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Placental defects are associated with male lethality in bare patches and striated embryos deficient in the NAD(P)H Steroid Dehydrogenase-like (NSDHL) Enzyme

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Abstract

NSDHL is a 3β-hydroxysterol dehydrogenase that is involved in the removal of C-4 methyl groups in one of the later steps of cholesterol biosynthesis. Mutations in the *Nsdhl* gene are associated with the X-linked male lethal mouse mutations bare patches (*Bpa*) and striated (*Str*), as well as with most cases of human CHILD syndrome. To begin to examine the pathogenesis of these disorders, we have determined that affected male embryos for several *Nsdhl* alleles die in midgestation, between E10.5 and 13.5, while the majority of affected male embryos for the most severe allele, $Nsdhl^{Bpa1H}$, die prior to E9.5. Although no consistent anomalies were identified in affected male embryos themselves, the labyrinth layer of the fetal placenta was always thinner, with fewer fetal vessels and decreased proliferation of labyrinth trophoblast cells. X-inactivation is non-random in females in most lineages of the rodent placenta with preferential inactivation of the paternal X chromosome. For primary defects involving these extraembryonic lineages, heterozygous females with a mutant maternal X chromosome would be expected to have an identical placental phenotype to that found in affected male embryos. We hypothesize that abnormalities in cells of the allantoic mesoderm that undergo random X-inactivation and form the endothelial lining of the fetal vessels of the labyrinth are associated with the male lethality, perhaps through disruption of an as yet unidentified signaling pathway.

Keywords: NSDHL; Placenta; X-inactivation; Cholesterol biosynthesis; Bare patches; CHILD syndrome

Introduction

The X-linked, male lethal mouse mutants, bare patches (*Bpa*) and striated (*Str*), result from mutations in the *Nsdhl* gene encoding a sterol dehydrogenase involved in the removal of C-4 methyl groups in one of the later steps of cholesterol biosynthesis [1]. Heterozygous *Bpa* females have a severe and often asymmetric skeletal dysplasia and are dwarfed compared to normal littermates. They also develop a patchy, hyperkeratotic skin eruption on postnatal days 5–7 that resolves, leaving "bare patches" arranged in a horizontal, striped pattern consistent with random X-inactivation. Occasional cataracts and/or microphthalmia have been detected in the original Bpa^{1H} allele [2–5]. Affected liveborn Bpa^{1H} males have never been recovered, and affected male embryos have been reported to die shortly after implantation [2]. Milder *Bpa* alleles, originally thought to be a distinct locus called striated (*Str*) [6], are indistinguishable from

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normal littermates until days 12–14 when the characteristic striping of the coat becomes apparent. We have identified mutations in *Nsdhl* in 7 *Bpa* and *Str* alleles, including a nonsense mutation (K103X) in the original Bpa^{1H} mutant mouse that produces a truncated protein that is predicted to be a null allele [1,7].

Heterozygous mutations in the human *NSDHL* gene were subsequently detected in several females with CHILD syndrome (congenital hemidysplasia with ichthyosiform nevus and limb defects, MIM #308050) [8–11]), a rare X-linked, male lethal malformation syndrome characterized by unilateral ichthyosiform skin lesions and limb reduction defects (reviewed in [5]). The unilateral distribution of lesions in CHILD syndrome, often with a midline demarcation, does not follow the typical pattern of X-inactivation and remains unexplained. While there is often asymmetry in the striping of the coat in *Bpa*/*Str* females, limb reduction defects and unilateral, diffuse skin lesions, as seen in human CHILD syndrome, have never been observed in affected mice (G. Herman, unpublished results).

Seven human disorders that involve enzyme defects in the conversion of lanosterol to cholesterol, so-called post-squalene cholesterol biosynthesis, have now been described. The first, Smith–Lemli–Opitz syndrome (SLOS), was reported in 1993 [12,13]. Since 1998, six additional disorders have been identified and include desmosterolosis [14–16], X-linked dominant chondrodysplasia punctata (CDPX2) [17,18], lathosterolosis [19,20], hydrops-ectopic calcification–moth-eaten (HEM) skeletal dysplasia [21], and Antley–Bixler syndrome [22], in addition to CHILD syndrome (for recent reviews see [23,24]). The incidence of these syndromes varies considerably. SLOS is by far the most common with an incidence of 1/40,000–1/50,000 in the United States, while only two cases each of desmosterolosis and lathosterolosis have been reported. There are some common features among the phenotypes of these disorders (summarized in [24]), and they are all associated with major developmental malformations, suggesting that perturbations of this pathway have potent teratogenic effects. In general, postnatal survival in the hemi- or homozygous state decreases and the clinical severity in surviving infants increases with enzymatic defects earlier in the pathway. Skeletal abnormalities are associated with each syndrome, although their severity and frequency vary, with occasional epiphyseal stippling in SLOS to a severe dysplasia incompatible with survival in HEM dysplasia. Mental retardation is associated with most cases of SLOS that survive beyond the newborn period, as well as with a single survivor each with lathosterolosis and desmosterolosis. Normal intelligence in the majority of females with CDPX2 and CHILD syndrome almost certainly relates to the phenomenon of random X-inactivation and functional mosaicism for the mutant gene, and, indeed, a recently reported male with a non-mosaic hemizygous mutation in the CDPX2 sterol isomerase gene exhibited mental retardation and seizures [25].

The pathogenesis of the malformations and decreased survival for patients with any of these disorders is yet to be elucidated. It has been speculated that decreased cholesterol or total cellular sterols, accumulation of toxic sterol intermediates, abnormal feedback regulation of earlier steps in the pathway including the synthesis of isoprenoids, or abnormal signaling by proteins in the hedgehog pathway may all play a role in pathogenesis. The survival to birth of some fetuses with SLOS null alleles and plasma cholesterol levels <10 mg/dl would argue that a simple lack of cholesterol cannot completely explain the embryonic lethality that occurs with some of these disorders. Distinct features in the phenotype for each disorder also suggest that at least some aspects of the pathogenesis may be unique for defects at each step.

The existence of naturally occurring or engineered mouse models for five of these disorders [1,17,20,26–28] is beginning to enable more detailed developmental and in vitro studies to examine the mechanisms of disease pathogenesis. As a first step toward understanding the pathogenesis of the male lethality in the X-linked cholesterol biosynthetic disorders, we have examined *Bpa*/*Str* male embryos to determine the timing and mechanism(s) of their death.

Methods

Mouse strains

The origins of the mutant murine *Nsdhl* alleles have been described [1,7]. Breeding stocks of each allele were maintained by mating heterozygous affected females to C57BL/6J$A^{w\text{-}J}$ × CBA (B6CBA) males (The Jackson Laboratory, Bar Harbor, ME). Affected females were identified at 5–7 days of age by their size and patches of hyperkeratosis (*Bpa* alleles) or by striping of the coat at 12–14 days of age (*Str* alleles). Mice carrying an X chromosomal *Lac*Z transgene that is subject to random X-inactivation [29] were obtained from frozen embryos stored at the Mammalian Genetics Unit, Harwell, UK. Transgenic mice of mixed genetic background have been crossed to C57BL/6J (The Jackson Laboratory). The presence of an Xce_b allele on the X chromosome carrying the transgene has been confirmed using the polymorphic markers *DXPas29* and *DXPas31* that flank the *Xce* and differentiate the three alleles, Xce_a, Xce_b, and Xce_c [30]. To demonstrate the pattern of cells that undergo random X-inactivation in female placentas, FVB females (Harlan, Indianapolis, IN) were mated to males carrying the *Lac*Z transgene, and placentas from female embryos were dissected from maternal deciduas at E10.5. X-gal staining of whole placentas was performed using standard methods [31]. The FVB X chromosome carries an