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INFANTILE-ONSET ACUTE PRIMARY ADRENAL INSUFFICIENCY: X-LINKED ADRENAL HYPOPLASIA CONGENITAL - A MOLECULAR ANALYSIS OF DAX1

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ABSTRACT

X-linked Adrenal Hypoplasia Congenital (X-linked AHC) occurred because of some abnormality for instance mutation or deletion in DAX1 on the X chromosome. This disorder, mostly emerges in male who have delayed or arrested in puberty as well as infertility. All these properties called hypogonadotropichypogonadism. Adrenal failure, which is the most significant phenotype of AHC resulting in glucocorticoid and mineralocorticoid deficiency subsequently influences the developmental transition of adult from fetus. DAX1 (dosage sensitive sex reversal-DSS) an important protein encoded by the gene NROB1 (orphan nuclear receptor) located in a specific region on the X chromosome. It is expressed throughout the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis suggesting its pivotal role in early human sexual development. It potentially interacts with many important cellular receptors like androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR). But, each of them are regulated by different mechanisms. Recent research reports on experimental animals highlight that the Dax1 can be alternatively spliced suggesting that functional role of Dax1 is more diverse and complex. Familial mutations in DAX1 are often associated with the pathological conditions like Adrenal hypoplasia congenital (AHC), hypogonadotropichypogonadism (HH). Unlike the mutations, the duplication of this gene resulting in dosage sensitive sex reversal (DSS). In this review, we summarize the molecular background of DAX1, biological function, impact of its mutation in AHC formation and clinical significance of the protein expression in HH.

INTRODUCTION

Genetic analysis of male patients with primary adrenal insufficiency revealed 13 novel mutations within the coding region of the NROB1 gene, which are predicted to inactivate the DAX1 function that were three nonsense mutations (c.312C>A, p.Cys104X, c.670C>T, p.Gln224X; and c.873G>A, p.Trp291X), five duplications (c.269_270dup, c.421_422dup, c.895_896dup, c.989dup, c.999_1000dup), and five deletions (c.483del, c.745_746del, c.734_740del, c.1092del, and c.1346del) [1].

The PREVALENCE of X-linked AHC is unknown. It has been widely estimated at 1:12,500 live births [2]. Some ratio express at less than 1:70,000 males [3]. Majority of the affected cases are newborns, aged >8 weeks, where some of them may exhibit the disease in later stage of childhood [4].

DAX1 (dosage sensitive sex reversal-DSS) is an important protein encoded by the gene NROB1 (orphan nuclear receptor) located in a specific region on the X chromosome. This protein is a well-studied molecular candidate involved in adrenal gland development and also responsible for steroidogenesis in adults. It is expressed throughout the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis suggesting its pivotal role in early human sexual development. It potentially interacts with many important cellular receptors like androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR). But, each of them are regulated by different mechanisms. However, the molecular mechanism of DAX1 in multiple stages of development is inadequately understood. Recent research reports on experimental animals highlight that the Dax1 can be alternatively spliced suggesting that functional role of Dax1 is more diverse and complex.

Clinical description

In the onset interval of one week to 3 years. Several cases is recognized between three weeks within 60% to nine years old with 40% [5]. The age of onset is fluctuated, they may have fertility problems or late puberty or delayed Onsted failure [6- 8]. In these individuals, residual glucocorticoid and mineralocorticoid activity present in the hypoplastic adrenal cortex may explain the late onset [9]. The failure of pubertal development may be caused by either abnormal hypothalamic or pituitary regulation of gonadotropin secretion, although the testicular steroidogenesis is largely intact, the functional maturity of Sertoli cells and also spermatogenesis are impaired. The type of mutation doesnot predict clinical phenotype [10]. The first symptom Adrenal insufficiency could be Hypoglycemia, which company with seizures, and then there are some common symptoms as it mentions before like: vomiting, feeding difficulty, dehydration, and shock caused by a salt-wasting episode. Mineralocorticoid deficiency may be the presenting feature of X-linked AHC in some cases [11, 12]. The most important role of Mineralocorticoids are the maintenance of fluid and electrolyte balance. Sometimes hyperpigmentation is appear in X-linked AHC, which caused by increased pituitary production of POMC (proopiomelanocortin), these pigmentation is gradually disappear with appropriate steroid therapy [9].

KEY WORDS

DAX1, NROB1, Adrenal hypoplasia congenital (AHC), Hypogonadotropichypogonadism (HH)

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Hypogonadotropichypogonadism (HH) is commonly associated with the expression of NROB1 in the hypothalamus and the pituitary. Its prospect the mutation DAX1-gene NROB1 responsible for HHG phenotypes. Which means the DAX1 is essential for development of function of hypothalamus- pituitary [13]. Azoospermia has been reported in individuals with classic X-linked AHC and severe oligospermia has been found in some individuals with partial forms of this condition [14]. There has been shortage of source and research about Fertility of individuals with AHC.

GENETICS OF AHC

The actual causes of AHC were unknown, prior to the identification of the DAX1 gene (*Dosage-sensitive sex-reversal, AHC, on the X chromosome, gene 1*). Initially, X-linked AHC was observed as contiguous genetic syndrome in some patients who already suffer with other genetic disorders like Duchene muscular dystrophy, glycerol kinase deficiency, ornithine transcarbamoyltransferase deficiency and mental retardation restricted to the locus Xp21.3-p21.2 [15]. In 1994, duplication of this genomic region was found to be linked with dosage-sensitive XY sex-reversal (DSS) in humans [16]. In the same year, DAX1 gene was successfully cloned and also experimental studies proved that loss-of-function mutations in the coding sequences of DAX1 could cause X-linked AHC and HHG [17]. In addition to that, DAX1 over expression were found to gain an induced sex-reversal in male mice highlighting that DAX1 gene is sole responsible for DSS [18]. DAX1 gene belongs to an orphan member of the nuclear receptor superfamily.

At present, the gene has been renamed as “NROB1” as per the uniform nomenclature system specific to the nuclear receptors where the protein product is alternatively referred as “DAX1”. NROB1 gene dosage is very crucial in normal human developmental process [19]. Functionally, the DAX1 interacts with wide range of molecular players of early development. On comparing with other nuclear receptors, the DAX1 protein has been found to be deficient of a normal DNA-binding domain [20]. The DAX1 protein also serves as a dominant-negative regulator to regulate the transcription of other nuclear receptors like steroidogenic factor 1(SF1). It also acts as an antagonist to SRY to establish that it is a potential anti-testis gene. DAX1 plays an essential role in the development of several hormone-producing tissues which include the hypothalamus, adrenal, pituitary glands, and reproductive structures like testes and ovaries. DAX1 serves a potential transcriptional regulator which controls the activity of various genes during embryonic development of hormone producing tissues. There are numerous reports which have highlighted that NROB1 gene mutations could result in both X-linked congenital adrenal hypoplasia (AHC) and hypogonadotropichypogonadism (HH) [21].

Mapping of Dax1 gene and protein structure

The genomic arrangement of NROB1 is simple with just two exons which have been partitioned with a single intron in between them [17, 22]. Exon 1 is quite larger than exon 2 where it spans around 1168 base pairs in length. A 3385 bp intron is preceded before the exon 2 which is 245 bp in size. The NROB1 gene is transcribed to produce an mRNA made up of 1413 nucleotides which encodes a functional DAX1 protein consist of 470 amino acids [23]. Exon 1 encodes the DNA binding domain (DBD) and partial ligand binding domain (LBD) where Exon 2 is responsible for the additional region of the LBD. The DAX1 protein has been classified as an orphan member under nuclear receptor superfamily. The members of nuclear receptor superfamily have an organized structure consisting of sub regions from A to E which makes a unique characteristic for each domain. Among these sub regions, region “A-B” is known as a modulator domain which varies in size and also exhibits evolutionary dissimilarity. These region constitutes a hormone independent transactivation domain called as “activation function 1(AF-1)”. The most highly conserved “C” region represents a DNA-binding domain (DBD) that contains two zinc fingers. More essentially, it aids to recognize hormone response elements and binding to promoters of target genes. This region also helps for receptor dimerization. Apart from A-B, C domain, the DAX1 also has two more notable domains namely “D” and “E”. The D region serves a potential docking site for corepressors where it also acts as a hinge between DBD and the ligand-binding domain (LBD). The E region is considered as second most highly conserved region that acts as LBD to attain various events like ligand binding, receptor dimerization and nuclear localization [21]. Among the nuclear receptor superfamily, the DAX1 is unusual and holds quite salient features [24]. The amino-terminal domain (NTD) has a novel structure consisting of 3.5 alanine/glycine rich repeats of a 65–70 amino acid motif that has no known homology to any other proteins [21]. The repeats show 33–70% identity to each other, and also contain cysteine residues in conserved positions that could potentially form zinc fingers [25]. DAX1 lacks the modulator domain (Region A/B), conventional DBD (Region C) and hinge region (Region D) but contains an AF-2 transactivation domain [21]. The carboxyl-terminal domain (CTD) of DAX1 shows peptide homology with strongest amino acid similarity to the LBD of other nuclear receptors complex. The CTD of DAX1 is very similar to the LBD of the testis receptor, COUP-TF, and retinoid X receptor (RXR) [24- 26].

MOLECULAR MECHANISMS OF DAX1 ACTIVITY

In general, nuclear receptors (NRs) play an inevitable role in steroidogenesis as well as maintenance of corresponding tissue functions. These NRs widely act as efficient transcription factors which could control and alter the core genetic network that is crucial for major processes like reproduction, development and

homeostasis. The NR activation is limited to various extracellular and intracellular signals [26]. Specifically, these receptors can be activated via the binding of hydrophobic compounds, i.e., steroids, retinoid, and thyroid hormones [26]. As an important member of NR, Dax1 also regulate expression of some novel genes which are critical for embryonic as well as postnatal development of vital hormone producing organs like hypothalamus, pituitary and adrenal glands. This significant role makes the DAX1 is key transcriptional repressor of the HPAG axis. It has been well known that DAX1 activity could modulate transcriptional repression of various other steroidogenic receptors like androgen (AR; NR3C4), estrogen (ER; NR3A1-2) and progesterone receptors (PR; NR3C3) and also liver receptor homologue-1 (LRH-1; NR5A2) [27, 28].

In addition to that, DAX1 could also act as a shuttling RNA binding protein between the nucleus and cytoplasm by it regulates mRNA levels of many genes [29]. Major in vitro experiments have highlighted that DAX1 could auto-regulate itself by recognizing the presence of a single stranded loop of its own promoter and also interacting with DNA hairpins. As an example, DAX1 inhibits a necessary step in steroid biosynthesis by blocking steroidogenic acute regulatory protein (StAR), an essential protein helps the cholesterol transfer to the inner mitochondrial membrane [29, 30]. DAX1 has also been reported to regulate genes that do not have hairpin structures by executing an inhibitory protein-protein interactions. Dax1 mediated inhibition of Steroidogenic factor 1 (SF1) is a well-known example inhibitory protein-protein interaction [31]. SF1 is an essential regulator of steroid hydroxylase gene expression which is abundantly expressed at the reproductive system which includes the HPAG axis [32]. The entire class of steroidogenic hydroxylases holds the SF1 responsive elements in their promoter regions [33]. In connection to this, promoter region of NROB1 also has a functional SF1 response element. However SF1 is not the single regulator for Dax1 expression since the feedback mechanism of Dax1 mediated SF1 regulation has been well established with animal models.

FUNCTIONAL ROLE OF DAX1 AND AHC

Familial mutations of NROB1 gene could result in the production of a defective DAX1 protein with impaired stability and altered activity that eventually exhibit a reduced transcriptional silencing ability in a disease condition known as congenital adrenal hypoplasia (AHC) [11]. These genetic mutations are believed to be the causative factor for direct transcriptional effect with a reduced capacity and also DAX1 mutants poorly interact with their target nuclear receptors and corepressor proteins [34]. Clinically, the AHC subjects show signs of developmental failure in the adult zone of the adrenal gland emphasizing the regulatory role of DAX1 in steroidogenesis. DAX1 protein is normally expressed during the entire development of adrenal cortex, but its exact role in adrenal morphogenesis is poorly understood [35]. Recent studies have elucidated that expression of DAX1 has been observed in all regions of hypothalamic-pituitary-adrenal-gonadal (HPAG) axis, which highlights that DAX1 could be functionally linked with maintaining pluripotency in the early development of adrenal gland. Collective evidences from experimental animal models suggest that DAX1 may have pleiotropic roles, with complex and distinct functions in development and adult function throughout the HPAG axis. Additionally, post transcriptional functions and other disruptive mechanisms of DAX1 in each of these tissues are known very less, especially in pathological conditions like AHC and HH [21].

PHENOTYPIC RELATIONSHIP WITH DAX1 EXPRESSION

Clinical subjects with AHC are phenotypically heterogeneous in relevant to the expression of DAX1 protein which has been identified in variety of tissues from the developing adrenal cortex, gonad, anterior pituitary, and hypothalamus, and also in adult adrenal cortex, Sertoli and Leydig cells in the testis, theca, granulosa, and interstitial cells in the ovary, anterior pituitary gonadotropes, and the ventromedial nucleus of the hypothalamus [36]. This pattern of expression suggests that DAX1 is involved in the development and function of the HPAG axis. Apart from these tissues the protein also present in intermediate level at cerebral cortex, spinal cord, thymus, heart, lung, and kidney [19]. In general, mutational spectrum of DAX1 contributes a distinguished phenotypic heterogeneity which may be varying between the same mutation as well as different mutations in an affected family. This could be an imperative phenomenon which suggests that environmental effects or modifier genes could alter the clinical manifestations of the disease. AHC is commonly diagnosed during infancy, contradictorily some patients may not exhibit the disease until later stage of their life. These abnormal mutations and downstream phenotypic expression have been reported in females. Occasionally, the mutation type or location may not be sufficient to predict disease severity and the early onset of adrenal insufficiency in affected individuals [37].

MUTATIONS OF DAX1

In the recent years, now it has been clearly demonstrated that AHC is absolutely caused by DAX1 gene mutations and other innumerable mutations including deletions, alterations of splice-sites, missense mutations, nonsense mutations and frameshift mutations [5]. These mutations cause a change in one of the building blocks (amino acids) of DAX1 which is tend to be basis for the production of an abnormally short protein that could be even a nonfunctional protein. Loss of function mutations of DAX1 leads to

severe adrenal insufficiency and hypogonadotrophic hypogonadism, which are the major characteristics of AHC. DAX1 mutations occurring naturally at specific location provide insights into the structural and functional relationships. In evidence of this, a little truncation of 9 amino acids from the carboxyl-terminus is sufficient to eliminate complete transcriptional repression by DAX1 [38]. The gene also harbors a large number of missense mutations which account for about one-quarter of other mutations and tend to cluster in the ligand binding-like domain at carboxyl-terminal [3]. It is also noted that amino-terminal region of the protein also yields few missense mutations possibly because of redundant function of the repeated LXXLL protein interaction domains [11]. Genomic deletion of genetic material in the X chromosome, specifically at NROB1 region may result in a condition called adrenal hypoplasia congenital with complex glycerol kinase deficiency. In addition to that affected individuals may show elevated levels of lipids in their blood, urine and also they may have complications in regulating blood sugar levels. In rare cases, the amount of genetic material may be deleted extensively which is common like as Duchenne muscular dystrophy (DMD). Like the deletions, the genomic duplication on the X chromosome, especially the region contains the NROB1 gene can cause a condition called dosage-sensitive sex reversal. The extra copy of NROB1 gene prevents the formation of male reproductive tissues where people identified with this duplication usually appear as a female though they are genetically male with both an X and a Y chromosome.

DIAGNOSIS

Clinical features of Adrenal hypoplasia congenital

The incidence of AHC is very rare, which accounts approximately 1 in 12,500 live births worldwide [39]. Many affected children exhibit the clinical symptoms like vomiting, poor feeding, hyperpigmentation, convulsions, vascular collapse that leads to unexpected mortality. This disorder also emerges with several biochemical abnormalities including hypoglycemia, hyponatremia, hyperkalemia, increased plasma ACTH, low level cortisol and aldosterone in serum [40]. Along with adrenal failure, hypogonadotropic hypogonadism (HH) is also an additional feature of X-linked AHC. "HH" is has been reported for the cause of delayed puberty in some cases already diagnosed with AHC [41]. But this can be successfully treated with testosterone replacement therapy. However, males identified with classic X-linked AHC are found infertile where the exogenous gonadotropin therapy is still unsuccessful [37].

AHC diagnosis

NROB1 is the only gene, which its mutation causes the x-linked AHC. Almost everyone with X-linked AHC has positive family background including a remarkable mutation in the NROB1 gene. AHC can be diagnosed with reliable physical and biochemical tests. Physical diagnosis of the disorder relies on the reports from anatomical observations and other clinical examinations. For the biochemical confirmation of the disorder, a routine hormone profiling in body fluids like blood and urine can be performed to measure levels adrenal hormones including cortisol, aldosterone and androgens. Identification of low cortisol and elevated ACTH levels after stimulation could be applied for early diagnosis of adrenal insufficiency [42].

There are genetically tests have been done for X-Linked AHC diagnosis, which the first step is sequencing of NROB1. If there is not any specific pathogenic variant, the next phase is deletion/duplication analysis. With sequencing analysis, we are able to diagnose the pathogenic and harmless variants. The pathogenic variant includes small intragenic deletions/insertions, missense, nonsense, and splice variant which are not identifiable as an exon or whole-gene deletions/duplications. It is proposed to take PCR before sequence analysis in order to investigate the epidemic deletions of one or more exons. Several methods used for diagnosis of exon or whole- gene deletion/duplications including: quantitative PCR, long- range PCR, multiplex ligation- dependent probe amplification (MLPA), and chromosomal microarray (CMA) [43]. Occasionally, X-linked AHC could cause unexplained deaths in male infants before recognition of the disorder in the family. To overcome this, rapid diagnostic methods can give hope to prevent sudden death after an early start of mineralocorticoid and glucocorticoid treatment. In recent days, advanced molecular diagnostic methods of AHC have been applied towards initiating rapid treatment schedules. Now it is clear that all affected individuals show the 100% positivity for family history with X-linked inheritance mode. Current molecular genetic approaches have opened up promising ways to identify disease causing mutation in DAX1 (also known as NROB1) which is directly associated to cause X-linked AHC [44].

Evaluation for the contiguous gene deletion syndrome

X-LINKED AHC may include the glycerol kinase deficiency (GKD) and in some ones the Duchenne muscular dystrophy (DMD), as a part of contiguous gene deletion syndrome. GKD diagnosed by the measurement of serum concentration of triglycerides and urine glycerol. If the serum concentration of creatine kinase (CK) is over normalized, DMD is suspicious and then diagnosis of diseases is done with the molecular genetic tests. If there is not any diagnosis of pathogenic variant the immunohistochemically staining of dystrophin on muscle biopsy will be running. If, on the base of clinical symptoms and levels of plasma creatine kinase and urine glycerol, there is any suspicion of complex glycerol kinase deficiency, then we propose FISH for NROB1 or a microarray. If there is not any identifiable deletion for NROB1 by FISH or microarray analysis, then the study proposes deletion/duplication analysis [43].

Prenatal diagnosis

X-Linked AHC: if we couldn't identify pathogenic variant in family members, the DNA analysis extracted from fetal cells obtained by amniocentesis (from the fifteenth up to eighteenth week) or chorionic villus sampling (between the tenth and twelfth week) is necessary. Complex glycerol kinase deficiency: for diagnosis, we can use the FISH or deletion/duplication analysis. The DNA analysis extracted from fetal cells obtained by amniocentesis (from the fifteenth up to eighteenth week) or chorionic villus sampling (between the tenth and twelfth week) is necessary [43]. In the following section of the research, there are some recommendations for evaluating the extremeness of disease and the need of a person given with the x-linked adrenal hypoplasia:

- ✓ The monitoring and checking of glucose, cortisol, and ACTH serum density
- ✓ Checking the performance of kidneys, including the measuring of serum BUN and creatinine
- ✓ The checking of serum and urinal density of electrolytes
- ✓ The search of vascular blood gases
- ✓ The check of aldosterone and plasma renin activity

TREATMENT OF AHC

Children who are involved, treated with special care. And considering the physiological processes observed in them, some patient need to hyperkalemia correction. Maintenance hormone treatment is promising because of replacement doses of glucocorticoids and mineralocorticoids allow patients to live the early ages. If steroid replacement therapy isn't sufficient the person maybe dies. Children treated with relevant hormonal therapy may show Hypogonadotrophypogonadism (HH) after maturity that can be easily distinguished from other causes of adrenal insufficiency [45]. In rare cases, the affected individuals display a deficiency of pituitary hormones like LH and FSH when the production of other hormones (ACTH, GH, TSH, and PRL) is normal. Hence the relevance of hypogonadotrophypogonadism to hypothalamic or pituitary dysfunction or both is unclear [46]. Clinical care of the affected children is more challenging and effective disease management is also very important. The patients may require intensive administration with appropriate genetic counseling and complete clinical course [47]. Acute Adrenal insufficiency attacks, can treat with closely monitor blood pressure, clinical status, blood sugar and electrolytes. In these cases, you may need correction of hypokalemia. These people have treated with management of saline, glucose, and hydrocortisone. If serum electrolytes do not improve, a mineralocorticoid added or solucortf will be increased. Enough sodium should also be provided.

After treating the initial acute attack, the patients need to begin replacement doses of glucocorticoids and mineralocorticoids, and nutritional supplements of sodium chloride, in younger children. Steroid doses should be increased during stress times, at this time may be required to prescribed sodium and glucose as well. Dying cause of acute adrenal insufficiency in people with x-linked adrenal hypoplasia congenital.

CONCLUSION

Recent advances in molecular diagnosis and clinical treatments have advanced our understanding of the AHC as well as HH disease at the earliest stage and also have extended patient life expectancy. Most of the X-linked AHC patients are infants and diagnosed with adrenal insufficiency during the first few months of life. In the later stage of the life, males are generally presented with idiopathic primary adrenal insufficiency where more than 50 % of them may have DAX1 mutations. Hence the NROB1/NrOb1 is marked as a central player in the molecular network of steroid hormone pathway and an effective modifier of HPAG axis development. Besides, pleiotropic role of DAX1 makes it a key molecule for the regulation of ER, AR, PR, LRH-1, and SF1 target genes throughout HPAG axis development.

However, the biological role of HPAG axis formation through the molecular mechanisms of DAX1 action is not explained clearly so far. The unanswered questions around DAX1 suggest that still there is a molecular mystery to be resolved by identifying an undiscovered function that is so crucial for the development of sexually dimorphic system like human.

CONFLICT OF INTEREST

There is no conflict of interest

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