

## NR0B1 (DAX1) Gene Analysis in X-linked Adrenal Hypoplasia Congenita (AHC)

**Disorder also known as:** Adrenal hypoplasia congenita with hypogonadotropic hypogonadism (AHC with HH); Cytomegalic form of AHC

**Includes:** 46, XY disorder of sexual development (dosage-sensitive sex reversal, DSS)

### Clinical Features:

Loss-of-function variants in the NR0B1 (DAX1) gene on chromosome Xp21 cause X-linked adrenal hypoplasia congenita (AHC). In males with X-linked AHC, the mature adult zone of the adrenal cortex fails to develop properly, resulting in a cortex that appears disorganized and contains large, cytomegalic cells that resemble cells in the fetal cortex. Typically, males with X-linked AHC develop salt-wasting primary adrenal failure in early infancy (~60%) or childhood (~40%), although later-onset cases presenting in adulthood have been described.<sup>1,2</sup> Individuals with variants in the NR0B1 gene also develop hypogonadotropic hypogonadism (HH), although the age of onset is variable. HH due to altered hypothalamic-pituitary-gonadal (HPG) activity may be observed in infancy, and greater than 10% of males with X-linked AHC have bilaterally undescended testicles. However, other individuals have normal HPG activity in infancy, and onset of HH is noted at the time of puberty. In rare cases, patients with DAX1 variants have been reported with spontaneous onset of puberty, although pubertal development is incomplete. Most males have azoospermia and remain infertile even after treatment with gonadotropins<sup>2</sup>, although a male with an NR0B1 variant was reported with preserved fertility.<sup>3</sup> While variants within the DAX1 gene result in isolated X-linked AHC, X-linked AHC may also occur as part of a contiguous gene deletion syndrome associated with mental retardation, glycerol kinase deficiency, and/or Duchenne muscular dystrophy, depending on the size of the deletion.

Most females who are heterozygous carriers of NR0B1 variants have normal adrenal function and no evidence of HH; however, several have been reported with delayed puberty,<sup>4</sup> and a female with a contiguous gene deletion including NR0B1 had primary adrenal failure due to skewed X-inactivation.<sup>5</sup> Additionally, one female was reported with a homozygous NR0B1 variant causing HH but apparently normal adrenal and ovarian function.<sup>3</sup>

Duplications of the NR0B1 gene and the surrounding genomic region, referred to as the dosage-sensitive sex reversal (DSS) region, do not cause X-linked AHC but instead result in a 46,XY disorder of sex development. Although most patients reported with duplications of the DSS region have complete gonadal dysgenesis causing XY sex reversal, partial gonadal dysgenesis with ambiguous genitalia has been described.<sup>6</sup> Duplications of DAX1 in 46,XX

individuals have no known clinical consequence, but the risk of transmission to 46,XY offspring is a significant consideration.

## Genetics:

Variants in NR0B1 are inherited in an X-linked manner. Most heterozygous female carriers are asymptomatic, although exceptions have been reported (see above). Germline mosaicism has been reported.<sup>7</sup> The NR0B1 gene, located on Xp21.3-21.2, is a member of the orphan nuclear receptor subfamily. The NR0B1 gene consists of two exons and encodes the 470 amino acid DAX1 protein. An alternatively spliced form of the gene, called NR0B1A, has also been described, although its function in adrenal and gonadal development is currently unclear.<sup>8,9</sup> The C-terminal region of the gene contains a characteristic ligand binding domain (LBD) typical of members of the nuclear receptor superfamily. However, the N-terminal portion of the gene contains an atypical DNA-binding domain (DBD) that consists of a 66-67 amino acid repeat motif that is different than the DBD found in other nuclear receptors.

## Test Methods:

For individuals with apparently isolated X-linked AHC, sequence analysis is performed by bi-directional sequencing of the two coding exons and the exon/intron splice junctions of the NR0B1 gene. Because sequencing cannot identify large deletions of the NR0B1 gene in females, targeted array CGH with exon-level coverage (ExonArrayDx) is available to evaluate for a whole or partial deletion in females having carrier testing for X-linked AHC. For patients with AHC and additional features suggestive of a contiguous gene deletion syndrome, whole genome array CGH (GenomeDx) is available to evaluate for a deletion of the NR0B1 gene and surrounding genomic region (see GenomeDx information sheet for more details: <http://www.genedx.com/site/genomedx>). For individuals with a 46,XY disorder of sex development, ExonArrayDx targeted array CGH can be performed to evaluate for a duplication of the NR0B1 gene. Variants found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method.

## Test Sensitivity:

Pathogenic variants in the NR0B1 gene were identified in 26/31 (84%) males with a clinical diagnosis of AHC, including 14 (45%) with a large deletion and 12 (39%) with an intragenic variant.<sup>10</sup> In a study of males with primary adrenal failure of an unknown etiology, 37/64 (58%) were found to harbor a variant in NR0B1,<sup>11</sup> including all 11 patients with both adrenal failure and HH.

Previous studies have identified duplications of NR0B1 in 4-5% of individuals with 46,XY partial or complete gonadal dysgenesis.<sup>10,13,14</sup>

## **Variant Spectrum:**

Large deletions including at least the entire NR0B1 gene account for 27-43% of variants causing X-linked AHC.<sup>10,11</sup> Specifically, 5-22% of variants in X-linked AHC are deletions of NR0B1 only, while 5-38% are contiguous gene deletions. Contiguous gene deletions extending telomeric to NR0B1 may include the ILRAPL1 gene, resulting in mental retardation. Deletions extending centromeric to NR0B1 may include the GK gene causing glycerol kinase deficiency, and possibly also the DMD gene causing Duchenne muscular dystrophy. Several males with X-linked AHC have been reported with partial deletions including only a single exon of the NR0B1 gene.<sup>10,12</sup> The remaining 47-73% of NR0B1 variants causing X-linked AHC are intragenic point variants.<sup>10,11</sup> Over 100 intragenic variants have been described, and there are no known variant hotspots. Frameshift (49%) and nonsense (28%) variants account for the majority and are scattered throughout the gene. Missense variants occur less frequently (~20%) and typically are located in highly conserved residues within the LBD.<sup>9</sup>

Duplications of NR0B1 are believed to be the cause of dosage-sensitive sex reversal (DSS) in patients with Xp21 duplications. The smallest duplication associated with a 46,XY disorder of sex development that has been reported was approximately 800 kb and included the four MAGEB genes adjacent to NR0B1.<sup>6</sup>

## **References:**

1. Achermann et al., (2001) *Molec Cell Endocrinol* 185:17-25.
2. Mantovani et al., (2002) *J Clin Endocrinol Metab* 87:44-48.
3. Merke et al., (1999) *NEJM* 340:1248-1252.
4. Seminara et al., (1999) *J Clin Endocrinol Metab* 84:4501-4509.
5. Shaikh et al., (2008) *J Med Genet* 45:e1.
6. Barbaro et al., (2008) *Clin Genet* 73:453-464.
7. Zhang et al., (1998) *Am J Hum Genet* 62:855-864.
8. Ho et al., (2004) *Mol Genet Metab* 83:330-336.
9. McCabe ERB (2007) *Mol Cell Endocrinol* 265-266:179-182.
10. Muscatelli et al., (1994) *Nature* 372:672-676.
11. Lin et al., (2006) *J Clin Endocrinol Metab* 91:3048-3054.
12. Salvi et al., (2002) *J Clin Endocrinol Metab* 87:4094-4100.
13. Bardoni et al., (1994) *Nat Genet* 7:497-501.
14. Morel et al., (January 2010) Studies of a cohort of 46,XY with DSD including steroid biosynthesis deficiencies Presented at Hormonal and Genetic Basis of Sexual Differentiation Disorder and Hot Topics in Endocrinology, Miami, FL.