HIGHLIGHT

Targeted disruption of the *Ahch* (*Dax-1*) gene: knockout of old concepts

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Mutations in the human transcription factor AHC cause the X-linked recessive form of adrenal hypoplasia congenita (1–3). Boys with adrenal hypoplasia congenita present with cortisol and mineralocorticoid deficiency in early childhood, and they fail to undergo puberty due to hypogonadotropic hypogonadism (HHG) (3, 4). In addition, a subset of these patients has a contiguous gene deletion syndrome involving several X-chromosomal genes (glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamoyl transferase deficiency, mental retardation). Duplication of the AHC locus on chromosome Xp21 also has dramatic consequences and results in a female phenotype in 46XY males, a mechanism referred to as 'dosage sensitive sex reversal'. The AHC gene, initially called DAX-1 (dosage-sensitive sex reversal – adrenal hypoplasia congenita on the X chromosome gene 1), consists of two exons and encodes an orphan nuclear receptor. The aminoterminal is formed of three and a half copies of a repeated motif, but lacks a typical zinc-finger DNA binding domain, while the carboxyterminal domain displays high homology to the ligand domain of nuclear receptors. AHC is expressed in the three layers of the adrenal cortex, ovarian granulosa and theca cells, testicular Sertoli and Leydig cells, pituitary gonadotropes, and in the hypothalamus (5, 6). Remarkably, there is developmental and spatial coexpression with another orphan nuclear receptor, the steroidogenic factor-1 (SF-1), a key regulator in the development and function of the reproductive axis (7). Functionally, AHC was found to be an inhibitor of SF-1-mediated transcription in transfection assays (8), and it can also directly repress certain target genes (9).

Consistent with the human phenotype, transgenic overexpression of the mouse homologue *Ahch* resulted in sex reversal by antagonizing the Y-chromosomal *Sry* gene (10). In the December 1998 issue of *Nature Genetics*, Richard Yu and colleagues present the targeted disruption of *Ahch* in transgenic mice. Several of their observations are surprising and challenge our concepts on sexual development (11). Using a conditional knockout with a Cre-*loxP* strategy, the carboxyterminal domain of *Ahch* was deleted. Exon 2 of *Ahch* was first flanked by *loxP* sites and a neomycin cassette was inserted downstream of it. This transgene was introduced in embryonic stem cells for homologous recombination. Mice expressing this transgene were then mated with

transgenic mice overexpressing the recombinase Cre, an enzyme that recognizes the *loxP* sites. By this strategy, exon 2 could be excised in preimplantation embryos. In males, this results in a deletion of the X-chromosomal *Ahch* gene (*Ahch* Δ 2/Y), while females are hemizygous for the mutation (*Ahch* Δ 2/*Ahch*).

At birth, the transgenic *Ahch* Δ 2/Y males did not differ from their wild-type littermates. However, at the onset of sexual maturation, the fetal adrenal cortex failed to regress. The zona glomerulosa and fasciculata were found to be normal, and corticosterone levels were within the normal range. This contrasts, in part, with the findings in humans with adrenal hypoplasia congenita. In these patients, the adrenal cortex also contains fetal cells, but the permanent zone of the adrenal cortex does not develop and it is replaced by large vacuolated cells (12). Expression of the P450 side-chain cleavage enzyme was present in the adrenal cortex of both the wild-type and the mutant animals, but reduced in the zona fasciculata. These results suggest that *Ahch* is required for the involution of the fetal adrenal cortex, but is not necessary for the development of the mature cortex and steroidogenesis in mice.

The testicles of Δ *Ahch*/Y mice were reduced by about 50% in volume, but there were no other malformations of the reproductive organs. Histologically, the germinal epithelium was not stratified normally, and older mice showed epithelial dysgenesis and degeneration. After 14 weeks, there was a complete loss in germ cells. This suggests that *Ahch* is not involved in the initiation of spermatogenesis, but essential for the maintenance of the germinal epithelium as well as Sertoli cell function. Moreover, Leydig cell hyperplasia and hypertrophy were observed adjacent to degenerated seminiferous tubules.

Pituitary glands from wild-type and mutant animals were similar in size and there were no differences in serum gonadotropin levels, cell numbers, or immunopositivity for pituitary hormones. Serum levels for luteinizing hormone, follicle-stimulating hormone and testosterone were not different in transgenic mice in comparison to their wild-type littermates. These findings are distinct from the human phenotype with combined hypothalamic-pituitary HHG (4). They suggest that the basis for the infertility in the transgenic Δ *Ahch*/Y mice is the result of a primary testicular defect affecting the germinal epithelium rather than a hypothalamic or pituitary defect.

In humans, females homozygous for AHC mutations have never been reported. This is most likely explained by the fact that males with adrenal hypoplasia congenita are infertile and have no offspring. In order to dissect the role of Ahch in ovarian development, Yu *et al.* (11) therefore generated females homozygous for the *Ahch* Δ 2 deletion. Surprisingly, female mice homozygous for the transgene had normal sexual maturation, ovulatory cycles and fertility. The only detectable phenotypic alteration was that a subset of the follicles displayed multiple oocytes within a single follicular structure. Ahch may have a role in follicular recruitment, granulosa cell proliferation, or follicle formation, but these observations indicate that it is not an ovarian determining factor as previously proposed (10). The study of Yu *et al.* (11) suggests that Ahch is essential for normal development and function of the testis, and the authors propose a model where AHC acts in a dose-dependent manner as a repressor of *Sry* and *Sf-1*, and as a positive regulator of spermatogenesis.

Since only exon 2 of *Ahch* was deleted, the possibility remains that the truncated AHC may result in a partial phenotype. Since mutations in the carboxyterminal part of AHC result in a complete phenotype in humans, it seems, however, rather unlikely that this could explain the differences between the human and the murine phenotype. Targeted disruption of exon 1 of *Ahch* may provide a definite answer to this question. In addition, the availability of mice carrying the Cre-loxP flanking exon 2 of *Ahch* will allow further dissection of the role of this transcription factor in different endocrine tissues. By mating these animals with mice expressing the Cre recombinase under the control of tissue specific promoters, *Ahch* can be deleted selectively in pituitary gonadotropes, adrenal cells, or in the germinal epithelium of the testis. Alternatively, it may be possible to rescue some of the phenotypic alterations by mating females homozygous for the *Ahch* Δ 2 deletion with transgenic male mice overexpressing AHC under the control of tissue-specific promoters in order to determine the role of Ahch in different cell types. The observation that the dose of AHC is critical for development of a normal testis leads to further interesting speculations: it does not, for example, seem unlikely that AHC could be involved in the pathogenesis of abnormal seminiferous tubules in the Klinefelter (XXY) syndrome.

The AHC (*Dax-1*) gene was cloned in 1994 and led to the elucidation of the molecular basis of patients with

adrenal hypoplasia congenita (1–3). As illustrated by the study of Yu *et al.* (11), the study of this transcription factor has been rewarding and provides fundamental insights into the development and function of the adrenal and gonadal axis. What will be the next piece in this puzzle?

References

- 1 Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W *et al.* An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature* 1994 **372** 635–641.
- 2 Muscatelli F, Strom TM, Walker AP, Zanaria E, Recan D, Meindl A *et al.* Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994 **372** 672–676.
- 3 Yu RN, Achermann JC, Ito M & Jameson JL. The role of DAX-1 in reproduction. *Trends in Endocrinology and Metabolism* 1998 **9** 169–175.
- 4 Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WFJ & Jameson JL. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism. Evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production. *Journal of Clinical Investigation* 1996 **98** 1055–1062.
- 5 Guo W, Burris TP & McCabe E. Expression of DAX-1, the gene responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism, in the hypothalamic-pituitary-adrenal/gonadal axis. *Biochemical and Molecular Medicine* 1995 **56** 8–13.
- 6 Ikeda Y, Swain A, Weber T, Hentges KE, Zanaria E, Lalli E *et al.* Steroidogenic factor 1 and Dax-1 colocalize in multiple cell lineages: potential links in endocrine development. *Molecular Endocrinology* 1996 **10** 1261–1272.
- 7 Parker KL & Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocrine Reviews* 1997 **18** 361–377.
- 8 Ito M, Yu R & Jameson JL. DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Molecular and Cellular Biology* 1997 **17** 1476–1483.
- 9 Zazopoulos E, Lalli E, Stocco DM & Sassone-Corsi P. DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. *Nature* 1997 **390** 311–315.
- 10 Swain A, Narvaez V, Burgoyne PGC & Lovell-Badge R. Dax1 antagonizes Sry action in mammalian sex determination. *Nature* 1998 **391** 761–767.
- 11 Yu RN, Ito M, Saunders TL, Camper SA & Jameson JL. Role of Ahch in gonadal development and gametogenesis. *Nature Genetics* 1998 **20** 353–357.
- 12 Hay ID, Smail PJ & Forsyth CC. Familial cytomegalic adrenocortical hypoplasia: an X-linked syndrome of pubertal failure. *Archives of Diseases in Childhood* 1981 **56** 715–721.

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