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Rare Variants, Autoimmune Disease, and Arthritis

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Abstract

Purpose of review—We review select studies of newly discovered rare variants in autoimmune diseases with a focus on newly described monogenic disorders, rheumatoid arthritis, and systemic lupus erythematosus.

Recent findings—Two new monogenic syndromes of inflammatory arthritis were discovered using whole exome sequencing: the COPA syndrome due to rare mutations in *COPA* and Haploinsufficiency of A20 (HA20) resulting from rare mutations in *TNFAIP3*. Targeted exon sequencing identified rare variants in *IL2RA* and *IL2RB* associated with rheumatoid arthritis. Rare variants in *TREX1* and other genes associated with monogenic interferonopathies are also associated with systemic lupus erythematosus.

Summary—Rare genetic variants contribute to the heritability of autoimmunity and provide key insight into both novel and previously implicated immunological pathways that are disrupted in autoimmune diseases.

Keywords

autoimmune arthritis; rare genetic variants; missing heritability; next generation sequencing; monogenic disorders; rheumatoid arthritis; systemic lupus erythematosus

Introduction

Autoimmune rheumatic diseases are known to cluster in siblings and families and to be strongly influenced by genetics [1]. Genome wide association studies (GWAS) have been instrumental in advancing our understanding of the genetic architecture of autoimmune diseases and demonstrate that most result from a number of complex interactions between polygenetic factors and environmental triggers [2]. GWAS has identified hundreds of common (i.e. allele frequency >5%) single nucleotide polymorphisms (SNP) linked to autoimmune diseases at biologically relevant genomic locations that include immune cell enhancers or regulators of immune differentiation [3,4**]. Many of the SNPs are associated

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with more than one autoimmune disease suggesting shared immunological pathways that are disrupted as immune tolerance is broken [3].

Despite the success of GWAS in identifying key immunoregulatory regions important for autoimmunity, it has been challenging to functionally validate many variants [2], 90% of which are noncoding and likely to involve subtle effects on immune dysregulation [5*]. The majority of autoimmune disease SNPs carry a low genotype relative risk of disease (OR < 1.2) and account for only a small minority of disease heritability [2]. These findings have spurred research efforts to uncover the ‘missing heritability’ of autoimmune disease not captured through GWAS by employing the powerful genetic technologies and research tools that are rapidly being developed [6].

Whole exome sequencing (WES) and whole genome sequencing (WGS) have revolutionized the search for rare variants associated with autoimmune disease [3,6]. These next generation sequencing strategies have been especially successful for identifying highly penetrant rare variants that are causative for Mendelian diseases, including those that have never previously been implicated in disease [7**]. Studies have recently identified rare variants in *CTLA4* [8,9] and *TNFAIP3* [10**] in new monogenic inflammatory syndromes. Importantly, common polymorphisms identified through GWAS in *CTLA4* and *TNFAIP3* have previously been linked to polygenic autoimmune diseases [11–14]. Taken together, these findings suggest the existence of a variety of SNPs at these gene loci and others that exhibit a range of functional effects from subtle to severe which are important to disease pathogenesis [15]. It also highlights the potential for GWAS and NGS studies to serve as complementary approaches in the study of the genetics disease.

Rare genetic variants have provided important insight into the genetic basis of autoimmunity and allowed for the discovery biological pathways that are central to disease [2]. In this review we seek to highlight select studies that have reported rare variants associated with autoimmune arthritis, both in complex diseases and Mendelian disorders. While the overall contribution of rare variants to the missing heritability of common disease continues to be explored [6,16*], there have been exciting new developments in rare variant discovery that promise to further shape our understanding of the genetics and pathophysiology of autoimmune disease.

COPA syndrome

Two groups independently performed WES and targeted sequencing in five families presenting with a syndrome of inflammatory arthritis and lung disease to discover rare, missense variants in the *Coatomer subunit alpha (COPA)* gene [7**]. This autosomal dominant, variably penetrant disease is now known as the COPA syndrome (OMIM #616414). Sequencing analysis pinpointed four crippling mutations in the five families all within exons 8 and 9 of *COPA*. Patients presented at a young age (< 3 years) with clinical features that included inflammatory arthritis of the small and large joints (e.g. hands, knees), pulmonary hemorrhage, and interstitial lung disease. Over time all of the COPA syndrome patients developed a combination of lung disease and non-erosive arthritis. Patients harbored high-titer autoantibodies including anti-myeloperoxidase antibodies and anti-nuclear

antibodies and a subset of patients had immune-mediated kidney disease, suggesting that the COPA syndrome is an autoimmune disease.

COPA is part of a seven subunit protein complex called COPI that is important for the retrograde movement of proteins from the Golgi apparatus to the endoplasmic reticulum (ER) [17]. All of the *COPA* mutations were located within a 14 amino acid region of the highly conserved WD40 domain of the COPA protein [7**]. In vitro analyses demonstrated that *COPA* mutations impair the binding of dilysine-tagged proteins that are destined for return to the ER by the COPI complex. This defect in binding was hypothesized to result in impaired protein trafficking and in turn increased ER stress. Functional studies in antigen presenting cells from mutant COPA patients confirmed higher levels of ER stress and activation of the unfolded protein response. Mutant COPA patient cell lines also demonstrated higher levels of mRNA transcripts encoding for cytokines that promote the differentiation and expansion of Th17 cells, a CD4 T helper cell lineage implicated in autoimmunity. The peripheral blood mononuclear cells (PBMC) from patients with COPA mutations were found to have a significant elevation in Th17 cells, suggesting that Th17 cells may indeed be pathogenic similar to the proposed role of Th17 cells in mediating tissue damage in RA [18].

An intriguing aspect of the COPA syndrome is that the disease manifests as autoimmunity in the joints and lungs even though the COPA protein is ubiquitously expressed. There may be unique properties about these organs such as shared antigen targets or specific cell types that render these tissues particularly susceptible to disease. Animal models may be required to understand the organ specificity of the disorder and to define mechanisms linking the lung and joints in autoimmune inflammation. Such models may also provide insight into whether inflammatory arthritis can be initiated or propagated in the lung, as has been proposed for rheumatoid arthritis [19]. A fundamental question that remains unanswered is how defects in intracellular transport lead to a break in immune tolerance and clinical disease. Additional areas for further investigation include studies to determine whether mutations in *COPA* or within shared biological pathways are defective in disorders of autoimmunity beyond the COPA syndrome, including granulomatosis with polyangiitis or other types of inflammatory arthritis.

Haploinsufficiency of A20

Investigators recently reported a novel inflammatory syndrome of arthritis, orogenital ulcers and ocular inflammation that is due to highly penetrant loss of function variants in A20 (OMIM #616744) [10**]. A20, a protein encoded by the *TNFAIP3* gene, is a potent inhibitor of the NF-KB signaling pathway [20]. Activation of NF-KB is important for the expression of several proinflammatory genes and SNPs in *TNFAIP3* have been linked to a number of autoimmune disorders including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [11,12]. Mice deficient in A20 were shown several years ago to be hypersensitive to TNF and die from multiorgan inflammation and cachexia [21]. In five families reported to present with a dominant syndrome of arthritis, ocular inflammation and orogenital ulcers, WES and targeted sequencing identified five truncating mutations in the A20 protein [10**]. Several patients were successfully treated with TNF inhibitors, as might

be anticipated given the role of TNF in the mouse studies [21]. Because the A20 mutant patients shared phenotypic features with Behcet's disease, targeted Sanger sequencing in over 700 Turkish and Japanese patients with Behcet's disease was performed leading to the discovery of an additional patient with a frameshift mutation in *TNFAIP3*. Most of the patients identified were female and presented with disease from age 7 months to 16 years. Other less common clinical manifestations included gastrointestinal inflammation, pathergy, and skin rash [10**].

A20 is a ubiquitin-editing protein that negatively regulates NF-KB signaling [20]. Five of the six *A20* variants mapped to the ovarian tumor domain (OTU) that mediates deubiquitinase activity [10**]. The mutations were shown to lead to truncated proteins that experimentally appeared to undergo rapid degradation. The sixth *A20* mutation identified resides in the fourth zinc-finger (ZnF4), which is responsible for E3 ubiquitin ligase activity. Interestingly, prior studies using knock-in mice engineered with point mutations that disrupt the functional activity of either the ZnF4 domain or the OTU domain showed that mice had increased inflammatory responsiveness to TNF and sensitivity to colitis [22]. In patients with A20 mutations, PBMCs demonstrated a reduction in wild-type A20 protein [10**]. Functionally, mutant proteins were unable to significantly suppress TNF-induced NF-KB activity and there was evidence of enhanced signaling in multiple steps in the NF-KB signaling cascade. The sera from patients had elevated levels of several proinflammatory cytokines including TNF, IL-1B, IL-6, and IL-17, consistent with persistent NF-KB activation. The investigators additionally identified a possible role for mutant A20 in activation of the Nlrp3 inflammasome, independent of an effect on NF-KB. The discovery of the haploinsufficiency of A20 syndrome (HA20) provides confirmatory evidence that A20 has an important role in human disease. Furthermore, patients with A20 mutations provide human biological evidence in support of a functional role of the more common *TNFAIP3* variants identified in polygenic inflammatory diseases.

Rare variants in rheumatoid arthritis

Genome wide association studies have been extremely successful in identifying genetic variants associated with rheumatoid arthritis (RA) susceptibility, with over 100 susceptibility loci identified thus far [23]. A study using polygenic risk scores to model the genetic architecture of rheumatoid arthritis suggests that common genetic variants with weak effects account for a portion of the missing heritability in RA, but low-frequency and rare causal variants also contribute to the underlying genetic risk [24].

One of the earliest rare variant associations reported in RA focused on *SIAE* (sialic acid acetyltransferase), which removes the 9-O-acetylation that is commonly found on sialic acids. In mice, the acetylation state of sialic acid influences peripheral B-cell activation, and mice lacking *Siae* spontaneously develop autoantibodies. Surolia et al. examined the role of *SIAE* in human autoimmune disease by sequencing all 10 exons of *SIAE* in 923 individuals with autoimmune disease, including 234 individuals with rheumatoid arthritis, and 648 controls, which were all of European descent. Five non-synonymous mutations of *SIAE* were observed in 7 affected individuals, and 2 nonsynonymous mutations were observed in controls (cumulative OR 8.31, 95% CI 1.69–40.87, $p=0.0056$). Three of these mutations

were found to cause a > 50% decrease in catalytic activity and profound decrease in secretion, while a fourth mutation caused a decrease in secretion alone [25]. While these findings suggested that rare variants of *SIAE* were associated with rheumatoid arthritis, subsequent studies did not replicate these results. Hunt et al. examined the *SIAE* alleles identified in Surolia et al.'s study in 66,924 individuals of European descent, and did not identify an increase in these alleles in individuals with autoimmune disease compared to controls [26]. In addition, Zhang et al. sequenced the 10 exons of *SIAE* in 444 individuals with RA and 647 controls of Han Chinese descent. Four known and three novel SNPs were identified, but no significant difference in the frequency of these SNPs were observed [27]. Variants of *SIAE* are suggested to be associated with primary biliary cirrhosis [28]. Thus, the role of *SIAE* in rheumatoid arthritis and other autoimmune diseases remains unclear.

To investigate whether rare variants of previously identified RA susceptibility genes contribute to disease risk, Diogo et al. performed deep exon sequencing and large scale genotyping of 25 RA risk loci identified by GWAS in 500 RA cases and 650 controls of European descent. Gene burden tests were used to assess the association of the 184 novel rare variants (out of a total of 281 total SNPs identified) not previously identified in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>) or the 1000 Genomes Project (<http://www.1000genomes.org/>). *IL2RA* (four non-synonymous variants) and *IL2RB* (6 non-synonymous variants) showed nominal evidence of association ($p < 0.05$) using these tests, with OR 5.75 for *IL2RA* and 2.9 for *IL2RB* when compared to 4,300 controls from the Exome Sequencing Project (<https://esp.gs.washington.edu/drupal/>) [29]. In a second study, this group examined the *TYK2* locus on chromosome 19p13 that was previously associated with RA. Conditional analyses applied to dense genotype data generated by the Immunochip (7,222 RA cases and 15,870 controls of European descent), Exomechip (4,726 RA cases and 13,683 controls of European descent), and direct sequencing of coding sequences confirmed the independent association of 3 protein-altering variants of *TYK2*: P1104A (minor allele frequency [MAF] 0.069–0.086), A928V (MAF 0.026–0.040), and I684S (0.004–0.008) [30]. Of note, a similar approach was utilized by Bang et al. to identify rare coding variants associated with RA. For this study, exon sequencing of 398 candidate genes was performed for 1,217 RA patients and 717 controls of Korean descent, followed by meta-analyses using a RA GWAS dataset (4,799 cases/controls) and RA ImmunoChip dataset (4,722 cases/controls). No rare coding variants with large effects sizes were identified [31]. These studies indicate that while rare variants within associated loci identified by GWAS can contribute to RA disease risk, they are not observed for all RA risk loci.

Rare variants in systemic lupus erythematosus

Systemic lupus erythematosus (SLE, OMIM #152700) is known to have a strong genetic component, based on the results of twin [32] and familial aggregation studies (sibling risk ratio $\lambda_s = 8$ –29) [33]. GWAS focused on common SNPs with minor allele frequency > 5%, have identified over 40 susceptibility loci in populations of European and Asian descent [33–37]. The most strongly associated SNPs are located within the major histocompatibility complex (MHC) region. Strong non-MHC genetic associations include SNPs in *STAT4*, *ITGAM*, and *IRF5*. Rare variants associated SLE have been identified through recognition of rare clinical syndromes and sequencing of targeted (“candidate”) genes.

Complement deficiency in SLE

The earliest rare genetic variants associated with SLE were deficiencies of proteins of the complement cascade. Homozygous hereditary deficiency of each of the initial proteins in the classical pathway has been associated with an increased risk of SLE, and the risk varies depending on the protein affected (C1q>C4>C2) [38]. SLE affects > 75% of individuals with C1q deficiency (71 reported cases of C1q deficiency reported thus far) [39]. Twelve non-synonymous causative mutations within C1q have been identified, with each causing a frameshift, premature termination, or amino acid substitution [40]. Deficiency of other proteins in the C1 complex—C1r and C1s—is more rare than C1q, and the majority of affected individuals have SLE [41]. The C4 locus is highly polymorphic, with copy number variation of the two C4 isoforms (C4A and C4B). Over 70% of individuals with total C4 deficiency, due to null alleles of both C4A and C4B (<100 reported cases), have SLE. Of note, the C4A null allele is frequent in the populations of European descent (MAF ~0.15 in unaffected and 0.31 in affected individuals) and is considered one of the most common genetic risk factors for SLE [42]. However, this allele is in strong linkage disequilibrium with the SLE risk gene *HLA-DR3*, and the contribution of the C4A null allele alone has yet to be determined. SLE occurs in 10–33% of individuals with homozygous C2 deficiency, which occurs in 1:20,000 individuals of Western European descent.

How complement deficiencies of the classical pathway predispose to SLE has not been clearly elucidated. Possible mechanisms for complement deficiencies to contribute to the development of SLE include impaired handling of immune complexes and/or apoptotic cells, impaired normal B-cell tolerance, and impaired cytokine production [43].

Interferonopathies and SLE

Interferon alpha is a key cytokine which helps regulates the innate immune response to viral infections. In SLE patients, increased serum levels of interferon alpha and increased expression of interferon-inducible genes in peripheral blood cells have been identified (called the “interferon signature” in SLE) [44,45]. Genome-wide association studies have also identified genetic variants in interferon regulatory factors such as *IRF5* that are associated with disease susceptibility [35].

TREX1 (3' repair exonuclease 1) is a major mammalian intracellular DNase that degrades viral and aberrant cellular DNA and is hypothesized to be a negative regulator of the STING-dependent type 1 interferon response. Mutations in *TREX1* are known to cause Aicardi-Goutières syndrome, an autosomal recessive inflammatory disorder of the brain and skin, characterized by increased interferon alpha levels in the cerebrospinal fluid. Some patients with Aicardi-Goutières syndrome also produce anti-nuclear antibodies. Based on these and other observations, Lee-Kirsch et al. investigated whether *TREX1* mutations were also associated with SLE. In their study, 417 SLE cases and 1,712 controls from the United Kingdom and Germany underwent sequencing of the coding region in *TREX1*. Eleven different non-synonymous and one 3'UTR variant were identified in the SLE cases while only 2 non-synonymous variants were identified in the controls (RR 25.3, 95% CI 5.6–232.4, *p* 1.7E-07) [46]. A separate study utilizing exome sequencing of a 4 year old Lebanese girl with early-onset SLE identified a rare, homozygous mutation in *TREX1*

(R97H) not identified by Lee-Kirsch et al. This mutation of a highly conserved amino acid residue results in an exonuclease with approximate 20-fold reduction in ability to degrade single-stranded DNA. Lastly, additional rare mutations of *TREX1* have been identified in SLE patients of African and Asian descent [47]. The mutations in *TREX1* associated with SLE and Aicardi-Goutières syndrome are thought to result in inappropriate accumulation of nucleic acids which can then trigger type I interferon signaling.

Variants in other genes associated with Aicardi-Goutières syndrome are also associated with SLE. Aicardi-Goutières syndrome is also caused by bi-allelic mutations in RNase H2 (ribonuclease H2), which cleaves RNA/DNA heteroduplexes and is critical for the removal of ribonucleotides misincorporated into DNA during replication. Günther et al. sequenced the genes encoding the 3 subunits for RNase H2 (*RNASEH2A*, *RNASEH2B*, *RNASEH2C*), in 600 SLE patients and 1,056 controls. Eighteen nonsynonymous and splice-site rare variants were identified for each of the 3 subunit genes in the SLE cases (combined $p=0.0011$, OR 2.00, 95% CI 1.32–3.06). Seventeen of the variants impaired RNase H2 function by impairing enzyme activity, complex stability, or altering subcellular localization. SLE patient fibroblasts with these mutations were shown to have increased levels of ribonucleotides in genomic DNA, as well as an enhanced response to UV light irradiation, a known trigger of cutaneous SLE flares [48].

Conclusion

Rare genetic variants have provided key insight into immunological pathways that are disrupted in autoimmunity. Gene editing tools are accelerating our ability to interrogate these pathways through functional studies in primary human cells or animal models to understand their contributions to clinical disease. As genomic technologies advance and additional rare variants are uncovered, our comprehension of both the genetic architecture of autoimmune disease and the important defects in biological networks they cause will continue to be enhanced. Ultimately, these advances have the potential to empower us to develop genetic risk profiles and design targeted therapeutics as we move ever closer to personalized approaches to treating our patients based on the genetics of autoimmune disease.

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**of outstanding interest

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Key points

- Genome wide association studies and next generation sequencing studies are complementary approaches to understanding the genetic architecture of autoimmune disease.
- Rare genetic variants associated with disease can be identified by sequencing members of families with highly penetrant forms of autoimmune disease, or by sequencing susceptibility genes found through genome-wide association studies.
- Highly penetrant variants with large effects such as those implicated in Mendelian disorders of autoimmunity provide strong biological evidence for specific defects in disease pathogenesis.
- Large sample sizes will continue to be needed to identify rare genetic variants with weaker effects.
- Rare genetic variants provide important insight into specific immunological pathways that may be dysregulated in autoimmune disease.