

Genetics of Rhinosinusitis

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Abstract Suggestion for a potential genetic basis to chronic rhinosinusitis (CRS) is afforded by degree of inheritability suggested from family and twin studies, existence of CRS in simple mendelian diseases, and development of sinusitis as part of the phenotype of certain gene “knockout” murine models. Genetic association studies are expected to identify novel genes associated with CRS and suggest novel mechanisms implicated in disease development. Although these studies are subject to methodologic difficulties, associations of CRS and polymorphisms in more than 30 genes have been published, with single nucleotide polymorphisms in 3 (*IL1A*, *TNFA*, *AOAH*) replicated. While the individual risk conferred by these single nucleotide polymorphisms remains modest, taken as a group, they suggest an important implication of pathways of innate immune recognition and in regulation of downstream signaling in the development of CRS. In a

demonstration of these techniques’ potential to identify new targets for research, the authors present a functional investigation of *LAMB1*, the top-rated gene from a pooling-based genome-wide association study of CRS. Upregulation of gene expression in *LAMB1* and associated laminin genes in primary epithelial cells from CRS patients implicates the extracellular matrix in development of CRS and offers a new avenue for further study.

Keywords Chronic rhinosinusitis · Endoscopic sinus surgery · Genetics · Nasal polyposis · Staphylococcus aureus · Staphylococcal superantigens · Gene association study · Pooling-based genome-wide association study · Asthma · Aspirin-intolerant asthma · Innate immunity · Toll-like receptor signaling · *IL1A* · *IL33* · *IL1RN* · *CACNG6* · *MMP9* · *IL10* · *TGFB1* · *IL22RA1* · *TNFAIP3* · *TNFA* · *NOS1* · *NOS1AP* · *TP73* · *CYSLTR1* · *ALOX5* · *ALOX5AP* · *LAMA2* · *PARS2* · *NAV3* · *LAMB1* · *CACNAII* · *KIAA1456* · *MUSK* · *TRIP12* · *AOAH* · *MSRA*

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Introduction

Chronic rhinosinusitis (CRS), a chronic inflammatory disease of the upper airways of uncertain etiopathogenesis, affects 6% to 13% of the population [1•]. It combines features of mucosal inflammation with colonization by specific bacterial pathogens. Despite the frequency of the disease, with its significant burden on patient quality of life and the impact on health care expenditures, our understanding of the factors underlying its development and persistence remains limited. The lack of an appropriate animal model for study has only compounded these difficulties.

Our current understanding of the disease has evolved over time. Initially perceived simply as a chronic bacterial

infection, biopsy studies of sinus tissue obtained during operation have implicated various inflammatory cells and proinflammatory cytokines in active disease [2]. More recently, our thinking has come full circle with a new focus on implicated bacterial pathogens in this disease. Pronounced differences between the bacterial flora of individuals with chronic sinusitis and healthy individuals have been identified, with increased recovery rates of *Staphylococcus aureus*, gram-negative organisms, and anaerobes in patients with CRS [3, 4]. Mechanisms by which these can contribute to the development of the disease have been elegantly demonstrated via the implication of immunomodulatory toxins produced by *S. aureus* in nasal polyposis [5••]. Enhanced bacterial resistance to host defenses and antibiotics has been attributed to novel bacterial survival strategies, such as the production of bacterial biofilms (aggregation of bacteria into colonies surrounded by polysaccharide matrix) [6••], or by intracellular persistence of bacteria [7•].

However, despite the progress made to date in our understanding of mucosal immunology and the microbiology, events occurring in early disease and factors contributing to the susceptibility to develop the disease remain largely unknown [8•]. Demographic associations have long been identified with the frequent coexistence of asthma and nasal polyposis. This is further supported by similarities in the histopathology of biopsy samples from individuals with both diseases, suggesting a common basis to the disorder [9]. However, common environmental or genetic factors contributing to susceptibility to development of the disease remain to be identified.

In this article, we review experimental work attempting to identify the genetic basis of CRS. It is hoped that the identification of genes associated with the disease will offer insights into the pathogenesis of this intriguing disorder and create a springboard for further research.

Is There a Genetic Basis to Chronic Rhinosinusitis?

A strong argument certainly can be made for the existence of a genetic component in at least certain forms of this disorder. The presence of nasal polyposis in cystic fibrosis (a common genetic disorder in which mutations in the *CFTR* gene impair chloride transport, conferring altered local immunity of the respiratory mucosa) provides an example in which genetically determined alterations of mucosal immunology contribute to the development of chronic sinusitis [10]. Animal studies involving mice that have been genetically modified to reduce function in specific genes (knockouts) also have described the development of sinusitis in various knockout models for genes other than *CFTR* [11].

In humans, family studies suggested a heritability of 14% to 42%, with highest reported heritability in the

asthma–nasal polyp–aspirin intolerance triad [12], while studies in twins show at least some concordance in the development of sinusitis between identical twins [13].

Taken together, this evidence suggests the existence of a potential genetic basis to CRS, which has motivated several investigators to attempt to identify the involved genes.

Techniques for Identifying Genes Implicated in Disease

In this article, we focus on genomics—techniques used to study the DNA of individuals that confer susceptibility to development of the disease—rather than those assessing levels of expression of mRNA (transcriptomics).

Two techniques are commonly used to attempt to identify genes implicated in disease. The first, linkage disequilibrium, studies markers of position (microsatellites or polymorphisms) on the genome of groups or families of related individuals to identify sections of the genome associated with the presence of disease [14•]. In these studies, results are presented as the logarithm (base 10) of odds score, with a positive score of 3.0 or greater suggesting a genetic basis. Depending on the number of markers used, this identifies an area of the genome for further in-depth study (fine mapping) to identify implicated genes. The precision of this technique varies depending on the number of markers employed, with a greater number of markers reducing the size of the genome for further fine mapping.

Recently, genetic association studies have increasingly focused on unrelated individuals characterized by the presence (affected) or absence (control) of the phenotypic trait of interest [15]. In these studies, genetic polymorphisms (base-pair substitutions in the DNA sequence, frequently called *single nucleotide polymorphisms* [SNPs]) are genotyped, and the frequency of the polymorphisms reported as the allelic frequency. These polymorphisms serve as markers of position in the genome and suggest—by their relationship to known genes—implicated genes. The availability of freely available, Internet-based resources cataloging these markers, such as the International HapMap database [16] and the UCSC (University of California, Santa Cruz) genome browser [17], has made these techniques available to even small research groups, while development of gene chips capable of genotyping several million SNPs simultaneously has made it possible to question the entire genome relatively easily. An important caveat to this is that the identified SNP is most likely not the causal genetic variation, but rather it is in close relation to the causative variation and can serve as a guide to its identification. Thus, SNPs under study are rarely in areas of the genome coding for the synthesis of amino acids (exons) and are potentially capable of causing a change in amino

acid sequence and consequent alteration of the secreted gene product. In fact, the overwhelming majority of SNPs are situated in regions of the gene not directly involved in protein generation (introns), or even outside the gene. Nevertheless, these may be implicated in the regulation of gene function, but this remains an area in which considerable work needs to be done.

For association studies, two approaches are currently used: the candidate gene approach and the genome-wide association study (GWAS). These differ mainly in experimental design and number of SNPs interrogated.

The candidate gene approach is a conventional hypothesis-based approach in which the investigator targets a biological function or process for study and genotypes a limited number of polymorphisms in these genes only. This yields information on potential genetic variants within genes and processes already implicated in disease by other means, but only rarely serves to identify novel mechanisms in disease development. The GWAS is a hypothesis-free approach to the study of the disease. Essentially, the investigator uses any one of a number of commercially available chips that assess more than 1 million SNPs across the entire genome. Identification of differences in allelic frequency can then implicate genes and biological processes not previously associated with disease, thus yielding valuable new insights into mechanisms of disease. This promise of new knowledge has led to the massive deployment of this technology in every disease area, but, unfortunately, these techniques have not yet yielded the quantum leap in our knowledge that was expected.

Pitfalls in Genetic Studies

Enthusiasm for these promising new technologies must be tempered by several potential difficulties in the performance and interpretation of the studies [18•].

Phenotyping

The disease characteristic (phenotype) under study must be carefully defined to ensure that the group under study is as homogenous as possible so as to maximize the genetic signal. However, even with the most conscientious investigators performing the most meticulous phenotyping, it remains possible that more than one molecular mechanism will underlie the development of a study phenotype (eg, nasal polypsis). Thus, genetic signals from one mechanism will be diluted by signals from the other(s), yielding weaker or conflicting signals. Addition of a biochemical or histologic marker, when available, may improve phenotyping.

Population Admixture

Considerable variation in allelic frequency exists between the different ethnic groups; thus, differing results may be reported between different ethnic groups. Population admixture within studied populations may be the cause as well, with contributions from several different groups contravening to the makeup of the group. This may be recognized or unrecognized and must be specifically addressed in the design and analysis of these studies for both study and control groups.

Population Size and Risk of Spurious Associations

Given the number of simultaneous tests (>1 million SNPs) performed in gene array studies, the possibility of an event occurring simply due to chance is considerable. Statistical testing is much more rigorous, with adjustment of *P* values for multiple testing essential. Also, because the effect size of the observed variation may be small, increasing risk of developing or having the disease by 1.5 times, group sizes must be large, with more than 1,000 patients per group common in GWAS.

As these three factors are the most common errors with early genetic studies, earlier reports must be carefully verified to ensure appropriate controls for these factors.

Gene–Environment Interactions

It is probable that genetic variation alone does not create disease as such but instead confers susceptibility to its development. Thus, this predisposition to development of disease may need to interact with an environmental factor to yield disease in a given individual. An extreme example of this would be a genetic polymorphism conferring susceptibility to development of tuberculosis, as this would lead to development of tuberculosis only if the individual was exposed to *Mycobacterium tuberculosis* [19•]. Another example of this is α -1-antitrypsin deficiency, in which genetic variations in the *SERPINA1* gene responsible for low levels of α -1-antitrypsin in secretions are associated with development of emphysema only in those individuals exposed to tobacco smoke [20].

Gene–Gene Interactions

Identified genetic mutations may not yield disease but may instead alter the function of a second, unrelated gene that is then responsible for disease development. Evidence for this phenomenon is suggested once again by cystic fibrosis, in which patients with the same mutation in the *CFTR* gene have considerable variability in the clinical phenotype [21•].

Rare Genetic Variants

Population association genetics is based on the premise of frequent polymorphisms in multiple genes contributing small but additive effects in the development of disease. However, in the instance of rare polymorphisms with a strong effect (high penetrance), the difference in observed allelic differences may be minimal and may be identified only by the study of genotypes.

Multiple Causal Variants Within the Same Gene

A study polymorphism may represent only a limited number of causal variations. For example, several hundred different mutations within the *CFTR* gene have been identified in cases of cystic fibrosis [21•]. The variation in allelic frequency associated with any one of these single mutations may not be sufficient to afford a level of detection and thus may only be identified by sequencing of the entire gene, or by using strategies to study multiple SNPs in the same gene simultaneously.

Implication of Multiple Genes in a Common Pathway

An extension of the difficulties encountered with multiple causal variants, alterations in function in any one of several genes within a pathway, such as Toll-like receptor (TLR) signaling, impact the function of the pathway, with a final common phenotype. Again, the study of a single gene thus may underestimate the importance of its impact on the function of the pathway [22, 23•].

Difficulty Demonstrating Biological Significance and Impact of Function of Identified Single Nucleotide Polymorphisms

Demonstration of a genetic association does not prove causality, which must be demonstrated by subsequent experiments showing altered function of the gene and mechanistic impact in the disease. However, experience from GWAS has frequently demonstrated that not only do identified polymorphisms not have a direct impact on protein coding, but that the genes identified frequently are not immediately of obvious biological significance within the model under study. More than anything else, this has been the main source of frustration with GWAS to date.

Replication

An important step in the confirmation of the significance of results is replication in a second, unrelated population. This helps ensure that the result is not a spurious association [24].

In other disease areas, genes identified in the first study failed to replicate successfully in a second, a phenomenon referred to as the *winner's curse* [25]. This may be secondary to differences in population structure, phenotype, ethnicity, or environment. Despite these difficulties, replication remains an important part of the confirmation of the relevance of unidentified association. Unfortunately, the number of groups working on chronic sinusitis is limited; thus, the number of replicated genes for the moment remains few.

Genetic Association Studies and Chronic Rhinosinusitis: What Is the Evidence?

In this section, we present and summarize the results from genetic association studies conducted to date on the different phenotypes of CRS. The individual results are presented in Table 1, reported as results from candidate gene or pathway studies, and published genes originating from a pooling-based GWAS.

Candidate Gene/Pathway Approach

The majority of work done to date has used the candidate gene or candidate pathway approach. The search has been limited primarily to mechanisms already identified as pertinent in chronic sinusitis, and has attempted principally to identify polymorphisms in genes for important factors or regulatory elements that are part of these mechanisms. To date, the focus of these efforts is principally on genes of innate immunity, the TLR pathway, and its regulatory elements or downstream products. Two of the polymorphisms identified—in *IL1A* [26] and *TNFA* [27]—have been replicated [27, 28], supporting their validity.

Taken individually, no single gene has emerged as uniquely causative of CRS. Although certain studies have reported high risk ratios, enthusiasm for these must be tempered by the small group size involved and the authors' reporting of a genotype-specific effect, rather than the allelic one commonly used. Second, very few of the SNPs identified have been in regions capable of altering amino acid sequences and secreted proteins. Nevertheless, a potentially interesting pattern does emerge. When the identified SNPs are modeled using Ingenuity (Redwood City, CA) pathway analysis software, a gene-centric network around the *TNF*, *IL1A*, and *NFκB* genes becomes evident, suggesting a central role of this network in the pathology (Fig. 1). This concept was recently validated in a paper by Rossin et al. [29], who demonstrated that for complex traits such as CRS, associated loci affect common molecular processes rather than distinct mechanisms for each association.

Table 1 Published significant gene associations in CRS

Gene ^a	SNP	Chromosome	Site	Population size (affected; controls)	Phenotype	Frequencies (case; control)	OR (95% CI) ^b	Nominal <i>P</i> value or <i>P</i> _{HWE}	Permutated <i>P</i> value	Replicated?	Reference (year)
Candidate gene approach											
<i>IL1A</i>	rs17651	2	Coding nonsynonymous	82; 106	Nasal polyposis			<0.001		Yes	Erbek et al. [27] (2007)
<i>IL1B</i>	rs16944	2	Promoter	82; 106	Nasal polyposis						
<i>TNFA</i>	rs361525	6	Promoter	82; 106	Nasal polyposis						
<i>TNFA</i>	rs1800629	6	Promoter	82; 106	Nasal polyposis					Yes	Karjalainen et al. [26] (2003)
<i>IL1A</i>	rs17651	2	Coding nonsynonymous	35; 210	Asthmatics with NP			0.005			
<i>IL33</i>	rs3939286	9	Unknown	284; 427	NP in AIA		1.53 (1.21–1.96)	0.00041			Buysschaert et al. [36*] (2010)
<i>IL1RN</i>	VNTR	2	Intron	88; 103	CRS			0.01			Cheng et al. [37] (2006)
<i>CACNG6</i>	rs192808	19	Intron	102; 429	NP in AIA	0.103; 0.045	2.88 (1.60–5.17)	0.0004	0.0029		Lee et al. [38] (2010)
<i>MMP9</i>	rs3918242	20	Promoter	203; 730	NP in AIA		1.62	0.023	0.022		Wang et al. [39] (2010)
<i>MMP9</i>	rs2274756	20	Coding	203; 730	Nasal polyposis			0.034			
<i>TNFA</i>	rs1800629	6	Promoter	97; 95	Nasal polyposis	0.186; 0.063	3.68 (1.27–10.7)	0.015			Batikhan et al. [40] (2010)
<i>IL10</i>	rs1800870	1	Promoter	498; 448	NP in AIA			0.007	0.21		Kim et al. [41] (2009)
<i>IL1B</i>	rs3087258	2	Near gene 5'	179; 153	CRS with NP			0.001			Bernstein et al. [42] (2009)
<i>IL10</i>	rs1800894	1	Near gene 5'	179; 153	CRS with NP			0.009			
<i>TNFA</i>	rs1800629	6	Promoter	179; 153	CRS with NP		1.86 (1.14–3.09)	0.01			
<i>TGFB1</i>	rs1800469	19	Promoter	203; 456	NP in AIA			0.001			Kim et al. [43] (2007)
<i>TNFAIP3</i>	rs3757173	6	Intron	206; 196	Refractory CRS	0.079; 0.125	1.67	0.0392			Cormier et al. [44] (2009)
<i>TNFAIP3</i>	rs5029938	6	Intron	206; 196	Refractory CRS	0.051; 0.095	1.95	0.0192			
<i>IL22RA1</i>	rs4292900	1	Flanking 5'UTR	206; 196	Refractory CRS	0.37; 0.25	1.75 (1.27–2.42)	0.0006			Mfuna-Endam et al. [45] (2009)
<i>IL22RA1</i>	rs4648936	1	Intron	206; 196	Refractory CRS	0.36; 0.25	1.71 (1.23–2.37)	0.0011			
<i>IL22RA1</i>	rs16829225	1	Near gene 5'	206; 196	Refractory CRS	0.19; 0.10	1.97 (1.28–3.05)	0.0014			
<i>IL22RA1</i>	rs10751768	1	Intron	206; 196	Refractory CRS	0.34; 0.45	0.64 (0.47–0.86)	0.0036			
<i>IL22RA1</i>	rs10794665	1	Near gene 3'	206; 196	Refractory CRS	0.36; 0.45	0.69 (0.51–0.93)	0.0144			
<i>IL22RA1</i>	rs11579657	1	Unknown	206; 196	Refractory CRS	0.20; 0.27	0.66 (0.46–0.93)	0.017			
<i>IL22RA1</i>	rs3936073	1	Unknown	206; 196	Refractory CRS	0.13; 0.20	0.63 (0.42–0.95)	0.0263			
<i>IL22RA1</i>	rs7418238	1	Intron	206; 196	Refractory CRS	0.16; 0.10	1.63 (1.03–2.56)	0.031			
<i>IL1A</i>	rs17561	2	Coding nonsynonymous	206; 196	Refractory CRS	0.31; 0.24	1.48 (1.06–2.05)	0.02		Yes	Mfuna-Endam et al. [28] (2010)
<i>IL1A</i>	rs1800587	2	UTR 5'	206; 196	Refractory CRS	0.32; 0.24	1.49 (1.08–2.07)	0.02			
<i>IL1A</i>	rs2048874	2	Near gene 5'	206; 196	Refractory CRS	0.10; 0.16	0.57 (0.37–0.89)	0.01			
<i>IL1A</i>	rs2856838	2	Intron	206; 196	Refractory CRS	0.35; 0.46	0.63 (0.47–0.85)	0.003			
<i>MET1</i>	rs38850	7	Intron	206; 196	Refractory CRS	0.37; 0.26	1.65 (1.18–2.32)	0.01	0.06		Castano et al. [46] (2010)
<i>IRAK-4</i>	rs4251580	12	Intron	206; 196	Refractory CRS	0.134; 0.199		0.0159			Tewfik et al. [47] (2009)
<i>IL1RL1</i>	rs10204137	2	Coding nonsynonymous	206; 196	Refractory CRS	0.32; 0.40	0.71 (0.51–0.97)	0.04	0.05		Castano et al. [48] (2009)
<i>IL1RL1</i>	rs10208293	2	Intron	206; 196	Refractory CRS	0.24; 0.31	0.69 (0.50–0.96)	0.03	0.03		

Table 1 (continued)

Gene ^a	SNP	Chromosome	Site	Population size (affected; controls)	Phenotype	Frequencies (case; control)	OR (95% CI) ^b	Nominal <i>P</i> value or <i>P</i> _{HWE}	Permuted <i>P</i> value	Replicated?	Reference (year)
<i>IL1RL1</i>	rs13431828	2	UTR 5'	206; 196	Refractory CRS	0.10; 0.17	0.56 (0.36–0.86)	0.008	0.008		
<i>IL1RL1</i>	rs2160203	2	UTR 3'	206; 196	Refractory CRS	0.19; 0.26	0.67 (0.47–0.96)	0.03	0.03		
<i>IL1RL1</i>	rs4988957	2	Coding synonymous	206; 196	Refractory CRS	0.32; 0.40	0.72 (0.53–0.97)	0.03	0.04		
<i>NOS1</i>	rs1483757	12	Intron	206; 196	Refractory CRS	0.393; 0.510	0.62 (0.46–0.85)	0.0023			
<i>NOS1</i>	rs9658281	12	Intron	206; 196	Refractory CRS	0.285; 0.353	0.66 (0.48–0.92)	0.0129			Zhang et al. [49] (2011, in press)
<i>NOS1AP</i>	rs4657164	1	Intron	206; 196	Refractory CRS	0.195; 0.128	1.66 (1.09–2.53)	0.0178			
<i>ALOX5AP</i>	rs17612127	13	Unknown	206; 196	Refractory CRS	0.025; 0.059	1.45	0.046	NS		Al-Shemari et al. [50] (2008)
<i>ALOX5</i>	rs3780894	10	Intron	206; 196	Refractory CRS	0.203; 0.139	1.67	0.019	NS		
<i>CYS1TRI</i>	rs321090	X	Intron	206; 196	Refractory CRS	0.195; 0.281	0.69	0.014	NS		
<i>TP73</i>	rs3765731	1	Intron	206; 196	Refractory CRS	0.2688; 0.3601	0.65 (0.47–0.89)	0.0081	0.0104		Tournas et al. [33] (2010)
From pGWAS											
<i>LAMA2</i>	rs2571584	6	Intron	206; 196	Refractory CRS	0.20; 0.37	0.43	0.000006			
<i>PARS2</i>	rs2873551	1	UTR 3'	206; 196	Refractory CRS	0.34; 0.51	0.49	0.000026			Bosse et al. [30••] (2009)
<i>NAV3</i>	rs1726427	12	Intron	206; 196	Refractory CRS	0.38; 0.23	2.08	0.000067			
<i>LAMB1</i>	rs4727695	7	Intron	206; 196	Refractory CRS	0.06; 0.16	0.35	0.000085			
<i>CACNA1I</i>	rs3788568	22	Intron	206; 196	Refractory CRS	0.31; 0.47	0.52	0.000118			
<i>KIAA1456</i>	rs11779957	8	Intron	206; 196	Refractory CRS	0.31; 0.17	2.12	0.000184			
<i>MUSK</i>	rs10817091	9	Intron	206; 196	Refractory CRS	0.37; 0.52	0.54	0.000218			
<i>TRIP12</i>	rs1035833	2	Intron	206; 196	Refractory CRS	0.21; 0.34	0.51	0.000230			
<i>AOAH</i>	rs4504543	7	Intron	206; 196	Refractory CRS	0.26; 0.40	0.52	0.000266		Yes	
<i>MSRA</i>	rs7001821	8	Intron	206; 196	Refractory CRS	0.44; 0.30	1.88	0.000296			
CRS in Chinese individuals											
<i>AOAH</i>	rs4504543	7	Intron	341; 328	CHB CRS without NP	0.13; 0.06	0.3	8.11×10^{-11}			Zhang et al. [31•] (2011, in press)
<i>IRAK-4</i>	rs4251431	12	Intron	323; 328	CHB CRS with NP	0.07; 0.10	0.61	0.03			
<i>IRAK-4</i>	rs1461567	12	Intron	323; 328	CHB CRS with NP	0.47; 0.54	0.77	0.03			
<i>IRAK-4</i>	rs3794262	12	Intron	323; 328	CHB CRS with NP	0.13; 0.18	0.67	0.02			
<i>IRAK-4</i>	rs4251559	12	Unknown	323; 328	CHB CRS with NP	0.44; 0.51	0.76	0.03			

^a Genes are identified by gene symbol as per HUGO nomenclature^b OR is presented in relationship to allele—rather than genotypic—values, unless otherwise stated

A1A—aspirin-induced asthma; CHB—chronic hepatitis B; CRS—chronic rhinosinusitis; HUGO—Human Genome Organisation; NCBI—National Center for Biotechnology Information; NP—nasal polyps; NS—not significant; OR—odds ratio; pGWAS—Post-Genome Wide Association Initiative; *P*_{HWE}—*P* value measuring significant deviation in number of heterozygotes from that expected under Hardy–Weinberg Equilibrium; SNP—single nucleotide polymorphism; UCSC—University of California, Santa Cruz; UTR—untranslated region; VNTR—variable number tandem repeat

(Location data from UCSC [<http://genome.ucsc.edu>] and NCBI build 36 [<http://www.pubmed.com>].)

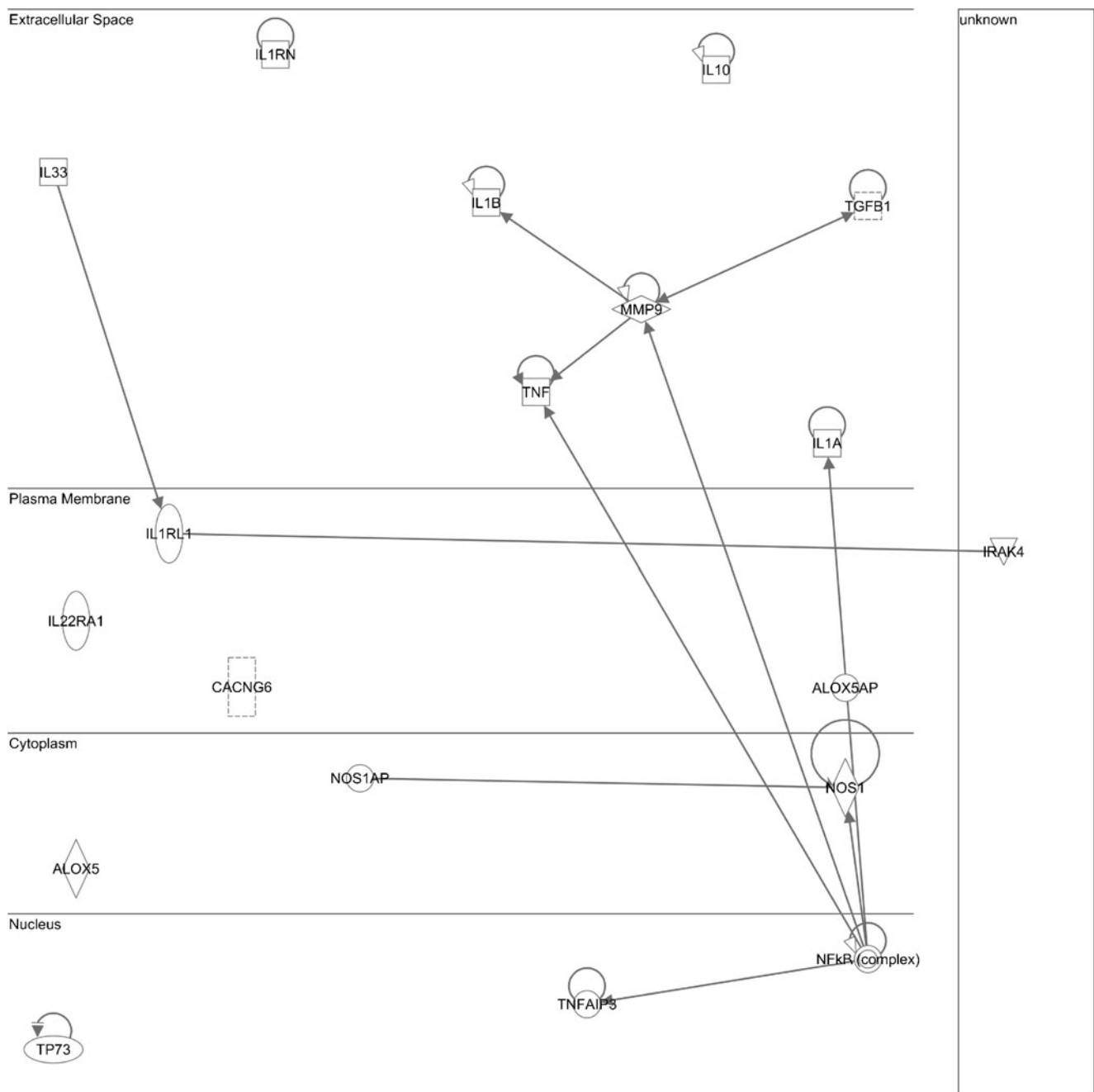


Fig 1 Ingenuity pathway analysis of identified single nucleotide polymorphisms (SNPs) in chronic rhinosinusitis. A gene-centric network around the *TNF*, *IL1A*, and *NFκB* genes can be seen, supporting their potential key role in this disorder. Although no

associations between SNPs in the *NFκB* gene and chronic rhinosinusitis have been described, it has been added to this illustration for clarity

Although we do not have direct documentation of the impact of these polymorphism functions, we note that polymorphisms within the pathway for innate immune recognition and regulation of response are numerous, suggesting the importance of this pathway in CRS. This supports previous descriptions of alterations in TLR expression and activity of downstream mediators in CRS. Clearly, this area warrants further study to identify the

functional impact of identified genetic polymorphisms in this pathway on CRS.

Genome-Wide Association Studies

Hypothesis-free, large-scale probing of the genome using genome-wide assessment of polymorphisms has been expected to yield new insights into disease mechanisms.

Although no true GWAS has been performed in CRS, pooling-based GWAS offer potential novel insights into mechanisms for development of disease via identification of novel genes.

A single pooling-based GWAS of CRS has been performed to date by our group and has identified polymorphisms in 400 genes, with a *P* value of greater than

0.05% [30••]. The top 10 reported associations are presented in Table 1. These genes suggest a potential role for interactions at the level of the basement membrane and extracellular matrix (laminin- α 2 [*LAMA2*] and laminin- β 1 [*LAMB1*]), mitochondrial function (prolyl-tRNA synthetase 2, mitochondrial [putative] [*PARS2*]), and lipopolysaccharide degradation (acyloxyacyl hydrolase [neutrophil]

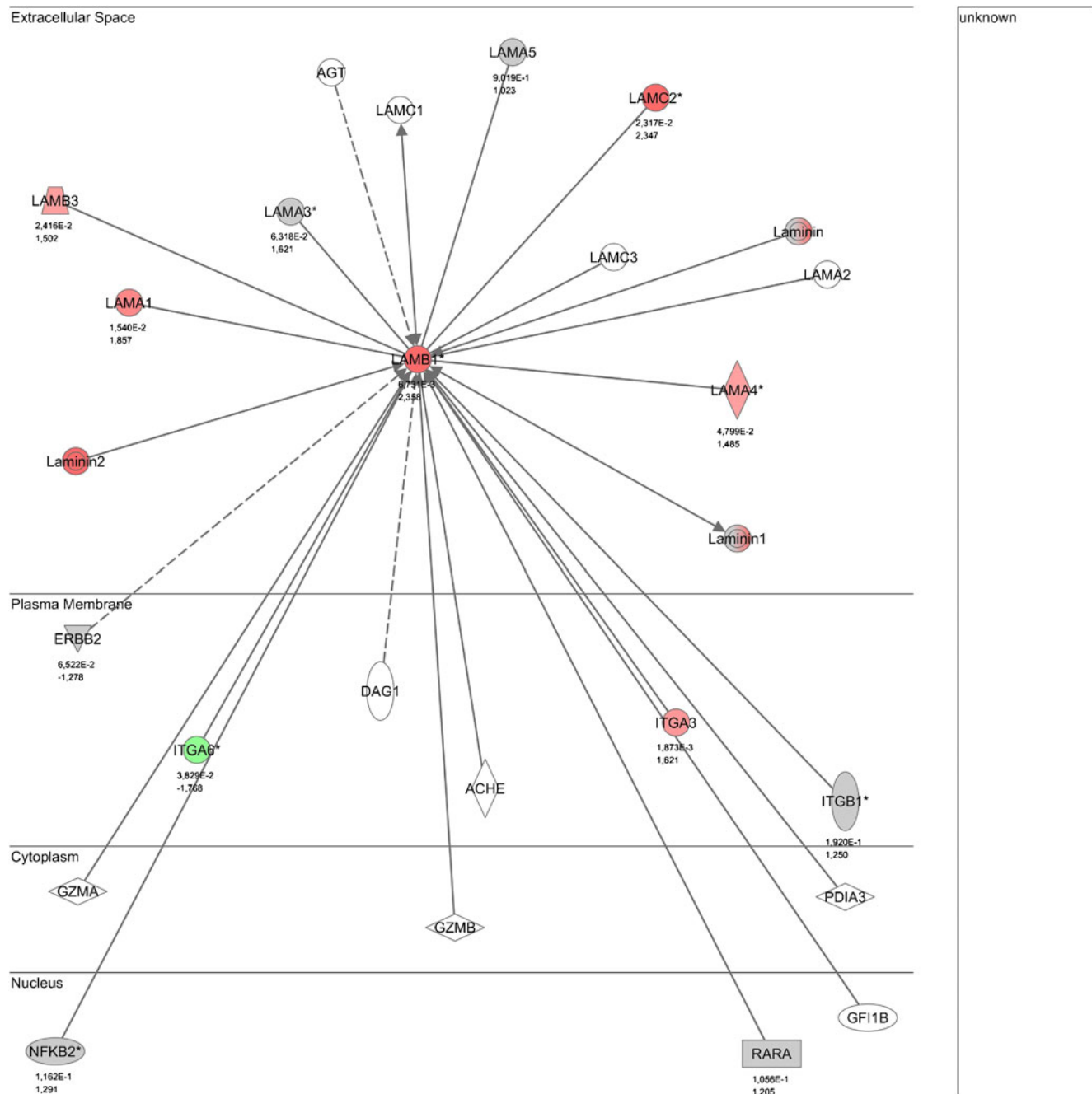


Fig 2 Ingenuity pathway analysis of gene expression results from epithelial cell cultures of 20 chronic rhinosinusitis (CRS) patients and 10 controls. Color indicates the expression level as compared with controls. Red shows upregulated genes in the CRS samples, and green shows downregulated genes. The first line under each gene indicates

that false-discovery rate (similar to a corrected *P* value), and the second line the fold change in expression. The *LAMB1* gene is upregulated in the individuals with CRS, as are several other related laminin genes. This suggests a potential role for the laminins in CRS and offers a base for a new avenue of research

[*AOAH*]). Confirmation for an association of *AOAH* with CRS is afforded via replication [31•]. In a Chinese population residing in China with CRS without nasal polyps, Zhang et al. [31•] replicated results for the same SNP in the *AOAH* gene. Interestingly, their study replicated with a much greater level of significance (8.11×10^{-11}) and a much higher risk (RR, 3.56) than the original study, which was a dramatic exception to the usual finding of a weaker association with lesser risk.

Additional genes identified by secondary analysis of our population according to severity also identified genes in *SERPINA1* [32] and *TP73* [33] as conferring an increased risk of severe, drug-resistant CRS. Direct impact on function has not been demonstrated, but nevertheless, two tantalizing observations are associated with these genes. A knockout mice model of *TP73* developed severe purulent rhinosinusitis, suggesting an important role for dysregulation of apoptosis in CRS [11]. Screening of a population with severe sinusitis identified low serum α -1-antitrypsin levels in a unexpectedly high percentage of CRS patients, again suggesting a role for this regulated α -1-antitrypsin metabolism [34]. However, homozygotes for the identified SNP did not show any genotype-specific alteration of level.

The identification of 2 genes of the extracellular matrix in our top 10 genes from the pGWAS (Post-Genome Wide Association Initiative) has motivated us to verify the potential role of extracellular matrix proteins in the pathogenesis of CRS. Differentiated primary epithelial cells from CRS patients were raised in an air-liquid interface and subjected to microarray analysis of gene expression. Interestingly, expression levels of *LAMB1* and of a related network of laminin genes were upregulated compared with those of controls, supporting a role for extracellular matrix genes in the development of this disorder (Fig. 2) [35•].

Conclusions

A genetic basis to CRS has long been suggested by the development of CRS in certain known genetic disorders and by family and twin data suggesting at least a modest hereditary basis to this disorder.

Genetic association studies offer the potential to identify the genetic basis of this complex disease. Although the complete picture of genetic contributions to susceptibility and development of this disorder remains to be articulated, studies reported to date have supported an implication for dysregulation of innate immune response in recognition, thus supporting the importance of environmental pathogens (eg, bacteria) in the development and persistence of this disorder. Pooling-based GWAS have also suggested new, promising pathways for the development of CRS that need to be exploited using conventional approaches and existing tools.

Although many potential pitfalls exist with the performance and interpretation of these studies, the number of genes identified and successful replication of findings nevertheless suggest that there is a future for this technique. The involvement of more groups with larger cohorts will help overcome current power considerations. However, we suspect that one of the greatest limitations to this technique remains the absence of an appropriate phenotype. Phenotyping individuals according to specific biomarkers related to underlying pathogenic mechanisms, such as molecular-based expression signatures, rather than current classification schemes based on clinical aspect may better differentiate patients and allow detection of the underlying genetic signal.

Additionally, the potential role of environmental modifiers (eg, bacterial pathogens) underlines the importance of better characterizing the bacteriology of CRS and the importance of a better understanding of potential interactions between bacteria and dysregulated mucosal immunity that are capable of causing disease or disease persistence.

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