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ANTIPHOSPHOLIPID SYNDROME (S ZUILY, SECTION EDITOR)

Genetics of Antiphospholipid Syndrome

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Abstract

Purpose of Review Antiphospholipid syndrome (APS) is a rare heterogenous disorder associated with the presence of antiphospholipid antibodies and can present in a wide variety of clinical manifestations including thrombosis and pregnancy complications. Although the etiology of APS remains poorly understood, there is strong support for considering APS as a complex genetic disease in which multiple genetic risk factors, in conjunction with environmental factors, affect its onset, progression, and severity. Here, we provide a comprehensive review of the current knowledge of the genetic basis of APS, which remains in its infancy.

Recent Findings Most genetic studies to date in APS were performed in small cohorts of patients. As a result, only few genetic associations reported are convincing. Several reports suggested genetic associations with HLA class II alleles in APS, and only two genetic loci outside of the HLA region (*STAT4* and *C1D*) reached the threshold for genome-wide level of significance ($P < 5 \times 10^{-8}$). In this review, we also shed light on the genetic differences among the diverse clinical subsets of APS and briefly discuss the role that DNA methylation changes might play in the pathophysiology of this disease.

Summary The genetic basis of APS remains poorly characterized. Larger collaborative multicenter studies using well-characterized patients are needed to comprehensively understand the role of genetic susceptibility in APS.

Keywords Antiphospholipid syndrome · Genetic · Antiphospholipid antibodies · APS · Lupus

Introduction

Antiphospholipid syndrome (APS) is characterized by venous or arterial thromboses and/or obstetrical complications in the presence of antiphospholipid antibodies (aPL) [1]. The antibodies include lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-β2-glycoprotein 1 antibodies (anti-β2GPI). While deep venous thrombosis, primarily in the legs, is the most common feature, all vessels could be affected leading to a wide range of manifestations including central nervous system complications and nephropathy [2].

The diagnosis of APS is based on the presence of at least one of both the clinical features and the detection of aPL [3, 4].

Although the prevalence of APS is not completely known, data published to date suggest that the prevalence of aPL in the general population ranges between 1 and 5% and only a minority of these individuals develop APS. A recent study estimated that the prevalence of APS is around 50 cases per 100,000 individuals [5]. Patients with APS are usually classified into two main groups. When APS occurs as an isolated disorder it is called primary APS. However, APS also can arise as a secondary manifestation of an autoimmune condition, mainly systemic lupus erythematosus (SLE, or lupus). In this case, the disease is referred to as secondary APS. In less than 1% of APS patients, the disease can develop into catastrophic APS (CAPS) which is an accelerated form of the disease that can lead to massive thrombotic complications causing organ failure [6].

The etiopathogenesis of APS remains unclear but accumulating evidence strongly suggests the role of genetic predisposition in its development. Support for a genetic component in APS comes from the observation of familial clustering of cases [7–9]. A recent study in which family histories of 108 APS patients were retrospectively evaluated described that the prevalence of APS or other autoimmune diseases was more

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common in these families than in the general population [10]. In addition, monozygotic twin studies and several genetic associations with the disease and its clinical manifestations have been documented [11, 12].

In recent years, advances in genomics have led to a growing number of studies trying to decipher the genetic architecture of autoimmune diseases. The identification of genetic loci associated with the development of APS and its specific clinical features will help to better understand the pathophysiology of APS and promises to impact disease classification and management. In this review, we provide a comprehensive update on our current understanding of the genetic component of APS.

Genetic Studies in Antiphospholipid Syndrome

Genetic Susceptibility Within the HLA Region

In line with most immune-mediated diseases, the human leukocyte antigen (HLA) region was the earliest genetic association described with APS. Initial genetic studies performed in APS were mainly focused on this region; thus, multiple independent studies addressing HLA associations in different populations have been published to date.

It is currently well established that HLA class II genes are associated with the disease. However, a variety of genes and alleles have been reported, producing a myriad of complicated and confusing data. One explanation for these inconsistencies may be the wide heterogeneity among studies in regard to the methods used, type of study, and groups of the subjects analyzed. In Table 1, we have summarized the results obtained from case-control studies in which the enrolled patients have been diagnosed with APS. A variety of *DR* and *DQ* alleles have been identified in multiple studies from different populations [13–18, 20–22]. In addition, Sanchez and colleagues reported the association of *DMA*0102* allele in a British population [19]. Overall, the specific genes and HLA alleles associated with APS remain difficult to identify largely due to small sample sizes and low statistical power in most studies, and the complexity of the HLA region. In addition, differences among ethnicities also can influence the results. Further analysis with larger cohorts and using multiple populations to confirm and localize the genetic association between HLA class II alleles and APS are warranted. The ascertainment of the causal variant(s) within the HLA region is a major challenge since its genetic architecture is considered the most complex in the human genome. This region harbors multiple genes encoding proteins related to the immune system and presents extensive linkage disequilibrium patterns which makes it difficult to identify causal genetic variants. Current technologies which allow dense single nucleotide polymorphisms (SNPs) genotyping followed by imputation of the classical alleles and amino acid variants in the HLA region have helped to better understand genetic associations in the HLA region with multiple autoimmune diseases [23], although the contribution of regulatory variants within this region is much less understood.

Genetic Susceptibility in Non-HLA Genes

To date, multiple candidate gene association studies to unravel the complex genetic basis of APS have been published. These studies demonstrate that genes outside the HLA region could contribute to disease susceptibility, clinical heterogeneity, and autoantibody production in APS. However, few consistent genetic associations with this rare disease have been identified and confirmed. Thus, we detailed in Table 2 all the genetic loci that have been suggested to be associated with APS.

The first genetic risk factor for APS described outside the HLA region was a polymorphism leading to an amino acid change (valine to leucine) at position 247 of β2-glycoprotein I (β2GPI) [37, 38]. However, a total of twelve independent studies analyzing this variant in different populations have been performed showing inconsistent results. To clarify these findings, Lee and colleagues performed a meta-analysis including 1507 APS patients and 1450 healthy individuals. The results revealed a significant association of β2GPI Val/Leu247 polymorphism and APS, thrombosis, and the presence of anti-β2GPI antibodies [25•]. Remarkably, in functional studies, this genetic variant has been found to correlate with anti-β2GPI production and with a strong reactivity with anti-β2GPI in patients with primary APS [39], supporting the evidence that this polymorphism in the gene encoding β2GPI (*B2GP1* or *APOH*) might be a genetic risk factor for APS.

Genetic studies performed in multiple autoimmune diseases over the last decade have revealed an extensive number of genetic risk loci shared among different diseases. Given the close relationship between APS and lupus, it is not surprising that multiple lupus-associated loci have been explored as candidate genes in APS. The gene encoding signal transducer and activator of transcription-4 (*STAT4*) is considered a common genetic factor for multiple autoimmune diseases, including lupus and rheumatoid arthritis [40]. Furthermore, it has been demonstrated that genetic polymorphisms in *STAT4* are associated with APS [26, 34]. Although the specific role that the disease-associated variant (rs7574865) might play in the pathophysiology of APS is unclear, this variant has been shown to correlate with increased sensitivity to interferon-α (IFNα) [41]. The gene encoding B lymphocyte kinase (*BLK*) has been also consistently reported as a genetic risk locus for multiple immune-mediated diseases including lupus [42, 43] and recently primary APS [26]. The disease risk polymorphism is known to be associated with reduced expression of BLK. BLK is a kinase protein which plays a role in B cell development and B cell receptor signaling [44]. Over the past decade,

Table 1 Case-control studies reporting the associations within the HLA region with antiphospholipid syndrome

Reference	Patients (*n*)	Controls (*n*)	Population	Gene/allele associations
Asherson et al. [13]	13 APS	69	British	*DR53/DR4 (DQ7)*
Camps et al. [14]	19 PAPS	261	Spanish	*DR4/DR7/DR53*
Vargas-Alarcon et al. [15]	21 PAPS	100	Mexican	*DR5*
Arnett et al. [16]	48 PAPS 196 SLE (70 SAPS) 18 other connective tissue disease (4 SAPS)	393	White American Black American Mexican American	*DR4-DQB1*0302* *DRB1*1302-DQB1*0604/05*
Bertolaccini et al. [17]	82 APS (53 PAPS and 29 SAPS)	177	British	*DRB*04-DQA1*0301/2-DQB1*0301/4*
Caliz et al. [18]	83 APS (53 PAPS and 30 SAPS)	177	British	*DRB1*1302-DQA1*0102-DQB1*0604/5/6/7/9*
Sanchez et al. [19]	51 APS 82 SLE (42 SAPS)	109	British	*DMA*0102*
Freitas et al. [20]	34 APS 89 SLE (35 SAPS)	166	Brazilian	*DRB1*03* *DQB1*0302/DQB1*0604*
Al Attia et al. [21]	7 PAPS	604	Arab	*DR4/DR7/DQ3*
Sugiura-Ogasawara et al. [22•]	115 obstetric APS	419	Japanese	*DQB1*0302*

APS antiphospholipid syndrome, *PAPS* primary APS, *SAPS* secondary APS, *SLE* systemic lupus erythematosus

new insights into the role of B cells in the pathogenesis of APS have been revealed, and trials using B cell-targeted therapy in APS are ongoing [45–47].

One of the most robust and consistent genetic associations described for lupus is with *IRF5*, encoding interferon regulatory factor 5. Yin and colleagues reported a suggestive weak genetic association of *IRF5* in primary APS [26]. In a subsequent work in which more subjects were enrolled, the results showed a significant association between *IRF5* and primary APS [35•]. The gene encoding protein tyrosine phosphatase non-receptor, *PTPN22*, appears to be one of the most important genes in autoimmunity. Despite that the functional variant *PTPN22*R620W* has been repeatedly described as a common risk factor for multiple immune-mediated diseases, its role in APS is still controversial [48]. In 2011, Castro-Marrero and colleagues analyzed the association of this polymorphism with primary APS in a small cohort of patients from Spain. The results of this study proposed that *PTPN22* does not confer risk to primary APS [33]. In contrast, a study performed by Ostanek and colleagues described the association of this functional variant with secondary APS in a European population [32].

Finally, a recent article reported a de novo variant within *MTOR*, encoding mechanistic target of rapamycin kinase (mTOR), in a Spanish patient with primary APS. This novel variant leads to an amino acid change of an aspartate for a valine in position 2412 of the protein, and in silico analysis predicted that this SNP produces a gain of protein function [30]. mTOR is a phosphatidylinositol kinase which plays a crucial role in multiple metabolic pathways, and increased mTOR activity has been related to cardiovascular diseases and autoimmunity [49]. Furthermore, activation of the mTOR complex pathway has been described in vascular lesions associated with the APS [50]. Although this polymorphism has been described in only one patient, additional studies should be performed to elucidate the possible role of this locus in APS.

Genetic Studies in Clinical Subsets of Antiphospholipid Syndrome

Thrombosis and obstetric complications are the two hallmarks of APS. Despite advances in understanding the pathologic processes underlying this disorder, the ability to identify individuals at greatest risk of thrombosis and/or pregnancy complications remains challenging. In this section, we will summarize known genetic contributions to specific disease subsets within APS.

Thrombosis

Thrombosis is the most frequent clinical manifestation in APS patients [51]. Although the genetic factors leading to the variable vascular involvement in APS patients are not completely known, some candidate gene association studies addressing this issue have been published. Jimenez and colleagues analyzed if platelet receptors might be involved by comparing the genotype distribution of variants in genes encoding platelet glycoproteins (GP) Ib-α, Ia/IIa, and IIb/IIIa according to the presence or absence of arterial thrombosis in Spanish APS patients. The results showed a combined effect of two polymorphisms in *GP Ia* and *GP IIIa* genes [27]. An independent work performed in Turkish APS patients also reported the association of GP genes, specifically *GP Ia*, *GP IIIa*, and

Table 2 Genetic associations of genes outside of the HLA region with antiphospholipid syndrome

Locus	Location	Gene name	Phenotype	Approach	Population	*N* (case/control)	*P* value	OR (95% CI)	Index SNP	SNP function	Ref
ANXA5	4q27	Annexin A5	Obstetric APS	Candidate gene	Dutch	73/107	< 5.00E−02	2.7 (1.0–6.7)	− 1 C ->T	5′-UTR	[24]
B2GP1 (APOH1)	17q23–24	B2 glycoprotein I gene	APS	Meta-analysis	Multiple populations	1507/1450	1.00E−02	1.31 (1.06–1.62)	Position 247	Val247Leu	[25•]
BLK	8p23.1	BLK proto-oncogene, Scr family tyrosine kinase	PAPS	Candidate gene	Italian	169/552	1.24E−06	1.92 (1.47–2.53)	rs2736340	5′-UTR	[26]
C1D	2p14	C1D nuclear receptor corepressor	Obstetric APS	GWAS	Japanese	115/419	4.64E−08	6.20 (2.96–13.0)	rs79154414	Intergenic	[22•]
CDH18	5p14.3	Cadherin 18	Obstetric APS	GWAS	Japanese	115/419	6.00E−06	2.63 (1.69–4.0)	rs12153263	Intronic	[22•]
FRMD4A	10p13	FERM domain containing 4A	Obstetric APS	GWAS	Japanese	115/419	6.00E−07	3.26 (2.01–5.28)	rs12570849	Intronic	[22•]
GATA3	10p14	GATA binding protein 3	Obstetric APS	GWAS	Japanese	115/419	8.00E−07	3.39 (2.05–5.62)	rs1020096	Intergenic	[22•]
GP Ia (ITGA2)	5q11.2	Integrin subunit alpha 2	Thrombosis APS	Gene candidate	Spanish	36/95*	^5E−03	^4.84 (1.67–13.96)	rs1126643	Synonymous	[27]
			Thrombosis APS	Gene candidate	Turkish	30/30*	2.30E−02	20.33 (0.96–427.54)	rs1126643	Synonymous	[28]
GP Ib (GP1BA)	17p13.2	Glycoprotein Ib platelet subunit alpha	Thrombosis APS	Gene candidate	Turkish	10/30[$]	2.40E−03	13.14 (2.24–76.84)	Kozak variant		[28]
GP IIIa (ITGB3)	17q21.32	Integrin subunit beta 3	Thrombosis APS	Gene candidate	Spanish	36/95*	^5E−03	^4.84 (1.67–13.96)	rs5918	Leu59Pro	[27]
IRF5	7q32.1	Interferon regulatory factor 5	PAPS	Candidate gene	Italian	169/552	3.17E−04	3.66 (1.71–6.6)	rs2070197	3′-UTR	[26]
LDLR	19p13.2	Low-density lipoprotein receptor	Thrombosis APS	Gene candidate	Spanish	90/557	3.54E−02	2.35 (1.25–3.33)	rs12983082	Intronic	[29]
LINC00299	2p25.1	Long intergenic non-protein coding RNA 299	Obstetric APS	GWAS	Japanese	115/419	6.00E−06	2.63 (1.72–4.0)	rs181132	Intronic	[22•]
MRPS23	17q22	Mitochondrial ribosomal protein S23	Obstetric APS	GWAS	Japanese	115/419	3.00E−06	2.75 (1.78–4.24)	rs1443267	Intronic	[22•]
MTOR	1p36.22	Mechanistic target of rapamycin kinase	PAPS	Whole-exome sequencing	Spanish	1	NA	NA	c.7235A>T	Asp2412Val	[30]
NGF	1p13.2	Nerve growth factor	Obstetric APS	GWAS	Japanese	115/419	4.00E−06	5.97 (2.55–14.0)	rs145365907	Intergenic	[22]
PCSK9	1p32.3	Proprotein convertase subtilisin/kexin type 9	Thrombosis APS	Gene candidate	Spanish	90/557	3.36E−02	1.84 (1.28–2.66)	rs562556	Val474Ile	[29]
PROCR	20q11.22	Protein C receptor	Thrombosis APS	Gene candidate	Spanish	50/42[$]	3E−0.3	0.23 (0.08–0.65)	H1 haplotype		[31]
PTBP3	9q32	Polypyrimidine tract binding protein 3	Obstetric APS	GWAS	Japanese	115/419	8.00E−06	2.15 (1.53–3.03)	rs6477918	Intergenic	[22]
PTPN22	1p13.2	Protein tyrosine phosphatase non-receptor type 22	SAPS	Candidate gene	Polish Caucasian	135/201	4.85E−02	NA	rs2476601	Arg620Trp	[32]
			PAPS	Candidate gene	Spanish	81/81	> 5.00E−02	NA	rs2476601	Arg620Trp	[33]
PTPRO	12p12.3	Protein tyrosine phosphatase receptor type O	Obstetric APS	GWAS	Japanese	115/419	1.00E−06	3.03 (1.92–4.76)	rs1024843	Intronic	[22]
RGS10	10q26.11	Regulator of G protein signaling 10	Obstetric APS	GWAS	Japanese	115/419	5.00E−06	3.16 (1.90–5.27)	r10886503	3′-UTR	[22]
SH3BP4	2q37.2	SH3 domain binding protein 4	Obstetric APS	GWAS	Japanese	115/419	9.00E−06	2.57 (1.68–3.93)	rs35176804	Intronic	[22]

Table 2 (continued)

Locus	Location	Gene name	Phenotype	Approach	Population	*N* (case/control)	*P* value	OR (95% CI)	Index SNP	SNP function	Ref
STAT4	2q32.2--q32.3	Signal transducer and activator of transcription 4	APS	Candidate gene	Japanese	74/414	< 1.00E−03	1.60 (1.12–2.29)	rs7574865	Intronic	[34]
			PAPS	Candidate gene	Italian	169/552	4.22E−08	2.12 (1.62–2.81)	rs7574865	Intronic	[35•]
SYCP2L	6p24.2	Synaptonemal complex protein 2 like	Obstetric APS	GWAS	Japanese	115/419	1.00E−06	3.22 (1.97–5.26)	rs2788869	Intronic	[22•]
TLR4	9q33.1	Toll-like receptor 4	Thrombosis APS	Gene candidate	Caucasian	110/220	3.80E−02	NA	rs4986790	Asp299Gly	[36]
						110/220	3.80E−02	NA	rs4986791	Thr399Ile	[36]
TSHR	14q31.1	Thyroid-stimulating hormone receptor	Obstetric APS	GWAS	Japanese	115/419	7.85E−08	6.18 (2.95–13.0)	rs2288493	3′-UTR	[22•]

APS antiphospholipid syndrome, *NA* not applicable, *PAPS* primary APS, *SAPS* secondary APS

*N = APS patient with thrombosis/APS patients without thrombosis

$ N = APS patients with arterial thrombosis/APS patients without thrombosis

^Values correspond to the coexistence of GP Ia and GPIIa risk alleles

GP Ib, with the risk of thrombosis [28]. Although future studies with larger cohorts are necessary to confirm these associations, the results suggest that platelet glycoproteins warrant further investigation in APS.

Pierangeli and colleagues found that the frequencies of *TLR4* Asp299Gly and Thr399Ile, two genetic variants in linkage disequilibrium known to impair TLR4-mediated inflammatory response, were significantly lower in APS patients with thrombotic manifestations in comparison with healthy controls [36]. Furthermore, a report of a family with multiple members positive for anti-β2GPI supports the protective role of these same *TLR4* variants [52]. In line with these findings, a subsequent independent study showed that the expression of TLR4 mRNA was increased in PBMCs from APS patients as compared with healthy individuals [53].

A recent work performed by Ochoa and colleagues revealed the association of two genes involved in cholesterol metabolism, *LDLR* (*low-density lipoprotein receptor*) and *PCSK9* (*proprotein convertase subtilisin/kexin type 9*) with the susceptibility of developing thrombotic manifestations in individuals with positive aPL [29]. Furthermore, polymorphism in *protein C receptor* (*PROCR*) gene (haplotype H1) has been described as protective against arterial thrombosis in APS patients, with the protective effect possibly attributed to lower levels of the soluble form of endothelial protein C receptor in haplotype H1 carriers [31].

Obstetric Antiphospholipid Syndrome

In 2017, a genome-wide association study (GWAS) of obstetric APS was performed. This study in which 154 Japanese patients and 419 healthy controls were included, reported the association of two new loci involved in thyroid function [22]: *thyroid-stimulating hormone receptor* (*TSHR*) gene (odds ratio = 6.18, $P = 7.85 \times 10^{-8}$) and *C1D nuclear receptor corepressor* (*C1D*) gene (odds ratio = 6.20 , $P = 4.84 \times 10^{-8}$), encoding a DNA-binding and DNA-dependent protein kinase-activating protein [54]. It should be noted that small sample sizes in GWAS studies and therefore lower power can result in inflation of association odds ratios detected. C1D interacts with Rac3 protein and acts as a corepressor for the thyroid hormone receptor. These genes have been also linked to other autoimmune diseases. *TSHR* variants have been associated with Graves' disease and C1D has been identified as an autoantigen in patients with the polymyositis-scleroderma overlap syndrome [55, 56]. Although thyroid antibodies and hypothyroidism have been described previously in APS patients [57], further studies to decipher the role of these genes are needed. In addition, the results of this work also revealed suggestive evidence of association in 13 additional genomic regions: *NGF* (*nerve growth factor*), *SYCP2L* (*synaptonemal complex protein 2 like*), *HLA-DRA* (*major histocompatibility complex, class II, DR alpha*), *GATA3* (*GATA*

binding protein 3), *FRMD4A* (*FERM domain containing 4A*), *LINC00299* (*long intergenic non-protein coding RNA 299*), *SH3BP4* (*SH3 domain binding protein 4*), *CDH18* (*cadherin 18*), *SYCP2L* (*synaptonemal complex protein 2 like*), *PTBP3* (*polypyrimidine tract binding protein 3*), *RGS10* (*regulator of G protein signaling 10*), *RPS23* (*ribosomal protein S23*), and *PTPRO* (*protein tyrosine phosphatase receptor type O*) [22].

Candidate gene studies analyzing the association of annexin 5, a Ca2þ-dependent placental anticoagulant protein, with obstetric APS have been performed. The possible role of annexin 5 in the pathology of APS is still controversial. Laat and colleagues described in 2006 a polymorphism within the *annexin A45* (*ANXA5*) gene as an independent risk factor for miscarriage in APS [24]. In addition, common haplotypes within the promoter of *ANXA5* have been linked to fetal loss [58–60].

Catastrophic APS

It is currently unclear why some patients with APS develop catastrophic APS (CAPS). It is possible to speculate that a genetic predisposition might be involved. The genetic susceptibility to develop CAPS has not been assessed to date, and the rarity of this condition makes this an enormous challenge. Large multicenter collaborative efforts will be necessary to begin to understand the possible genetic basis of this life-threatening form of APS.

The Genetics of Antiphospholipid Antibody Production

Since the continuous presence of aPL is a requirement for the diagnosis of APS, understanding the genetic basis of aPL production might help to define the molecular mechanisms involved in APS.

To unravel the genetic bases of aPL production, Kamboh and colleagues performed a GWAS focused on identifying the susceptibility loci for the occurrence of the three main aPLs (LA, aCL, and anti-β2GPI) in a mixed cohort of lupus patients and healthy controls, with results adjusted for disease state (lupus). Although no SNP reached the threshold for genome-wide level of significance, several suggestive genomic regions were reported [61]. It is worth it to mention the suggestive association of the *B2GP1* locus with the presence of anti-β2GPI, and suggestive associations with multiple variants located within the HLA region, which is consistent with previous studies. In addition, other suggestive associated genes encode proteins involved in the immune response providing new candidate genes for further studies [61].

A second GWAS investigated the association between genetic polymorphisms and the presence of aPL in a population-based cohort of around 5000 individuals from Germany. The results of this GWAS showed two loci associated with the presence of anti-β2GPI surpassing the genome-wide level of

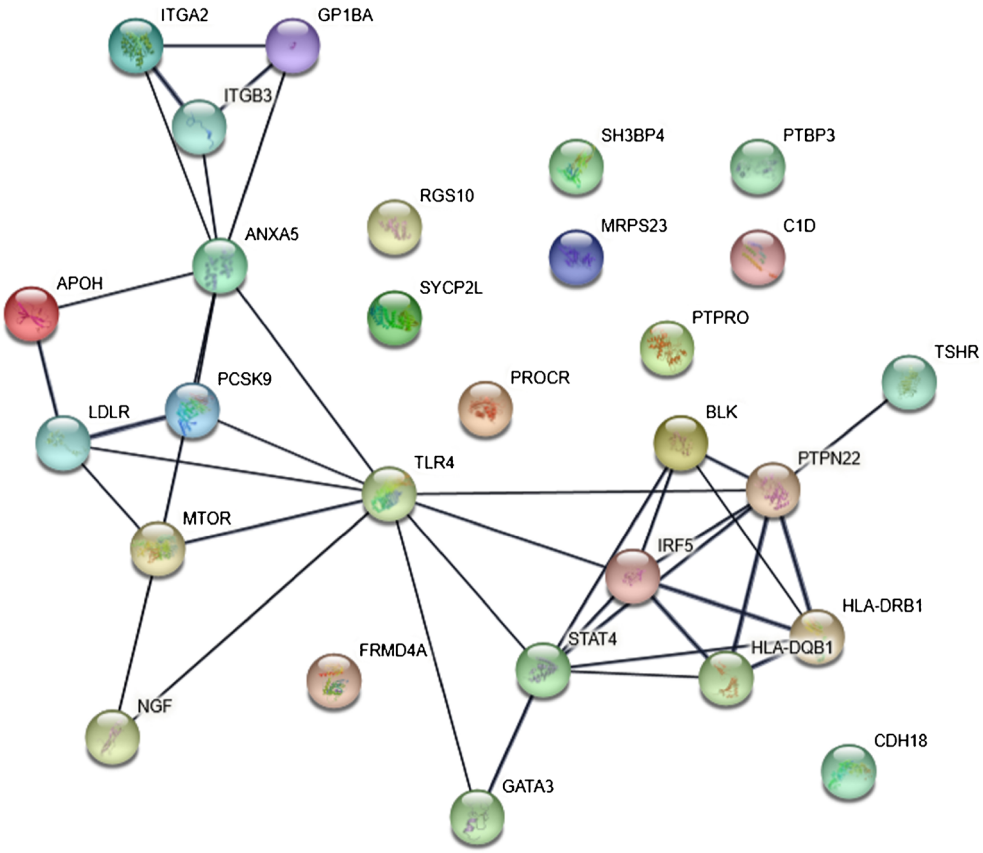

Fig. 1 Protein-protein interaction network of susceptibility genes reported to be associated with APS. The thickness of the lines represents the confidence of data supporting these interactions. The analysis was performed using STRING V10.0 (STRING https://string-db.org/)

significance: *B2GP1* (odds ratio = 1.79, $P = 1.4 \times 10^{-11}$) and *MACROD2* (odds ratio = 1.87, $P = 1.63 \times 10^{-9}$). It should be noted that the results did not show association of these two loci with LA or aCL, and that the associations with *B2GP1* and *MACROD2* were detected in a small set of 42 patients positive for anti-β2GPI IgG, and 40 patients positive for anti-β2GPI domain 1 IgG, respectively [62]. Interestingly, *MACROD2* locus was identified as a suggestive association with the presence of anti-β2GPI in the first GWAS [61]. This gene, located on chromosome 20, encodes a deacetylase involved in removing ADP-ribose from mono-ADP-ribosylated proteins. Although the mechanisms by which this gene could be involved in the pathogenesis of APS are still unknown, this gene has been described as a susceptibility factor for thyroid-associated orbitopathy in Graves' disease patients [63]. In addition to these two genome-wide significant associations, several other loci were described as suggestive associations with the presence of different antibodies [62].

DNA Methylation Studies

The study of epigenetics as a mechanism to elucidate the interplay between environmental factors and genetic susceptibility has contributed significantly to the current understanding of the pathogenesis of immune-mediated diseases. Indeed, the association of DNA methylation changes with lupus has been extensively reported, and it has been suggested that epigenetic variability might contribute to clinical heterogeneity in this disease [64]. Similarly, characterizing the DNA methylation profile of APS might help to understand the molecular mechanisms underlying disease pathophysiology and progression.

Weeding and colleagues performed a genome-wide DNA methylation analysis of neutrophils from patients with primary APS versus healthy individuals. A total of 42 differentially methylated sites, 17 hypomethylated, and 25 hypermethylated, were found. It is worth it to mention that several of these differentially methylated sites were located within the HLA region. The most hypomethylated gene in primary APS patients was *PTPN2*, which is a known genetic risk locus in multiple autoimmune diseases [65•]. A gene ontology analysis of hypomethylated loci in APS revealed significant association with pregnancy-related genes [65•]. These results are in concordance with the pathology of the disease given that pregnancy morbidity is one of the hallmarks of APS. Importantly, the methylation profile of neutrophils in primary APS patients appears to be distinct from that observed in lupus patients, with no differentially methylated sites shared between the two diseases [65•]. A recent candidate gene methylation study using whole blood samples revealed significant hypomethylation and hypermethylation in APS of methylation sites in *interleukin 8* (*IL8*) and *coagulation factor III* (*F3*) genes, respectively. Furthermore, hypomethylation of *IL8* was more robust in APS patients with a history of thrombotic events [66]. These recent findings suggest a potential role for methylation changes as diagnostic and prognostic tools in APS.

Conclusion

Recent insights into the genetic component of APS are promising to contribute to our knowledge and understanding of the possible molecular pathways involved in the development of this disease. In Fig. 1, the results of a protein-protein interaction analysis of the proteins encoded by the genes reported to be associated with APS (Table 2) are represented. Most of these genes are related to the immune response. Specifically, they are involved in antigen receptor-mediated signaling, interferon-gamma-mediated signaling, T cell receptor signaling, and regulation of B cell receptor signaling pathways. These results provide new attractive areas for further investigations. However, despite the advances achieved, adequately powered genome-wide association studies involving larger cohorts will be necessary to comprehensively uncover the genetic basis of APS and understand the pathophysiology underlying this rare disease. International collaborations and consortia will be warranted. In addition, efforts to decipher the genetic risk factors involved in the diverse clinical features of APS will help to identify patients at risk, and therefore improve the management, for the different manifestations of APS.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors

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