Polymyositis/Dermatomyositis

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5.1 Introduction

The idiopathic inflammatory myopathies (IIM) represent a group of autoimmune disorders, all of them characterized by weakness of proximal muscles but showing heterogeneous system manifestations, including skin rashes, interstitial lung disease, and malignancy [1]. According to the presence of extramuscular and histopathological features, IIM can be subdivided into different subsets, including dermatomyositis (DM),

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polymyositis (PM), inclusion body myositis (IBM), immune-mediated necrotizing myopathies (IMNM), and DM/PM overlapping with other connective tissue diseases, which are correlated with the presence of disease-specific autoantibodies [1].

Although IIM are rare diseases, they represent the most common cause of acquired muscle disease in adults. The incidence of myositis has been estimated in 50–100 cases per million [1]. The precise cause of IIM is still unclear, but as other autoimmune diseases, these disorders present a complex etiology in which exposure to environmental risk factors results in chronic immune activation in individuals with a predisposing genetic background along with other epigenetic mechanisms. This genetic basis is supported by the identification of increased risk of autoimmune diseases in first-degree relatives of patients with IIM and rare case reports of familial aggregation [2, 3].

In this chapter, we summarize the current knowledge about the genetic architecture of IIM, with a particular focus on DM and PM, as well as the recent progress in the elucidation of the epigenetic factors influencing their development.

5.2 Immunopathogenesis of DM/PM

The pathogenesis of DM/PM is not fully understood. Both immune and nonimmune processes have been implicated in the development of muscle damage. In addition, muscle weakness seems to be mediated by different mechanisms in the different subgroups of IIM.

On one hand, the complement cascade has been shown to play a crucial role in the DM pathogenesis. Specifically, it has been proposed that the disease begin when putative antibodies directed against endothelial cells activate the C5b-9 complement membrane attack complex (MAC). Once activated, MAC is deposited on the surface of endothelial cells leading to necrosis of these cells, capillary ischemia, and muscle-fiber destruction, which results in the characteristic perifascicular atrophy found in muscle biopsy of DM patients [4]. In addition, the complement activation induces the production of pro-inflammatory cytokines, chemokines, and adhesion molecules, which are responsible for the recruitment of activated lymphocytes, including CD4+ T cells, B cells, and plasmacytoid dendritic cells (pDCs), to the site of inflammation. Moreover, increasing evidences suggest a relevant role of pDCs in DM through the production of type 1 interferons (IFNs) [5]. On the other hand, CD8+ T cells are considered as the primary effector cells mediating muscle damage in PM. These lymphocytes can invade healthy non-necrotic muscle fibers that aberrantly express major histocompatibility complex (MHC) class I molecules and release perforin granules toward the surface of the muscle fibers causing necrosis [4].

Nonimmune mechanisms have also been implicated in the IIM pathogenesis. The overexpression of MHC class I occurring in muscle fibers of myositis patients seems to act as a potential inducer of endoplasmic reticulum (EM) stress, which results in nuclear transcription factor κ-beta (NF-κB) activation and the subsequent production of pro-inflammatory cytokines [6]. Additionally, two potent inducers of autophagy, Toll-like receptor (TLR) 3 and TLR4, were found significantly overexpressed in IIM [7]. Moreover, these receptors co-localized with LC3, an autophagy-related protein, in IIM patients, thus suggesting a role of the autophagy pathway in myositis [7].

5.3 Genetics of DM/PM

In the last years, two main approaches have been used to elucidate the genetic basis of these disorders, both of which involve genotyping of genetic variations, mainly single-nucleotide polymorphisms (SNPs), in cases and controls. Initially, the genetic component of IIM was explored by candidate-gene association studies, which examine a set of SNPs within *loci* selected on the basis of their biological functions (functional candidates) or their location within genomic regions previously implicated by association or linkage studies (positional candidates). In contrast to this strategy, the more recently developed genome-wide association studies (GWASs) investigate the entire genome in a hypothesis-free manner, thus revolutionizing the possibilities of identifying the genetic component of complex diseases [8]. Subsequently, the success of GWAS spurred the design of targeted genotyping platforms, such as the Immunochip. Immunochip is specifically designed for fine-mapping of established autoimmune-associated *loci*, with a particular focus on the human leukocyte antigen (HLA) region [9]. In the last years, the application of the GWAS and Immunochip strategies to the study of the genetic basis of IIM together with international collaborative efforts to gather larger cohorts of IIM patients has led to significant advances in the knowledge of the molecular mechanisms underlying these disorders.

5.3.1 HLA Associations

The HLA region, encompassing 7.6 Mb on chromosome 6p21, is the most gene dense region within the human genome, including genes involved in the immune response. Similarly to that observed for other autoimmune diseases, allelic variants within the HLA region represent the strongest and most consistent genetic associations for IIM.

During the last years, several studies have investigated the role of the HLA in the IIM susceptibility. As result of these studies, the 8.1 ancestral haplotype (8.1 AH) was identified as the highest risk factor within this region [10–12] (Table 5.1). 8.1 AH, formed by the combination of the alleles HLA-A*01:01, -C*07:01, -B*08:01, -DRB1*03:01, -DQA1*05:01, and -DQB1*02:01, represents an evolutionary highly conserved haplotype that contains hundreds of immunologically relevant genes. Nevertheless, the independent impact of alleles forming the 8.1 AH could not be fully elucidated, probably due to the strong linkage disequilibrium (LD) within this region.

In 2006, a large immunogenetic study of HLA allelic associations with particular myositis-specific autoantibody (MSA) and myositis-associated autoantibody (MAA) groups of IIM patients identified the 8.1 AH-associated alleles B*08:01 and

Table 5.1 Main risk HLA alleles for overall idiopathic inflammatory myopathies (IIM), dermatomyositis (DM), and polymyositis (PM) in different populations

Population	IIM	DM	PM	Study
European	8.1 AH (**DRB1*03:01** and **B*08:01**)	8.1 AH (**B*08:01,** AA_DQB1_57, DQB1*04:02)	8.1 AH (**DRB1*03:01** and **B*08:01**)	[10–12]
Chinese	–	DQA1*01:04 DRB1*07	–	[15]
Japanese	DRB1*08:03	–	DRB1*08:03	[16]
African American	A*68:02 **DQA1*05:01** **DRB1*03**	**DRB1*03:01** DQA1*06:01	**DQA1*05:01**	[17]

IIM idiopathic inflammatory myopathies, *DM* dermatomyositis, *PM* polymyositis, *AH* ancestral haplotype

HLA alleles included in the 8.1 AH are marked in bold. Independent signals within the HLA region are shown in brackets

DRB1*03:01 as the main HLA risk markers [11]. In addition, several HLA susceptibility factors appeared to be specifically associated with particular MSA and MAA serogroups [11].

More recently, two large-scale genetic analyses have thoroughly explored the role of the HLA in different subgroups of Caucasian IIM patients by imputation of classical HLA alleles and amino acids from SNP genotype data [10, 12]. In 2015, a GWAS performed on 1710 patients with DM, PM, and juvenile DM (JDM), and DM, JDM, or PM cases with anti-histidyl-tRNA synthetase (anti-Jo-1) autoantibodies confirmed the previously described association between the 8.1 AH and myositis [10]. However, the stratified analysis according to the different clinical phenotypes showed that the DRB1*03:01 allele was the strongest signal in DM and JDM patients, whereas the B*08:01 allele represented the main risk factor for PM and myositis with anti-Jo autoantibodies.

Interestingly, an Immunochip study published a year later, which included the larger cohort of IIM patients analyzed so far, specifically 2566 patients with DM, JDM, PM, and IBM, yielded contradictory results [12]. In this study, DRB1*03:01 and B*08:01 were identified as independent association signals in PM, whereas three independent signals, B*08:01, amino acid position 57 of DQβ1, and DQB1*04:02, were detected in the DM subgroup. Regarding the JDM subset, DRB1*03:01 was identified as the strongest association and the C*02:02 allele (which is not included in the 8.1 AH) showed an independent effect after conditioning on DRB1*03:01. On the other hand, in this Immunochip study, the association with DRB1*03:01 was presumably attributed to amino acid residues that are only present at this classical allele, asparagine at position 77 and arginine at position 74 [12]. Interestingly, position 74 is structurally involved in the peptide-binding groove of the HLA-DRβ molecule, and the presence of arginine has been reported to modify the three-dimensional structure of pocket 4 of the binding cleft in a way that probably enables presentation of autoantigens [13, 14].

Although 8.1 AH is a risk factor for IIM among individuals of European origin, such association has not been clearly established in other ethnic groups (Table 5.1).

A study performed in Chinese population reported a susceptibility effect of DQA1*01:04 and DRB1*07 and a protective effect of DRB1*03 in DM patients [15]. In Japanese IIM patients, the strongest signal within the HLA region corresponded to the DRB1*08:03 classical allele [16]. Finally, a study performed in African Americans evidenced both shared and distinct immunogenetic susceptibility factors between this ethnic group and European American patients [17]. However, these studies included small sample sizes and lacked replication cohorts. Therefore, studies on well-powered case-control sets are required to definitively confirm or discard these associations.

On the other hand, the class III region of the HLA complex includes genes encoding several components of the complement system (C4, C2, and factor B), which is involved in the muscle damage occurring in DM. Most human chromosomes carry two *C4* genes, *C4A* and *C4B*; however, the number of expressed *C4* genes can range from none to four. Remarkably, the 8.1 AH is associated with a single *C4B* and the absence of a *C4A* gene, which leads to a genetic defect of complement function. A candidate-gene study published in 2016 analyzed gene copy-numbers (GCNs) for total *C4*, *C4A*, *C4B*, and HLA-DRB1 genotypes in 105 JDM patients and 500 healthy controls, all of them from European origin [18]. They found an association of presence of HLA-DRB1*03:01 and deficiency of *C4A* with JDM. However, the co-existence of both DRB1*03:01 and *C4A* deficiency conferred higher risk than either individual risk factor. In addition, they also observed that JDM patients with *C4A* deficiency had higher levels of multiple serum muscle enzymes compared with controls [18]. These findings suggest that deficiency of *C4A* represents a risk factor for JDM.

5.3.2 Non-HLA Associations

5.3.2.1 Genome-Wide Association Studies in DM/PM Research

Four GWAS on IIM, two in European population and two in Asian population, have been published so far [10, 19–21]. The application of this approach to the study of the genetic background of IIM has led to the discovery of several novel risk *loci* for both DM and PM (Table 5.2). However, the impact of GWAS on deciphering the genetic component of IIM has not been as relevant as for other autoimmune diseases. This may be due to a relatively weaker genetic influence on IIM predisposition or, more likely, to a higher disease heterogeneity compared to other immune-mediated disorders.

In 2013, the International Myositis Genetics Consortium (MYOGEN) published the first GWAS on DM, including 1178 cases and 4724 controls of European origin [20]. This study confirmed the HLA region as the main genetic risk factor for DM but failed to identify genome-wide associations outside this region. However, the analysis of 141 genetic variants previously associated with autoimmune disorders, specifically rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes, Crohn's disease, thyroid disease, gluten-sensitive enteropathy, and multiple sclerosis, led to the identification of three new risk loci for DM, phospholipase

Table 5.2 Main non-HLA susceptibility factors for overall idiopathic inflammatory myopathies (IIM), dermatomyositis (DM), and polymyositis (PM)

Susceptibility locus	Chromosomic region	Phenotype	Population	Approach	Overlap with ADs	Study
YDJC/ UBE2L3	22q11.21	IIM	European	Immunochip	CeD, CD, JIA, MS, Ps, RA, SLE, UC	[12]
DGKQ	4p16.3	IIM	European	Immunochip	PBC, SJO	[12]
STAT4	2q32.2	IIM	European, Japanese	Immunochip, candidate-gene	CeD, CD, JIA, MS, PBC, RA, SLE, T1D, UC, SSc, SJO	[12, 40]
MGAT4A	2q11.2	IIM	European	Immunochip	–	[12]
PRR5L/ TRAF6	11p12	IIM	European	Immunochip	RA	[12]
CCL17	16q21	IIM	European	Immunochip	–	[12]
EOMES	3p24.1	IIM	European	Immunochip	MS, RA	[12]
CD28	2q33.2	IIM	European	Immunochip	CeD, RA, T1D	[12]
RPL31P10	12p13.31	IIM	European	Immunochip	–	[12]
BLK	8p23.1	DM/PM	European, Chinese, Japanese	GWAS, Immunochip, candidate-gene	RA, SLE, SSC, SJO	[12, 20, 23, 24]
CCL21	9p13.3	DM/PM	European, Chinese	GWAS, Candidate-gene	RA	[20, 25].
TNFAIP3	6q23.3	DM/PM	Chinese	Candidate-gene	CeD, CD, MS, PBC, Ps, RA, SLE, T1D, UC, SSc, SJO	[42]
IRF5	7q32.1	DM/PM	Chinese	Candidate-gene	CD, PBC, RA, SLE, UC, SSc, SJO	[42]
PLCL1	2q33.1	DM	European, Chinese	GWAS, Candidate-gene	CD, UC	[20, 22]
TYK2	19p13.2	DM	European	GWAS follow-up study	AS, CeD, CD, JIA, MS, PBC, Ps, RA, SLE, T1D, UC	[31]
CPN1	10q24.2	DM	Chinese	GWAS	–	[21]
PIP	7q34	DM	Chinese	GWAS	–	[21]

Table 5.2 (continued)

Susceptibility locus	Chromosomic region	Phenotype	Population	Approach	Overlap with ADs	Study
PTPN22	**1p13.2**	**PM**	**European**	**Candidate-gene, Immunochip**	**CD, JIA, RA, SLE, T1D, UC, SSc**	**[38, 12]**
LOC728073/ RPL38	17q25.1	PM	European	Immunochip	–	[12]
UBE3B/ MMAB	12q24.11	PM	European	Immunochip	–	[12]
NAB1	2q32.2	PM	European	Immunochip	PBC	[12]
IL18R1	2q12.1	PM	European	Immunochip	CeD, CD, PBC, UC	[12]
SLC26A1/ IDUA	4p16.3	PM	European	Immunochip	PBC	[12]
RGS1	1q31.2	PM	European	Immunochip	CeD, MS, T1D	[12]
ROPN1L/ ANKRD33B	5p15.2	DM+JDM	European	Immunochip	–	[12]
PTTG1/ ATP10B	5q33.3	DM+JDM	European	Immunochip	SLE, SJO	[12]
GSDMB	17q21.1	DM+JDM	European	Immunochip	CD, MS, PBC, RA, SLE, T1D, UC	[12]
WDFY4	10q11.23	CADM	Japanese	GWAS	RA, SLE	[19]

IIM idiopathic inflammatory myopathies, *DM* dermatomyositis, *PM* polymyositis, *JDM* juvenile dermatomyositis, *CADM* clinically amyopathic dermatomyositis, *GWAS* genome-wide association study, *ADs* autoimmune diseases, *AS* ankylosing spondylitis, *CeD* celiac disease, *CD* Crohn disease, *JIA* juvenile idiopathic arthritis, *MS* multiple sclerosis, *PBC* primary biliary cirrhosis, *Ps* psoriasis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *T1D* type 1 diabetes, *UC* ulcerative colitis, *SSc* systemic sclerosis, *SJO* Sjögren syndrome
Loci associated at the genome-wide significance level are indicated in bold

C like 1 (*PLCL1*), B lymphoid tyrosine kinase (*BLK*), and chemokine (C-C motif) ligand 21 (*CCL21*). Subsequently, candidate-gene studies performed in Asian population confirmed the association between *PLCL1* and DM [22] and described associations between *BLK* and *CCL21* and other myositis subgroups, specifically *BLK* was associated with DM and PM in Japanese and Chinese patients [23, 24], and *CCL21* was associated with PM in a Chinese cohort [25].

PLCL1 encodes a protein involved in an inositol phospholipid-based intracellular signaling cascade. Although its exact role in the pathogenesis of DM is not clear, this protein acts as a component in the phospho-dependent endocytosis process of GABA A receptor and regulates the turnover of receptors, thus contributing to the maintenance of the muscle tone [26]. *BLK* encodes a tyrosine kinase of the src family of proto-oncogenes which is involved in B-cell receptor signaling and B-cell development. Several lines of evidence support a key role of B lymphocytes in IIM, such as the presence of this cell type in DM skin and muscle biopsies [27], the existence of disease-specific autoantibodies [1], and the efficacy of anti-B-cell therapies [28]. Interestingly, the most strongly associated polymorphism in the genome-wide scan was in high LD with a genetic variant reported to downregulate both *BLK* mRNA and protein expression in naïve B cells [29]. Regarding *CCL21*, this gene encodes a protein that inhibits hemopoiesis and stimulates chemotaxis for thymocytes and activated T cells. In addition, this cytokine plays a role in mediating homing of lymphocytes to secondary lymphoid organs and acts as a high-affinity ligand for chemokine receptor 7 that is expressed on T and B cells [30].

Since this first GWAS revealed the existence of genetic overlap between DM and other autoimmune diseases, a follow-up study to test association between IIM (adult and juvenile DM and PM) and immune-related variants not captured through the DM GWAS was subsequently performed [31]. Using this candidate-gene association approach, tyrosine kinase 2 (*TYK2*) was identified as a new genetic risk *locus* for DM. *TYK2* encodes a member of the Janus kinases (JAKs) protein family with a crucial role in interleukin and IFN signaling [32].

In 2015, the MYOGEN Consortium published another GWAS including 1710 IIM cases and 4724 controls of European origin, which represented the first genome-wide scan in PM and myositis patients with anti-Jo-1 autoantibodies [10]. As mentioned above, this study confirmed the association between the 8.1 AH and IIM and identified specific HLA associations with the different IIM subgroups. Nevertheless, although the number of patients was increased with respect to the first GWAS, no genetic signals at the genome-wide level of significance were observed outside the HLA region.

Two large-scale genotyping studies in IIM patients of non-European origin have also been recently published. In 2016, a GWAS performed in 127 patients with DM and 1566 healthy controls from Han Chinese population identified two non-HLA signals that almost reached genome-wide significance [21]. These signals mapped on the prolactin-induced protein (*PIP*) and cytochrome P450 family 11 subfamily B member 1 (*CPN1*) *loci*. PIP has been shown to bind to CD4, a T-cell co-receptor molecule with a crucial role in activation of Th cells [33, 34], whereas the *CPN1* gene encodes a member of the cytochrome P450 superfamily of enzymes involved in de novo synthesis of glucocorticoids, essential regulators of T-cell development and function [35]. On the other hand, this GWAS failed to replicate previously reported IIM associations in Han Chinese population, including those described at the *PLCL1*, *BLK*, and *CCL21 loci*. However, these results should be taken with caution, considering the limited sample size of this study.

Finally, 576 IIM cases, including PM, DM, and clinically amyopathic DM (CADM), and 6270 controls from Japan were analyzed in a very recent GWAS by Kochi and colleagues [19]. No significant association signals for IIM, PM, or DM patients were found. However, when CADM patients were evaluated independently, an intronic variant within the WDFY family member 4 (*WDFY4*) gene appeared to be associated with this specific subgroup. *WDFY4* encodes a protein expressed in dendritic cells, neutrophils, B cells, and macrophages, and it has been previously identified as a susceptibility *locus* for RA [36] and SLE [37].

5.3.2.2 Findings from the Immunochip Approach

In 2016, the Immunochip platform was used to analyzed the largest cohort of IIM patients to date, including 2566 cases with DM, JDM, PM, and IBM and 15,651 controls of Caucasian descent [12]. Protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*) was identified as a genetic risk factor for IIM, representing the only association at genome-wide level of significance in myositis thus far. Although an association between *PTPN22* and IIM was detected in the overall analysis, stratification by clinical subgroups revealed that this signal was mainly driven by the PM subset. A role of this gene in PM predisposition was previously reported in a candidate-gene association study including 381 British patients with adult or juvenile IIM [38], which overlapped with part of the samples included in the Immunochip study. *PTPN22* encodes a tyrosine phosphatase known as LYP that is involved in several signaling pathways associated with the immune response, including the T-cell receptor (TCR) pathway and the humoral activity of B cells. The PM-associated SNP (rs2476601) has been implicated in several autoimmune diseases, representing the clearest example of common genetic risk factor in autoimmunity. This SNP is a functional variant that results in a non-synonymous arginine (R) to tryptophan (W) amino acid change at position 620. Although its functional consequences have not been clearly elucidated yet, it has been reported that humans carrying the rs2476601 risk allele displayed enhanced B lymphocyte autoreactivity, deregulated TCR signaling, and reduced capacity for TLR-induced type 1 IFN production [39].

Furthermore, nine suggestive associations within the *YDJC/UBE2L3*, *DGKQ*, *STAT4*, *MGAT4A*, *PRR5L/TRAF6*, *CCL17*, *EOMES*, *CD28*, and *RPL31P10 loci* were also identified through the Immunochip approach [12]. Notably, the association between *STAT4* (signal transducer and activator of transcription 4) and IIM was described in a previous candidate-gene association study performed in Japanese population [40], supporting the implication of this gene in the myositis susceptibility. The protein encoded by *STAT4* is a member of the STAT family of transcription factors, which is crucial for mediating responses to interleukin-12, interleukin-23, and type 1 IFNs and regulating the differentiation of T helper cells into the Th1 and Th17 lineages [41].

Finally, specific non-HLA associations with the PM and DM/JDM subgroups were identified for seven (*LOC728073/RPL38*, *UBE3B/MMAB*, *NAB1*, *BLK*, *IL18R1*, *SLC26A1/IDUA*, *RGS1*) and three (*ROPN1L/ANKRD33B*, *PTTG1/ATP10B*, *GSDMB*) *loci*, respectively [12]. Although these signals did not reach genome-wide significance, this finding points to the existence of genetic differences between clinical subgroups.

During the last years, polymorphisms at common susceptibility *loci* in autoimmunity, such as *TNFAIP3*, *IRF5*, *IFNG*, *IL1*, or *TNF*, have been implicated in the IIM susceptibility by candidate-gene association studies [42–44]. However, no SNPs within these regions showed evidence of association with IIM in the Immunochip study, even though this platform is designed for dense genotyping of immune-related loci. This seems to indicate that they could be false-positive associations, mainly taking into account the low statistical power of these genetic studies. However, considering that some of these signals, such as those located within the *TNFAIP3* and *IRF5 loci* [42], were detected in non-European population, they may represent population-specific associations, and, therefore, a role in the IIM susceptibility cannot be discarded.

5.4 Biological Insights from GWAS and Immunochip Associations

Over the last few years, the translation of GWAS/Immunochip findings into biological implications has been challenging, mainly due to the difficulty of identifying causal variants as well as by the fact that most of the disease-associated SNPs are located in noncoding regions. Nevertheless, substantial effort has been recently directed toward the functional characterization of the large amount of genetic data generated by large-scale scans. In this line, different in silico approaches have been developed to understand biological meaning behind GWAS and Immunochip findings. These methods are usually based on the search for overrepresentation of disease-associated genes in protein-protein interaction networks or specific biological pathways, as well as for overlapping of associated variants with regulatory elements, such as DNase I hypersensitive sites, histone modifications associated with active promoters/enhancers, transcription factor-binding sites (TFBSs), or expression quantitative trait *loci* (eQTL), among others.

A number of approaches have been conducted to characterize the biological functions connected to myositis susceptibility. A first application of network-based methods in the context of IIM was reported in 2016. Following the Immunochip study, Parkes and colleagues conducted protein-protein interaction (PPI) and pathway enrichment analyses using both proteins encoded by genes associated with IIM (significant and suggestive signals) and autoantibody targets, in order to identify molecular mechanisms involved in myositis [45]. PPI analysis identified several genes, including *TRAF6*, *HSPA1A/B*, *UBE3B*, and *PSMD3*, as potentially relevant for IIM. TRAF6 (TNF receptor-associated factor 6) acted as a hub protein interacting with both autoantibody targets and IIM-associated proteins. This protein is a member of the TNF receptor-associated factor (TRAF) protein family that is involved in the development, homeostasis, and/or activation of B cells, T cells, macrophages, and dendritic cells, as well as in the organogenesis of thymic and secondary lymphoid tissues [46]. In addition, TRAF6 function is crucial for both a proper activation of the immune response and the maintenance of immune tolerance [46]. Remarkably, a pivotal role of TRAF6 in homeostasis of satellite cells, a stem cell population responsible for myofiber regeneration upon injury, has been recently described [47]. On the other hand, the remaining four genes identified in the PPI analysis encode proteins related to the ubiquitin proteasome pathway (UPP). Furthermore, pathway enrichment analysis evidenced ubiquitination as a relevant mechanism in IIM. It has been proposed that ER stress activates downstream UPPs in myositis, thus activating NF-kB, which leads to upregulation of MHC class I, production of pro-inflammatory cytokines, and suppression of myoblast differentiation [6]. Consequently, UPP inhibition has been proposed as a potential therapeutic target for myositis [48].

On the other hand, Kochi and colleagues performed in silico analyses using both publicly available datasets and in vitro studies to examine the causal mechanism of the CADM-associated variant in the Japanese GWAS [19]. First, an eQTL analysis using data from the Geuvadis project showed that the *WDFY4* SNP acted as a splicing QTL for a truncated *WDFY4* isoform in lymphoblastoid B cells [19]. In addition, using trans-eQTL and weighted parametric gene set analyses, the authors determined that the associated variant correlated with an increase expression of NF-κB associated genes, such as *TYK2* and *PTPN6*. They also demonstrated that the truncated isoform interacted with several pattern recognition receptors, especially MDA5, thus increasing NF-κB activation and apoptosis [19]. This finding is consistent with the presence of anti-MDA5 autoantibodies characteristic of patients with CADM [49] and suggests a role of both inflammatory signals and apoptosis in the pathogenesis of this condition.

5.5 Epigenetics Studies in DM/PM

Although the etiology of DM/PM is still unknown, it has become clear that both genetic and environmental factors may interact in the pathophysiology of these disorders. Epigenetic mechanisms, which include DNA methylation, histone modifications, nucleosome positioning, and noncoding RNA regulation, among others, represent the link between environmental triggers and genetic modulation. In this regard, several epigenetic studies have been conducted in PM and DM so far.

Genome-wide methylation profile of muscle biopsies from 20 JDM patients and four controls identified 27 genes differentially methylated, which allowed to distinguish cases from controls using supervised hierarchical clustering [50]. The set of differentially methylated genes was enriched in transcription factors and cell cycle regulators, including homeobox and *WT1* genes. These results were subsequently confirmed by pyrosequencing in patients with JPM and other IIM. Considering these findings, Wang and colleagues proposed that children with IIM may have an enhanced ability to self-renewal of damaged muscles and that this repair process would be facilitated through epigenetic modifications of homeobox and *WT1* genes [50].

Moreover, increased levels of a group of microRNAs (miRNAs) known as myomiRs, including miR-1, miR-133a/b, miR-206, miR-208, miR-208b, miR-486, and miR-499, were found in skeletal and cardiac muscle [51]. These muscle-specific miRNAs regulate diverse aspects of muscle function, and, therefore, they could be involved in muscle diseases. Indeed, a high number of miRNAs have been found to be deregulated in IIM.

Specifically, using array technology, Eisenberg and colleagues identified a high number of miRNAs deregulated in ten major muscular disorders, including DM and PM [52]. Specifically, they detected 37 miRNAs upregulated and 1 downregulated in PM patients and 35 miRNAs upregulated and 2 downregulated in DM. The set of miRNAs deregulated in patients with DM was enriched in pathways related to the immune response [52]. In addition, five miRNAs, miR-146b, miR-155, miR-214, miR-221, and miR-222, were consistently deregulated across all the muscle diseases analyzed, indicating a common regulatory mechanism for all of them [52]. Remarkably, miR-146b and miR-155 play key roles in the immune system [53]. Moreover, these two miRNAs, together with miR-146a (also involved in innate immunity [53]), miR-21, miR-432, and miR-378, were also overexpressed in muscle biopsies from myositis patients in two subsequent studies [54, 55], supporting their role in IIM.

On the other hand, a decreased expression of several miRNAs known to be absolutely critical for adult muscle differentiation and maintenance, specifically miR-1, miR-133a, miR-133b, and miR-206, has been detected in muscle biopsy samples from patients with myositis [54, 55].

Finally, long noncoding RNAs (lncRNAs) have also been implicated in IIM. A transcriptomic profiling of lncRNAs performed in muscle tissue identified 1198 lncRNAs deregulated (322 upregulated and 876 downregulated) in DM patients compared with controls [56]. Then, by constructing mRNA-lncRNA co-expression networks, they predicted the target genes of the differentially expressed lncRNAs. Interestingly, a lncRNA named linc-DGCR6-1 appeared to target USP18, a type 1 IFN-inducible protein considered as a key regulator of IFN signaling. In addition, an upregulated expression of USP18 protein was observed in perifascicular atrophy myofibers of DM patients using immunohistochemistry staining [56].

5.6 Conclusions

The past decade has seen a huge advance in the identification of genetic factors that predispose individuals to complex diseases, largely thanks to the development of high-throughput genotyping platforms such as GWAS and Immunochip. IIM have benefited from that genome-wide era, which has led to the discovery of several consistent genetic risk *loci*. However, despite the international collaborative efforts carried out, genetic studies conducted in IIM were still underpowered to detect variants with moderate effects, and only genome-wide associations at the *HLA* and *PTPN22 loci* have been identified thus far. In addition, the existence of genetic heterogeneity among the different myositis subgroups has probably also been an obstacle to the identification of robust susceptibility signals. Therefore, further genetic studies in larger and more homogeneous cohorts would definitively improve the consistency of the results, thus providing information on the missing heritability of IIM. In addition, the identification of the functional implications and mechanisms of the associated genetic *loci* will be critical to translate genetic findings into medical practice.

On the other hand, recent studies have shown that epigenetic regulation also plays a pivotal role in the IIM pathogenesis; this seems to indicate that certain environmental factors would trigger epigenetic modifications in genetically susceptible individuals, who would develop myositis. Integration of genetics and epigenetics has emerged as a useful approach to unravel the mechanisms behind complex diseases. Thus, a better understanding of the interplay between both factors will allow to obtain a clearer picture of the molecular network involved in the IIM pathogenesis.

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