The murine \textit{tub} (rd5) mutation is not associated with a primary axonemal defect

Abstract Some genetic syndromes causing loss of hearing and vision, such as some forms of Usher’s syndrome, also cause reduced sperm cell motility, bronchiectasis, and other pathologies involving cilia- and flagella-bearing cells. In some Usher’s patients, ultrastructural defects of axonemes within photoreceptor ciliary bridges, nasal cilia, and sperm cell flagella have been found, indicating a primary defect of axonemal conformation. Mice homozygous for the \textit{tub} (rd5) mutation exhibit progressive retinal degeneration, sensorineural hearing loss, reduced fertility, and obesity, and presently represent the only animal model with neuroepithelial degeneration of both cochlea and retina without other neurological abnormalities. They provide a good phenotypic match to human genetic sensory syndromes, particularly human sensory/obesity syndromes, such as Alstrom’s and Bardet/Biedl, although no human candidate genes have been identified. Because of their unique phenotype, \textit{tubby} mice are an appropriate model in which to look for a primary axonemal defect. We studied the axonemal ultrastructure of photoreceptors and sperm cells and performed functional testing of sperm in \textit{tub/tub} mice before and after the onset of obesity. Approximately 15% of photoreceptor axonemes appeared abnormal in \textit{tub/tub} animals, compared to 0% in controls. Both \textit{tub} homozygotes and controls exhibited approximately 10% abnormal sperm cell axonemes, and no differences in sperm cell motile function were found at any age. The modest occurrence of axonemal defects in photoreceptors of \textit{tub/tub} animals is likely to be a secondary effect of retinal degeneration. We conclude that the \textit{tubby} phenotype is not associated with a generalized defect of cilia- and flagella-bearing cells and that the \textit{tub} mutation does not primarily affect axonemal structure.

Key words Retina · Cilia · Sperm · Usher’s syndrome · Alstrom’s syndrome · Bardet/Biedl syndrome · Cochlea · Photoreceptors · Mouse C57BL/6J

Introduction

Some genetic syndromes, such as certain forms of Usher’s, Alström’s, and Bardet/Biedl, are characterized by retinal photoreceptor and cochlear hair-cell loss, combined with reduced fertility (Gorlin 1995; Millay et al. 1986; Schuknecht 1993; Sebag et al. 1984). Alström’s and Bardet/Biedl syndromes also feature obesity. These characteristics may be accompanied by no other neurological abnormality and by normal intelligence, suggesting that the primary defect involves cellular structures and/or functions common to only a few cell types. In addition to sensory cell loss and reduced fertility, some Usher’s patients exhibit bronchiectasis (Bonneau et al. 1992). One cell structure common to cochlear hair cells, photoreceptors, sperm cells, and lumenal cells of the bronchi is the axonemal core of cilia and flagella. Cochlear hair cells transiently express cilia during development (Sobkowicz et al. 1995); photoreceptors contain a ciliary bridge linking the inner and outer segments; sperm have flagella; and epithelial cells of the airways possess motile cilia. Appropriate arrangement of microtubules and their associated proteins in motile cilia and flagella is necessary for coordinated movement, while the role of the ax-
ominal remnant of photoreceptors is obscure. Direct ultrastructural observations of axonemes from sperm cells, nasal cells, and photoreceptors in fact indicate that the primary defect in some forms of Usher’s is one of axonemal conformation (Arden and Fox 1979; Barrong et al. 1992; Berson and Adamian 1992; Hunter et al. 1986). About 60% of the axonemes in photoreceptors may be abnormal in Usher’s, compared to about 2–13% found in forms of non-syndromic retinal degeneration, suggesting that the smaller percentage is likely to be secondary to the degeneration. Similarly, while a normal sample of sperm may include 10–40% of cells with axonemal defects, this fraction in Usher’s may be 40–60%. The observable functional consequence is a reduction in motility, while sperm production may be normal.

Recent progress in our understanding of genetic sensory-deficit syndromes includes the delineation of at least six different Usher’s genes (Kimberling and Moller 1995) and the characterization of one these, the USH1B gene (Weil et al. 1995). Progress on USH1B was in part possible because of the identification of its murine homolog, sh-1 (Gibson et al. 1995; Steel 1995), although this gene defect does not cause retinal degeneration in mice. Recent evidence also indicates that human USH2A may have as a homolog the murine retinal degeneration gene rd3 (Piek-S-Dahl et al. 1997) which – in frustrating symmetry – does not appear to cause hearing loss in mice. While these models retain immense value for the study of syndromic sensory cell loss, they also highlight the potential value of another mouse model, the tubby mouse. Mice homozygous for the tub (rd5) mutation exhibit progressive sensorineural hearing loss, retinal degeneration, and obesity, unaccompanied by any motor or balance disorders (Heckenlively et al. 1995; Ohlemiller et al. 1995, 1997). Physiological responses of the ear and eye are measureable but never normal (Heckenlively et al. 1995); histopathology is apparent in the retina by two weeks and in the cochlea by one month (Ohlemiller et al. 1997). Homozygotes reproduce for only a short period, typically 2–4 months of age, prior to the onset of obesity. By six months, tub/tub animals are on average twice as heavy as heterozygous littermates (Coleman and Eicher 1990). The major features of the tub gene – sensory loss, poor fertility, and obesity – make it a noteworthy phenotypic match to some human sensory deficit/obesity syndromes, most notably Alstrom’s and Bardet-Biedl. No clear human candidate genes have been identified, however, although homology to human USH1C has been proposed (Heckenlively et al. 1995). The tub gene was recently isolated and characterized (Kleyn et al. 1996; Noen-Trauth et al. 1996). Its product is a novel protein of unknown function expressed primarily in the brain, eye, testis, and large intestine.

Regardless of which human genetic syndrome it best models, the tubby mouse is an appropriate model in which to examine the possibility that a primary genetic defect of axonemal conformation is responsible for its unique phenotype. We have addressed this question in two of the affected functions, vision and reproduction, by ultrastructural examination of photoreceptor and sperm cell axonemes and by functional testing of sperm.

Materials and methods

Animals and genotyping

All procedures were approved by the Central Institute for the Deaf and Washington University Animal Care and Use Committees. The study included a total of 28 C57BL/6J tub/tub, tub/+ and +/+ mice bred in our animal colony and derived originally from mating pairs purchased from the Jackson Laboratory (TJL). Except as noted, comparisons were between tub/tub and tub/+ animals. Each of the pleiotropic effects of the tub mutation reliably occur together, such that by four months of age one could use retinal degeneration, pronounced cochlear degeneration distinguishable from the progressive hearing loss seen in the C57BL/6J strain (Henry 1983), or obesity as a marker for homozgyosity. In younger animals, however, the effects of tub are more subtle. Clear anatomical differences in the retina are usually manifest as shortened photoreceptor outer segments by two weeks, although this can be confused with incomplete development. Identification of tub/tub animals at two weeks instead relied principally upon linkage (engineered by TJL) of the tub and + allele, respectively, with the hemoglobin beta-chain “s” and “p” alleles (HbbS and HbbP) (Coleman and Eicher 1990). The HbbS and HbbP forms migrate to opposite poles of an electric field, permitting identification of tub homozygotes with 95% accuracy by the qualitative migration pattern of their hemoglobins in electrophoresis. Hbb and/or retina typing were used to verify the genotype of all tub/tub and tub/+ animals.

Assessment of structure

Animals were injected with Ketamine (40 mg/kg i.p.) and perfused transcardially with saline followed by 2.0% paraformaldehyde/2.5% glutaraldehyde in 0.08 M cacodylate buffer. Eyes and caudal epididymis/vas deferens were removed, followed by the removal of cornea and lens from each eye. All tissues were then immersed overnight in fixative containing 1 or 4% tannic acid. Specimens were post-fixed in buffered 1% osmium tetroxide, dehydrated in an ascending acetone series, and embedded in Epon-Araldite. The animals used for ultrastructural assessment were sacrificed in the course of a separate study on the progression of retinal and cochlear sensory cell loss (Ohlemiller et al. 1997).

Retina

Because retinal degeneration begins prior to maturity in tub/tub animals, we focused our observations at 13–14 days of age. The sample included three 14-day littermates (two tub/+ and one tub/tub), a 13 day tub/tub, and one six month tub/tub animal. Embedded retinas were sectioned at 1 μm tangential to the curvature of the eye, then thin sectioned for transmission electron microscopy (TEM) at a depth intended to yield photoreceptor ciliary bridges in cross-section. Sections were stained with uranyl acetate and lead citrate. Regions containing multiple cilia were photographed within a single histological section to prevent scoring the same photoreceptor twice. Individual cilia, in which the ring of microtubule doublets could be clearly viewed, were scored by examination of magnified negatives on a light table. To be scored as “normal”, it was required that a cilium contain an ordered circular array of nine microtubule doublets (+9+0) without extra microtubules.

Sperm cells

The distinct drop in the fecundity of tub/tub mice after 4 months of age seems to coincide with the onset of obesity, raising the possibil-