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DAX1: Increasing Complexity in the Roles of this Novel Nuclear Receptor

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Summary

DAX1 (NR0B1) is a nuclear receptor with a characteristic C-terminal ligand binding domain, but an atypical DNA binding domain. Mutations in the *DAX1* gene cause adrenal hypoplasia congenita (AHC) establishing its biological importance. Recent studies highlight the complexities of *DAX1* regulation and function. There is considerable phenotypic variability in AHC suggesting the existence of *DAX1* modifier genes and environmental influences on *DAX1* function. The findings of an alternatively spliced DAX1A, more common than DAX1 in all tissues except testis, of DAX1 homodimers, and of DAX1 heterodimers with a number of transcription factor partners including DAX1A and SHP point to an expanded transcription regulatory network under *DAX1* control. Model organisms (mice and zebrafish) are being used to identify other DAX1 functions and modifier genes to understand the pathogenesis of AHC and the lack of genotype-phenotype correlation.

Keywords

DAX1; NR0B1; Adrenal hypoplasia congenital; DAX1A; DAX1 homodimerization; DAX1 modifier genes

DAX1 and Adrenal Hypoplasia Congenita (AHC)

Mutations in *DAX1 (NR0B1)*, which is encoded in the Xp21 chromosomal region a, can lead to adrenal insufficiency with glucocorticoid and mineralocorticoid deficiency (McCabe, 2001). These mutations are responsible for many, but not all, patients with the cytomegalic form of adrenal hypoplasia congenita (AHC). These mutations also cause hypogonadotropic hypogonadism (HH) which is consistent with the expression of *DAX1* in the hypothalamus and pituitary.

Duplications of the region of the X chromosome containing *DAX1* cause dosage sensitive sex reversal (Bardoni et al., 1994; McCabe, 2001). Transgenic XY mice with additional copies of the mouse DAX1 ortholog expressed at high levels do not have male to female sex reversal, but do show delayed testicular development (Swain et al., 1998). When these investigators crossed the transgenic female mice with *poschiavinus* males who have a weaker male sex determining *Sry* allele, they did observe complete sex reversal.

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DAX1 is an unusual member of the nuclear receptor superfamily (McCabe, 2001). It is an orphan receptor. The C-terminal portion has the structure characteristic of a ligand-binding domain (LBD). However, the N-terminal portion is an atypical DNA-binding domain (DBD) with 3.5 amino acid repeats. DAX1 is a negative regulator that interacts with steroidogenic factor 1 (SF1, NR5A1) to inhibit SF1-mediated transactivation of numerous genes involved in the development of the hypothalamic-pituitary-adrenal-gonadal axis and in the biosynthesis of steroid hormones (Iyer and McCabe, 2004). One example is the effect of DAX1 to block the synergistic SF1-Wilms Tumor gene (WT1) heterodimerization that appears to play a role in sexual differentiation (Vilain and McCabe, 2004).

Because DAX1 is such an unusual nuclear receptor, and because of new information we will describe showing an interaction with DAX1, we feel it is important to introduce briefly the only other mammalian member of the NR0B family, the small heterodimer partner (SHP; NR0B2). SHP contains one of the *DAX1* N-terminal repeats, and also interacts with other nuclear receptors as a negative regulator (Iyer et al., 2006).

The purpose of this brief review is to introduce the reader to the phenotypic complexity of AHC as well as the functional complexity in DAX1 action. We will discuss phenotypic variability among patients with *DAX1* mutations and speculate on reasons for this variability. We will describe recent reports from our group and others describing an alternatively spliced form of DAX1, as well as homodimerization of this unusual nuclear receptor, and will consider how these findings contribute to the complexity of DAX1 action. We will also present our work in model organisms, including mice and zebrafish, that are intended to help us document and dissect the phenotypic and functional complexity.

DAX1 Mutations and Phenotypic Variability

There is considerable phenotypic variability associated with *DAX1* mutations (Phelan and McCabe, 2001;Zhang et al., 2001). This reflects a combination of influences including genetic (modifier genes, and variability in expressivity and penetrance) and environmental (intercurrent illnesses and other stressors) (Clipsham and McCabe, 2003;Dipple and McCabe, 2000a and 2000b;Dipple et al., 2001). This phenotypic variability reflects the manner in which biological networks are designed (Dipple et al., 2001;Clipsham and McCabe, 2003). These robust, scale-free, hub and spoke networks are designed such that other nodes in the network can act as modifiers. One example of the phenotypic variability involves the original two brothers described with the contiguous gene syndrome including Becker muscular dystrophy, glycerol kinase deficiency, AHC and mental retardation (Guggenheim et al., 1980) who had complete deletion of *DAX1*. The younger brother died of AHC at 33 months and the older boy first presented with AHC at six years of age. A series of 17 AHC patients presented clinically at a median age of three weeks with a range of one week to three years (Peter et al., 1998). Two brothers and their maternal uncles presented with AHC between 17 and 32 years of age (Brochner-Mortensen, 1956).

DAX1 mutations are distributed throughout the gene regions encoding the C-terminal and N-terminal domains of the protein (Phelan and McCabe, 2001). Variability of phenotype can occur with the same mutation within a family (Bernard et al., 2006). Two daughters and their father had the same mutation. One daughter presented with AHC at eight years, four months of age. The other daughter had not shown symptoms by five years of age, and the father was phenotypically normal. This particular mutation, C200W, which is in the central or “hinge region” between the DBD and LBD, resulted in a change in the subcellular localization of DAX1, shifting it from the nucleus to the cytoplasm. This is a relatively mild import defect. The patient’s cells maintain 80% of wild-type activity. The *DAX1* continues to interact functionally with *SF1*. Since the nuclear import of *DAX1* involves direct interaction with

SF1, the authors speculate that *SF1*-independent interactions of *DAX1* could be responsible for the import defect in C200W mutations. Thus the phenotypic variability in this family may be due to the increased sensitivity of a relatively mild molecular defect to the influences of modifiers.

Alternatively Spliced *DAX1A*

An alternatively spliced form of *DAX1*, called *DAX1A*, has recently been discovered (Ho et al., 2004;Hossain et al., 2004). Quantitative RT-PCR showed more *DAX1A* than *DAX1* in all tissues in which it is expressed except testis where they are nearly equal. Previous research using antibodies to *DAX1* were actually measuring the sum *DAX1* and *DAX1A*. The N-terminal domains are unique among nuclear receptors, and therefore were used to generate discriminating antibodies.

Given the excess of *DAX1A* transcripts in all tissues except testis, the heterodimerization of DAX1 and DAX1A (see below), and the phenotypic consequences of a *DAX1* knockout (KO) mouse in which *DAX1A* could still be expressed (Yu et al., 1988), it is very likely that DAX1A has a function in the adrenal and other tissues. Yu et al. (1998) showed that targeted KO of exon 2 in the murine ortholog of *DAX1*, *Ahch*, which would not disrupt *Ahch1A*, the *DAX1A* ortholog, resulted in progressive loss of the germinal epithelium of the testis and male sterility. The absence of an adrenal phenotype in this KO mouse (Yu et al., 1998) and the presence of a protein of the appropriate molecular weight for Ahch1A (Niakan et al., 2006), could be a model of expression of DAX1A in the absence of DAX1 (Niakan and McCabe, 2005) and could be interpreted to suggest that DAX1A may be important for adrenal development.

DAX1 Homodimerization

DAX1 heterodimerizes with other nuclear receptors, including SF1, ligand-activated ERα, SHP and DAX1A (Iyer and McCabe, 2004;Iyer et al., 2006). Iyer et al. (2006) also showed that *DAX1* homodimerizes by quantitative yeast two-hybrid assay and by *in vitro* co-immunoprecipitation. We showed that *DAX1* homodimerizes in mammalian cells (HEK293 cells and HeLa cells), and established that the homodimers form both in the nucleus and cytoplasm (in HEK293 cells). We confirmed the *DAX1-SF1* interaction in HEK293 cells and demonstrated that *DAX1* homodimers decrease upon heterodimerization with *SF1* or with ligand-activated *ERα*.

DAX1 heterodimerizes with *SF1* through its N-terminal LXXLL motifs (Suzuki et al., 2003). Iyer et al. (2006) showed that DAX1 may form an antiparallel homodimer and identified the residues that were involved in *DAX1* homodimerization using site-directed mutagenesis of LXXLL motifs and the AF-2 domain. The motifs involved in *DAX1* homodimerization include the LXXLL domains (positions 13–17, 80–84, and 146–150) and AF-2 (MMLEML) domain (positions 461–466). *DAX1* and *SHP* homodimers may serve as a holding reservoir and/or may have other functions, such as binding to a homodimer response element that is not bound by the monomer.

Model Organisms to Identify Other *DAX1* Functions and Modifier Genes

DAX1 is expressed in mouse blastocysts (Clipsham et al., 2004;Niakan et al., 2006). Niakan et al. (2006) showed that *Dax1* was expressed in E5.5 throughout the embryo except in the proximal visceral endoderm and that this differential pattern of expression continued through E7 in mice. siRNA knockdown of *Nr0b1* suppressed embryonic development, suggesting that *Dax1* may be involved in maintaining a more primitive state.

Another vertebrate animal model useful for analyses of complex developmental processes is the zebrafish (*Danio rerio*). The advantages of the zebrafish include: high fecundity (mature females lay several hundred eggs at weekly intervals); short generation time (three-four months); rapid development; external fertilization; translucent embryos; and easy maintenance. Zhao et al. (2006) compared human *DAX1* and zebrafish *dax1* and found that the gene structure was absolutely conserved. *dax1* was first detected at 26 hours post-fertilization (hpf) in the developing hypothalamus. Transient *dax1* expression occurred in the adrenal region around 31 hpf. *In situ* hybridization showed *sf1* (*ff1b*) at 19 hpf. Knockdown of *dax1* function by morphilino downregulated expression of the steroidogenic genes *cyp11a* and *star*. *dax1* may function downstream of *ff1b* during zebrafish interrenal development since *dax1* morphilino RNAi did not alter the *ff1b* gene expression in the interrenal organ, and knockdown of *ff1b* activity abolished the interrenal expression of *dax1*.

Complexity of *DAX1*

A number of recent observations on the molecular actions of *DAX1* and its biological activities suggest a greater level of complexity than previously appreciated. *DAX1* mutations can cause adrenal hypoplasia congenita with wide phenotypic variability, suggesting the existence of modifier genes and environmental factors that impact on *DAX1* function. The identification of a novel splice variant of *DAX1*, the finding that *DAX1* homodimerizes and the discovery of new heterodimer partners for SF1 all point to a greatly expanded regulatory network under *DAX1* control. Finally, recent studies in mice and zebrafish identify other developmental roles for *DAX1*. A full understanding of these complexities may help explain the mechanisms through which DAX1 controls the development of the hypothalamic-pituitary-adrenal-gonadal axis and the phenotypic variability of AHC mutations.

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