V76D mutation in a conserved γD-crystallin region leads to dominant cataracts in mice

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Abstract. During a large-scale ENU mutagenesis screen, a mouse mutant with a dominant cataract was detected and referred to as Aey4. Aim of this study was the morphological description of the mutant, the mapping of the mutation, and the characterization of the underlying molecular lesion. The slit-lamp examination revealed a strong nuclear cataract surrounded by a homogeneous milky opacity in the inner cortex. The histological analysis demonstrated remnants of cell nuclei throughout the entire lens. The mutation was mapped to Chromosome 1 by a genome-wide linkage making the six γ-crystallin encoding genes and the closely linked βA2-crystallin encoding gene to relevant candidate genes. Finally, a T→A exchange in exon 2 of the γD-crystallin encoding gene (symbol: Ctrygd) was demonstrated to be causative for the cataract phenotype; this particular mutation is, therefore, referred to Ctrygd 675. The alteration in codon 76 leads to an amino acid exchange of Val→Asp. Val at this position is highly conserved; it is found in all mouse and rat γD/E/F-crystallins as well as in the human γA- and γD-crystallins. It may be replaced solely by Ile, which is present in all bovine γ-crystallins, in the rat and mouse γA/B/C-crystallins, as well as in the human γB/C-crystallins. It is predicted that the exchange of a hydrophobic side chain by a polar and acidic one might influence the microenvironment by a dramatic decrease of the isoelectric point by 1.5 pH units in the 10 amino acids surrounding position 76. The Ctrygd 675 additionally demonstrates the importance of the integral of the Ctryg gene cluster for lens transparency.

Introduction

Large-scale ENU mutagenesis screens have been demonstrated to be a source for mouse mutants with clinically important phenotypes (Ehling et al. 1985; Hrabé de Angelis and Balling 1998; Justice et al. 1999; Hrabé de Angelis et al. 2000; Nolan et al. 2000; Brown and Balling 2001). One of the phenotypes we are looking for are opacities of the ocular lens (cataracts). The method was demonstrated to be quick and sensitive (Kratochvílová and Ehling 1979), and about 200 different cataract mutants have been collected (Favor and Neuhäuser-Klaus 2000).

Among the proteins that are responsible for lens transparency, the superfamily of the β/γ-crystallins is most prominent. They were considered for a long time to be present mainly in the ocular lens. However, just recently, expression of the βB2-crystallin mRNA and protein was reported also in retina, brain, and testis (Magabu et al. 2000; Graw et al. 2001b). The common characteristic of all β- and γ-crystallins is the so-called Greek-Key motif. Crystallography has shown that each of the β- and γ-crystallins is composed of two domains, each built up by two Greek Key motifs (for reviews see Slingsby and Clout 1999; Graw 1997; Wistow and Prisandorsky 1988). The γ-crystallin encoding Ctyg genes are mainly located in a cluster of six highly related genes (Ctygα → Ctygβ) on mouse Chromosome (Chr) 1; the 7th Ctyg gene (Ctyg6) is mapped on mouse Chr 16. Several mutations in Ctyg genes have been characterized and are causative for cataracts (for reviews see Graw 1999; Francis et al. 2000; He and Li 2000).

The mutant Aey4 was found as a dominant cataract by slit-lamp screening of mice after paternal treatment with ethynitrosourea (ENU). We demonstrate here linkage of this mutation close to the Ctryg gene cluster on mouse Chr 1. Molecular analysis revealed that the Ctrygd gene is affected in this particular mutant line.

Materials and methods

Animals. Mice were kept under specific pathogen-free conditions at the GSF-Research Center and monitored within the ENU-mouse mutagenesis screen project (Hrabé de Angelis et al. 2000; Hrabé de Angelis and Balling 1998). Male C3HBe/FbJ mice were treated with ethynitrosourea (ENU; 160 mg/kg) at the age of 10 weeks, according to Ehling et al. (1985), and mated to untreated female C3HeB/FeJ mice. The offspring of the ENU-treated mice were screened at the age of 4–6 months with the aid of a slit lamp (SLM30, Zeiss, Oberkochen, Germany) for the presence of cataracts (Kratochvílová and Ehling 1979). Mice with lens opacities were tested for a dominant mode of inheritance. Homozygotes were obtained by brother × sister mating.

Morphological analysis. For gross documentation, lenses were enucleated under a dissecting microscope (Leica MZ APO, Bensheim, Germany) and photographed. For histological analysis, eye globes were fixed for 3 h in Carnoy’s solution and embedded in JB-4 plastic medium (Polysciences Inc., Eppelheim, Germany). Serial transversal 2-μm sections were cut with a dry glass knife (Ultratome OMU3, Reichert, Walldorf, Germany), stained with methylene blue and basic fuchsin, and evaluated with a light microscope (Axioskop, Zeiss). Images were processed with the Adobe software packages Illustrator and Photoshop (Adobe, Unterschleissheim, Germany).

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Fig. 1. Morphological description of Aey/4 lenses. Lenses of 12-week-old Aey/4 mice demonstrate a strong nuclear and milky cortical cataract (a). Both mutant genotypes show smaller lenses than the wild type (left); the homozygotes (right) are more severely affected than the heterozygotes (middle). Histological comparison of eyes of 8-day-old wild types (b, c) and homozygous Aey/4 mice (d, e) indicate the opaque cataractous core of the lens and persistence of remnants of cell nuclei in the Aey/4 mutants (d). The clefts and vacuoles in the anterior part of the lens might be due to sectioning; however, since it is never observed in the wild types, it reflects a weaker adherence of the cells in this region of the cataractous lenses. The magnification of the lens bow region (e) indicates that the nuclei are fragmented at the outer cortex and not fully degraded in the deeper zones. Obviously, other parts of the eye are not different between wild-type and mutants. C, cornea; L, lens; LB, lens bow; LE, lens epithelium; R, retina; the bars represent the given distance.

Mapping. Homozygous carriers (1st generation) were mated to wild-type C57BL/6J mice; the offspring (2nd generation) were backcrossed to the wild-type C57BL/6J mice. DNA was prepared from tail tips of 46 cataractous offspring of the 3rd generation (G3) according to standard procedures; DNA was adjusted to a concentration of 50 ng/μl. Genome-wide linkage analysis was done as described previously (Graw et al. 1999).

Isolation of RNA, DNA, and PCR conditions. Genomic DNA was prepared from spleen or tail tips of 3-week-old mice according to standard procedures. RNA was isolated from lenses (stored at −80°C) of newborn mice. cDNA synthesis and PCR with genomic DNA or cDNA as template were performed essentially as reported previously (Klopp et al. 1998, 2001). In addition, for amplification of the Cryg4 exons 1 and 2 including their flanking regions, we used