Genetics of chronic rhinosinusitis

Kyle B. Mitts, DO, and Eugene H. Chang, MD *Tucson, Ariz*

Key words: *Genetics, chronic rhinosinusitis, phenotype, endotype, transcriptome*

Chronic rhinosinusitis (CRS) is a common condition affecting 12% of the US population, with annual direct costs exceeding $8 billion. It is defined as the presence of chronic inflammation of the sinonasal mucosa with greater than 12 weeks of sinonasal symptoms and accompanied by endoscopic or radiologic findings.[1] Although the pathophysiology of CRS is multifactorial, the disease frequently occurs in different individuals of the same family. Twin studies and the co-occurrence with asthma suggest a genetic component.[2] The identification of genes associated with CRS is critical to understanding the pathogenesis of disease. Several published studies have identified and comprehensively -reviewed genes associated with CRS.[1,2] However, the objective of this review was to introduce the reader to various strategies to identify susceptibility genes, review the concepts of endotype-phenotype interactions, describe the role of gene expression in CRS tissues, and provide relevant examples of specific genes associated with CRS in this context.

THE ROLE OF THE CF TRANSMEMBRANE CONDUCTANCE REGULATOR GENE IN CRS

In 1989, a collaborative effort from the laboratories of Drs Collins, Riordan, and Tsui identified the CF transmembrane conductance regulator (*CFTR*) gene, in which mutations in both copies of the *CFTR* gene resulted in cystic fibrosis (CF). *CFTR* is a classic example of a monogenic gene disorder related to CRS. Nearly all persons with 2 CFTR mutations and CF will develop CRS. There is a high association between CFTR function and CRS severity, and deletion of the CFTR protein in a transgenic porcine model replicates the human CRS phenotype. Interestingly, genetic screening for CFTR in the CRS population has identified that CFTR heterozygotes with a single CFTR mutation and those without CF have a 3-fold increased risk to develop CRS. Genetic screening in CF is also critical to the precision treatment of CF. For example, ivacaftor, a small molecular compound, specifically targets the G551D-CFTR mutation by increasing CFTR function, significantly improving quality of life and outcomes, and reversing CFTR-associated sinus disease.[3]

From the Department of Otolaryngology-Head and Neck Surgery, University of Arizona College of Medicine, Tucson.

Disclosure of potential conflict of interest: E. H. Chang receives funding from the National Institutes of Health (grant no. R01 AI146131). K. B. Mitts declares no relevant conflicts of interest.

Received for publication December 16, 2019; revised January 24, 2020; accepted for publication January 27, 2020.

Available online January 31, 2020.

Corresponding author: Eugene H. Chang, MD, 1501 N Campbell Ave, Tucson, AZ 85713. E-mail: echang@oto.arizona.edu.

J Allergy Clin Immunol 2020;145:777-9.

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2020.01.029

However, CFTR-related CRS composes only a small fraction of those with CRS and the identification of multiple genes associated with CRS highlights the complex and heterogeneous nature of this disorder.

GENOME-WIDE SCREENING OF SUSCEPTIBILITY GENES IN CRS

Genome-wide linkage and association studies rely on mapping regions of the human genome that correspond to the CRS phenotype. Genome-wide linkage studies for CRS require populations with a similar genetic background, and Pinto et al[4] identified a genetic locus on chromosome 7q31.1-7q32.1 that influenced CRS susceptibility in 8 related individuals with CRS by screening a total of 291 persons from the Hutterite community. Genome-wide association studies for CRS use high-throughput genotyping platforms that cover 300,000 to more than 1 million single nucleotide polymorphisms. Genome-wide association studies allow for the ability to detect risk variants with modest effect sizes, and do not require the use of homogeneous subjects. However, genome-wide association studies do require large sample sizes, a clear disease phenotype, and significant expenses in genotyping costs (Fig 1). In best-case scenarios, in which genetic associations result in large effect sizes, at least 2000 cases and 2000 controls are recommended to detect causal gene variants. Cormier et al[5] circumvented some of these costs by using a pooling-based strategy in which individual DNA samples were pooled into groups on the basis of the presence of *Staphylococcus aureus* in nasal swabs and CRS. This technique identified 23 single nucleotide polymorphisms located within or near 21 genes associated with *S aureus* colonization and CRS.

PHENOTYPES AND ENDOTYPES OF CRS

The success of genome-wide association studies in CRS depends on a clear definition of the CRS phenotype. CRS phenotypes have been broadly defined as CRS with or without nasal polyposis, and subphenotypes include allergic fungal rhinosinusitis and CRS-related aspirin-exacerbated respiratory disease. CRS phenotypes cannot elucidate mechanisms of disease; thus, endotypes based on unique pathomechanisms from affected tissues have been described. Tomassen et al[6] divided 173 patients with CRS on the basis of tissue immune markers in a phenotype-free approach. They identified several clusters associated with (1) T_H2- and eosinophil-driven inflammation, (2) neutrophilic and proinflammatory cytokines, (3) T_H17-T_H22-related markers, and (4) T_H1, IFN-γ markers. When they related these endotypes to CRS phenotypes, they determined that IL-5 was a primary marker associated with CRS with nasal polyposis and asthma.[6] Endotype markers in CRS with nasal polyposis have supported the use of biologic therapies that specifically target these receptors (IgE, IL-4, IL-5, and IL-13), and several studies have highlighted their potential role in the treatment of CRS with nasal polyposis and

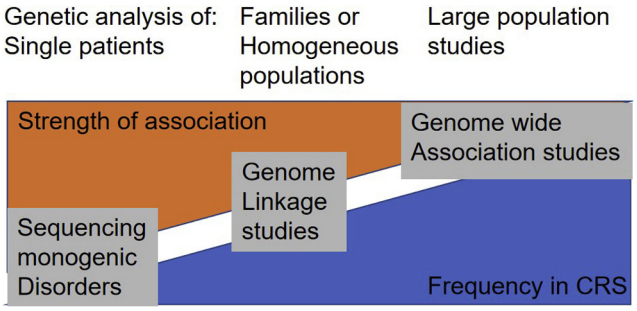

FIG 1. Strategies to identify genes associated with CRS. Monogenic disorders, such as CF, are highly associated with CRS yet comprise only a small portion of the total frequency of patients with CRS. The identification of these single genes can be identified by direct sequencing if the gene is known, or linkage studies to determine genetic loci in homogeneous populations. In complex heterogeneous disorders, genome-wide association studies can identify multiple genes that may individually have a lower association to CRS but represent a higher frequency of those with the disease.

asthma. One example of a unique testable phenotype in patients with CRS was described by Farquhar et al,[7] in which supertasters of the bitter phenylthiocarbamide compound were associated with less frequent sinus infections and improved nasal symptoms. This phenotype was based on findings in which airway tissues expressing genetic variants in the *TAS2R38* gene encoding for the phenylthiocarbamide receptor were linked to significant differences in the ability to clear and kill bacteria.

TRANSCRIPTOME SIGNALS IN CRS

Genetic studies identifying the cause of CRS have focused on DNA changes in the human genome, whereas transcriptome studies identify the genes that are actively expressed at any given time by analyzing RNA signals. Transcriptome analyses, therefore, bridge the gap between the genetic code and the functional molecules that distinguish cell behavior. Although nearly every cell contains the same genome, different cells can show distinct patterns of gene expression between health and disease. The analysis of CRS tissues and their associated gene expressions can be analyzed in several ways. Gene set analysis, in which a list of genes is associated with the disease set, are a common tool that provides an unordered and unstructured collection of genes associated with CRS on the basis of significantly altered differential expression of genes. The use of microarray analysis is a cost-effective method of analyzing the expression of thousands of known genes; however, the advent of total RNA-sequencing can identify new genes at the expense of greater cost and bioinformatic analyses. Pathway analysis is a complex model that describes a given disease process or mechanism, and the ability to look at gene-gene interactions based on known pathway databases (such as gene ontology terms or the Kyoto Encyclopedia of Genes and Genomes) (Fig 2). Pathway analyses in CRS have identified many genes associated with defined endotypes in CRS. Some of these identified genes include pathways related to T_H2 response, eosinophilia, regulatory T cells, eicosanoid metabolism, innate immunity and host defense, and epithelial-mesenchymal transitions and barrier function.[8] However, a major limitation in transcriptome analyses has been the use of surgical tissues from patients with advanced disease. Subsequently, we are unable to determine whether these gene expression changes are causative for CRS, or an effect of progressive disease. The recent discovery of minimally invasive methods to collect transcriptome data from sinonasal mucus and brushings can facilitate the longitudinal collection of samples and address the critical need to identify genetic and environmental factors that can affect the development of CRS from its onset.

LONGITUDINAL STUDIES OF CRS PATHOGENESIS

Our group recently published a study of the pathogenesis of CRS by identifying risk factors for CRS in the longitudinal

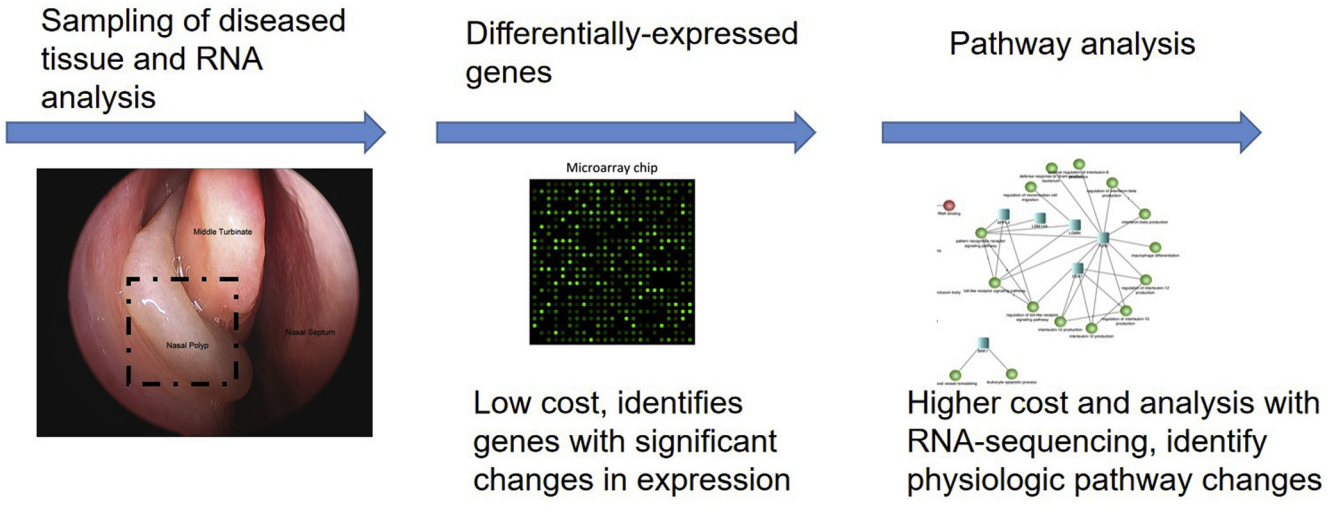

FIG 2. Methods to analyze gene expression in CRS. Gene expression, as measured by RNA, can be extracted from diseased tissue in CRS samples. Microarrays allow low-cost analysis of known differentially expressed genes that are either significantly overexpressed or underexpressed in the tissue sample. Total RNA-sequencing can identify differential expression of known and novel genes comparatively, but with increased cost and bioinformatic time. Pathway analyses combined gene expression changes with known pathways to determine how genes coordinate and interact and can aid in the mechanisms involved in disease.

Tucson Children's Respiratory Study cohort. The Tucson Children's Respiratory Study cohort is the longest-running birth cohort designed to study airway disease. We identified an early-onset chronic sinusitis group by analyzing subjects who were initially diagnosed with sinusitis by age 8 years, and then proceeded to have a diagnosis of CRS at age 22 to 32 years. This early-onset chronic sinusitis group was characterized by atopy, allergic rhinitis, wheeze/asthma, eczema, and a history of greater than 4 colds in early life when compared with groups without early-onset chronic sinusitis.[9] Rhinovirus infections are the most common virus infections detected in colds, childhood asthma, and viral causes of CRS. We recently reported that a novel single nucleotide polymorphism in the cadherin-related family member 3 gene, the receptor for rhinovirus C, was associated with adult CRS in a multicohort study. This suggests that genome-virome interactions may be a common risk factor for CRS and asthma.[10] Studies designed to identify molecular mechanisms of early-life phenotypes may distinguish gene pathways that are critical in the pathogenesis of disease.

CONCLUSION AND FUTURE DIRECTIONS

We are in an exciting era in the field of genetics in CRS. Technological and scientific advances in genetic sequencing, RNA-sequencing, transcriptome analyses, and the identification of longitudinal populations with CRS will allow in-depth studies of the role of genes and relevant pathways in CRS. In this article, we provide specific examples including CFTR genetic screening and its role in precision medicine, the use of biologics to target inflammatory receptors in CRS, novel screening methods to identify TAS2R38 variants that impact bacterial killing in CRS, and the identification of CDHR3, a receptor for rhinovirus C associated with childhood asthma and adult CRS. These examples offer potential areas of investigation that may identify genotype-specific variants of CRS and develop targeted therapies to halt the progression of disease.

REFERENCES

1. Orlandi RR, Kingdom TT, Hwang PH, Smith TL, Alt JA, Baroody FM, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. Int Forum Allergy Rhinol 2016;6:S22-209.
2. Hsu J, Avila PC, Kern RC, Hayes MG, Schleimer RP, Pinto JM. Genetics of chronic rhinosinusitis: state of the field and directions forward. J Allergy Clin Immunol 2013;131:977-93.e1-5.
3. Chang EH. New insights into the pathogenesis of cystic fibrosis sinusitis. Int Forum Allergy Rhinol 2014;4:132-7.
4. Pinto JM, Hayes MG, Schneider D, Naclerio RM, Ober C. A genomewide screen for chronic rhinosinusitis genes identifies a locus on chromosome 7q. Laryngoscope 2008;118:2067-72.
5. Cormier C, Endam LM, Filali-Mouhim A, Boisver P, Boulet LP, Boulay ME, et al. A pooling-based genomewide association study identifies genetic variants associated with *Staphylococcus aureus* colonization in chronic rhinosinusitis patients. Int Forum Allergy Rhinol 2014;4:207-15.
6. Tomassen P, Vandeplas G, van Zele T, Cardell L-O, Arebro J, Olze H, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. J Allergy Clin Immunol 2016;137:1449-56.e4.
7. Farquhar DR, Kovatch KJ, Palmer JN, Shofer FS, Adappa ND, Cohen NA. Phenylthiocarbamide taste sensitivity is associated with sinonasal symptoms in healthy adults. Int Forum Allergy Rhinol 2015;5:111-8.
8. Li C, Shi L, Yan Y, Gordon BR, Gordon WM, Wang DY. Gene expression signatures: a new approach to understanding the pathophysiology of chronic rhinosinusitis. Curr Allergy Asthma Rep 2013;13:209-17.
9. Chang EH, Stern DA, Willis AL, Guerra S, Wright AL, Martinez FD. Early life risk factors for chronic sinusitis: a longitudinal birth cohort study. J Allergy Clin Immunol 2018;141:1291-7.e2.
10. Chang EH, Willis AL, McCrary HC, Noutsios GT, Le CH, Chiu AG, et al. Association between the CDHR3 rs6967330 risk allele and chronic rhinosinusitis. J Allergy Clin Immunol 2016;139:1990-2.e2.