.192$, respectively). As previously described in literature, the production of proinflammatory substances by S. aureus is connected to type 2 host inflammation. Further, Kanemitsu et al.26 have analysed the correlation between sensitization to mould and/or S. aureus enterotoxins and surgical outcomes of patients with CRS. The authors reported all biomarkers related to type 2 inflammation significantly higher in patients with moulds or S. aureus sensitization compared to control group ($p<0.05$). Notably, our study found a link between the presence of S. epidermidis and improved nasal perspectives outcomes (SNOT-22 and SS-I), suggesting that S. epidermidis may play a beneficial role in protecting the nasal environment and preventing the colonization of harmful bacteria. Moreover, in our study S. epidermidis colonization in the dupilumab group was associated to higher SS-I and SNOT-22 scores compared to S. aureus group ($p<0.05$), for the first time linking the nasal cavity colonization of S. epidermidis to better outcomes for patients when combined with dupilumab treatment. Our results reflect the different influence dupilumab treatment can have depending on the bacterial species present and how outcomes can vary significantly depending on the microbiological profile. Lastly, for the Nasal Polyp Score (NPS) there were no significant differences observed, with excellent results at follow-up regardless of treatment or species identified.

Limitations

The longitudinal aspect of the study posed a challenge, as a 6-month follow-up period may not be sufficient to observe long-term changes in the microbial profile or assess the enduring consequences of the treatments. The size of the study sample might also limit the statistical power to detect meaningful differences between treatment groups, likewise the culture method – in our culture-based study – may represent a limiting factor for the isolation of fastidious strains like anaerobic species or other low-represented populations. Furthermore, the potential influence of uncontrolled or unmeasured variables on the microbial profile, such as the patients' overall health, lifestyle factors, use of other medications, or differences in post-treatment care, must be considered. Inter-patient variability can also complicate the assessment of treatment effects as microbial profiles can differ significantly among individuals. These challenges underline the complexity of designing and conducting prospective studies, and the need for careful interpretation of our results. This forward-looking study focusing on the microbial profile in patients with chronic rhinosinusitis (CRS) undergoing treatment

with Dupilumab or surgical procedures holds value, yet it is not without potential constraints. The study's longitudinal design posed a hurdle, with a 6-month follow-up period that may not be long enough to fully capture the long-term shifts in the microbial profile or to evaluate the enduring effects of the treatments. This concern about the duration of the study naturally leads to questions about the size of the study sample. The limited participant pool could restrict the statistical strength needed to identify significant differences between the treatment groups, making it more difficult to draw robust conclusions from the observed data. Adding another layer of complexity to this issue are the unregulated or unmeasured factors that could have influenced the microbial profile. Factors such as the overall health status of the patients, lifestyle habits, administration of other drugs, or variations in post-treatment care, all could have played a role in the outcomes we observed. One of the most significant challenges in this study, and indeed in many similar studies, is the issue of inter-patient variability. The assessment of treatment effects can be complicated by the fact that microbial profiles can exhibit substantial differences among individuals, making it harder to draw general conclusions from the data. These challenges, taken together, underscore the intricacies linked with the design and execution of prospective studies. They highlight the need for a cautious interpretation of results and indicate areas where future studies could be strengthened to provide even more robust and reliable data.

5. CONCLUSION

This study shines light on the potential influence of nasal microbiota on both the development and treatment outcomes of Chronic Rhinosinusitis (CRS). These findings suggest the potential benefits of personalized CRS treatments, which would be tailored to the patient's individual nasal microbiota profile. Despite these promising insights, additional research is required to validate these associations and explore the practicality and effectiveness of microbiota-focused therapies in managing CRS. The outcomes of Dupilumab therapy may not be uniform across patients, but rather, are influenced by their unique SNP profiles. Certain SNPs, we found, seem to correlate with a more favorable response to this therapy. Notably, Dupilumab, known for its potential anti-inflammatory properties, might lead to disparate outcomes in patients harboring different SNPs. Patients undergoing endoscopic surgery displayed contrasting responses, underscoring the complex interaction between genetic variants and treatment efficacy. These observations highlight the potential of crafting personalized treatment plans that take into account the individual SNP genotypes of patients. The findings of our study suggest the feasibility of creating targeted treatment protocols rooted in the SNP genotypes identified at the time of diagnosis. This could pave the way for more personalized management strategies in dealing with chronic rhinosinusitis. Despite this promising lead, the necessity for larger, prospective studies persists. These studies are indispensable for corroborating the impact of SNP genotypes on long-term clinical efficacy and disease progression, fully comprehending the role of genotypes in determining treatment outcomes for chronic rhinosinusitis demands further examination.

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