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Molecular pathogenesis of lipoid adrenal hyperplasia and adrenal hypoplasia congenita☆

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Abstract

Congenital lipoid adrenal hyperplasia (lipoid CAH) is the most severe form of CAH in which the synthesis of all gonadal and adrenal cortical steroids is markedly impaired. Lipoid CAH may be caused by the defect in either the steroidogenic acute regulatory (StAR) protein or the P450scc. More than 34 different mutations in StAR gene have been identified. Clinically, most of the patients manifest adrenal insufficiency from 1 day to 2 months of age, but some patient show delayed onset of adrenal insufficiency. Affected 46, XY subjects do not show pubertal development, whereas affected 46, XX subjects undergo spontaneous feminization, breast development and cyclical vaginal bleeding at the usual age of puberty.

X-linked adrenal hypoplasia congenital (AHC) is a rare congenital adrenal disorder characterized by severe adrenal insufficiency and hypogonadotropic hypogonadism. More than 80 different several intragenic mutations of DAX-1 have been identified. The failure of pubertal development may be caused by either abnormal hypothalamic or pituitary regulation of gonadotropin secretion. In addition, although the testicular steroidogenesis is largely intact, the functional maturity of Sertoli cells and also spermatogenesis are impaired. The type of mutation does not predict clinical phenotype. Thus, unified mechanism how DAX-1 gene defect gives rise to adrenal insufficiency, hypothalamic/pituitary hypogonadism and impaired spermatogenesis remains established.

Keywords: Lipoid adrenal hyperplasia (lipoid CAH); StAR; P450scc; Adrenal hypoplasia congenita; DAX-1

1. Introduction

Congenital adrenal insufficiency presents as a life-threatening crises and is lethal if left untreated with glucocorticoids and mineralocorticoids. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, congenital lipoid adrenal hyperplasia (lipoid CAH) and adrenal hypoplasia congenital (AHC) are the most common and present severe adrenal insufficiency in newborn period. The genes that involved in the function and development of adrenal glands are increasingly identified. This gives insights into the understanding of the molecular pathophysiology of congenital adrenal disorders and also permits a rapid accurate diagnosis for the disorder [1]. The mutations in the gene of steroidogenic acute regulatory (StAR) protein and subsequently the cholesterol side chain cleavage enzyme (P450scc) are identified for the potentially lethal disease in humans known as congenital lipoid adrenal hyperplasia [2–12]. As a cause of X-linked AHC, the DAX-1 (dosage-sensitve sex reversal, adrenal hypoplasia congenita, critical region on the X chromosome, gene [1] is identified [13,14]. In this presentation, we will describe recent understanding of molecular pathogenesis in lipoid CAH and AHC.

2. Congenital lipoid adrenal hyperplasia

Lipoid CAH is the most severe form of congenital adrenal hyperplasia, which is characterized by inborn error of steroid hormone biosynthesis that disrupts the synthesis of all adrenal and gonadal steroids and leads to the accumulation of cholesterol esters [3,15]. The true incidence of lipoid CAH is unknown, but it is clearly much higher in people of Japanese, Korean and Palestinian ancestry. Affected individuals are phenotypically female and have severe salt wasting. It is shown that mitochondria isolated from affected adrenal glands and gonads of lipoid CAH patients fail to convert cholesterol to pregnenolone. The endocrine profiles and in vitro studies clearly indicate that the metabolic lesion in this disorder is localized to the first

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step of steroidogenesis [16,17]. The cholesterol side chain cleavage enzyme (P450scc) supported by its electron transport system consisting of NADPH-adrenodoxin reductase and adrenodoxin catalize the conversion of cholesterol to pregnenolone in mitochondria of adrenal and gonads. The steroidogenic acute regulatory protein also functions as a labile protein factor in steroidogenesis mediate cholesterol transport within mitochondria [18]. As a cause of lipoid CAH, the gene for steroidogenic acute regulatory protein was eventually identified [2]. StAR facilitates the transport of cholesterol into mitochondria. StAR is expressed in the adrenals and gonads but not in the placenta, and placental biosynthesis of progesterone, which requires P450scc, is unaffected in lipoid CAH. Placentally produced progesterone is essential for the maintenance of human pregnancy. The human corpus luteum of pregnancy secretes progesterone only during the first trimester, during which there is a luteo-placental shift to the placental production of progesterone. Therefore, mutations in P450scc were suggested to be incompatible with term gestation. Nevertheless further molecular genetic analysis in the few patients with lipoid CAH in which mutations in the StAR gene have not identified may indicate another cause of lipoid CAH such as P450scc, unique protein that interact with StAR, or in the conveyance of cholesterol prior or subsequent to StAR action. Consequently in two cases with a syndrome that is clinically indistinguishable from lipoid CAH either heterozygous or compound heterozygous mutations in P450scc gene were identified [11,12]. Thus, both of the defects of StAR and P450scc are now considered to be responsible for lipoid CAH, but most lipoid CAH may be caused by the mutations in the StAR gene.

3. Human StAR mutation

More than 34 different mutations in the StAR gene have been identified until now [19] (Fig. 1). The mutations are present in all exons. The mutations in intronic region are also found in intron 1 and 4. The mutations causing premature translational termination or altering of the StAR reading frame are common, and they substantially alter the structure of the StAR protein. All missense mutations are found in the carboxy-terminal 40% of the amino acid StAR protein. The Gly 258 End mutation in exon 7 is very common in Japanese and Korean [4,9]. In Japanese this mutation is identified in 62% of the alleles and in over 80% of the patients. The Arg 182 Leu mutation in exon 5 is common among Palestinian patients. Most of the mutant StAR mutation except M225T proteins did not enhance the conversion of cholesterol to pregnenolone, indicating that the mutant StAR proteins are inactive. M225T mutant retained 43% activity of the wild type. The mutation in the StAR gene has not been identified in the few patients with lipoid CAH. Thus, the other yet unidentified, key regulatory elements in the promoter and/or to unique proteins that interact with StAR, or in the conveyance of cholesterol prior or subsequent to StAR action may be also responsible for the lipoid CAH.

4. Clinical and laboratory findings in the patients with the defect of StAR gene

Affected individuals are phenotypically female and have severe salt wasting. The age of onset of adrenal insufficiency is during the neonatal and early infantile period. All patients have a generalized pigmentation at the time of diagnosis, elevated levels of ACTH and plasma renin activity (PRA), decreased levels cortisol, urinary 17-hydroxycorticosteroids and urinary 17-ketosteroids [15]. None of the patients with a 46, XY karyotype manifests any pubertal changes despite their chronological age. In contrast, the patients with a 46, XX karyotype manifest secondary sexual characteristics with the development of breast tissue, pubic hair and irregular menstruation, suggesting heterogeneity of the functional defect in gonadal steroidogenesis in lipoid CAH [20,21] (Fig. 2). Spontaneous thelarche and subsequent menarche manifests following after the increased LH and estradiol levels. Ovaries in post-pubertal stage are enlarged with many cysts occupying the entire volume of the ovary

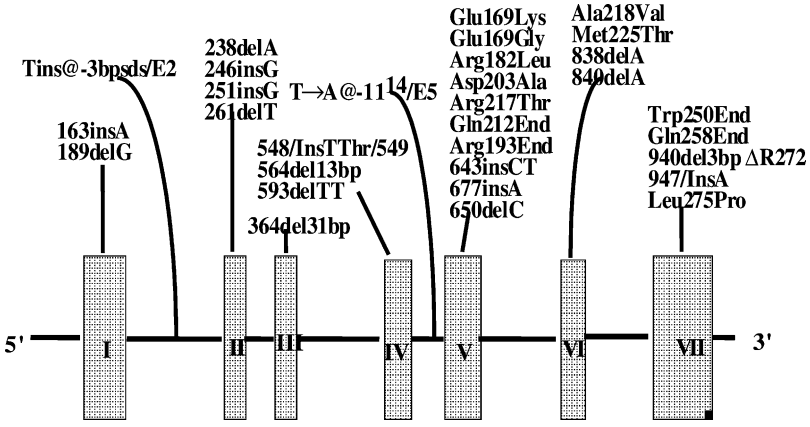

Fig. 1. Mutations in the StAR gene identified in lipoid CAH. Schematic representation of StAR to show the range of naturally occurring mutations.

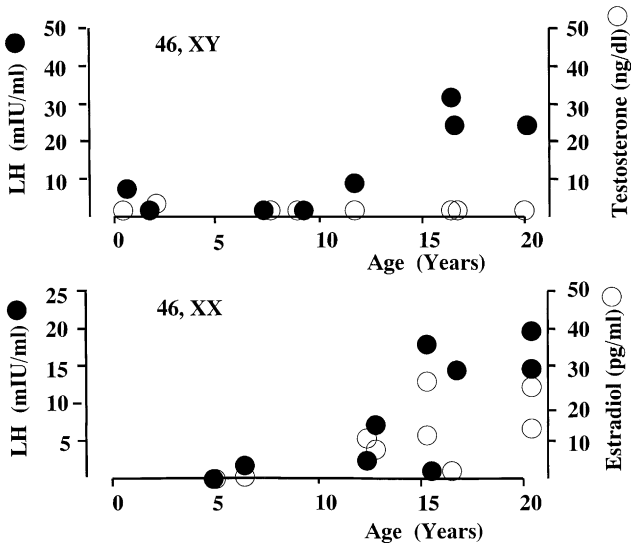

Fig. 2. Cross-sectional change of LH, testosterone and estradiol in the patients with the defect of StAR gene. Upper column shows 46, XY subjects and lower column shows 46, XX subjects. Closed circle represent LH and open circles represent testosterone in XY subjects and estradiol in XX subjects.

and hypertrophied ovarian stroma. In contrast, ovaries in the prepubertal stage are normal size and there is no cystic change, indicating that ovarian cysts are very common in lipoid CAH female and supporting that ovarian steroidogenic tissues are spared the early and persistent stimulation that dooms the testicular and adrenal cortical cells to damage from cholesterol engorgement. Adrenal insufficiency manifests at birth in most of the patients with lipoid CAH. The affected patient with compound heterozygous mutation of M225T and common Japanese Q258X Gly 258 End mutation show delayed onset. He had no clinical symptoms until 2 months of age and survived without treatment 4 months, at which time the clinical picture was comparatively mild. This infant is 46, XY patient had mild virilization of the external genitalia, consisting of mild clitoromegaly, minimal posterior labial fusion, and mild rugation of the labia. Because of these findings, this infant was followed closely and had normal basal and ACTH-stimulated cortisol values at 1 and 3 months of age, but eventually experienced a salt-losing crisis at 10 months, associated with hyponatremia, hyperkalemia, and grossly elevated ACTH and plasma renin values.

5. Human P450scc mutation

Mutations in P450scc gene may also create a syndrome that is clinically indistinguishable from lipoid CAH. Two cases with either heterozygous or compound heterozygous mutations in P450scc gene have been identified [11,12]. First index patient has 46, XY karyotype and born after an uneventful pregnancy. Inguinal hernias were diagnosed at 2 years of age, and inguinal masses were resected. When first seen at age 4 year, he was lethargic and had hyperpigmentation. He had clitoromegaly, no labial fusion, and separate vaginal and urethral openings. Gonads were not palpable. Plasma ACTH was extremely elevated, cortisol was low, PRA was elevated and plasma aldosterone was low. ACTH administration did not increase serum cortisol. Salt-restricted diet did not stimulate urinary aldosterone excretion. Administration of GnRH increased LH and FSH. Human CG stimulation did not increase serum testosterone levels. Antibodies to P450scc, P450c21 and P450c17 were not detected in her serum, ruling out the common form of autoimmune Addison's disease. Computed tomography and ultrasonographic examinations revealed no hypertrophy of the adrenal glands, and no uterus. Vaginography demonstrated a blind vaginal pouch. This patient's StAR gene and the SF-1 gene were homozygously normal. The exons of her gene for P450scc was then sequenced and identified heterozygousty for a six-base insertion in exon 4 (Fig. 3). This mutation, which may have occurred by slipped strand mispairing, inserts codons for Gly and Asp between Asp 271 (GAC) and Val 272 (GTG) without altering the P450scc reading frame. Analysis of the patient's genomic DNA from hair follicles and urinary sediment confirmed that this mutation was heterozygous and was inconsistent with mosaicism. Leukocyte genomic DNA from her mother and father did not contain this mutation, suggesting that the patient's mutation occurred de novo. This in-frame insertion altered P450scc enzymatic activity and did not act as a dominant negative mutant. Any other mutations were not identified by direct sequencing of all other exons, splice sites and 620 bases of 5′-flanking DNA. The reason why this patient developed incomplete virilization and mild adrenal insufficiency despite retaining 50% of normal enzymatic activity remains examined. However, it can be probably explained by the same "two-hit" mechanism that accounts for the pathophysiology of lipoid CAH [3]. P450scc is the rate-limiting enzyme in steroidogenesis. This is a very slow enzyme, turning over only 1 mol of cholesterol per mole of P450scc per second. Thus, haploinsufficiency of P450scc may prevent the elaboration of an appropriate steroidogenic response. The insufficient steroidogenesis will lead to increased trophic stimulation of the adrenals by ACTH and of the gonads by gonadotropins, stimulating the uptake of low-density lipoproteins and cholesterol. This cholesterol accumulates, eventually causing cell death. The principal difference with lipoid CAH is that haploinsufficiency of P450scc permits more steroidogenesis than the low levels of StAR-independent steroidogensis seen in StAR deficiency, so the progression from the mutation-induced impairment in steroidogenesis (the first hit) to the loss of steroidogenesis from cell death (the second hit) is slower. Katsumata identified recently second patient with a compound heterozygous mutation in P450scc gene [12]. This 46, XX patient had one mutation, a maternally inherited mutation which resulted in markedly reduced P450scc activity and the other mutation which is de no novo mutation in the paternal allele, did not

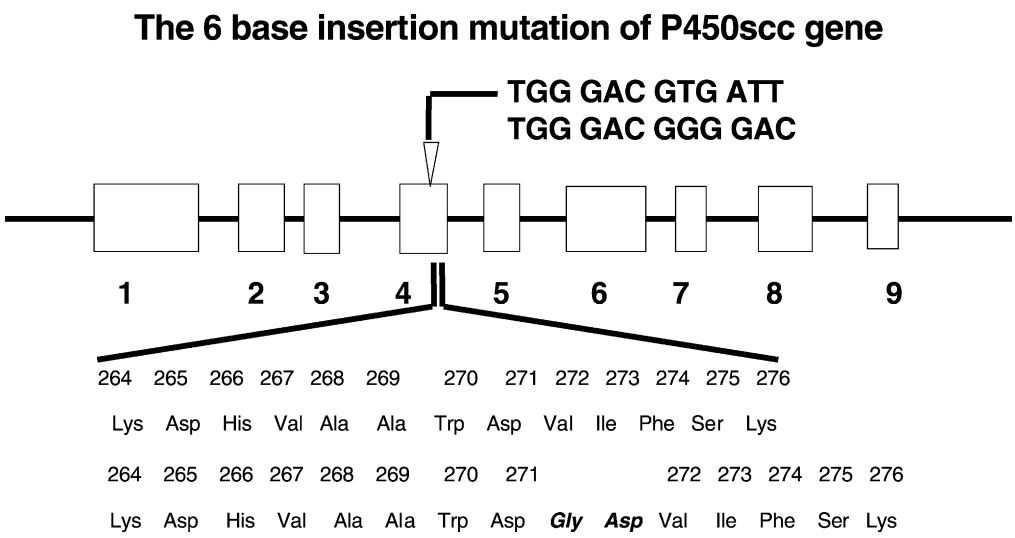

Fig. 3. Mutations in the P450scc. Schematic representation of the mutation sites and part of the amino acid sequence of P450scc. The 6 bp insertion adds two amino acids (Gly and Asp) in-frame between Asp 271 and Val 272.

affect the P450scc activity. The patient had a partially preserved ability to synthesize adrenal steroid hormones and showed delayed appearance of adrenal insufficiency. Thus, it is true that P450scc is also responsible for lipoid CAH.

6. X-linked adrenal hypoplasia congenita

Adrenal hypoplasia congenita is an inherited disorder that most often presents in infancy with adrenal insufficiency and severe salt wasting. Isolated hypogonadotropic hypogonadism is also a feature of this disorder, but it is recognized only after treatment with adrenal steroids allowed survival beyond childhood. Careful clinical management of the affected children is important, because rapid and life-threatening deterioration of adrenal function frequently follows a symptomatic period during infancy. Adult adrenal cortex consists of three different zones of zona glomerulosa, zona fasiculata and zona reticularis. These three zones develop from definitive zone of the fetal adrenal cortex after birth. The primary form of AHC appears as X-linked and autosomal recessive disorder with different adrenal morphologies. The adrenal glands in the X-linked form lack the definitive zone of the adrenal cortex and are characterized by large vacuolated cells resembling fetal adrenocortical cells.

The locus for X-linked AHC has been mapped to Xp21.3-21.2 by deletion studies of male patients with a contiguous gene syndrome, including AHC, glycerol kinase deficiency, and Duchenne muscular dystrophy. The gene order Xpter-AHC-GKD-DMD-cen was established from the deletion studies. The gene, termed DAX-1 (dosage-sensitive sex reversal). Adrenal hypoplasia congenital critical region on the X-chromosome, gene [1] (NR0B1) is identified responsible for X-linked AHC [13]. Thus, AHC may be caused by deletion of the Xp21 DAX-1 gene as part of a contiguous gene syndrome or by intragenic mutations. The locus for autosomal recesive AHC remains unidentified.

DAX-1 is a member of the nuclear receptor family in which no ligand has been identified to regulate its function. and is expressed in the adrenal cortex, gonads, ventromedial hypothalamus and pituitary gonadotropes. This gene consists of two exons separated by a 3.4 kb intron. The cDNA sequence of this gene contains an open reading frame of 1410 bp, predicting a protein of 470 amino acids. DAX-1 shares structural homology with the C terminus of other nuclear receptors, it lacks the zinc-finger DNA-binding domain that is characteristic of most nuclear receptors. Instead of the zinc fingers, the N-terminus of DAX-1 contains multiple copies of a unique 66 amino acid repeat motif that appears to mediate protein–protein interactions. Each of the three repeats contains a leucine-rich receptor-binding motif, known as the LXXLL motif. DAX-1 acts in part by repressing the transcription of other nuclear receptors, including steroidogenic factor 1 (SF-1), estrogen receptor and androgen receptor. Amino acid sequences of DAX-1 have high homology between these species of human, monkey, pig, rat and mouse.

7. Human DAX-1 mutation

AHC may be caused by deletion of the Xp21 DAX-1 gene as part of a contiguous gene syndrome or by intragenic mutations. More than 80 different mutations in DAX-1 have been reported [13,14,22–35] (Fig. 4). The majority of the mutations are frameshift and nonsense mutation. These mutations are distributed throughout the DAX-1 coding region, being predicted to result in the structural important changes of the DAX-1 protein, thereby disrupting its function. Seventeen

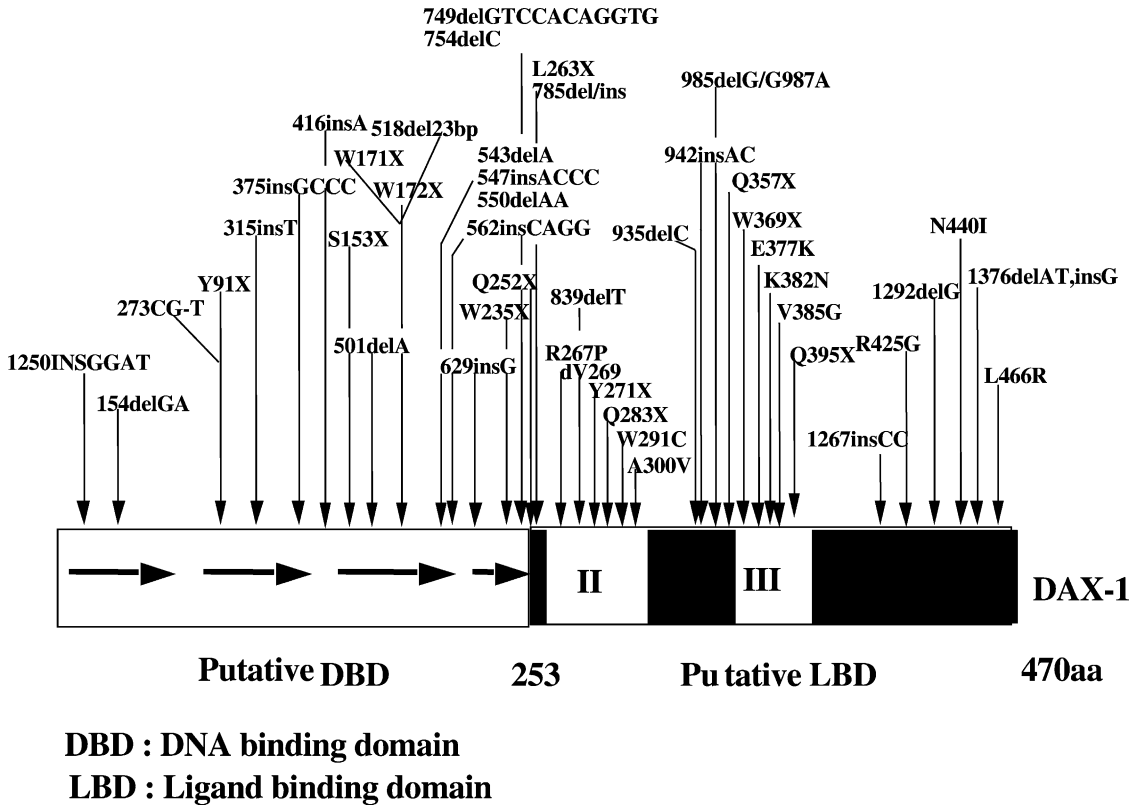

Fig. 4. Mutations in the DAX-1 gene in CAH. Arrows indicate positions of localization of the premature stop codon caused by nonsense or frameshift, missense or deletion mutations. The repeat motif structure of the amino-terminal of DAX-1 is shown by arrows.

missense mutations are now identified and are located only in the C-terminal presumptive ligand-binding domain. All these findings suggest that the C-terminal half of the DAX-1 protein, especially the terminal 4–11 amino acids may be more important than the N-terminal half for DAX-1 protein function [23,33]. DAX-1 is shown to be present in the cytoplasma as well as the nucleus, reflecting its ability to shuttle between the two compartments. One of the LXXLL motifs in DAX-1 is also shown to be essential for its nuclear localization through interaction with SF-1, and its AF2 domain also contributes to its nuclear localization.

8. Clinical manifestation in X-linked AHC

X-linked AHC is characterized by impaired development of the adult zone of the adrenal cortex, leading to adrenal insufficiency. Isolated hypogonadotropic hypogonadism that is also a feature of this disorder may be caused by impaired production of either hypothalamic GnRH or pituitary gonadotrope production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [22]. The AHC patients have normal Leydig cell function, but have some defect of spermatogenesis [28]. In our experience, long term hCG treatment increased testosterone significantly, whereas the administration of hCG and HMG did not increase his testicular volumes at all. No evidence of spermatogenesis was obtained by semen analysis. Biopsied testis showed the presence of the prepubertal immature state of Sertoli cells and absence of germ cells.

Clinical presentation of adrenal insuffciency and hypogonadotropic hypogonadism is recognized recently to variable [29–32,34–37]. Several boys presented in neonatal life with salt-losing states, whereas others had a more insidious presentation with adrenal failure later in childhood [29]. In some families where two siblings have the same DAX-1 mutation, they present adrenal insufficiency at different age. There is little correlation between the type of DAX-1 mutation and age at presentation. Spontaneous onset of puberty has been also reported in several patients with DAX-1 mutation [36,37]. Thus, variability in the presentation of patients with AHC raises the issue that additional genetic and epigenetic factors are important in determining the clinical course of X-linked AHC. DAX-1 is a nucleo-cytoplasmic shuttling protein associated with ribonucleoprotien structures in the nucleus and polyribosomes in the cytoplasm. It was shown that cytoplasmic localization of DAX-1 AHC mutants directly correlates with the magnitude of the reduction of their transcriptional repression activity. In this context, a defect in human organogenesis, AHC, may be caused by impaired nuclear localization of DAX-1, resulting in an impairment in the transcriptional repression [38].

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