

Review

Recent advances in the molecular genetics of congenital and acquired primary adrenocortical failure

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Introduction

In recent years there have been many advances in our understanding of the molecular pathogenesis of both congenital and acquired adrenocortical failure (autoimmune or otherwise). As well as providing some fascinating insights into adrenal gland development and steroid hormone biosynthesis, the recognition of these various distinct disorders, either at a clinical or molecular genetic level, often has implications for the management of the patient and their immediate family. In this paper, we review the salient clinical and molecular features of the various causes of primary adrenocortical failure (Table 1).

Genetics of autoimmune Addison's disease

Autoimmune Addison's disease (AAD) is a chronic disorder of the adrenal gland, characterized by insufficiency of adrenocortical hormones due to autoimmune destruction of steroidogenic adrenocortical cells (Oelkers, 1996). The cytochrome P450 enzymes involved in steroidogenesis, including 21-hydroxylase (Baumann-Antczak *et al.*, 1992; Winqvist *et al.*, 1992), 17-hydroxylase (Krohn *et al.*, 1992) and side chain cleavage enzyme (Winqvist *et al.*, 1993) have been identified as primary autoantigens in AAD. With the overall decrease in the prevalence of tuberculosis, AAD has emerged as the most common cause of primary adrenal failure in developed countries (Nerup, 1974; Kong & Jeffcoate, 1994). Nevertheless, AAD is a relatively rare endocrinopathy; recent epidemiological studies showing an estimated prevalence in the general European population of about 100 per million (Kong & Jeffcoate, 1994; Laureti *et al.*, 1999).

The results of human leucocyte antigen (HLA) typing, taken together with the clinical heterogeneity of presentation and different modes of inheritance, have suggested distinct

pathogenic bases for different forms of AAD. Thus, AAD may occur in isolation, as a manifestation of the autoimmune polyendocrinopathy type 1 syndrome (APS1), or as part of the autoimmune polyendocrinopathy type 2 (APS2) syndrome (Table 1) (Spinner *et al.*, 1968; Neufeld *et al.*, 1981; Maclaren & Riley, 1986).

Autoimmune polyendocrinopathy type 1 syndrome: a monogenic autoimmune disorder

In the autoimmune polyendocrinopathy type 1 (APS1) syndrome, which is also known as the autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) syndrome, AAD occurs in association with autoimmune hypoparathyroidism, chronic mucocutaneous candidiasis and other organ-specific autoimmune disorders. These include type 1 diabetes, primary gonadal failure, pernicious anaemia, chronic active hepatitis and hypothyroidism (Neufeld *et al.*, 1981; Ahonen *et al.*, 1990). The age of onset of AAD in APS1 is typically between 11 and 15 years, although other manifestations of APS1 (e.g. hypoparathyroidism and candidiasis) are likely to present at an earlier age. APS1 is a monogenic disorder with autosomal recessive inheritance, affecting both sexes with equal prevalence (Ahonen, 1985), and is rare in most populations. However, because of founder effects, it is relatively common in the Finnish population and Iranian Jews with estimated prevalences of 1/25 000 and 1/9000, respectively (Ahonen, 1985; Zlotogora & Shapiro, 1992). Initial genetic studies of APS1 focused on HLA, and no association of this disorder with any specific HLA haplotype has been found (Neufeld *et al.*, 1981; Maclaren & Riley, 1986; Aaltonen *et al.*, 1993; Huang *et al.*, 1996). The gene underlying APS1 was subsequently localized on chromosome 21q22 by linkage analysis (Aaltonen *et al.*, 1994) and identified by positional cloning (Finnish-German APECED consortium, 1997; Nagamine *et al.*, 1997). This gene, designated autoimmune regulator-1 (AIRE-1), encodes a 545 amino acid protein that has two plant homeodomain (PHD)-type zinc-finger motifs, suggesting a role as a transcription factor (Finnish-German APECED consortium, 1997; Nagamine *et al.*, 1997). AIRE-1 mRNA is expressed in lymphoid tissues including thymus, lymph node and spleen, and possibly also in other tissues including the adrenal cortex (Finnish-German APECED consortium, 1997; Nagamine *et al.*, 1997), which suggests that it may have an important role in the development of a normal immune response.

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Table 1 Genetic causes of primary adrenocortical failure

Disorder	Genes (chromosomal locations)
Autoimmune Addison's disease (AAD)	
APS1	AIRE-1 gene (21q22)
APS2 and isolated AAD	Complex trait. HLA (p21), CTLA-4 (2q33), other genes
Adrenoleukodystrophy	ALD gene (Xq28)
Adrenal hypoplasia congenita (AHC)	
X-linked AHC	DAX-1 gene (Xp21)
SF-1 linked AHC	SF-1 gene (9q33)
Autosomal recessive AHC	Unknown gene
IMAGe syndrome	Unknown gene
Congenital adrenal hyperplasia	
21-hydroxylase deficiency	CYP21 gene (6p21)
3 β -hydroxysteroid dehydrogenase deficiency	3 β -HSD2 gene (1p13)
11 β -hydroxylase deficiency	CYP11 β 1 gene (8q22)
17 α -hydroxylase deficiency	CYP17 gene (10q24–25)
Congenital lipid adrenal hyperplasia	StAR gene (8p11)
Familial ACTH resistance syndromes	
Familial glucocorticoid deficiency	ACTHR gene (18p11)
Triple A syndrome	Linked to 12q13
Kearns–Sayre syndrome	Mitochondrial DNA

To date, 29 different mutations of the AIRE-1 gene have been identified in APS1 patients from different populations (Finnish-German APECED consortium, 1997; Nagamine *et al.*, 1997; Myhre *et al.*, 1998; Pearce *et al.*, 1998; Rosatelli *et al.*, 1998; Scott *et al.*, 1998; Wang *et al.*, 1998; Heino *et al.*, 1999; Ward *et al.*, 1999; Bjorses *et al.*, 2000). Due to founder effects, certain AIRE-1 mutations have been found to be common in some populations. For example, in Finnish APS1 patients, a particular nonsense mutation in exon 6 (R257X) accounts for >80% of mutant AIRE-1 alleles (Finnish-German APECED consortium, 1997; Nagamine *et al.*, 1997; Bjorses *et al.*, 2000). This is also the predominant mutation in the Italian APS1 population (Scott *et al.*, 1998). In the UK population, we have found that a 13 base-pair deletion in exon 8 (964del13, which has also been designated del1085–1097) comprised >70% of mutant AIRE-1 alleles (Pearce *et al.*, 1998) (Fig. 1), and a nonsense mutation, R139X, accounts for nearly all Sardinian cases of APS1 (Rosatelli *et al.*, 1998). The identification of AIRE-1 mutations, particularly mutations such as R257X, 964del13 and R139X occurring as the predominant mutation in different populations, will aid in genetic diagnosis of APS1 and screening of unaffected family members of APS1 patients (Fig. 1).

Isolated autoimmune Addison's disease and autoimmune polyendocrinopathy type 2 syndrome: complex genetic traits

The autoimmune polyendocrinopathy type 2 (APS2, Schmidt)

syndrome is the association of AAD with autoimmune thyroid disease and/or type 1 diabetes. APS2 has a predilection for middle-aged females (Neufeld *et al.*, 1981), with an average age of onset between 35 and 40 years. APS2 accounts for about 50% of autoimmune adrenocortical failure in our own UK series. Other autoimmune disorders, such as primary gonadal failure, pernicious anaemia, vitiligo and alopecia may also occur in APS2. Hypoparathyroidism and chronic candidiasis are absent (Neufeld *et al.*, 1981).

In contrast to APS1, the genetic basis of non-APS1 AAD (isolated AAD and APS2) has been less clearly defined. There are several reports showing concordance of these disorders in individual monozygotic twin pairs (Smith *et al.*, 1963; Heggarty, 1968; Simmonds & Lister, 1978; Russell *et al.*, 1991) and familial clustering (Hewitt, 1957; Frey *et al.*, 1973; Anderson *et al.*, 1980; Fairchild *et al.*, 1980). In addition, there is an increased prevalence of other autoimmune disorders, including autoimmune thyroid disease and type 1 diabetes, in patients with AAD and their family members, suggesting a close genetic relationship between these disorders (Nerup, 1974; Anderson *et al.*, 1980; Neufeld *et al.*, 1981; Kasperlik-Zaluska *et al.*, 1994; Zelissen *et al.*, 1995). In common with the majority of organ-specific autoimmune diseases, it is thought that isolated AAD and APS2 are inherited as complex traits, with many loci conferring variable degrees of susceptibility in different populations (Vyse & Todd, 1996).

Although multiple susceptibility genes are involved in the pathogenesis of complex traits, individual disease alleles are

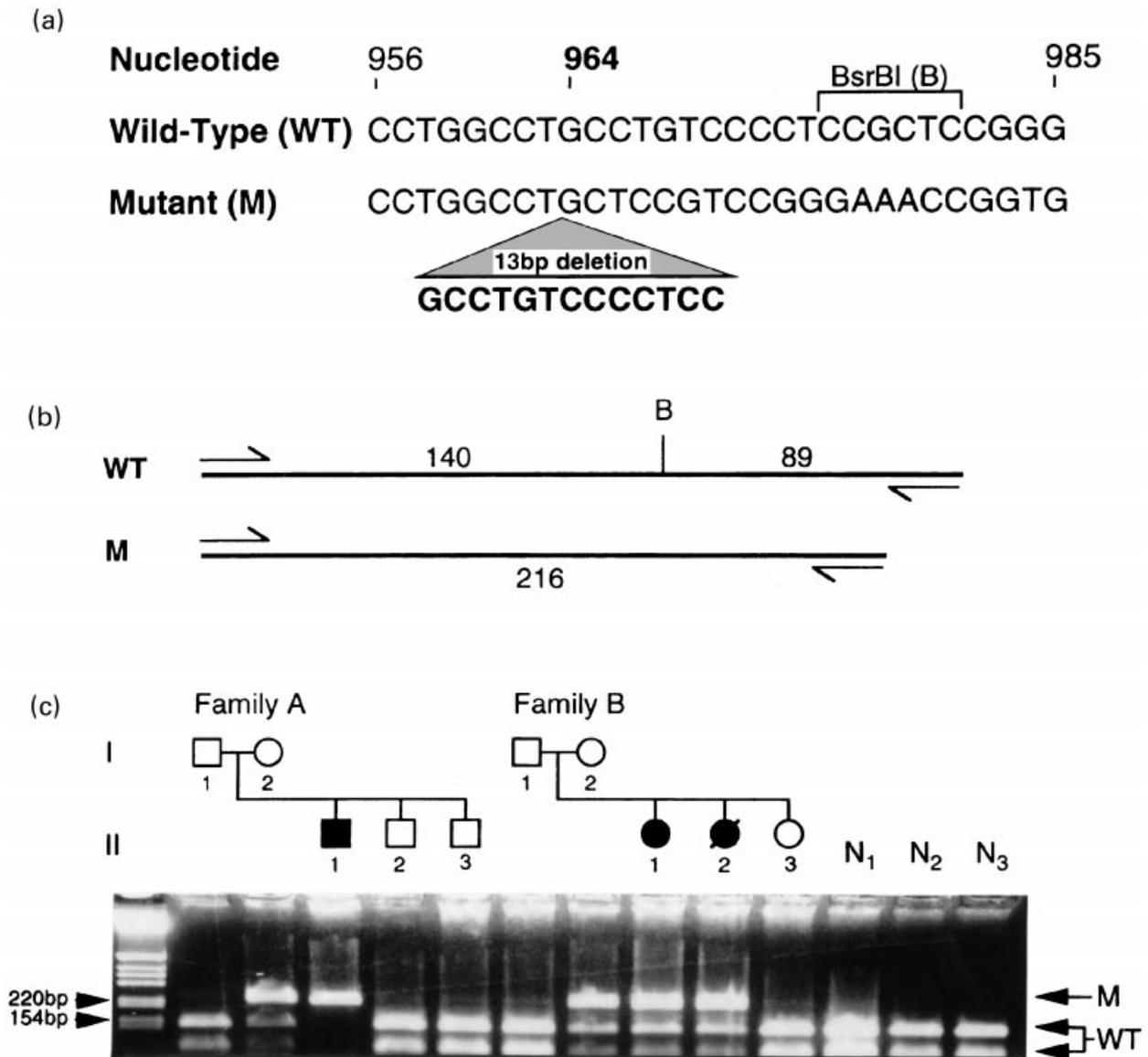


Fig. 1 Demonstration of a 13 base-pair deletion (964del13) at nucleotide 964 in the autoimmune regulator gene (AIRE-1) in two UK families with autoimmune polyendocrinopathy type 1 (APS1). (a) shows the wild-type (WT) and the mutant (M) (964del13) DNA sequences. This deletion abolishes the recognition site for the restriction enzyme *BsrBI* shown above the wild-type sequence. (b) shows a restriction map of the wild-type and mutant PCR product with the enzyme *BsrBI* (B). (c) shows the results of PCR amplification of exon 8 followed by *BsrBI* digestion in the two APS1 families (A and B). Subject II-1, family A has only the mutant 216 bp band, and neither of the wild-type digestion products, demonstrating that he is homozygous for the 13 bp deletion. His mother I-2, can be seen to be heterozygous for this deletion. In contrast, his father I-1, has only the wild-type 140 and 89 bp products. Paternity could not be refuted by the analysis of 14 microsatellite polymorphisms. Therefore, this mutation in the paternal allele in subject II-1, family A was due to a *de novo* mutation. The two younger brothers of the proband of family A, II-2 and II-3 who were aged 6 and 2 years, respectively, were demonstrated not to have this mutant allele. Regular screening for adrenal failure with six monthly Synacthen testing was therefore discontinued. The 964del13 mutation is also demonstrated to be heterozygous in subjects II-1 and II-2 family B who are affected with APS1, and in their mother I-2. Their unaffected younger sister II-3 and three unrelated normal subjects (N₁₋₃) are shown to have only the wild-type digestion products. These affected sisters were found to be compound heterozygotes having also inherited a single basepair deletion at nucleotide 1264 of AIRE-1 from their father. (Reproduced with permission, from Pearce *et al.*, 1998.)

Study	Country	No. of patients	Polymorphism/ allele	Relative risk
HLA and related genes				
Thomsen <i>et al.</i> , 1975	Denmark	32	B8	7.0
Maclaren & Riley, 1986	USA	34	DR3	12.1
			DR4	8.9
Latinne <i>et al.</i> , 1987	Europe	34	DR3	3.4
Boehm <i>et al.</i> , 1991	Germany	72	DR3	3.4
Weetman <i>et al.</i> , 1991	UK	33	DR3	3.6
Partanen <i>et al.</i> , 1994	Finland	12	DRB1*0301	24.0
			DQA1*0501	27.0
			DQB1*0201	17.0
			DPB1*0101	12.0
Badenhoop <i>et al.</i> , 1995	Germany	49	DQA1*0501	3.0
Betterle <i>et al.</i> , 1996	Italy	22	DR3	2.3
			DR5	2.1
Huang <i>et al.</i> , 1996	USA	40	DR3-DQB1*0201	2.9
Gambelunghe <i>et al.</i> , 1999	Italy	28	MIC-A5-1	6.5
			DR3/DQ2	4.8
CYP21 gene				
Partanen <i>et al.</i> , 1994	Finland	12	CYP21A del	25.0
Peterson <i>et al.</i> , 1995	Finland	12	+L ¹⁰ ,R ¹⁰² ,S ⁴⁹⁴	8.9
CTLA-4 gene				
Donner <i>et al.</i> , 1997	Germany	76	CTLA-4 A/G	ns*
Kemp <i>et al.</i> , 1998	UK	39	CTLA-4[AT] <i>n</i>	2.2
	Norway	43		ns
	Finland	8		ns
	Estonia	12		ns
Vaidya <i>et al.</i> , 2000	UK	90	CTLA-4 A/G	1.6
AIRE-1 gene				
Vaidya <i>et al.</i> , 2000	UK	90	964del13	ns

* Only significant in patients carrying HLA DQA1*0501 allele; ns, not significant.

neither necessary nor sufficient to cause the disease. Therefore, some individuals carrying a high risk allele of a susceptibility gene may not develop the disease (incomplete penetrance) while other individuals without the susceptibility allele may have the disease (phenocopy). This lack of correlation between genotype and phenotype causes great difficulties in defining the susceptibility loci for complex disorders (Lander & Schork, 1994; Vyse & Todd, 1996). Genetic analysis is particularly difficult in a rare complex trait such as AAD, where it is impossible to collect sufficient families with multiple affected members to perform conventional familial linkage studies. Thus, not surprisingly, genetic analyses in non-APS1 AAD have been essentially limited to population-based case-control association studies of candidate genes, often using small number of patients (Table 2). While case-control association studies may be sensitive in detecting loci with small effects, they can yield flawed conclusions if patients and control subjects are derived from genetically heterogeneous populations (population stratification), especially if sample sizes are small (Lander & Schork, 1994).

Studies of candidate genes in isolated autoimmune Addison's disease and autoimmune polyendocrinopathy type 2 syndrome

Human leucocyte antigen (HLA). HLA molecules play a key role in determining T cell responses to antigens, and various HLA alleles have been shown to be associated with many T cell mediated autoimmune disorders. Initial studies reported an association of AAD with HLA-B8 (Thomsen *et al.*, 1975; Eisenbarth *et al.*, 1978; Eisenbarth *et al.*, 1979). Later, a stronger association of AAD was found with HLA-DR3, which is in linkage disequilibrium with HLA-B8. Maclaren & Riley (1986) showed that HLA-DR3 and/or HLA-DR4 conferred susceptibility to AAD, except when the disease occurs as a component of APS1. The relative risk of AAD for Caucasian subjects who carried both HLA-DR3 and HLA-DR4 alleles was found to be as high as 46.8. Several subsequent studies have confirmed the association of AAD with various alleles within the HLA-DR3 carrying haplotype, including B8, DRB1*0301, DQA1*0501, DQB1*0201, DQ2 (Latinne *et al.*, 1987; Boehm

Table 2 Selected association studies of candidate genes in non-APS1 Addison's disease

et al., 1991; Weetman *et al.*, 1991; Partanen *et al.*, 1994; Badenhop *et al.*, 1995; Betterle *et al.*, 1996; Huang *et al.*, 1996; Gambelunghe *et al.*, 1999) (Table 2). This haplotype is also associated with type 1 diabetes and Graves' disease. In contrast, the association of HLA-DR4 with AAD is far less convincing, as several studies failed to reproduce the association (Latinne *et al.*, 1987; Boehm *et al.*, 1991; Weetman *et al.*, 1991; Huang *et al.*, 1996). Huang *et al.* (1996) showed that the association of AAD and the DR4-DQB1*0302 haplotype was due to the presence of concurrent type 1 diabetes and/or antibody evidence of pancreatic β -cell autoimmunity (which are associated with DR4 carrying haplotypes). However, a recent study showed a significant increase in the frequency of transmission of the HLA-DR4 haplotype (DRB1*0404, DQ8) from parents to children affected with AAD (irrespective of presence or absence of associated type 1 diabetes or anti-islet antibodies), but not to unaffected children (Yu *et al.*, 1999). Although the sample size in this study was small (seven families), it does suggest a possible role of this HLA haplotype in conferring risk to development of the disease in some populations.

Other genes within the HLA complex have also been studied for an association with AAD. However, due to strong linkage disequilibrium of genes within this region it is difficult to determine the independent role of a particular gene in conferring susceptibility to the disease. It has been shown that the association of AAD with a polymorphism of the tumour necrosis factor- β (TNF β) gene located in the class III major histocompatibility complex (MHC) region is due to linkage disequilibrium with the class II HLA genes (Partanen *et al.*, 1994). Similarly, it is likely that the recently reported association of AAD with a microsatellite polymorphism in the MHC class I chain-related (MIC-A) gene is a result of linkage disequilibrium, rather than a primary association (Gambelunghe *et al.*, 1999).

21-Hydroxylase (CYP21) gene. The gene encoding steroid 21-hydroxylase, designated CYP21, is located in the MHC class III region and is mutated in congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (see below). As 21-hydroxylase is a major autoantigen for AAD, its gene (CYP21) may play a role in conferring susceptibility to AAD. An association between AAD and the CYP21 polymorphisms has been reported; however, due to strong linkage disequilibrium between CYP21 and genes within the HLA region, the independent effect of this gene in disease susceptibility is difficult to ascertain (Partanen *et al.*, 1994; Peterson *et al.*, 1995). Recently, Nikoshov *et al.* (1999) studied the binding of sera from AAD subjects to several naturally occurring mutants of the 21-hydroxylase enzyme. One missense mutation (R483P) in the carboxyl-terminal domain of 21-hydroxylase, which is found in CAH, was found to alter binding of AAD

autoantibodies. However, this mutant allele was not found in the CYP21 gene sequence from 17 AAD subjects. These studies suggest that the commonly occurring CYP21 mutations that cause CAH in homozygotes or compound heterozygotes are not major susceptibility alleles for AAD.

Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene. The cytotoxic T lymphocyte antigen-4 (CTLA-4) is a costimulatory molecule that is expressed on the surface of activated T lymphocytes, and which negatively regulates T cell activation. CTLA-4 knockout mice develop a massive lymphoproliferative disorder with splenomegaly, lymphadenopathy and autoimmunity (Waterhouse *et al.*, 1995), providing evidence for a negative regulatory role of CTLA-4 in the immune response. In recent years, studies have demonstrated linkage and association of this locus with several autoimmune endocrine disorders including type 1 diabetes (Nisticò *et al.*, 1996; Donner *et al.*, 1997b; Marron *et al.*, 1997), Graves' disease (Yanagawa *et al.*, 1995; Donner *et al.*, 1997b; Kotsa *et al.*, 1997; Heward *et al.*, 1999; Vaidya *et al.*, 1999), and autoimmune hypothyroidism (Donner *et al.*, 1997a; Kotsa *et al.*, 1997).

Three case-control studies have examined the CTLA-4 polymorphisms for association with AAD. Donner *et al.* (1997a) found an association of the G allele of a single nucleotide polymorphism (CTLA-4 A/G) in exon 1 of the CTLA-4 gene with AAD in a subgroup of patients carrying a specific HLA allele, DQA1*0501. Kemp *et al.* (1998) studied a microsatellite polymorphism (CTLA-4[AT]*n*) in exon 4 of the CTLA-4 gene in AAD patients from four European countries. They found an association of the 106 bp allele of CTLA-4[AT]*n*, which is in linkage disequilibrium with the G allele of CTLA-4 A/G, in a cohort of 39 English AAD patients but not in AAD subjects from the Norwegian, Finnish or Estonian populations. Using a larger cohort of AAD patients from the UK, our group has confirmed that the G allele of the CTLA-4 A/G polymorphism confers susceptibility to AAD (Vaidya *et al.*, 2000). The contribution of CTLA-4 in conferring susceptibility to AAD is modest (relative risk 1.6–2.2) (Kemp *et al.*, 1998; Vaidya *et al.*, 2000), and this association was not found in several populations studied (Kemp *et al.*, 1998). Such genetic heterogeneity of CTLA-4 in different populations has also been shown in other autoimmune endocrinopathies, including type 1 diabetes (Nisticò *et al.*, 1996) and Graves' disease (Barbesino *et al.*, 1998; Vaidya *et al.*, 1999). Nevertheless, CTLA-4 is the first non-MHC linked locus for non-APS1 AAD, and these findings confirm the complex genetic nature of its pathogenesis.

Autoimmune regulator-1 (AIRE-1) gene. Homozygous or compound heterozygous mutations of the AIRE-1 gene cause AAD in the context of APS1, therefore AIRE-1 is a candidate susceptibility gene for isolated AAD and APS2. Although subjects carrying one mutant AIRE-1 allele are generally normal, it is possible that heterozygous AIRE-1 mutations

interacting with susceptibility alleles at other loci may predispose to the development of isolated AAD and APS2, as multiple susceptibility loci are likely to be involved in complex traits. We have found that only one of 90 unrelated non-APS1 AAD patients from the UK was a heterozygous carrier of the 964del13 AIRE-1 mutation, which is similar to the frequency of this allele in a healthy control UK population (Vaidya *et al.*, 2000). However, the presence of the 964del13 mutation in one AAD subject, together with another recent report showing a heterozygous missense mutation (V301M) within AIRE-1 in an APS2 patient (Soderbergh *et al.*, 2000), suggests that it may be too early to exclude a minor role for this gene in non-APS1 AAD.

Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is a rare X-linked recessive disorder characterized by primary adrenocortical failure, demyelination within the central or peripheral nervous system, and sometimes testicular failure (Moser, 1997). In males, adrenal failure most often presents before the age of

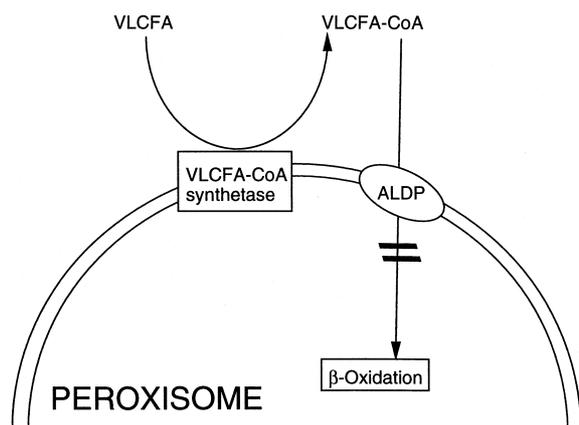


Fig. 2 Putative mechanism of peroxisomal very long chain fatty acid (VLCFA) accumulation in adrenoleukodystrophy (ALD). The ALD protein (ALDP) is a member of the ATP-binding cassette transporter family and is located on the peroxisomal membrane as a dimer. VLCFA undergo β -oxidation preferentially in peroxisomes, rather than in mitochondria, where the majority of β -oxidation of shorter-chain fatty acids occurs. The VLCFA coenzyme A synthetase (VLCFA-CoA synthetase) converts VLCFA to activated VLCFA-CoA, which is then thought to be transported into the peroxisome via the ALDP for degradation by β -oxidation. In ALD, the mutations in the ALDP gene lead to a failure of peroxisomal transport and cellular accumulation of VLCFAs. This may be particularly marked in steroidogenic and myelin producing cells, leading to VLC-cholesterol ester accumulation. The exact mechanism by which accumulated VLCFAs cause cellular dysfunction is unclear, but may involve disruption of normal cell membrane morphology.

15 years (Jorge *et al.*, 1994), however, it can manifest at any age and ALD cannot be excluded solely on the basis of the age of onset of adrenal failure (Korenke *et al.*, 1997). Approximately 10% ALD patients also have adrenal failure without nervous system involvement. The commonest neurological presentation (45%) is the rapidly progressive childhood cerebral ALD, which manifests before the age of 10 years, and often leads to severe disability and death within a few years. The slowly progressive adult type (35%), also known as adrenomyeloneuropathy, presents most commonly between ages 20 and 40 years, and affects mainly the spinal cord. Up to 20% of heterozygous females also develop a late-onset, mild form of neurological disorder resembling adrenomyeloneuropathy (Moser, 1997).

Recent studies have demonstrated that ALD, which affects about 1 in 20 000 males, is not as uncommon a cause of adrenal failure as previously thought (Sadeghi-Nejad & Senior, 1990; Aubourg & Chaussain, 1991; Jorge *et al.*, 1994; Laureti *et al.*, 1996; Aubourg, 1997; Laureti *et al.*, 1998). One study of Italian males with idiopathic Addison's disease showed up to a 35% prevalence of ALD (Laureti *et al.*, 1996). Adrenal failure is associated with both childhood cerebral ALD and adult adrenomyeloneuropathy, and can precede the onset of the neurological manifestations by several years. Thus a diagnosis of ALD should be considered in all males with primary adrenal failure, even in the absence of neurological symptoms, particularly if adrenal autoantibodies are negative (Laureti *et al.*, 1998). The principal biochemical abnormality in ALD is the accumulation of saturated unbranched very long chain fatty acids (VLCFAs) in blood and tissues due to impaired peroxisomal β -oxidation (Fig. 2). In ALD, the adrenal glands show a marked accumulation of cholesterol esterified with VLCFA, and undergo progressive atrophy as the disease advances (Powers *et al.*, 1980). Overt adrenal failure in heterozygous females is rare, but subclinical glucocorticoid deficiency after corticotropin-releasing hormone stimulation, occurs in about 60% (el-Deiry *et al.*, 1997).

The gene responsible for ALD is located on chromosome Xq28 and encodes a peroxisomal membrane protein with significant homology to the ATP-binding cassette superfamily of transporters (Mosser *et al.*, 1993). The precise function of the ALD transporter remains unknown, but it is thought that this protein is involved in the transfer of VLCFAs into the peroxisomes, where these are metabolized into shorter-chain fatty acids (Fig. 2). Over 200 disease-causing mutations in the ALD gene have been identified, which include deletions, missense, nonsense, frameshift and splice defect (reviewed in Moser, 1997; Dubois-Dalcq *et al.*, 1999; Smith *et al.*, 1999). The majority of these mutations are unique to a single family, but patients from the same family may present with differing spectra of disease manifestations (i.e. cases of both

childhood cerebral ALD and adrenomyeloneuropathy). However, a two-base-pair (AG) deletion in exon 5 is common and occurs in about 20% of unrelated kindreds (Moser, 1997). Recently, ALD gene knockout mice have been developed, which show accumulation of VLCFA in the brain and adrenal glands together with a decrease in β -oxidation *in vitro*, but these mice appear clinically normal up to 12 months (Kobayashi *et al.*, 1997).

The diagnosis or exclusion of ALD as the cause of adrenal failure has several implications. The incidence of *de novo* mutation of ALD gene is less than 8% (Fanen *et al.*, 1994; Feigenbaum *et al.*, 1996) therefore the diagnosis of a case of ALD frequently results in identification of other cases in the family. Newer therapeutic approaches are emerging in an attempt to reverse or arrest the clinical progression of ALD. A diet low in VLCFA and enriched in erucic and oleic acid esters (Lorenzo's oil) normalizes plasma VLCFA within 2 months (Rizzo *et al.*, 1989), but does not improve the symptoms in ALD patients who have already developed a neurological deficit (van Geel *et al.*, 1999). Whether this diet can prevent or delay neurological involvement in patients without neurological symptoms is still under investigation (Moser, 1997). Bone marrow transplantation has been found to be effective in preventing neurological deficits in ALD patients with minimal or no neurological symptoms (Aubourg *et al.*, 1990). These observations highlight the importance of early diagnosis of ALD by measuring plasma VLCFA level before the manifestation of overt neurological symptoms. Recently, Lovastatin (Singh *et al.*, 1998) and 4-phenylbutyrate (Kemp *et al.*, 1998) have been shown to normalize plasma VLCFA, and are under investigation as possible therapeutic agents. In addition, identification of heterozygous carrier females is also important for genetic counselling. Prenatal diagnosis of ALD is now possible with VLCFA assay and mutational analysis (Moser & Moser, 1999).

Adrenal hypoplasia congenita

Adrenal hypoplasia congenita (AHC) is an X-linked recessive disorder characterized by primary adrenal failure with low serum concentration of glucocorticoids, mineralocorticoids, and adrenal androgens that are unresponsive to ACTH (Mitchell & Rhaney, 1959; Weiss & Mellinger, 1970). In AHC, there is a failure of development of the permanent adult adrenocortical zones, and only large vacuolated cells resembling fetal adrenocortical cells are present. The incidence of AHC has been reported as one per 12 500 births (Lavery *et al.*, 1973), but this is likely to be an overestimate. There is a bimodal distribution in the age at onset of AHC with the majority presenting in the first 2 months of life with severe salt wasting, and the others between the ages of 2–9 years with

insidious adrenal failure (Reutens *et al.*, 1999). Boys who manifest in neonatal period with salt wasting may sometimes be confused with congenital adrenal hyperplasia due to 21-hydroxylase deficiency, but androgens and 17-hydroxyprogesterone are not elevated in AHC. AHC is frequently associated with delayed puberty due to hypogonadotropic hypogonadism (Prader *et al.*, 1975; Hay *et al.*, 1981), which is caused by abnormalities of gonadotropin secretion at both the hypothalamic and pituitary level (Habiby *et al.*, 1996). In addition, affected males with AHC may also have a defective spermatogenesis that is unresponsive to gonadotrophins (Seminara *et al.*, 1999). Rarely, AHC can present with adrenal failure later in adulthood (Tabarin *et al.* 2000), suggesting that AHC can not be excluded on the basis of age of onset of adrenal failure.

The locus for AHC was mapped to chromosome Xp21 by studies of male patients with contiguous gene deletion syndromes, which include AHC, Duchenne muscular dystrophy, mental retardation and biochemical evidence of glycerol kinase deficiency (Francke *et al.*, 1987). The gene for AHC, designated DAX-1 (Dosage sensitive sex reversal-AHC critical region on the X chromosome), was subsequently identified, and encodes a member of the nuclear hormone receptor family (Muscatelli *et al.*, 1994; Zanaria *et al.*, 1994). The C-terminal half of DAX-1 protein has high homology to the ligand binding domain of the nuclear hormone receptor family. However, in contrast to other nuclear hormone receptors, DAX-1 lacks zinc-finger DNA binding domain in the N-terminal and instead has three and a half repeats of a 65–67 amino acid motif (Zanaria *et al.*, 1994). DAX 1 plays a key role in the development of the adrenal gland and hypothalamo–pituitary–gonadal axis, and is expressed in the adrenal, testis, ovary, pituitary and hypothalamus (Zanaria *et al.*, 1994; Guo *et al.*, 1995; Hanley *et al.* 2000). Co-localization of DAX-1 and steroidogenic factor-1 (SF-1) and phenotypic similarity in their mutations (see below) suggest that these two proteins act in the same pathway of endocrine development (Ikeda *et al.*, 1996; Hanley *et al.*, 2000). So far, over 30 different DAX-1 gene mutations have been identified in AHC, and these mutations have been found to be responsible for both adrenal hypoplasia and associated hypogonadotropic hypogonadism. The majority of DAX-1 mutations are deletions or premature stop codons resulting in a loss of the C-terminal region. Recently, isolated hypogonadotropic hypogonadism has been reported in a female due to a homozygous DAX-1 mutation apparently resulting from an embryonic gene conversion event (Merke *et al.*, 1999). Apart from its use in genetic counselling, the molecular diagnosis of AHC in a child with adrenal failure has therapeutic implications, as it will predict hypogonadotropic hypogonadism later in the prepubertal period and allow timely initiation of androgen replacement therapy.

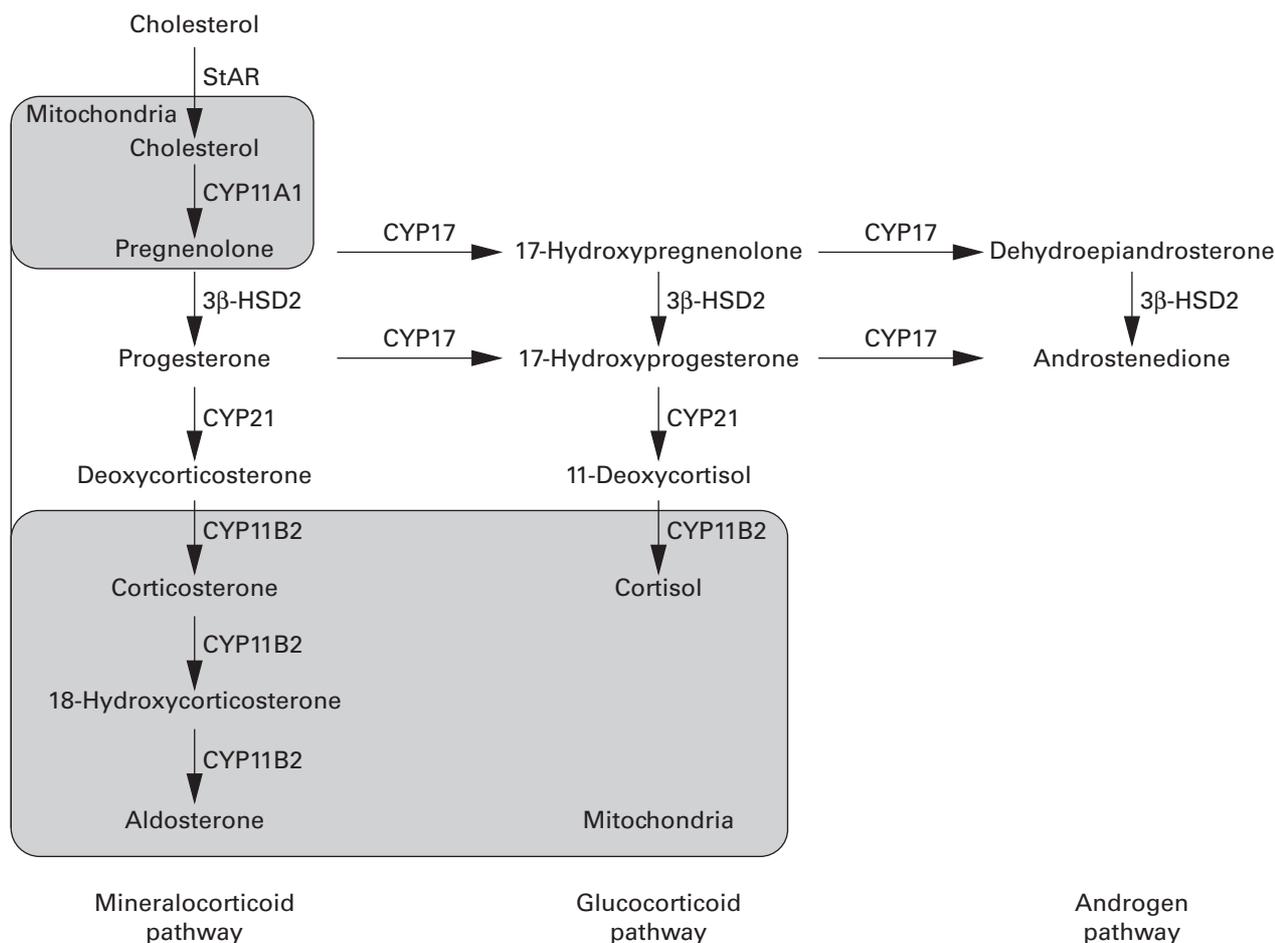


Fig. 3 Steroid biosynthesis pathway in the adrenal cortex. Enzymes involved in different steps of adrenal steroidogenesis are shown, which include CYP11A1 (cholesterol side-chain cleavage enzyme), 3β -HSD2 (3β -hydroxysteroid dehydrogenase), CYP17 (17α -hydroxylase/ $17,20$ -lyase), CYP21 (21-hydroxylase), CYP11B2 (aldosterone synthetase) and CYP11B1 (11β -hydroxylase). The StAR (steroidogenic acute regulatory) protein regulates the transfer of cholesterol to the inner mitochondrial membrane where the cholesterol side-chain cleavage complex is located. Various forms of congenital adrenal hyperplasia result from the defective secretion of different steroidogenic enzymes due to mutations in the genes encoding these enzymes.

Three other forms of AHC have been identified. Neonatal primary adrenal failure and XY sex reversal has been described in a phenotypically female patient, due to a heterozygous 2-bp mutation in the DNA binding domain of SF-1 (Achermann *et al.*, 1999). The adrenal morphology in this case remains unknown. SF-1 is an orphan nuclear receptor involved in regulation of steroidogenesis, reproduction and male sexual differentiation. The targeted disruption of this gene in mice results in adrenal and gonadal aplasia and XY sex reversal in homozygotes, consistent with the human phenotype (Luo *et al.*, 1994). A further rare autosomal recessive form of AHC is characterized by 'miniature adult' adrenal glands with a permanent cortical zone but a diminished fetal zone (Burke *et al.*, 1988). The genetic basis of this form of AHC remains unknown. Similarly, the genetic basis

of the recently described IMAGE syndrome (intrauterine growth retardation, metaphyseal dysplasia, AHC, and genital anomalies) is also unknown (Vilain *et al.*, 1999). Sequence analysis of DNA from patients with this syndrome revealed no mutation in the DAX-1 or SF-1 coding sequences (Vilain *et al.*, 1999).

Congenital adrenal hyperplasia

21-hydroxylase deficiency

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders due to the defects of the enzymes involved in different stages of adrenal steroidogenesis (White *et al.*, 1987; Speiser & White, 1998; New & Wilson, 1999) (Fig. 3).

The most common form of CAH, accounting for more than 90% of the cases, is due to the deficiency of the 21-hydroxylase enzyme. This enzyme converts 17-hydroxyprogesterone to 11-deoxycortisol in the glucocorticoid synthesis pathway, and progesterone to deoxycorticosterone in the aldosterone synthesis pathway. Therefore, a variable degree of impairment of both cortisol and aldosterone synthesis occurs in CAH due to 21-hydroxylase deficiency. The defective synthesis of cortisol leads to ACTH-driven adrenocortical hyperplasia and excess production of the steroid precursors, particularly 17 α -hydroxyprogesterone. These steroid precursors are also diverted to adrenal androgen synthesis resulting in the increased secretion of adrenal androgens, including androstenedione, dehydroepiandrosterone and testosterone. 21-Hydroxylase deficiency manifests in a wide range of presentations. In the 'classical salt wasting' type, affected infants present with severe dehydration, hypotension, hyponatraemia, hyperkalaemia and hypoglycaemia due to cortisol and aldosterone deficiency in the first few weeks of the life. In females, it is usually accompanied by varying degree of virilization of the external genitalia, which often leads to the early detection of this condition. In contrast, the diagnosis of this condition in male infants demands a high index of suspicion. The diagnosis of 21-hydroxylase deficiency is confirmed by raised plasma 17 α -hydroxyprogesterone and a characteristic pattern of increased urinary adrenocorticosteroid metabolites. The plasma cortisol levels may be low or in the low-normal range (White *et al.*, 1987). In the milder 'classic simple virilizing' type, CAH manifests with virilization of external genitalia without salt wasting. The 'nonclassic' or 'late onset' type CAH occurs around puberty or in adult life with the features of mild hyperandrogenaemia. The worldwide incidence of the 'classical' 21-hydroxylase deficiency is estimated to be about 1/15 000 live births (New & Wilson, 1999).

The gene encoding 21-hydroxylase, CYP21, together with its homologous pseudogene, CYP21P, is located in the MHC class III region on chromosome 6p21, alternating with the serum complement genes, C4A and C4B. Deletions and various point mutations of the CYP21 gene have been identified in 21-hydroxylase deficiency (reviewed in Speiser & White, 1998; Wedell, 1998). Most of the disease-causing mutations of CYP21 have been found to be normal sequences of the pseudogene, CYP21P. Either one of the two types of meiotic recombination between CYP21 and CYP21P are thought to be responsible for the phenomenon: first, misalignment and unequal crossing over, which results in large deletions of CYP21, and second, the gene conversion event leading to the transfer of small deleterious mutations from CYP21P to CYP21 (Speiser & White, 1998; New & Wilson, 1999). More than 95% of CAH due to 21-hydroxylase deficiency is caused by CYP21 gene deletions and the mutations with CYP21P sequences,

making mutational analysis suitable for diagnostic purpose (Day *et al.*, 1995). However, about 5% of cases of 21-hydroxylase deficiency results from rarer population specific mutations. The incidence of *de novo* CYP21 mutations causing CAH is estimated to be about 1%. (Wedell, 1998). There is a good correlation between genotype and phenotype in most cases of 21-hydroxylase deficiency, and genotypes are a useful predictor of clinical outcome (Speiser *et al.*, 1992; Speiser & White, 1998; Wedell, 1998). One group of mutations (deletions, R356W, Q318X, L307insT, Cluster E6) results in total inactivation of the 21-hydroxylase enzyme resulting in the severe 'salt wasting' disease. The second group of mutations (particularly, I172N) usually results in simple virilization without salt wasting, and the final group (such as V281L, P30L) is most often associated with the 'nonclassic' type. There are, nevertheless, several reports where a close genotype-phenotype correlation has not been observed (Wilson *et al.*, 1995).

Of the other rare steroidogenic enzyme defects leading to CAH, only 3 β -hydroxysteroid dehydrogenase deficiency is associated with adrenocortical failure. In 3 β -hydroxysteroid dehydrogenase deficiency, there is a defect in the conversion of steroid precursors with Δ^5 , 3-hydroxy configuration (pregnenolone, 17 α -hydroxypregnenolone and dehydroepiandrosterone) to their corresponding Δ^4 , 3-ketosteroids (progesterone, 17 α -hydroxyprogesterone, Δ^4 androstenedione) (Fig. 3). Male neonates with this disorder usually present with pseudohermaphroditism and adrenal insufficiency. In females, adrenal failure alone or with mild virilization occurs. This condition is caused by mutations in the 3 β -HSD2 gene (Simard *et al.*, 1995). CAH due to 11 β -hydroxylase and 17 α -hydroxylase deficiencies predominantly present with mineralocorticoid excess and hypertension, with overt glucocorticoid deficiency being rare. 11 β -hydroxylase deficiency, in addition, also leads to increased androgen secretion, affected females presenting with ambiguous genitalia and affected males with precocious puberty. This disorder is caused by mutations in CYP11 β gene, located on 8q22 (White *et al.*, 1994; Peter *et al.*, 1999). 17 α -Hydroxylase deficiency also causes abnormalities of sexual differentiation; affected females present with amenorrhoea and failure of sexual development, and karyotypic males with female genitalia. Different mutations including small deletions, duplications and point mutations have been found in the CYP17 gene in this condition (Yanase, 1995).

Lipoid congenital adrenal hyperplasia

Lipoid congenital adrenal hyperplasia (Lipoid CAH), is the most severe form of CAH, characterized by impaired synthesis of all adrenal and gonadal steroid hormones (Hauffa *et al.*, 1985; Miller, 1997). Severe adrenal insufficiency in lipoid CAH

leads to severe salt-losing crisis, hyponatraemia, hyperkalaemia, dehydration and hyperpigmentation in the neonatal period. 46XY genetic males are born with female genitalia because of the failure of testicular steroidogenesis. However affected 46XX females may undergo normal feminization and have cyclical vaginal bleeding (Bose *et al.*, 1997; Fujieda *et al.*, 1997). The metabolic defect in lipid CAH occurs in the conversion of cholesterol to pregnenolone, which is the first step of steroidogenesis. There is a massive accumulation of cholesterol and its ester in the adrenal cortex. Lipid CAH is more common in Japanese and Koreans than in other ethnic populations (Bose *et al.*, 1996).

Lipoid CAH is caused by mutations in the steroidogenic acute regulatory protein (StAR) gene, which is located on chromosome 8p11 (Lin *et al.*, 1995; Sugawara *et al.*, 1995). The StAR protein is expressed abundantly in the adrenal cortex and gonads (Sugawara *et al.*, 1995). This protein regulates steroid synthesis by playing a key role in the transfer of cholesterol to the inner mitochondrial membrane where the P450 side-chain cleavage complex is located (Lin *et al.*, 1995; Stocco, 2000). In humans several different mutations of StAR gene have been identified in lipid CAH. The mutation Q258X accounts for more than 80% the affected StAR alleles from Japanese and Korean patients (Lin *et al.*, 1995; Bose *et al.*, 1996; Nakae *et al.*, 1997; Yoo & Kim, 1998), and a carrier frequency of this mutation in these populations has been estimated as approximately 1 in 200 (Bose *et al.*, 1996; Yoo & Kim, 1998). The R182L mutation occurs commonly in the Palestinian population (Bose *et al.*, 1996).

Miller and colleagues (Bose *et al.*, 1996; Miller, 1997) have hypothesized a two stage mechanism for the pathogenesis of lipid CAH. According to this model, mutations in the StAR gene result in the disruption of StAR protein-dependent steroidogenesis in the fetal testis and adrenal glands (first stage). A small amount of steroid secretion, however, continues through StAR-independent steroidogenesis. The defect in StAR-dependent steroidogenesis leads to the persistent overstimulation of tropic hormones and an increased uptake of cholesterol by the steroidogenic cells. Eventually massive accumulation of cholesterol esters in the steroidogenic cells destroys these cells, and the steroid secretion is completely disrupted (second stage). In contrast to fetal testis, the fetal ovaries lack steroidogenic enzymes, and gonadotrophin-dependent ovarian steroidogenesis occurs first only around the time of puberty in XX females. Therefore, these XX females may undergo normal pubertal development through StAR-independent steroidogenesis until the ovarian accumulation of cholesterol ester (stage 2) results in hypogonadism.

Corticotropin resistance syndromes

At least three syndromes of corticotropin (ACTH) resistance

have been defined by a combination of clinical and molecular genetic means. Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder in which children suffer from recurrent hypoglycaemia sometimes resulting in infantile convulsions, pigmentation, recurrent infections and failure to thrive (Thistlethwaite *et al.*, 1975). A greatly elevated ACTH level is found with a low concentration of circulating cortisol, but electrolyte disturbances and dehydration are absent, consistent with intact mineralocorticoid production. Homozygous or compound heterozygous inactivating mutations of the G protein-coupled ACTH receptor (melanocortin-2 receptor) (Mountjoy *et al.*, 1992) are found in about 40% of FGD kindreds (Clark *et al.*, 1993; Tsigos *et al.*, 1993; Clark & Weber, 1998). These mutations result in a failure of adrenocortical organization with absent zonae fasciculatae and reticulatae, and hence glucocorticoid insufficiency. Although most of these FGD families have different ACTH receptor mutations which are spread throughout the gene, a recurrent missense mutation (S74I) has been identified in several UK pedigrees (Clark & Weber, 1998). No mutation in the ACTH receptor gene can be found in the remaining 60% of cases of FGD, and segregation studies exclude linkage to the ACTH receptor region in several kindreds (Weber & Clark, 1994). This demonstration of genetic heterogeneity suggests that two distinct forms of FGD are likely to exist.

Allgrove's syndrome, also known as the triple A syndrome, is characterized by the triad of adrenocortical failure due to ACTH resistance, achalasia and alacrimia (Allgrove *et al.*, 1978). Patients present in childhood with severe hypoglycaemia, pigmentation, failure to thrive, weakness and adrenal crisis. It is frequently associated with progressive neurological dysfunction, which includes autonomic, sensory, and motor neuropathy, deafness, and mental retardation (Moore *et al.*, 1991). In contrast to FGD, in which electrolyte disturbance does not occur, mineralocorticoid deficiency eventually develops in about 15% of subjects with this condition (Grant *et al.*, 1993). Allgrove syndrome is inherited as an autosomal recessive disorder and the gene for this disorder, which is yet to be identified, has been mapped to chromosome 12q13 near the type II keratin gene cluster (Weber *et al.*, 1996; Stratakis *et al.*, 1997). In contrast to FGD, no ACTH receptor gene mutation has been found in Allgrove's syndrome (Tsigos *et al.*, 1995; Wu *et al.*, 1998), providing further evidence for genetic heterogeneity in these syndromes of ACTH resistance.

Kearns–Sayre syndrome

Kearns–Sayre syndrome is a multisystem mitochondrial cytopathy with a wide variety of clinical manifestations, including ocular myopathy, pigmentary retinopathy, deafness, encephalopathy, lactic acidosis, stroke-like episodes, epilepsy,

myoclonus, heart block and ataxia. Several different endocrinopathies, including growth hormone deficiency, thyroid disorder, hyperaldosteronism, hypogonadism, diabetes mellitus and hypoparathyroidism may occur in this syndrome (Harvey & Barnett, 1992). Adrenal failure has been associated with Kearns–Sayre syndrome in two cases (Artuch *et al.*, 1998; Boles *et al.*, 1998). This disorder is maternally inherited and a variety of deletions of mitochondrial DNA are found in most cases.

Conclusion

The recent advances in the molecular pathogenesis of both congenital and acquired adrenocortical failure have clinical implications for both children and adult patients presenting with these disorders. Such subjects should be thoroughly evaluated for additional clinical features, e.g. other autoimmune disorders, gonadal failure, neurological dysfunction, as well as for evidence of past or current mycobacterial infection and metastatic disease in adults. In the absence of autoantibodies or other additional features to suggest the cause of the adrenal failure, further investigation for the various monogenic disorders is warranted. This may be particularly important in phenotypical males, and could result in the early diagnosis of adrenoleukodystrophy or adrenal hypoplasia congenita, which will both have prognostic and therapeutic implications for the patient and their families. Similarly the genetic background for autoimmune Addison's disease has started to be defined with the identification of the autoimmune regulator-1 gene, which has already allowed accurate genetic testing for siblings of probands with autoimmune polyendocrinopathy type 1 syndrome. Slow progress is being made towards defining the susceptibility loci for sporadic (non-APS1) autoimmune Addison's disease, and in the future this will allow better understanding of the pathogenesis of autoimmune Addison's disease, and may provide guidance in screening for other associated autoimmune disorders. The elucidation of the molecular basis for these various disorders has also cast light on some of the basic mechanisms behind normal adrenal development and steroid hormone biosynthesis.

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