Congenital adrenal hypoplasia and DAX-1 gene mutations

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INTRODUCTION

Congenital adrenal hypoplasia (AHC) is a rare disorder. Two distinct forms have been described based on the pathological features of the adrenal glands. The miniature form also called anencephalic or secondary form is characterized by small adrenal glands with normal architecture. This form is secondary to ACTH-deficiency and is usually sporadic although few cases of autosomal inheritance have been described [1]. The primary form is characterized by absence of the permanent (e.g. adult) zone of the adrenal cortex and the presence of large vacuolated cells resembling fetal adrenocortical cells [1, 2]. The X-linked inheritance of the « primary » form of CAH was identified during the late fifties but its association with hypogonadotrophic hypogonadism was recognized only in the mid seventies in patients who survived beyond childhood after treatment with adrenal steroids [1, 3].

DISCOVERY OF DAX-1

Families with the X-linked form of AHC show a pattern of X-linked recessive transmission in which only males are affected. Heterozygous females are unaffected but are carriers. The AHC locus was mapped initially to a critical region in Xp21 by determining the deletion break-
points in males with contiguous gene deletion syndromes (glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine carbamyltransferase deficiency and mental retardation). The dosage-sensitive sex-reversal (DSS) locus was also mapped to a region of Xp21 that overlaps the AHC critical region. DSS occurs in XY individuals carrying a duplication of this region and causes genetic males to undergo sex-reversal [3]. In 1994, the human DAX-1 gene was cloned and identified as the gene responsible for both X-linked AHC and the associated HHG [4, 5].

THE DAX-1 GENE AND ITS PRODUCT

The DAX-1 gene has a simple genomic structure composed of two exons and a single intron [4]. The C-terminal portion of the DAX-1 protein is homologous to the dimerization and ligand-binding domain of nuclear hormone receptors. Therefore, the product of the DAX-1 gene is considered to be a member of the nuclear hormone superfamily. However, no ligand is known to bind the putative ligand-binding domain of DAX-1, which is thereby considered to be an orphan receptor. The DAX-1 protein has a unique N-terminal domain that consists of a repeating motif of 66-67 amino acids. This region does not resemble any known protein; however, it may participate to the regulation of transcription of target genes by binding to hairpin loop structures in DNA [6, 7].

Expression of the homologue of the DAX-1 gene in mice (Ahch) is restricted to the three layers of the adrenal cortex, Leydig and Sertoli cells in the testis, ovarian theca and granulosa cells, pituitary gonadotropes, and the hypothalamic ventromedial nucleus [8, 9]. This tissue distribution is in accordance with the endocrine systems affected by mutations in the DAX-1 gene. DAX-1 shares other major links with reproductive function and phenotypic sex determination. The gene lies inside the critical region on the X chromosome, the duplication of which causes dose-sensitive sex reversal in XY individuals as mentioned previously [10]. This sex-reversal is also noted after transgenic overexpression of Ahch in mice [33]. DAX-1 is implicated in sex determination through inhibition of the action of the testis-determination factor SRY [11, 12].

A number of issues concerning the function of DAX-1 remain unsolved. Whether or not it possesses an endogenous ligand and the mechanisms through which it plays its physiological role in the development of the adrenal cortex and gonadotrophic axis remain speculative.

Complex interactions between DAX-1 and SF-1, a key mediator in the development of the adrenal glands, pituitary gonadotrope cells and ventromedial hypothalamus [13] have been demonstrated, suggesting they interact in a common genetic pathway. DAX-1 and SF-1 are co-expressed in the same tissues suggesting a direct physical interaction. Protein-protein interactions between SF-1 and DAX-1 have been demonstrated in vitro and in transfected cell lines [14, 15]. In all cases, DAX-1 represses the transcriptional activity of SF-1 [3]. The transcriptional silencing domain in DAX-1 is localized to the carboxyterminus of the protein corresponding to the putative LBD [3, 14, 16]. The molecular mechanism of this inhibition is not fully understood. DAX-1 may recruit corepressor to SF-1 such as N-CoR [15] or the recently described Alien [17]. Alternatively, since the DAX-1 interaction region in SF-1 is also required for interaction with the SCR-1-NcoA-1 family of coactivators, another mechanism for DAX-1 action could be competition with coactivators for a common binding site on SF-1 [3, 16]. In addition, a SF-1 response element has been identified in the DAX-1 promoter [18] and SF-1 activates Ahch, the DAX-1 mouse homologue [19].

Remarkably, all mutations associated with AHC reported to date alter the structure of the carboxy-terminus of the DAX-1 protein [20-22] (OMIM 300200). Given its transcriptional silencing activity in vitro and the phenotype of patients with DAX-1 mutation, a repressor role for DAX-1 in the adrenal and reproductive axes is difficult to understand. It is postulated that, while necessary for normal development of the adrenal glands and gonadotrophic axis, DAX-1 may also serve as a « brake » in normal maturation. Alternatively, if a ligand for DAX-1 exists, its binding to DAX-1 may reverse the transcriptional repression.

Apart from interactions with SF-1, DAX-1 also inhibits directly the transcriptional activity of SF-1 target genes such as the steroidogenic acute regulatory protein (StAR), P450sc, 3 beta-hydroxysteroid-dehydrogenase genes involved in adrenal steroidogenesis and consequently DAX-1 blocks steroidogenesis [6, 23]. This effect depends on the presence of hairpin structure in the promoter region of target genes to which DAX-1 binds [7].

MUTATIONS IN THE DAX-1 GENE

All mutations associated with AHC reported to date alter the structure of the carboxy-terminus of the DAX-1 protein (20-22, OMIM : 300200). The majority of these are frameshift or non-sense (stop codon) that result in a truncated protein. Only 11 different missense mutations in DAX-1 associated with AHC have been reported to date [for review see [21]]. Structural consequences of these mutations have been speculated based on the
molecular modelling of the putative DAX-1 C-terminus assuming that it is highly homologous to the LBD domain of several nuclear hormone receptors with known crystal structure [7, 22]. A direct relationship between AHC and the loss of repression of SF-1-responsive genes and/or SF-1 mediated transactivation in vitro, has been demonstrated for several naturally occurring mutations [14, 16, 20, 21]. However, apart a single case report [21], there is no obvious relationship between the putative structural consequences of these mutations and the clinical phenotype [20]. This is exemplified by the variability in the onset and the degree of adrenal insufficiency within the same family as well as the gonadotropins secretion pattern and suggests that other epigenetic or nongenetic factors influence the clinical course of AHC [20, 24, 25].

NEW PHENOTYPES OF AHC

Recently, DAX-1 mutations have been found in several men and women who have less typical phenotypes. We described the novel phenotype of a man who presented with apparently isolated primary adrenal insufficiency at 28 years of age [21]. Examination revealed undiagnosed incomplete HHG of hypothalamic origin that had allowed significant pubertal development to occur. Genomic analysis revealed a novel Ile439Ser missense mutation in the carboxy-terminus of DAX-1. Consistent with the patient’s mild phenotype, this mutation only partially inhibits the ability of DAX-1 to act as a repressor in several transient gene expression assays of DAX-1 function. This unique case extends the clinical spectrum of AHC to include delayed onset primary adrenal insufficiency in adulthood and milder forms of HHG.

Given this phenotype in which reproductive abnormalities preceded adrenal symptoms, Achermann et al. hypothesised that DAX-1 mutations might cause idiopathic familial or sporadic HH or constitutional delay of puberty (CD) among patients lacking a history of overt adrenal failure [37]. DNA sequence analysis of over 100 such patients suggests, however, that mutations in DAX-1 are unlikely to be a common cause of such conditions.

Knockout studies have recently revealed an unsuspected male-specific function of Ahch [10]. Male Ahch knockout mice exhibit retention of the fetal zone of the adrenal glands but have normal corticosterone production. In addition, and contrary to the findings observed in humans, mutant mice do not develop HHG. However, impaired spermatogenesis is seen and is suggestive of a primary Sertoli cell defect that occurs independently of abnormal gonadotropin and testosterone production, resulting in complete loss of germ cells by 14 weeks [19]. Whether or not DAX-1 mutations affect spermatogenesis in humans, independently of gonado-

CLINICAL AND BIOLOGICAL FEATURES OF « CLASSICAL » AHC

Boys with X-linked AHC usually develop adrenal failure during the first few weeks of life. The median age at presentation of patients with confirmed DAX-1 mutations is three weeks [20]. Rare cases have been described of a delayed onset in adrenal failure for several years [25-29] or progressive deterioration of adrenal function following an asymptomatic period during infancy [24, 30]. However, in all cases reported to date, unequivocal symptoms of adrenal failure appeared before the age of 10. Salt-losing crises in the neonatal period are common. Symptoms of primary adrenal insufficiency such as nausea, weight loss, hypotension and hyper-pigmentation are seen in boys who present later in childhood. A history of adrenal insufficiency or unexplained childhood death among brothers or boys in the mother’s family is frequently encountered although one third of AHC patients reported to date have no other affected family members and have de novo DAX-1 mutations [20, 27, 31]. Laboratory investigations are consistent with primary adrenal insufficiency including hyperkalemia, low cortisol, 17-hydroxyprogesterone and aldosterone plasma concentrations with contrasting elevated ACTH and renin plasma concentrations.

Although the pituitary-gonadal axis may be preserved early in infancy [32-34], failure to undergo puberty due to hypogonadotrophic hypogonadism is a hallmark of cases with DAX-1 mutations. Partial gonadotrophin deficiency through most of childhood has recently been reported in 3 males with adrenocortical insufficiency, in whom early- or mid-puberty had been reached spontaneously [35]. However, gonadotrophin deficiency rapidly progressed in these patients between the age of 15 and 18, resulting in failure to progress in puberty and dramatically low serum testosterone concentrations. This suggests that DAX-1 as a critical role in the maintenance but not the onset of the gonadotropic axis. Whether HHG results from hypothalamic or primary pituitary dysfunction has been a matter of debate. Recent studies suggest that, in accordance with the pituitary and hypothalamic expression of DAX-1 [2], mutations impair gonadotropin production by acting with variable intensities at both the hypothalamic and pituitary levels [24, 25, 36].

Apart from symptoms of adrenal and hypothalamic/hypophyseal involvement, features of a contiguous deletion gene may also be present (OMIM : 300200).
tropin production, remained unknown until recently. The patient that we described previously had, in contrast to the incompleteness of HHGs, a severe oligo-
spermia [21]. Oligospermia that did not improve after 10 months of gonadotropin therapy. The finding that the serum inhibin B concentration in this patient was dramatically lower than expected in view of the serum immunoreactive FSH concentration provides additional support for the view that the DAX-1 mutation causes a primary defect in Sertoli cell function [38]. Warnet et al. recently described a case with similar testicular reproductive features [39]. Furthermore, Seminara and al. reported a family in which the proband underwent a testicular biopsy revealing rare spermatogonia at the age of 27 after 7 years of low dose hCG therapy [24]. Despite the administration of steadily progressive doses of hCG and hMG during 3 years, the patient remained azoospermic. A relative resistance of Leydig cells to hCG due to impaired production of Sertoli cell-secreted factor has also recently been suggested in one case report [40]. All together, these data suggest that abnormalities of spermatogenesis and consequently infertility, independent of gonadotrophin production, may also comprise part of the clinical spectrum of DAX-1 mutations.

Normal adrenal function, in a woman who is homo-
ygous for a DAX-1 mutation through gene conversion [41] and extreme pubertal delay, but normal fertility, among heterozygous female carriers of DAX1 mutations [24] have also been recently described.

CONCLUSIONS

Several recent reports of patients expand the clinical spectrum of X-linked AHC to include delayed onset of adrenocortical insufficiency in adulthood, subclinical HHG, intrinsic defects in spermatogenesis in men and possibly delayed puberty in women. Additional longitudi-
dlal studies in human will be necessary to confirm poor prognosis of AHCh-associated infertility.

Observation of patients with missense mutations, ani-
mal and in vitro models of DAX-1 mutations will be use-
ful to define the relationship between the structure and function of the DAX-1 protein and its functional role. Last, no point mutations in the coding region of DAX-1 have been described in sporadic cases and familial cases of X-linked AHC associated with HHG [5] suggesting genetic heterogeneity. The genetic abnormalities involved in these cases and the recently described syndrome of X-linked AHC with normal puberty remains to be deter-
mined [42].

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