Chapter 4
Autoimmune Addison's Disease: Genetic Aetiology and Pathophysiology

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Introduction

Autoimmune Addison's disease (AAD) is a rare endocrine disorder with a reported prevalence of 100–210 cases per million in Caucasian populations [1–4]. Like other autoimmune diseases, it is more prevalent amongst women, with a female-to-male ratio of 1.3–3.5:1 [1], with the exception of individuals younger than 30 years of age where there is no gender difference [5]. AAD is most commonly diagnosed in individuals between their third and fifth decades of life. In European countries, the disease has a reported incidence of 4.7–6.2 per million per year. Both prevalence and incidence of AAD have been increasing in recent years raising the possibility that, as yet undefined, environmental factors may play a role in the pathophysiology of the disease [5, 6].

Historically, tuberculous adrenalitis was a frequent cause of primary adrenal insufficiency [7] and remains a problem in developing countries, but in recent decades, autoimmunity has become the commonest aetiology in developed countries [8–10], reflecting an increase generally in autoimmune conditions in the population. AAD results from destruction of the adrenal cortex by an aberrant immune response. It accounts for over 80% of cases [9–11]. Other causes of primary adrenal insufficiency can be categorised into three distinct groups: impaired steroidogenesis, defects in adrenal gland development and adrenal cortex destruction by other disease processes (Box 4.1).

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Box 4.1 Causes of primary adrenal insufficiency

Impaired steroidogenesis

- Congenital adrenal hyperplasia (*CYP21*, *CYP11B1*, *HSD3B2* and *CYP17* mutations)
- Familial glucocorticoid deficiency due to mutations in genes involved in DNA replication and mitochondrial redox homeostasis (*MCM4, NNT, TXNRD2, PRDX3, GPX1*) [12, 13]
- Smith-Lemli-Opitz syndrome (*DHCR7* mutations) [14]
- Lipoid adrenal hyperplasia due to steroidogenic acute regulatory protein (*StAR gene*) and *CYP11A1* mutations [13]
- Drug induced (mitotane, ketoconazole, metyrapone, etomidate, aminoglutethimide)

Adrenal gland dysgenesis or hypoplasia

- *DAX1* mutation
- *SF1* mutation
- ACTH receptor pathway defects (*MC2R* and *MRAP* mutations) [13]

Adrenal destruction

- Autoimmunity
- Infection, e.g. tuberculosis
- Haemorrhage
- Adrenal metastases
- Primary adrenal lymphoma
- Sarcoidosis
- Amyloidosis
- Drug induced (mitotane)
- Adrenoleukodystrophy (*ABCD1* gene mutations)

A number of observations support strong heritability of AAD; these include concordance of AAD in mono- and dizygotic twins [15–17] and clustering of AAD within families [18, 19]. In addition, AAD is frequently observed in association with other autoimmune conditions in the context of autoimmune polyglandular syndrome type 2 (APS2). APS2 is defined as AAD coexisting with autoimmune thyroid disease and/or type 1 diabetes and/or another autoimmune condition such as vitiligo or pernicious anaemia and is present in 50–65% of individuals with primary adrenal insufficiency [20–22].

Although it has been long recognised that AAD is a highly heritable disorder, the rarity and complexity of this condition make its investigation challenging [23].

Pathogenesis of AAD

AAD is characterised by selective destruction of steroid hormone-producing cells in the adrenal cortex by T-cell-mediated inflammation (adrenalitis). The steroidogenic enzymes become the autoantigens against which an autoimmune response is generated. In the disease process, all three hormone-producing cell layers, the *zona fasciculata, zona reticularis* and *zona glomerulosa*, are gradually destroyed. The primary autoantigen in AAD is the 21-hydroxylase (21-OH) enzyme, and circulating 21-OH autoantibodies can be detected in approximately 85% of subjects with AAD. They may occur years prior to the development of clinically significant steroid deficiency [24, 25]. The other targets of the autoimmune process include the 17-alpha-hydroxylase enzyme and the cholesterol side-chain cleavage enzyme [26, 27]. Autoantibodies directed against the two latter enzymes are more commonly associated with AAD occurring in the context of autoimmune polyglandular syndromes [27, 28]. Steroid 21-OH autoantibodies are predominantly of the IgG_1 isotype and target the carboxy terminal of the enzyme [29]. While the detection of these antibodies can be used to cement the diagnosis of AAD in an individual with adrenal insufficiency, their role in the pathogenesis of the disease remains unclear. Although *in vitro* studies have demonstrated that these antibodies inhibit enzymatic activity of the 21-OH enzyme by preventing its interaction with cytochrome P450 oxidoreductase, these findings have not been corroborated *in vivo* [30]. The presence of such antibodies in some individuals with no detectable reduction in steroid concentration would argue against such an interaction [31]. In addition, 21-OH antibodies can cross the placenta as IgG antibodies; however, there have been no reports of transient hypoadrenalism in offspring born to AAD mothers. This suggests that the presence of adrenal antibodies in the serum alone is insufficient to cause autoimmune adrenal insufficiency [32]. In keeping with this is the intracellular location of the steroidogenic enzymes in intact cells, precluding their direct interaction with circulating autoantibodies. Recently, it has been shown that 21-OH-specific CD4 and CD8 T cells are abundant in AAD subjects many years after diagnosis and their immunoactivation generates persistent cytolytic T-cell populations, with the potential to destroy 21-OH-expressing cells [33]. Interestingly, T-cell immune responses in AAD subjects cluster around just a few 21-OH immunodominant antigenic determinants: HLA-B8-restricted epitope $21\text{-}OH_{431\text{-}438}$, HLA-A2-restricted epitope at position $21\text{-}OH_{342\text{-}350}$ and HLA-DRB1*0401 restricted epitope at position $21\text{-}OH_{342\text{-}361}$ [33–35].

In AAD, like in other organ-specific autoimmune disorders, three stages can be identified: potential, preclinical and clinical. In the potential phase, adrenal autoantibodies are present, but adrenal steroidogenesis is normal and no clinical features of the disease can be found. It appears that adrenal autoantibodies are very rare in the general population. In a number of population studies including apparently healthy individuals, approximately 32,000 people were tested, and 21-OH

autoantibodies were detected in only 430 individuals, giving a prevalence of 13 in 100,000 [36]. Subjects with positive 21-OH antibodies have an approximately 20% cumulative risk of developing clinically apparent AAD [37]. The reported positive predictive value of adrenal antibodies for development of clinically apparent AAD varies from 0 to 90% [38–40]. These huge discrepancies can be partly explained by varying follow-up duration and differences in the populations recruited. It appears that the highest risk for development of AAD in the presence of adrenal autoantibodies occurs in paediatric populations; the reported cumulative risk ranges from 20 to 100% [40–43]. The very high risk of development of AAD in children reported in some studies is due to the inclusion of subjects with APS1 syndrome, which in itself is associated with a prevalence of AAD of up to 90%. Amongst adult individuals, higher titres of autoantibodies are associated with higher risk for development of AAD, and these individuals progress more rapidly than those with low titres [44]. However, individual responses to an ongoing autoimmune process vary hugely as illustrated by a case study of a woman with a 9-year history of hyperpigmentation, elevated ACTH concentrations and high 21-OH antibody titres but a normal cortisol response to administration of synthetic ACTH [45]. In addition, some individuals shown to have 21-OH antibodies revert to being antibody negative [37]. A study by Baker et al. suggests that this variability in controlling the autoimmune response might have a genetic basis with the HLA-B15 haplotype conferring protection from progression to AAD in antibody-positive individuals [46].

In the subclinical phase, adrenal function gradually becomes impaired, but this is not sufficient to produce overt clinical manifestations of the disease. The first biochemical evidence of impaired steroidogenesis in the adrenal cortex appears to be increased ACTH concentration followed by raised plasma renin activity, accompanied by normal or low plasma aldosterone concentration [47]. However, one group has reported that abnormalities in the plasma renin-aldosterone axis occur first [25]. The discrepancies in these observations could be related to different dietary habits, salt intake in particular, and/or treatment with medications influencing renin such as ACE inhibitors or angiotensin receptor blockers in the populations studied. Finally basal and/or stimulated cortisol concentration becomes abnormal (<550 nmol/l after 250 μg of tetracosactide—Synacthen administration). Overall it appears that the *zona glomerulosa* is the adrenal cell subtype that is most sensitive to autoimmune destruction. We postulate that this may be due to a lack of protective high glucocorticoid concentrations in the *zona glomerulosa*, which are present in the *zona fasciculata*, that can potentially inhibit local immune responses.

Finally, in the clinical phase of the disease, symptoms and signs develop; these include hyperpigmentation, fatigue, weight loss, hypotension and salt craving. This usually occurs when at least 90% of the functioning adrenocortical cells have been destroyed [48]. However, it appears that progression to the clinical phase, even in individuals with evidence of organ-specific autoimmunity and impaired steroidogenesis, is not inevitable. This is illustrated by four case reports. Three patients achieved spontaneous partial remission in established AAD [49–51], and one patient achieved long-term remission of subclinical AAD following high-dose glucocorticoid therapy for another condition [52].

Genetic Basis of APS1 Syndrome

AAD can occur in the context of a rare, monogenic syndrome known as autoimmune polyglandular syndrome type 1 (APS1) also referred to as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). The syndrome is characterised by at least two out of the following: AAD, hypoparathyroidism and mucocutaneous candidiasis [53]. These conditions usually become apparent in a specific order: fungal infection of the skin, nails and mucous membranes occurs typically in infancy, followed by hypoparathyroidism in early childhood and adrenal insufficiency predominantly in teenagers and young adults. Other APS1-associated conditions include premature gonadal failure, type 1 diabetes mellitus, hypothyroidism, alopecia, vitiligo, pernicious anaemia, autoimmune hepatitis, hypoplasia of the dental enamel and nail dystrophy [54–56]. Intrestingly, Graves' disease is very rare in the context of APS1. The syndrome is inherited in an autosomal recessive fashion and results from mutations in the autoimmune regulator (*AIRE)* gene localised on chromosome 21q22. The product of the *AIRE* gene is predominantly expressed in the thymus and concerns development and maintenance of self-tolerance [57]. Over 60 pathogenic mutations in the *AIRE* gene have been described; the majority of these result in a truncated protein product [58]. In keeping with recessive inheritance, affected individuals can be either homozygous for an identical mutation or can be compound heterozygotes. There are reports of genotype-phenotype correlations [59]. The APS type 1 syndrome is most prevalent in Iranian Jewish (1:9000), Sardinian (1 in 14,000), Finnish (1 in 25,000) and Norwegian (1 in 90,000) populations [59–62]. Recently, families with dominant inheritance of a milder APS1 phenotype have been described owing to heterozygous *AIRE* mutations that inactivate the normal *AIRE* allele (dominant-negative mutation) [63]. Affected individuals have a heterogeneous presentation with later onset and lower penetrance compared to the classical APS1 syndrome. Dominant-negative *AIRE* mutations are associated with various forms of organ-specific autoimmunity; in particular, vitiligo and pernicious anaemia are commonly present.

Genetic Basis of Human Non-APS1 AAD

Unlike APS1, isolated AAD and AAD in the context of APS2 are not inherited in a Mendelian fashion. Their pathogenesis is thought to be due to a complex interplay between genetic and environmental factors. The genetic basis for the disease is complex and involves multiple genetic susceptibility variants. It appears that no single susceptibility variant is sufficient to cause the disease and a "critical" genetic load is required to initiate the pathogenic process. A sibling recurrence risk ratio (the ratio of risk of disease manifestation, given that one sibling is affected, compared with the disease prevalence in the general population) in non-APS1 AAD is approximately 160–210, considerably higher to that seen in type 1 diabetes or Graves' disease, which have sibling recurrence risk ratios of 15 and 10, respectively [64, 65].

This suggests a very strong genetic influence in the pathophysiology of AAD. In addition, the clustering of autoimmune conditions with AAD in the context of APS2 suggests that there are common susceptibility loci for these disorders.

Molecular Genetic Studies

A number of candidate gene case-control studies have been conducted in cohorts of patients with AAD. The number of AAD cases in the cohorts studied is significantly smaller than in the genetic studies carried out in other autoimmune disorders such as type 1 diabetes or autoimmune thyroid disease because of the relative rarity of this condition. Candidate genes are selected based on underlying biological plausibility (they are either implicated in an associated autoimmune disease such as autoimmune thyroid disease or type 1 diabetes, or they are associated with rare, monogenic variants of the disease, such as APS1). To date, a number of susceptibility variants to AAD have been identified (Table 4.1). However, with the exception of MHC locus (in particular, DRB1), the susceptibility variants discovered thus far contribute only a small amount towards an individual's overall genetic susceptibility to AAD.

Autoimmunity is thought to arise as a result of aberrant responses within both the adaptive and innate immune systems. The adaptive immune system refers to antigen-specific immune responses involving antigen recognition and processing, forming "immune memory", and concerns its key cells—lymphocytes. Innate immunity refers to non-specific defence mechanisms against pathogens occurring within hours of an antigen's appearance and comprises a number of cell types. Amongst the functions of the innate immune system are pathogen recognition, production of cytokines and chemokines leading to immune cell recruitment, complement cascade activation and activation of the adaptive immune system via antigen presentation. In patients with AAD, similarly to other autoimmune diseases, aberrant responses in both innate and adaptive immunity are implicated. However, the majority of susceptibility loci identified to date encode proteins involved in antigen presentation and T-cell activation.

MHC Risk Alleles

The major histocompatibility complex (MHC) in humans, the human leukocyte antigen (HLA) complex, is located on chromosome 6p21 and comprises multiple genes involved in immune processes. Amongst those are HLA class I (HLA-A, HLA-B and HLA-C) and HLA class II (HLA-DRB1, HLA-DQB1, HLA-DQA1, HLA-DPB1 and HLA-DPA1) genes which encode antigen-presenting molecules and are the most important determinants of polygenic autoimmune disease risk. HLA proteins are expressed on the surface of antigen-presenting cells and display

Table 4.1 Case-control studies of candidate genes in patients with non-APS1 AAD

Gene or marker	Population	Number of patients	Odds ratio	P value
MHC loci				
HLA-B*08	Norway [66]	414	2.6	4×10^{-11}
HLA-DR3-DQ2	Norway [67]	94	3.6	$<10^{-7}$
HLA-DR3-DQ2	Finland, Russia, Estonia [68]	69	14.5	<0.0001
HLA-DR4-DQ8*0404	Norway [67]	94	NR	$<10^{-5}$
HLA-DR4-DQ8*0401				$<10^{-4}$
HLA-DRB1*0301	Norway [66]	414	22.13	6×10^{-20}
HLA-DRB1*0404				
Other loci				
MICA*5.1	Italy [69]	28	6.52	0.0015
	USA [70]	46	22.5	$<10^{-5}$
	Norway [66]	414	1.78	0.0003
CYP21Adel	Finland [71]	12	25.0	NR
CYP21 L10, R102, A494	Finland [72]	12	8.9	NR
CLEC16A (rs12917716)	Norway [73]	332	0.72	0.0004
	UK [73]	210	1.06	0.71
	Combined [73]	542	0.81	0.006
CYP27B1-126C > A (rs4646536)	Germany [74]	124	1.18	0.006 (genotype)
				0.3354 (allele)
	UK [75]	104	1.71	0.003
	6 European cohorts [76]	1955	0.9	0.03
VDR Fok1 (exon2)	Germany [77]	95	NR	0.0351 (genotype)
				0.3390 (allele)
FCRL3 (rs7528684)	UK [78]	200	1.61	0.0001
PTPN22 (rs2476601)	UK [79]	104	1.69	0.031
	Germany [80]	121	1.03	0.9878
	Norway [81]	302	1.39	0.016
	UK [82]	251	1.63	0.008
	Poland [82]	87	1.84	0.010
CTLA4 A > G (exon 1)	UK [83]	90	1.64	0.008
	Norway, UK, Germany, Spain and Italy [84]	1002	1.37	0.002
CTLA4 J030G > A	UK [85]	40	1.9	0.02
	Norway [85]	94	1.4	0.04
	Combined [85]	134	1.5	0.03
NLRP1 (rs12150220)	Norway [86]	333	1.25	0.007
	Poland [87]	101	1.5	0.015
PD-L1 (rs1411262)	UK [88]	315	1.33	0.032
	Norway [88]	342	1.34	0.026
	Combined [88]	657	1.32	3.03×10^{-3}

(continued)

Table 4.1 (continued)

Gene or marker	Population	Number of patients	Odds ratio	P value
STAT4 (rs4274624) (rs10931481)	6 European cohorts [76]	1955	1.27	<0.0001
		1262	1.23	0.0007
GATA3	6 European cohorts [76]	1955	0.9	0.03
NFκB1 (rs10026278) (rs230532) (rs4698861)	UK [76]	309	0.69	0.0034
			0.65	0.00041
			0.63	0.00017
Il-23A	Italy [76]	280	2.37	0.0028
GPR174 (rs3827440)	UK [89]	286	1.34	0.03
Il-2 (rs3136534)	Poland [90]	223	0.71	0.003
BACH2 (rs3757247)	UK [91]	358	1.44	1.4×10^{-6}
	Norway	317	1.41	0.0015
NFATC1 (rs754093)	Norway [92]	384	NR	0.48
	Sweden [92]	367	NR	0.033 (genotype)
				0.07 (allele)
	UK [92]	346	NR	0.15
	Combined [92]	1097	NR	0.02

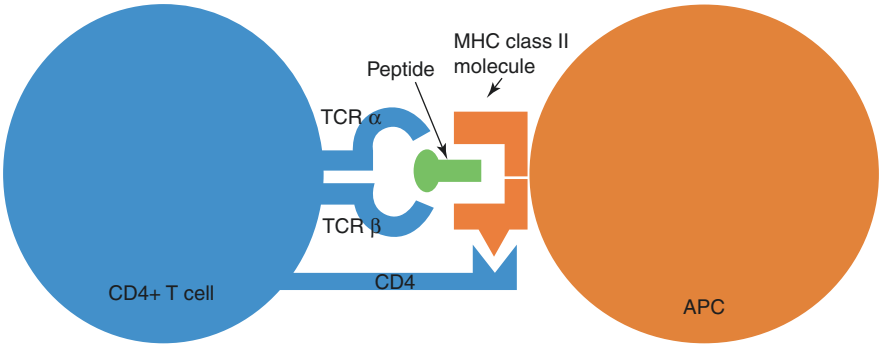

Fig. 4.1 Schematic representation of antigen presentation by MHC class II molecules to CD4+ T cell. The antigen-MHC complex is recognised by the T-cell receptor. CD4 is a co-receptor that binds to a non-polymorphic region of the MHC and assists in T-cell activation. APC, antigen-presenting cell; TCR, T-cell receptor

peptides (both self and non-self) for recognition by T cells. Aberrant activation of T cells in response to self-antigens leads to development of autoimmune disease (Fig. 4.1). Although the exact immunopathology of AAD remains to be established, it has been shown that MHC II molecule expression on adrenocortical cells is highly upregulated in the active phase of the disease [93].

Similar to other autoimmune conditions, the challenge in finding causal variants within the MHC for AAD lies in the fact that this region contains the largest number of polymorphisms in the entire genome [94] and that there is strong and extensive

LD amongst alleles throughout this locus [95]. A number of variants within the MHC class II genes are associated with several autoimmune conditions. In particular, a strong association between autoimmunity and allelic variability in HLA-DR and DQ molecules, which present exogenous antigens for recognition by CD4+ helper (Th) cells, exists. Allelic polymorphism at these loci results in variant proteins that allow self-peptides to enter the antigen-binding groove more readily. The association between HLA class II molecules and AAD has been recognised for three decades [96]: in a seminal study by Maclaren and co-workers, susceptibility to AAD was linked to HLA-DR3 and HLA-DR4 alleles. Subsequently, these findings were replicated in a number of AAD cohorts [66, 67, 71, 97, 98]. A particularly high-risk genotype for AAD has been identified as DR3-DQ2/DR4-DQ8 [67]. To date, the HLA class II alleles DRB1*0301 and DRB1*0404 have been shown to confer the highest risk for AAD with odds ratios (OR) of 2.9 and 3.3, respectively [66]. An especially large risk increment occurs in compound heterozygotes possessing both these haplotypes (OR = 22). The DRB1 alleles occur in strong linkage disequilibrium (LD) with other alleles associated with AAD: DQB1*02 and DQB1*0302 (OR 1.8 and 1.5, respectively) [66]. In addition, a number of haplotypes conferring protection from AAD have been also discovered: DRB1*0401-DQ8 [67] and DRB1*0403 [99]. Thus far, none of the susceptibility loci identified are specific to AAD with the possible exception of HLA-DRB1*0404. The possible explanation for this is that peptides from 21-OH might bind particularly well and be presented to autoreactive T cells by this HLA class II molecule [35].

Polymorphisms in the *CYP21A2* (cytochrome P450, family 21, subfamily A, polypeptide 2) gene, which encodes the 21-OH enzyme and is located within MHC class III region, have been associated with AAD. *CYP21A2* is 600 kb away from the HLA-DRB1 locus; therefore, the association of its polymorphisms and AAD has been attributed to long-range LD with MHC class II alleles [72]. A recent study confirmed that no specific variants of *CYP21A2* are associated with AAD. Instead, *CYP21A2* polymorphisms are in LD with the high-risk haplotype *HLA-DRB1* locus and do not contribute to the disease susceptibility independently [100].

The genes that appear to be independently associated with AAD susceptibility include HLA-B (OR 2.6 for HLA-B*08) and MHC class I-related chain A (*MICA*) (OR 1.8 for MICA*5.1) [66]. Homozygosity for the MICA*5.1 allele in the presence of the high-risk HLA genotype DR3-DQ2/DR4.4-DQ8 confers extreme risk for AAD development [101].

Non-MHC Risk Alleles

The *CIITA* gene encodes a protein functioning as a HLA class II transactivator, the master control factor for MHC class II expression. Mutations in *CIITA* result in a severe monogenic immunodeficiency disease known as bare lymphocyte syndrome. Allelic variability in this gene has been associated with conditions such as

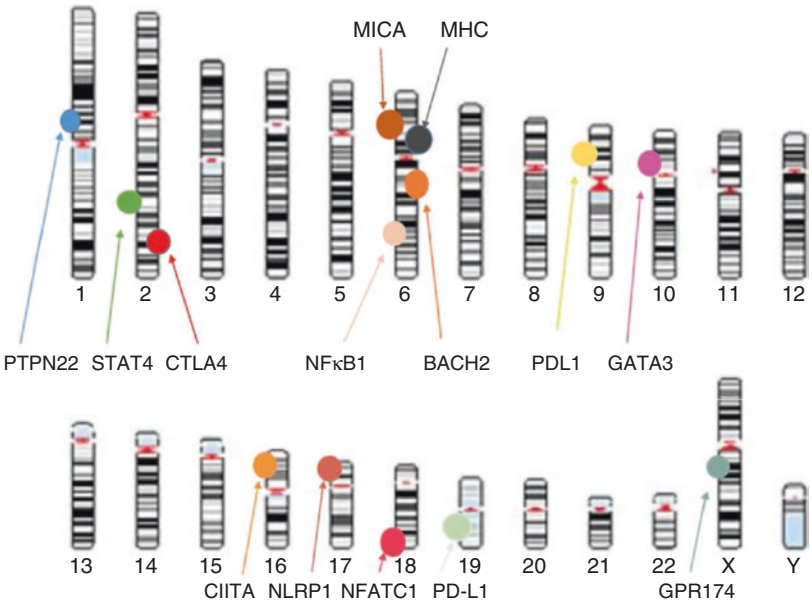

Fig. 4.2 Schematic representation of genes associated with autoimmune Addison's disease that are implicated in T-cell proliferation and activation

rheumatoid arthritis [102], systemic lupus erythematosus [103] and coeliac disease [104], amongst others. Polymorphisms in the promoter, as well as in intron 3, of this gene have been reported to be associated with AAD susceptibility [73, 105] Although the mechanism by which these polymorphisms confer disease susceptibility remains unknown, it has been hypothesised that they could influence the levels of tissue selectivity of HLA class II expression (Fig. 4.2).

T-Cell Proliferation, Differentiation and Activation Genes

Activation of T lymphocytes, the key cells of the adaptive immune system, requires simultaneous engagement of the T-cell receptor by MHC class II peptides and costimulatory molecules. The cytotoxic T-lymphocyte-associated antigen 4 (*CTLA4*) gene located on chromosome 2q33 encodes CD152, a costimulatory molecule which acts as a vital negative regulator of T-cell activation and proliferation [106]. CTLA4 competes with costimulator CD28 for binding to B7 on antigen-presenting cells. The critical role of this molecule is demonstrated in *CTLA4* knockout mice which die prematurely, at an age of 2–3 weeks, due to severe lymphoproliferation, lymphocytic infiltration and destruction of major organs [107]. In humans, mutations in the *CTLA4* gene result in an immune dysregulation syndrome [108]. *CTLA4* gene polymorphisms have been linked with susceptibility to a number of autoimmune diseases including autoimmune thyroid disease [109, 110],

type 1 diabetes mellitus [111, 112], rheumatoid arthritis [113] and coeliac disease [114]. The association between *CTLA4* gene variants and AAD has been reported in a number of studies in different European populations [83–85, 115, 116]. The most commonly described *CTLA4* polymorphisms in AAD populations are non-synonymous polymorphism in exon 1 region of *CTLA4* gene +49 A → G (*Ala17Thr*) [83, 84, 115], (AT)n dinucleotide repeat polymorphism within the 3′ untranslated region [116] and G or A alleles of the JO30 SNP downstream of this gene [85]. Polymorphisms in the latter are postulated to be the causative variants affecting the relative amount of the soluble and membrane-bound CTLA4 and therefore enabling CD28 to access more of its ligand, resulting in T-cell activation [117]. In contrast to this hypothesis, a number of studies have demonstrated that individuals with autoimmune conditions have, in fact, increased serum levels of soluble CTLA4 isoforms [118, 119] suggesting that the complexity of CTLA4-CD28 interaction and signalling is incompletely understood. Another mechanism of negative immune regulation by CTLA4-positive cells is the ability of CTLA4 to capture and degrade CD80 and CD86 (ligands shared with the stimulatory receptor CD28) from antigen-presenting cells [120].

Another key autoimmunity gene, *PTPN22* on chromosome 1p13, encodes a tyrosine phosphatase which is a crucial regulator of immune homeostasis, inhibiting T-cell receptor (TCR) signalling. The association of a missense SNP (1858C > T) in *PTPN22*, encoding an arginine to tryptophan substitution at amino acid 620, has been identified in type 1 diabetes [121], rheumatoid arthritis [122], systemic lupus erythematosus [123] and Graves' disease [79]. For a number of autoimmune diseases, this allelic variant ranks as the most important non-MHC single gene contributor to disease susceptibility. A number of studies have implicated this variant in susceptibility to AAD [79, 81, 82]. The functional effect of 1858 C > T polymorphism has not been fully elucidated. Some studies suggest that this variant results in increased suppression of TCR signalling [124, 125], while others suggest the opposite [126, 127]. One study suggested that proteolytic binding and cleavage of Arg620Trp is increased with consequent reduction in LYP levels in T and B cells leading to lymphocyte and dendritic cell hyperresponsiveness and autoimmunity [127].

Programmed death ligand-1 (PD-L1, CD274, B7-H1) is a costimulatory molecule binding the PD-1 moiety of T cells, leading to downregulation of cytokine production and T-cell activation, thereby inducing immune tolerance. PD-L1 variants have been implicated in susceptibility to AAD, although the effect on risk is very modest (OR 1.34 for the allele with the strongest association) [88].

Interleukin-2 (Il-2) and its receptor are important determinants of the immune response. Il-2 is a potent T-cell growth stimulator and influences T-cell differentiation, in particular formation of the regulatory T cell (T_{reg}) lineage. T_{reg} cells are crucial in maintaining self-tolerance due to their ability to suppress autoreactive T cells which escape negative selection in the thymus. *IL2RA* gene encodes the alpha subunit (CD25) of the IL2 receptor, a unique subunit conferring high affinity to IL2. Polymorphic variants of *IL2* (4q27) and *IL2RA* (10p15.1) genes have been associated with type 1 diabetes and rheumatoid arthritis [128–130]. The C minor allele in *IL2* conferred protection from AAD in a Polish cohort. However, there was no

association found between *IL2* polymorphisms and Norwegian patients with AAD [73]. Similarly, an association between *IL2RA* polymorphisms and susceptibility to AAD was found in Norwegian subjects, but this finding has not been replicated in British or Polish series [73, 90]. Although there is known genetic heterogeneity between various AAD populations, the association between AAD and *IL2* or *IL2RA* needs further replication in larger cohorts.

STAT4 (signal transducer and activator of transcription 4) on chromosome 2q32 encodes a transcription factor which is implicated in Th-1 and Th-17 differentiation and activation. Polymorphisms of *STAT4* have been shown to be associated with rheumatoid arthritis [131–133], systemic lupus erythematosus [122] and type 1 diabetes [134]. A meta-analysis by Mitchell et al. revealed a significant association between *STAT4* polymorphisms and AAD in European populations [76]. The allelic variability identified in AAD maps to intron 3 of the *STAT4* gene and is in moderate-to-strong linkage disequilibrium with *STAT4* polymorphisms identified in the other autoimmune conditions. The exact mechanism by which polymorphisms in this gene lead to autoimmune disease remains unknown.

The *GATA3* gene on chromosome 10p14 encodes a C2C2-type zinc finger transcription factor which regulates a number of steps in T-cell development and differentiation. In particular, this transcription factor has been shown to be the Th2 lineage master regulator [135] and could therefore contribute to T-cell dysregulation present in autoimmune disease. A recent meta-analysis demonstrated an association between *GATA3* polymorphisms and AAD in European cohorts [76]. The minor G allele at *rs3802604* was protective for AAD (OR 0.9). This finding is, however, in contrast to the known association of the same allele with susceptibility to rheumatoid arthritis [136] possibly reflecting immunopathogenic differences between these two conditions.

The *BACH2* gene on chromosome 6q15 plays a vital role in CD^{4+} T-cell differentiation; in particular it is crucial in the formation of T_{reg}, which are the key cells in maintaining immune tolerance. Polymorphisms at the *BACH2* locus have been associated with type 1 diabetes, generalised vitiligo, autoimmune thyroid disease, Crohn's disease and coeliac disease [137–141]. Recently, an association between the minor T allele at *rs3757247* in the *BACH2* locus and susceptibility to AAD was described in UK and Norwegian cohorts [91].

The first linkage study in multiplex AAD families implicated regions on chromosomes 6 (corresponding to the *HLA* region), 7, 9 and 18 in the susceptibility to AAD [92]. A follow-on study, looking at 64 SNPs underlying the linkage peaks on chromosomes 9 and 18 conducted in case-control cohorts from the UK, Norway and Sweden, revealed nominal association with three independent SNPs in chromosome 18 and AAD. One of these encodes a non-synonymous variant (*pCys751Gly*) in exon 9 of the *NFATC1* (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1) gene. Upon T-cell activation, family members of NFAT translocate to the nucleus where they can activate target genes and as such play a central role in gene transcription during the immune response [142]. NFATC1 has been shown to play a role in the regulation of PD-1 expression, a cell surface receptor functioning as an immune checkpoint and reducing T-cell activation [143].

The *GPR174* (*G protein-coupled receptor 174*) gene at Xq21.2 consists of one exon encoding a protein which belongs to the G protein-coupled receptor superfamily and is

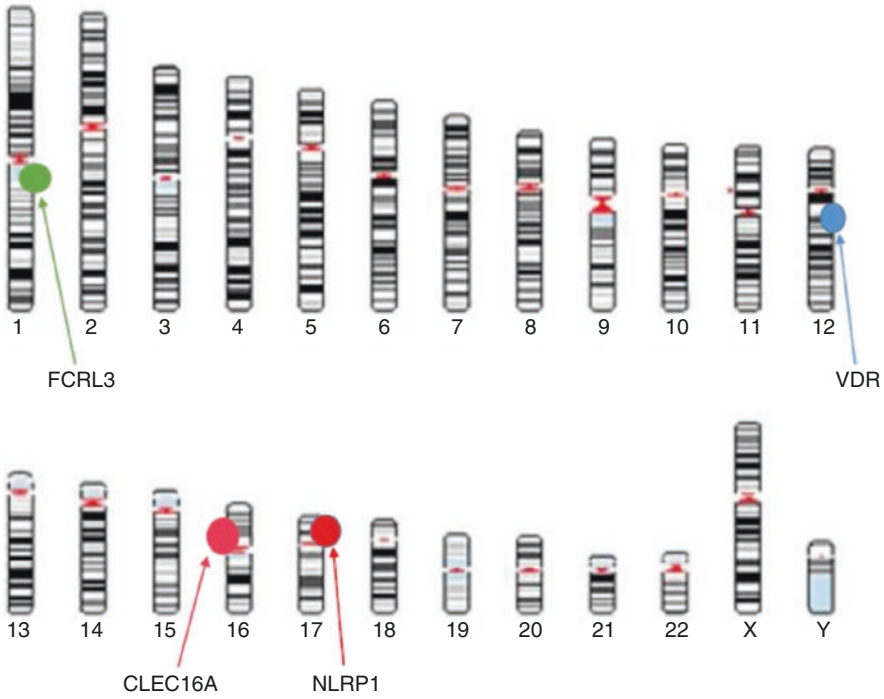

Fig. 4.3 Schematic representation of genes found to be associated with autoimmune Addison's disease which are implicated in proliferation and activation of B cells and antigen-presenting cells

involved in immune cell signal transduction. Recently a lysophosphatidylserine (LysoPS) was found to be a ligand for GPR174, and the interaction between the two has been shown to stimulate an increase in the intracellular cyclic adenosine monophosphate (cAMP) [144]. cAMP has been previously demonstrated to be a potent negative regulator of T-cell immune function [145]. These findings offer a plausible link between *GPR174* polymorphisms and autoimmunity. Polymorphisms in *GPR174* have been found to be associated with Graves' disease [146] [147]. A serine to proline non-synonymous variant in *GPR174* has been associated with AAD [89]. The localisation of *GPR174* on chromosome X and its role in AAD autoimmunity are particularly interesting given the gender bias observed in this disorder, although it is unlikely that a single gene is responsible for the higher susceptibility of females to develop AAD (Fig. 4.3).

Genes Implicated in B Lymphocyte and Antigen-Presenting Cell Proliferation and Activation

Vitamin D has been recognised for its effects on the immune system. On a molecular level, the active form of vitamin D, 1,25-dihydroxyvitamin D, leads to reduced expression of HLA class II molecules on endocrine cells and inhibits T-cell proliferation [148, 149]. 1,25-Dihydroxyvitamin D is also implicated in innate immunity

by inhibiting differentiation of dendritic cells which are potent antigen-presenting cells [150]. Polymorphisms in the vitamin D receptor (*VDR*) gene, located on chromosome 12q12-14, have been studied in a number of autoimmune conditions including type 1 diabetes and autoimmune thyroid disease with conflicting results [151–154]. Although specific genotypes in VDR have been associated with AAD risk in a relatively small German cohort, no such association was found for an individual VDR allele [77]. Additional studies are required to confirm VDR as a susceptibility locus for AAD. In contrast to this, a promoter polymorphism in the *CYP27B1* gene (*−1260C > A*) has been shown to be associated with AAD in two independent cohorts [74, 75]. More recently, an intronic SNP, *rs4646536*, in *CYP27B1*, was associated with AAD in a large meta-analysis of several European cohorts [76]. CYP27B1 hydroxylase catalyses the conversion of 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D. A promoter polymorphism in *CYP27B1* might affect enzyme transcription and thus the rate of final hydroxylation of 1,25-dihydroxyvitamin D.

The Fc receptor-like 3 gene (*FCRL3*) located on chromosome 1q21 encodes an orphan cell surface receptor belonging to the immunoglobulin receptor superfamily, expressed predominantly on B lymphocytes. A polymorphism in the *FCRL3* promoter region (*FCRL3_3*C*) has been implicated in susceptibility to rheumatoid arthritis, autoimmune thyroid disease and systemic lupus erythematosus in Asian cohorts [155]. Contrary to the findings in other autoimmune conditions, a study of a UK AAD cohort found that the *FCRL3_3*C* variant confers protection from the disease [78]. The allele most associated with disease risk in this cohort was found to be *FCRL3_3*T* (OR = 1.61). Based on functional studies of this locus, *FCRL3_3*T* is predicted to result in lower promoter activity. Such contradictory findings for one haplotype conferring both protection and disease susceptibility in different populations illustrate the complexity of the genetic underpinning of polygenic autoimmune diseases including AAD.

NLRP1 (nuclear localisation leucine-rich-repeat protein 1) is a regulator of the innate immune response. NLRP1 belongs to the NOD-like receptor family and participates in recognising microbial products, such as lipopolysaccharide, and assembly of inflammasomes, cytoplasmic protein complexes mediating pro-inflammatory responses via cytokine activation [156]. Polymorphisms of the *NLRP* gene have been reported to confer risk for a number of autoimmune conditions including vitiligo [157], type 1 diabetes [86], coeliac disease [158] and rheumatoid arthritis [159]. A coding variant of *NLRP1* (*Leu155His*) has been associated with AAD in two European cohorts [86, 87]. Surprisingly, different alleles were found to confer risk of AAD in Polish (minor allele A) and Norwegian (major allele T) populations.

The *CLEC16A* (*C-type lectin domain family 16*) gene encodes a protein of unknown function but which is almost exclusively expressed in immune cells such as dendritic cells, B lymphocytes and natural killer cells. This makes it a plausible susceptibility gene for autoimmunity. A polymorphism in the *CLEC16A* gene (intronic SNP *rs12917716*) was found to be associated with AAD in a Norwegian cohort with an OR of 0.71. Comparable effects of *CLEC16A* SNPs were previously demonstrated in cohorts of subjects with type 1 diabetes (OR 0.65 to 0.83) [160, 161].

Processes Affecting Gene Expression

Gene expression can be altered by both common copy number variation (CNV) and epigenetic modification such as DNA methylation. As a result, different phenotypes can develop despite similar genetic profiles.

CNV in the human genome has been recently identified as a source of genetic diversity and has been shown to influence disease susceptibility [162]. Recently, CNVs in two genes, *UGT2B28* and *ADAM3A,* have been found to be associated with AAD [163]. However, the mechanism by which this association confers susceptibility to the disease remains unknown.

Abnormal DNA methylation is commonly observed in autoimmune disorders. It has been suggested that hypermethylation (addition of methyl groups to oligonucleotides by DNA methyltransferases) of promoter regions silences genes, whereas intronic hypermethylation is involved in gene activation. DNA methylation has been shown to be one of the mechanisms involved in transcriptional control of genes such as *FOXP3*, *Interferon Gamma* and *AIRE* which in turn influence T-cell differentiation and function. A recent study identified multiple hypomethylated gene promoter regions in DNA isolated from CD4 T cells from AAD subjects [164]. A multitude of differently methylated regions have been localised in genes implicated in immune modulation and autoimmunity suggesting that this epigenetic modification plays a role in the immunopathogenesis of this disease. This is likely to be an area of research going forward.

Genetics of Canine Addison's Disease

Autoimmune hypocortisolism is highly prevalent in a number of dog breeds including collies, poodles, terriers and retrievers. Canine hypoadrenalism shares some susceptibility loci with human AAD including MHC (DLA, dog leucocyte antigen), *PTPN22*, *NLRP1* and *AIRE* [165]. In addition, allelic variability in IL-16 and GC has also been implicated in canine Addison's disease. Similar to humans, most of the allelic variability associated with the increased risk pertains to genes implicated in T-cell receptor pathways.

Environmental Factors in Pathophysiology of AAD

We have recently suggested a potential seasonal periodicity, with excess risk for development of AAD in individuals born in winter months and a protective effect when born in summer. Exposure to seasonal viral infection in the perinatal period and vitamin D exposure related to UVB radiation intensity are the postulated environmental factors underpinning this association [166].

Another interesting concept is that of physical or psychological stress as a trigger of autoimmune processes. In fact, many retrospective studies have found that a high proportion of patients with various autoimmune conditions reported emotional stress prior to disease onset [167]. However, data pertaining to the role of stress in the pathophysiology of AAD are lacking.

Summary

Our current concept of AAD aetiopathogenesis is that it results from an interplay between as yet unidentified environmental factors and genetic susceptibility loci. The susceptibility genes discovered to date encode proteins that are involved in the activation and regulation of antigen-specific T cells; however, these have only a modest effect in terms of disease risk contribution and are commonly associated with other autoimmune disorders. Further work is required to gain a better understanding of the genetic architecture of this interesting autoimmune condition.

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