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Strategies for Evaluating Idiopathic Inflammatory Myopathy Disease Susceptibility Genes

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Abstract

This review outlines the progress that has been made in understanding the genetics of the idiopathic inflammatory myopathies in the previous two years, with a particular focus on dermatomyositis and polymyositis. A recent genome wide association study in dermatomyositis has confirmed the importance of the major histocompatibility region in this disease, and suggested a shared genetic etiology with other autoimmune disorders. This has led to an ongoing study of additional immune-related loci using the Immunochip array. Candidate gene studies have identified novel risk associations in non-Europeans, such as STAT4 and HLA-DRB1 in the Japanese population. Evidence for gene-environment interactions has come from two recent studies implicating smoking status and statin use with HLA alleles. This review also touches on future approaches to genetic research in myositis, including bioinformatics tools to identify causal variants, HLA imputation from existing genetic data and statistical methods to investigate shared effects between subgroups of myositis.

Keywords

Myositis; Dermatomyositis; Idiopathic inflammatory myopathies; Genetics; Genome wide association study; Candidate gene; Gene-environment interaction

Introduction

The idiopathic inflammatory myopathies (IIM) broadly comprise dermatomyositis (DM), polymyositis (PM) and DM/PM overlapping with other connective tissue diseases. However, there is increasing evidence that IIM represent a growing spectrum of heterogeneous autoimmune conditions. There is ongoing debate regarding whether inclusion body myositis (IBM) represents part of this spectrum. Research into IIM has proved difficult because of their rarity. These conditions are characterized clinically by the presence of

isolated proximal muscle weakness, or weakness occurring in association with a number of extramuscular manifestations, either alone or in characteristic groups and of varying severity. The presence of these extramuscular features subdivides IIM into well characterized subsets which are predictable by knowledge of an individual patient's myositis specific/associated antibody status. Furthermore, an individual's human leukocyte antigen (HLA) genotype predicts which myositis specific/associated antibody, and hence which clinical phenotype will likely develop. IIM are thus complex genetic diseases initiated by immune activation following specific environmental trigger events in genetically predisposed individuals. To date, the strongest genetic associations in the IIM have consistently been shown to be within the major histocompatibility complex (MHC), although candidate gene studies have identified shared genetic susceptibility with other autoimmune diseases [1].

Over the last decade coordinated international efforts have been made to recruit sample cohorts of sufficient size to enable studies on more homogeneous sub-populations within the IIM spectrum, so as to try to identify further specific immunogenetic risk factors. This review discusses significant recent advances in our understanding of IIM genetics from genome wide, candidate gene and gene-environment interaction perspectives. This review also examines the current landscape of genetic research in other more common autoimmune diseases, and how this may relate to future genetic research studies in IIM.

Genome wide and targeted association studies show that dermatomyositis has common genetic etiology with other autoimmune diseases

Genome wide association studies (GWAS) have succeeded in identifying common genetic variation contributing to a large number of clinical diseases and normal physiological traits [2]. These studies have contributed greatly to our understanding of a number of autoimmune disorders, including rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), type 1 diabetes, systemic lupus erythematosus (SLE) and multiple sclerosis.

The first GWAS and the largest genetic association study thus far undertaken in IIM was reported in 1178 adult and juvenile dermatomyositis patients of European ancestry, recruited through the international Myositis Genetics Consortium (MYOGEN)[3]. This study confirmed involvement of the MHC region on chromosome 6, although no loci outside the MHC reached genome wide significance ($p < 5 \times 10^{-8}$). Additional analysis of 141 single nucleotide polymorphisms (SNPs) previously associated with RA, SLE, type-1 diabetes, Crohn's disease, thyroid disease, gluten-sensitive enteropathy and multiple sclerosis was undertaken to assess genetic overlap with other autoimmune disorders. These results identified three genes possibly associated with DM; phospholipase C-like 1 (PLCL1), B lymphoid tyrosine kinase (BLK), and chemokine (C-C motif) ligand 21 (CCL21), none of which had been reported previously in DM.

A recent study sought to extend these findings to identify novel genetic risk factors for adult and juvenile DM and PM, by investigating genetic variants associated with 10 autoimmune disorders and not examined in the aforementioned DM GWAS (Jani et al. 2014 – under review). Outside the MHC region, a non-synonymous amino-acid changing SNP in the

TYK2 gene was identified as a novel locus associated with DM and IIM overall. TYK2 encodes a member of the Janus tyrosine kinase protein family, is involved in cytokine receptor signaling, and has been associated with RA, JIA, SLE, type 1 diabetes and multiple sclerosis.

Collectively, these results suggest that DM and other autoimmune disorders share common genetic risk factors consistent with the familial clustering of multiple autoimmune diseases, and also indicate possible genetic heterogeneity between DM and PM. These studies support the analysis of additional immune-related loci in larger IIM cohorts using denser SNP genotyping arrays such as the Immunochip.

The Immunochip, a dense SNP genotyping array, has been widely used in several autoimmune disorders

The Immunochip is a custom-made Illumina array containing nearly 200,000 SNPs mapping to 186 loci previously genetically associated with 12 autoimmune diseases. The use of this array has led to high impact publications for several autoimmune disorders [4-6]. In addition to detection of novel or rare allele associations, the Immunochip allows more comprehensive mapping of genetic variation at immunobiologically relevant regions, localizing causal variants and associations to single genes or gene regulatory elements, and detecting multiple independent signals at established loci. The putative common genetic etiology of IIM with other autoimmune disorders has resulted in a concerted international effort to recruit further patients through the MYOGEN consortium, and genotyping is ongoing using the Immunochip platform. Additionally, the Immunochip also contains dense SNP coverage across the MHC region which has allowed accurate imputation of classical HLA alleles, SNPs and amino acids to identify novel associations and validate existing associations with different clinical subgroups and auto-antibody serotypes [6;7].

Candidate gene studies have identified novel associations in non-European populations

Prior to the advent of dense SNP genotyping arrays, most of the genetic studies carried out in IIM were focused candidate gene studies. In these studies, candidate genes or genetic regions of interest were selected from previous studies of IIM or other autoimmune diseases. Some of the most interesting recent candidate gene findings have emerged from non-European populations, such as from Japanese individuals with IIM.

HLA-DRB1 is identified as a risk locus for anti-MDA-5 positive dermatomyositis in the Japanese population

Interstitial Lung Disease (ILD) can be a life-threatening complication in myositis and is particularly associated with the presence of antibodies against amino-acyl transfer RNA synthetases (anti-aaRS). In the Japanese population however, a rapidly progressive form of ILD has also been associated with clinically amyopathic dermatomyositis (CADM) and serological specificity against melanoma differentiation-associated gene 5 (MDA-5) [8]. The association of anti-MDA-5 antibodies with HLA-DRB1 genotype has been studied in a

Japanese population of 17 individuals with anti-MDA-5 positive DM, where 94% carried either the HLA-DRB1*01:01 or HLA-DRB1*04:05 alleles [9]. This combined frequency was significantly higher than in individuals with myositis without ILD or anti-aaRS autoantibodies (odds ratio [OR] 42.7, 95% confidence interval [CI] 4.9-370.2, $p = 1.1 \times 10^{-5}$). The HLA-DRB1*04:05 allele has been previously associated with the presence of anti-aaRS in the Japanese population [10], whereas HLA-DRB1*03:01 is associated with anti-aaRS in Caucasian populations. The associated HLA-DRB1*01:01 and HLA-DRB1*04:05 alleles both contain part of the “shared epitope” amino acid sequence QRRRAA at positions 70-74 of the β 1 subunit and third hypervariable region of the HLA-DR molecule, suggesting these positions may be important in antigenic peptide presentation [11].

Polymorphism in STAT4, an established autoimmune locus, is associated with myositis in a Japanese population

The signal transducer and activator of transcription 4 (STAT4) gene is an established locus for several autoimmune diseases in different populations, including RA, SLE, systemic sclerosis, celiac disease and primary biliary cirrhosis[2]. In response to growth factors and cytokines, including interleukin-1, interleukin-23 and type-1 interferons (IFN- α and IFN- β), STAT4 acts as a transcription factor to drive the T-helper type 1 (Th1) and Th17 response [12]. These proinflammatory Th17 cells play an important role in autoimmune inflammatory diseases such as RA and SLE [13]. Activation by the type I interferon pathway is of particular interest in IIM as IFN- α and IFN- β inducible proteins and genes are highly over-expressed in DM muscle tissue, suggesting interferon α/β -mediated innate immune mechanisms in DM [14]. A STAT4 SNP, rs7574865, previously associated with autoimmune disease, was investigated for association with adult-onset PM and DM in the Japanese population [15]. This SNP was genotyped in 460 Japanese IIM patients (273 PM and 187 DM) and 683 controls. The rs7574865T allele conferred risk of PM and DM (OR 1.37, 95% CI 1.16-1.64, $p(\text{corr})=0.0012$), and with both the DM and PM subgroups independently. A gene dosage effect was also noted with an OR of 1.2 for carriers of one risk allele increasing to 2.0 for carriers of two risk alleles. This STAT4 polymorphism was also investigated for association to ILD in the first group of 138 patients with myositis, including 79 with ILD, and identified association of the rs7574865T allele to ILD compared to a control group (OR 1.59, CI 1.10–2.28, $p=0.039$). The contribution to disease risk may be higher in Asian populations compared to Caucasians due to the higher frequency of the risk allele in the Asian population. Population differences in effect size have been shown previously, for example with the RA risk loci PTPN22 and PADI4.

Gene-Environment Interactions have been shown to play a role in Myositis

The etiology of IIM is likely due to interplay of environmental factors in genetically susceptible individuals. An interesting concept of Autoimmune/inflammatory syndrome induced by adjuvants has been proposed, where as a result of environmental stimuli, autoimmune-like diseases are described in individuals often with particular HLA genotypes [16]. With respect to myositis, a putative environmental stimulus is currently unproven;

however two recent studies have implicated smoking status and statin use with HLA alleles as possible gene-environment interactors.

HLA class II and autoimmune mediated statin-induced myositis

Muscle symptoms resulting from statin use form a spectrum of severity ranging from mild myalgia to myositis and fulminant rhabdomyolysis. While statin-associated myopathy is commonly self-limiting, in a subset of patients, a rare autoimmune necrotizing myopathy occurs where muscle manifestations become self-sustaining even after cessation of the offending statin. Such patients experience a higher creatine kinase (CK) than polymyositis and muscle biopsies shows features of a necrotizing myopathy with macrophagic infiltration, a lesser degree of inflammatory T-cell infiltrates, and up-regulation of hydroxymethylglutaryl-coenzyme A reductase (HMGCR) on regenerating muscle fibres [17]. Circulating serum autoantibodies to HMGCR suggests that statin exposure is likely to play an important pathological role in the genesis of this condition [18]. HLA class I and II associations have been described where twenty-eight cases (20 US Caucasian, 8 African American) with anti-HMGCR positive statin-induced myositis associated autoimmune myopathy were broad HLA-typed and compared to a) a cohort with mild self-limiting statin intolerance identified from an interventional statin study and b) a larger control cohort [19]. The combination of HLA-DR11, DQA5, DQB7 alleles was significantly increased in anti-HMGCR positive Caucasian patients compared to controls (70% vs 17%; OR 11.7, 4.0-35.3, $p=4.1 \times 10^{-7}$). Each of these HLA phenotypes was also significantly associated with risk of anti-HMGCR status: DR11 (70% vs 18%; 10.4, OR 3.6-31.4, $p=1.2 \times 10^{-6}$), DQA5 (75% vs 40%; OR 4.5, 1.5-14.5, $p=0.0022$), DQB7 (75% vs 35%; OR 5.5, 1.8-17.6, $p=5.5 \times 10^{-4}$). Only the frequency of HLA-DR11 was significantly increased in the smaller African American cohort compared to controls (88% vs 21%; OR 26.4, 3.1-590.3, $p=0.0002$). It was therefore hypothesised that the HLA-DRB1*11 antigen confers risk for anti-HMGCR positive statin-induced myositis. None of the 51 subjects with statin-limited intolerance were anti-HMGCR antibody positive; a lower frequency of the HLA-DR11, DQA5, DQB7 combination was noted in this group compared to the anti-HMGCR antibody positive subjects (21 vs 70%, $p=5.4 \times 10^{-4}$). The previously reported rs4149056 C risk allele in the SLCO1B1 gene, strongly associated with statin myopathy (prevalence 0.54 in those developing statin myopathy) was also investigated in anti-HMGCR positive statin exposed subjects (frequency 0.14), but was not significantly different compared to control populations (0.14-0.22) [17]. These findings suggest a different mechanism underlying self-limiting statin sensitivity. Although the numbers are low and findings require replication, a gene-environment interaction is suggested for the development of anti-HMGCR associated myopathy via presentation of HMGCR-derived peptides by HLA-DRB1*11:01.

Interaction between HLA-DRB1 alleles and smoking for development of anti-Jo-1 antibodies

HLA-DRB1*03 is known to be a risk factor in IIM, and the association is enriched further in anti-Jo-1 positive subjects who often have concomitant ILD [20]. In RA, smoking is known to be a risk factor for the development of anti-CCP positive antibodies in patients with HLA alleles forming the shared epitope [21]. The Jo-1 antigen is preferentially expressed in lung tissue [22][23], which then lends the question whether an analogous gene-environmental

interaction takes place in anti-synthetase syndrome. This hypothesis was studied further in a multi-centre European study of 557 Caucasian IIM patients recruited with information on Jo-1 antibody status, HLA-DRB1 genotype and smoking history [19]. The frequency of smoking was increased in anti-Jo1 positive compared to negative cases (50% vs 36%; OR 1.83, 1.18-2.83, $p=0.004$). The frequency of HLA-DRB1*03 was increased in smokers compared to non-smokers, significantly so in the Hungarian cohort (59% vs 25%, OR 4.39, 95% CI 1.96 to 9.92, $p<0.00001$). Finally, an interaction between smoking status with HLA-DRB1*03 positivity was investigated. The frequency of anti-Jo-1 was increased in HLA-DRB1*03-positive smokers compared to HLA-DRB1*03-negative non-smokers (42% vs 8%; OR 7.75, 4.21 to 14.28, $p<0.0001$) and HLA-DRB1*03-positive non-smokers (42% vs. 31%; OR 1.61, 0.91-2.83, $p=0.08$). Thus, the data suggests that the risk of development of anti-Jo-1 antibodies is further increased in HLA-DRB1*03 positive smokers. The study requires replication and further exploration across other HLA alleles and myositis specific antibody subgroups.

Future approaches to IIM genetics; fine-mapping, sequencing and functional annotation

Since the first wave of GWAS in 2007 [24], effort has focused on both detecting further loci and refining associations to causal functional variants. Strategies to increase sample numbers through trans-ethnic meta-analyses, increase variants tested through imputation, and detect novel variants through large scale sequencing studies have continued to reveal significant associations. In 2010, there were 31 associated risk loci for RA [25]; by 2013 this had increased to 101 [7], 42 of which were reported in a recent trans-ethnic meta-analysis. Interestingly, most of these associations revealed similar allele frequencies and odds ratios between populations. This may be particularly useful in a rare disease such as myositis where combining Caucasian cohorts with other ethno-geographic cohorts may significantly increase the power of these studies.

Functional annotation to identify causal variants

Associations from GWAS frequently fall within non-coding regulatory regions that may influence gene expression. Large publicly available datasets exist to facilitate the interpretation of associated variants. Online expression QTL (eQTL) databases such as Genevar [26], RegulomeDB [27] and GTEx [28] catalogue these datasets and can be used to explore the effect of SNPs on gene expression. Datasets containing functional annotation from chromatin immunoprecipitation sequencing (ChIP-Seq), DNase hypersensitivity, and histone modification studies also help to predict functional consequences of a SNP, localise a signal or identify crucial cell types in a given disease [29]. This has led to increasing awareness and understanding of the complexity of gene expression and regulation, and the importance of spatial and temporal studies to fully elucidate the pathogenic mechanisms underlying disease. It will be important to utilise these bioinformatics databases to inform future functional studies in IIM.

HLA imputation/fine-mapping

The ability to impute HLA alleles from genotype data has allowed for large scale fine-mapping studies within the MHC region. With a 4-digit resolution accuracy of approximately 95% using Immunochip data, this is now a common and accepted method to localise disease associations to specific amino acids within classical HLA proteins [30]. This method has been used in many autoimmune diseases. Most successfully, it has localised the majority of the HLA association with anti-citrullinated protein antibody (ACPA) positive RA to five amino acids within three HLA proteins. These amino acids all fall within the peptide binding grooves of the proteins, and explain more of the disease association than with classical HLA associations. When teasing out HLA associations in IIM subgroups, clinical heterogeneity may be an issue as certain HLA alleles may be driving associations not related to the phenotype being investigated. A recent study in ACPA-positive RA developed a method to correct for these effects using known risk loci for potential confounding diseases as covariates [31]. This approach may be useful in myositis, where for example, a strong DR11 association with anti-HMGCR antibodies in statin myopathy patients may influence associations with polymyositis in a clinically heterogeneous cohort.

Next generation sequencing

The decreasing cost of sequencing has enabled more disease focused studies targeting coding regions. While revealing many de novo and very low frequency variants, they have rarely revealed rare coding variants with strong genetic effect. A recent study that sequenced 25 GWAS risk genes in 24,892 individuals comprising six autoimmune diseases indicates that the missing heritability for autoimmune diseases may not be rare variants in coding-regions [32].

Shared effects between autoimmune diseases

Investigating genetic overlap has been a focus for many diseases, but for autoimmunity in particular. The Immunochip consortium was set up specifically to address this. Overlap may be defined as the same variant in different diseases, different variants within the same gene/loci in the different diseases, or the same variant having the opposite effect in different diseases. Methods have been developed to estimate the genetic similarity between diseases [33-35] based on these definitions. Comparing associated loci is sensitive to issues such as varying power or heterogeneity between the two diseases you are comparing. For example, in an Immunochip study of autoimmune thyroid disease, five of six regions associated with Graves' disease were not associated with Hashimoto's thyroiditis. Even though there was a significant lack of power to detect these associations in Hashimoto's thyroiditis, it was shown to be more likely a genuine absence of association rather than lack of power [33;36]. Genetic overlap has been investigated between clinical phenotypes such as Crohn's-disease and ulcerative-colitis in inflammatory bowel disease and in serological subtypes such as ACPA positive and negative rheumatoid arthritis [5;37]. In a heterogeneous disease such as myositis it will be important to investigate the genetic differences between clinical subgroups and serological profiles.

Conclusions

Substantial genetic risk for IIM resides within the MHC. However, studies in other autoimmune diseases have shown that there are a significant number of genes outside this region potentially contributing risk. In IIM, while candidate gene studies have implicated a few loci, it is the large scale GWAS and ImmunoChip studies that will tell us more about the genetic architecture of IIM. Learning from approaches in other diseases and using available bioinformatics tools, we are able to look forward to the next stage in IIM genetics research to further understand these complex and heterogeneous diseases.

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