Mamm Genome (2013) 24:95–104
DOI 10.1007/s00335-013-9447-1

A mutation in the start codon of γ-crystallin D leads to nuclear cataracts in the Dahl SS/Jr-Ctr strain

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Received: 14 November 2012 / Accepted: 17 December 2012 / Published online: 13 February 2013

Abstract Cataracts are a major cause of blindness. The most common forms of cataracts are age- and UV-related and develop mostly in the elderly, while congenital cataracts appear at birth or in early childhood. The Dahl salt-sensitive (SS/Jr) rat is an extensively used model of salt-sensitive hypertension that exhibits concomitant renal disease. In the mid-1980s, cataracts appeared in a few animals in the Dahl S colony, presumably the result of a spontaneous mutation. The mutation was fixed and bred to establish the SS/Jr-Ctr substrain. The SS/Jr-Ctr substrain has been used exclusively by a single investigator to study the role of steroids and hypertension. Using a classical positional cloning approach, we localized the cataract gene with high resolution to a less than 1-Mbp region on chromosome 9 using an F_1(SS/Jr-Ctr × SHR) × SHR backcross population. The 1-Mbp region contained only 13 genes, including 4 genes from the γ-crystallins (*Cryg*) gene family, which are known to play a role in cataract formation. All of the γ-crystallins were sequenced and a novel point mutation in the start codon (ATG → GTG) of the *Crygd* gene was identified. This led to the complete absence of the CRYGD protein in the eyes of the SS/Jr-Ctr strain. In summary, the identification of the genetic cause in this novel cataract model may provide an opportunity to better understand the development of cataracts, particularly in the context of hypertension.

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Introduction

Cataracts account for approximately 50 % of the world's blindness and affect more than 20 million Americans (Ryskulova et al. 2008; Zambelli-Weiner et al. 2012). In general, cataract formation involves the aggregation of lens proteins, resulting in the clouding of the lens that disrupts the flow of light to the retina and leads to decreased clarity, dull color contrast, and impaired vision (Michael and Bron 2011). There are several types of cataracts, including nuclear, cortical, and subcapsular. Nuclear cataracts are the most common and typically take years to develop and primarily occur in the elderly. There are several medical conditions, such as diabetes, hypertension, trauma, or exposure to certain drugs, that contribute to the development of cataracts (West 2007). In contrast, congenital cataracts, which can be nuclear, cortical, or subcapsular, appear at birth or develop in early childhood (Churchill and Graw 2011). These can result from prenatal infection or be directly linked to specific genetic mutations. There have been numerous studies in humans and animal models that have identified genetic mutations in a number of genes,

such as crystallins, gap junction proteins, membrane transport and channel proteins, and transcription factors, that lead to cataracts (Graw 2009a; Huang and He 2010).

The Dahl salt-sensitive (SS/Jr) rat is a widely studied model of salt-sensitive hypertension. The model has been used extensively to study the pathophysiology of hypertension (Zicha et al. 2012) as well as the genetic basis of hypertension (Garrett et al. 1998; Joe et al. 2009) and associated kidney disease (Garrett et al. 2006, 2007; Williams et al. 2012). Originally, the Dahl S model was maintained as an outbred stock but was later inbred by John Rapp by more than 20 generations of brother–sister mating (Rapp and Dene 1985). At some point, animals within the colony demonstrated the appearance of cataracts, presumably the result of a spontaneous mutation (Rapp 1986). Despite the development of cataracts in these animals, they appeared to be phenotypically similar with regard to the development of salt-sensitive hypertension compared to noncataract Dahl S rats (Rapp 1986). Ultimately, the Dahl S animals that exhibited cataracts were provided exclusively to Elise Gomez-Sanchez and later denoted as SS/Jr-Ctr.

The SS/Jr-Ctr strain has been used extensively for studies investigating the role of steroids in hypertension (Gomez-Sanchez et al. 1996, 2010; Oki et al. 2012), but there has been only one report characterizing the cataracts exhibited in this strain (Margo et al. 1987). Light and electron microscopy analysis of eyes from the SS/Jr-Ctr strain demonstrated lens capsular defects and marked degenerative changes of lens fibers when examined at an advanced age. Lens fibers showed cell membrane ruptures and dissipation, liquefied lens proteins, and "dark" bodies inferring necrosis, followed by shrinkage and calcification at 30–40 days after birth, and finally separation from the zonules and luxation (Margo et al. 1987). However, the specific genetic defect that leads to the development of cataracts in the SS/Jr-Ctr has not been addressed.

The purpose of our study was to identify the gene and specific mutation(s) that are responsible for the development of cataracts in the eyes of the SS/Jr-Ctr strain and whether the genetic mutation could have some impact on the cardiovascular and renal complications exhibited by the strain. To achieve this goal, we performed a systematic genome-wide linkage analysis for cataract status as well as related traits, sequenced candidate genes, and ultimately demonstrated by Western blot analysis that the gene mutation leads to an apparent knockout at the protein level.

Materials and methods

Animals, study design, and segregating population

All experiments were approved by the Institutional Animal Care and Use Committee. The Dahl salt-sensitive (SS/Jr) and the spontaneously hypertensive rat (SHR//NHsd) inbred strains are maintained at the University of Mississippi Medical Center. The SS/Jr-Ctr strain was obtained from E. Gomez-Sanchez at G. V. (Sonny) Montgomery VAMC, Jackson, MS.

A segregating population was derived from SS/Jr-Ctr and SHR to map the cataract mutation. SS/Jr-Ctr males ($n = 4$) were bred to SHR females ($n = 7$) to generate F_1(SS/Jr-Ctr × SHR). F_1 animals ($n = 57$) were backcrossed to SHR ($n = 19$), generating an F_1(SS/Jr-Ctr × SHR) × SHR backcross population ($n = 146$), denoted as BC. At day 21, the BC population was characterized with respect to body weight (BW), eye weight (EW), presence or absence of cataract, eye-to-eye distance (EED), eye-to-nose distance (END), and histology.

Genotyping and sequencing

DNA was obtained from a spleen or tail biopsy and prepared using a Wizard SV 96 Genomic DNA kit (Promega, Madison, WI). The BC population was genotyped using two approaches: (1) whole-genome single-nucleotide polymorphism (SNP) genotyping (Kbioscience, UK). A total of 112 SNP markers were tested throughout the genome; and/or (2) microsatellite analysis ($n = 10$) using standard PCR-ethidium bromide-gel electrophoresis or a fluorescent-based approach on a Beckman Coulter CEQ8000 XL capillary sequencer (Beckman Coulter, Brea, CA) (Garrett et al. 2006, 2007).

Primers were designed from known sequences (www.ncbi.nlm.nih.gov or www.ensembl.org) to amply the coding regions of the γ-crystallin (*Cryg*) family *b–e* (Table 1). All primers were tagged with either M-13 Forward (5′ GTA AAACGACGGCCAGT 3′) or Reverse (5′ CAGGAAACAGCTATGAC 3′) for sequencing analysis. PCR was performed using SS/Jr, SS/Jr-Ctr, F_1(SS/Jr-Ctr × SHR), and SHR genomic DNA samples, purified using a PureLink PCR Purification Kit (Invitrogen, Carlsbad, CA) and prepared for fluorescence-based DNA sequencing on a CEQ8000 using a DTCS Quick Start Kit (Beckman Coulter). Sequencing reads were assessed for quality and aligned to the BN reference sequence using the DNASTAR's Lasergene v7.2 software package (DNASTAR, Inc., Madison, WI). SNPs were visually confirmed in trace files and identified variants were verified by direct sequencing from genomic DNA isolated from at least three rats per strain.

Western blot analysis

Whole eyeballs from SS/Jr, SS/Jr-Ctr, F_1(SS/Jr-Ctr × SHR), and SHR rats were homogenized in RIPA lysis buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and normalized, and then 50–100 μg of total protein were

Table 1 Genomic DNA primers used to amplify γ-crystallin gene family (*Crygb-Cryge*)

Primer name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
Crygb Exon1–2	TGTGTGATTTCCTGTGGAGG	TGCTCTAATTGAAACGACTTGG
Crygb Exon3	GAGGTGTCGGGACAGGAAAC	GGAAGTTCCTTCCCATCTCTG
Crygc Exon1–2	TCCGCTAACACAGCAACAAC	GACAAACAAACCCTATTCTCTGG
Crygc Exon3	CGTTGTTTAGCTTGGGTGAG	TGTAGGGAGAGTTTACAGGTGG
Crygd Exon1–2	CCTTTTGTGCTGTTCCTGC	GGCAACTGCAGTCAGGGTC
Crygd Exon3	ATGGCTTGATGCAGCTGAG	CCAATAAACATGAGCAAACAGG
Cryge Exon1–2	TTGTGCTGTTCCTGCCAAC	CGGCCACAGATGTTACTAGC
Cryge Exon3	TGGGTTTCCAAGTTCAGGTC	CATCCTTTCTTTGCTGTGCC

All *Crygb-e* genes consist of three exons. The first primer pair was used to amplify exon 1–2 (including intron 1) and the second primer pair was used to amplify exon 3

evaluated using standard polyacrylamide gel electrophoresis on 4–10 % Mini-PROTEAN TGX gels (Bio-Rad Laboratories, Hercules, CA) as previously described (Williams et al. 2012). Samples were transferred to PDVF membranes using the Trans-Blot Turbo System (Bio-Rad Laboratories). Blots were probed with monoclonal anti-CRYGD antibody (Sigma-Aldrich, St. Louis, MO) using 1:500 dilution and 1:000 goat anti-mouse (HRP) as the secondary. Pierce ECL substrate (ThermoScientific, Waltham, MA) was prepared according to the manufacturer's instructions and added to membranes. The membranes were imaged using a ChemiDoc XRS + System (Bio-Rad). Equal loading of protein was confirmed by GAPDH (MAB374; Millipore, Billerica, MA) on the same blot probed with CRYGD.

Histological assessment

Whole eyeballs isolated from SS/Jr and SS/Jr-Ctr were fixed in buffered formalin, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E). Unstained sections were stained with Von Kossa stain (American Mastertech Scientific, Lodi, CA) to confirm calcium deposits observed under H&E. Histological images were captured using Nis-Elements image analysis software ver. 3.03 (Nikon Instruments Inc., Melville, NY) on a Nikon 55i microscope with DS-Fi1 5-megabyte Color C digital camera (Nikon).

Statistical and genome-wide linkage analyses

Linkage analysis was performed using Windows QTL Cartographer V2.5_010 (Li et al. 2006; Wang et al. 2012). The BC population was analyzed using the interval mapping function for continuous traits (BW, EW, EED, and END) and category trait analysis for cataract status. The LOD threshold for significance was calculated by permutation testing (1:1,000). Additional statistical comparisons of phenotypic data (e.g., eye weight) were evaluated using an independent *t* test (SPSS, Inc., Chicago, IL), and $p < 0.05$ was considered to be statistically significant. All data are presented as mean ± standard error (SE).

Results

The presence of cataracts in the lenses of SS/Jr-Ctr animals was immediately obvious from the time their eyes opened (~day 15–16). Eyes from SS/Jr-Ctr animals demonstrated an opaque white color in the center of the lens, while the lenses from wild-type SS/Jr animals appeared transparent (Fig. 1). Eyes from SS/Jr-Ctr animals (or heterozygous animals) were significantly smaller than eyes from SS/Jr, regardless of sex (Fig. 2). The distances from eye to eye and eye to nose were also significantly reduced in the SS/Jr-Ctr rat compared with those in the SS/Jr rat. Histological assessment of eyes from SS/Jr-Ctr revealed the clear presence of a nuclear cataract, disruption of lens fibers, and calcification, whereas SS/Jr eye sections appeared normal (Fig. 3).

To map and identify the causative genetic variant(s) responsible for cataracts in the SS/Jr-Ctr strain, a segregating population was developed using the SS/Jr-Ctr rat and the spontaneously hypertensive rat (SHR). The SHR was selected as a contrasting strain because linkage analyses had been performed previously to identify quantitative trait loci (QTL) for cardiovascular and renal traits in similar segregating populations (Garrett et al. 2003, 2006). All F_1(SS/Jr-Ctr × SHR) animals developed cataracts, thus confirming that the causative mutation was completely dominant. An F_1(SS/Jr-Ctr × SHR) × SHR population was evaluated for several traits, including body weight (BW), eye weight (EW), eye-to-eye distance (EED), eye-to-nose distance (END), and the presence or absence of cataracts. A total of 112 SNP markers were tested across the entire genome and genome-wide interval

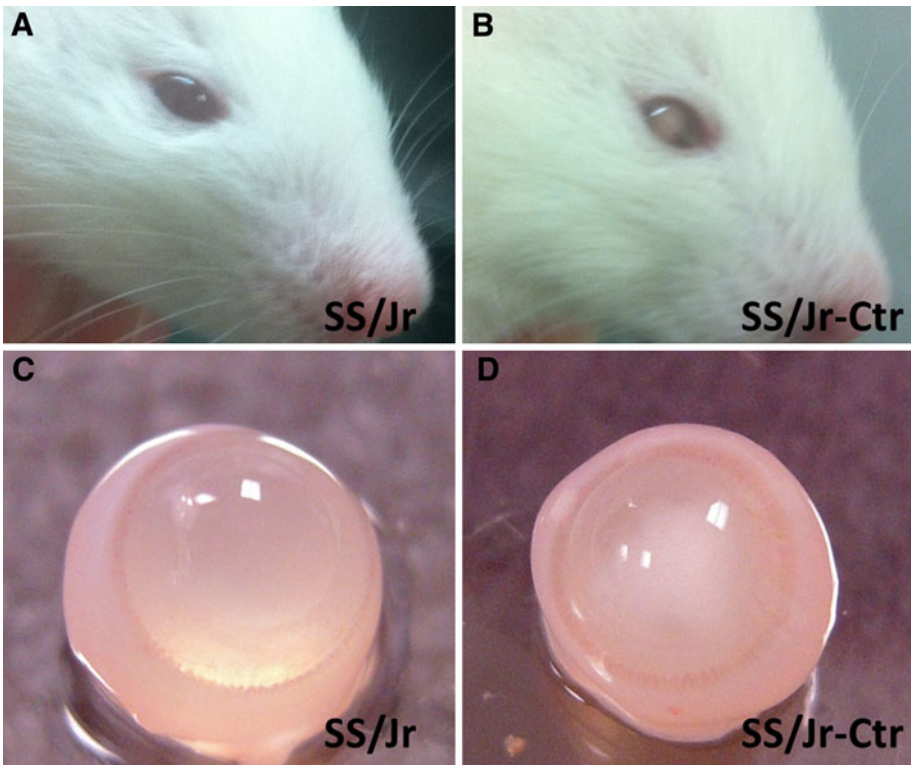

Fig. 1 Gross appearance of nuclear cataract at 21 days of age. **a** Normal appearance of the eye in wild-type SS/Jr strain. **b** Appearance of cataract in the eye of the SS/Jr-Ctr strain which exhibits spontaneous cataracts. Gross dissection of the eye from **c** wild-type and **d** cataract animals. The lens of the SS/Jr eye is clear and transparent, whereas the lens from the SS/Jr-Ctr eye is opaque and cloudy

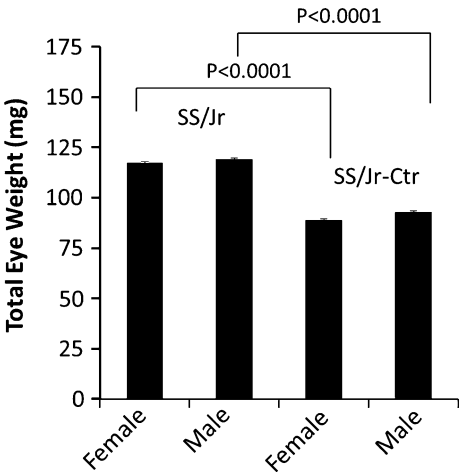

Fig. 2 Measurement of eye weight in SS/Jr and SS/Jr-Ctr animals. Total eye weight (left eye + right eye) is shown for each strain and sex. For either sex, eyes from SS/Jr-Ctr were significantly smaller than eyes from SS/Jr ($n = 6$)

mapping was performed. A single, highly significant (LOD > 28) locus was identified on chromosome 9 for cataract status (Fig. 4). QTLs for EW (left, right, or total) were also identified at the same location as that for the cataract status, but no QTL was observed for EED or END. A suggestive QTL for EW (LOD = 2.6) was also observed on chromosome 15.

To better define the region on chromosome 9 containing the cataract mutation, animals were classified by genotype at multiple markers (haplotype analysis) (Fig. 5a). Five distinct haplotypes were observed in the region between 40.2 and 68.1 Mbp. Animals with haplotype 1 were homozygous SHR/SHR and exhibited no cataracts. Haplotype 2 carried the S allele between 40.2 and 45.3 Mbp and exhibited no cataracts, whereas animals with haplotypes 3–5 all carried the S allele at 59.7 Mb and exhibited cataracts, suggesting that the location of the cataract mutation was tightly linked to the marker at 59.7 Mbp (range = 45.3–68.1 Mbp). Three key recombinant animals were further genotyped using additional microsatellite markers to refine the location of the cataract mutation (Fig. 5b). All three animals exhibited cataracts and shared the same genotype (S/SHR) only at microsatellites *D9Rat85* (63.68 Mbp) and *D9Mit2* (63.71 Mbp). Based on these data and the recombination interval for two of the animals (85867 and 85633), the cataract mutation could be localized to the region from 62.9 to 63.8 Mbp, or ~900 kbp (Fig. 5b).

Table 2 provides a summary of the 13 genes that reside in the refined genomic interval (~900 kbp). Five genes (D4A9A7_RAT, *Fam119a*, F1M4U0_RAT, LOC100363697, and *Pikfyve*) were denoted as uncharacterized proteins and another four genes (*Creb*, *Fzd5*, *Plekhm3*, and *Idh1*) appear to have no biological role in the eye or lens. However, there were four genes in the interval that belong to the γ-crystallin family (*Crygb*, *-c*, *-d*, and *-e*), and mutations in these genes have been previously linked to the development of cataracts. The coding regions of all four *Cryg* genes were sequenced. No sequence differences were observed in *Crygb*,

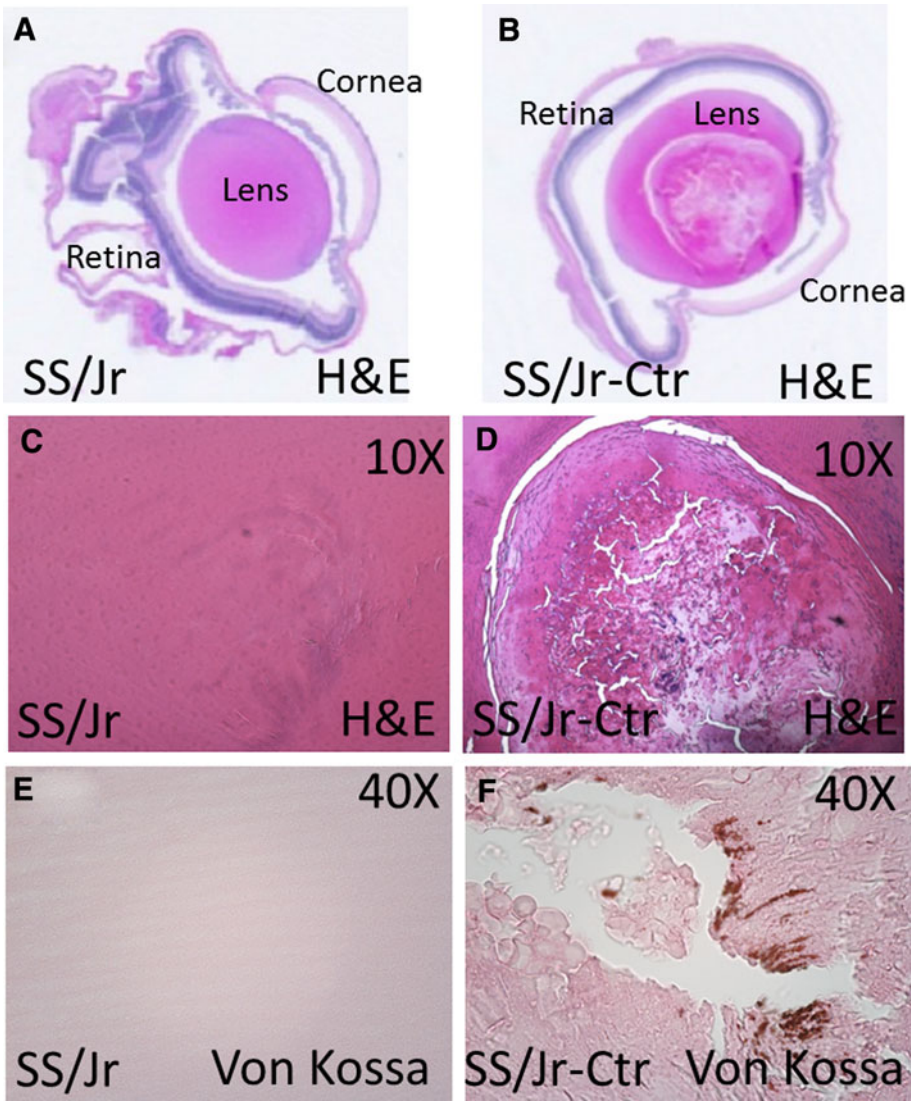

Fig. 3 Representative whole-eye and high-resolution images of lenses from SS/Jr and SS/Jr-Ctr at 21 days of age. **a**, **b** Longitudinal histology section through whole eye (1×, H&E) that demonstrates the normal appearance of key anatomical structures in each strain, aside from obvious lens fiber changes observed in the eye from SS/Jr-Ctr. **c**, **d** Higher-magnification images (10×, H&E) showing normal lens appearance in the eye from SS/Jr and disruption of lens fibers in the eye from SS/Jr-Ctr. **e**, **f** Lens from SS/Jr-Ctr also demonstrates distinct calcification (40×, Von Kossa stain), whereas wild-type SS/Jr lens has a normal appearance

-*c*, or -*e* between the SS/Jr and SS/Jr-Ctr rats. However, a single-base mutation was observed in the start codon of exon 1 in the *Crygd* gene (Fig. 6). The start codon (ATG) in SS/Jr encodes a methionine, whereas in SS/Jr-Ctr it is expected to encode a valine (GTG). Western blot analysis of eye homogenates demonstrated no CRYGD protein in SS/Jr-Ctr, but it was clearly observed in wild-type SS/Jr animals (Fig. 6). Eyes from heterozygous animals [F_1(SS/Jr-Ctr × SHR)] demonstrated an ∼50 % reduction in the amount of CRYGD protein compared to wild-type SS/Jr animals. Western blot analysis of homogenates from various organs demonstrated that the CRYGD protein appears specific to the eye.

Discussion

Though the Dahl SS/Jr-Ctr strain has been around since the mid-1980s, no attempt had been made to elucidate the underlying genetic cause of cataracts in the model. Using the classical positional cloning approach, the causative gene was localized with high resolution to a less than 1-Mbp region on chromosome 9, which contained only 13 genes, including 4 genes from the *Cryg* family. A novel point mutation in the start codon (ATG → GTG) of the *Crygd* gene was identified by sequencing. In eukaryotes, the start codon almost always codes for methionine (ATG) and alternative start codons (non-ATG) are rare (Hwang et al. 2005). The *Crygd* ATG start codon (methionine) is also conserved across all mammals (mouse, human, dog, etc.) and vertebrate species (data not shown). Thus, it was expected that the *Crygd* gene transcript would not be translated efficiently into protein in the SS/Jr-Ctr strain. Western blot analysis confirmed that the CRYGD protein was not expressed in the eye of SS/Jr-Ctr but was clearly observed in the wild-type SS/Jr and SHR. Therefore, we conclude that the Met1Val substitution is almost certainly

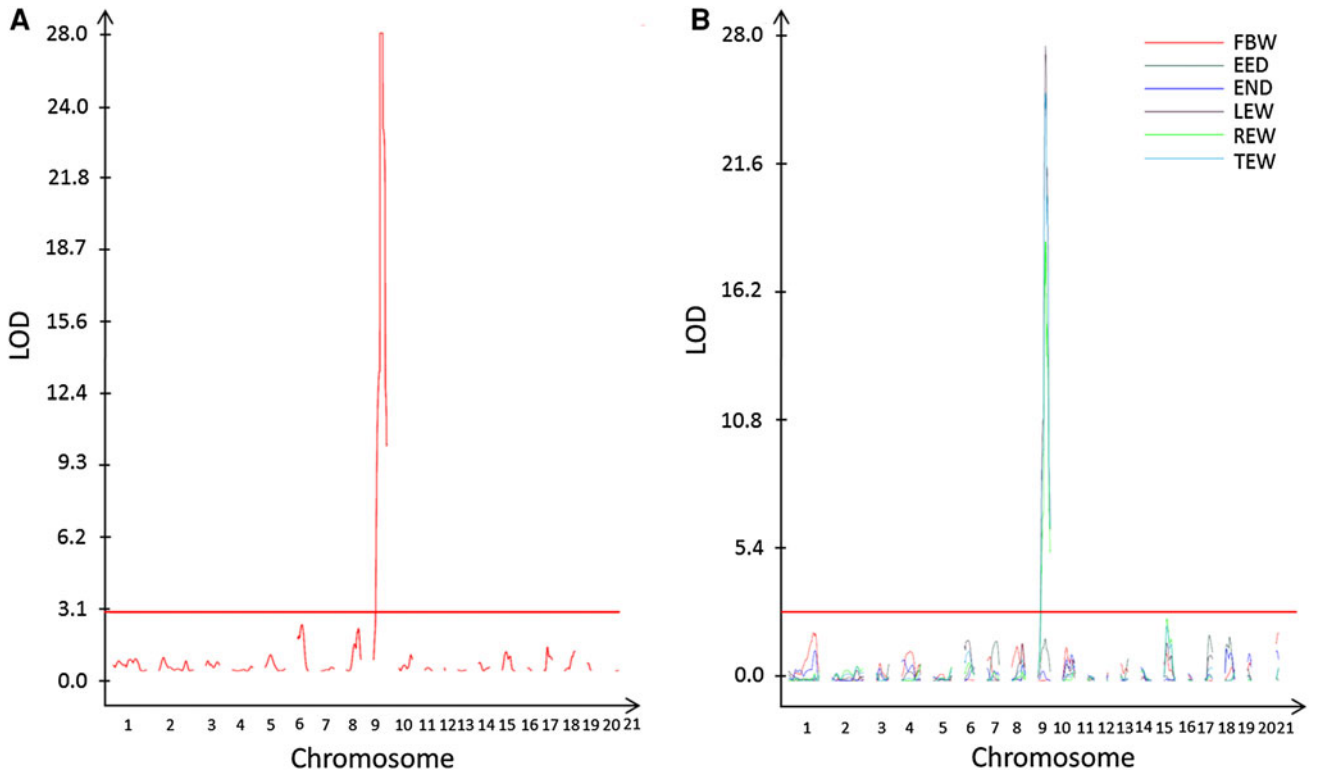

Fig. 4 Genome-wide linkage analysis for cataract status in F_1(SS/Jr-Ctr × SHR) × SHR population at 21 days of age. **a** LOD plot of cataract status (category trait analysis-presence or absence of cataracts). **b** LOD plot of continuous traits, including body weight, eye weight (left, right, and total), eye-to-eye distance, or eye-to-nose distance. The chromosome location is shown on the *x* axis and LOD score is shown on the *y* axis. The significance threshold for significant quantitative trait locus (QTL) was determined by permutation testing and is denoted by the line around LOD ~ 3

the cause of cataracts in the SS/Jr-Ctr strain, although definitive evidence could be established only by additional experiments such as a gene knockout.

The lens of the eye is composed of fiber cells that undergo differentiation that involve changes in cell shape, expression of crystallin proteins, and degradation of cellular organelles that ultimately lead to the transparency of the lens (Michael and Bron 2011). Crystallins are the predominant proteins of the lens and are divided into two major families, α and β/γ (Andley 2007). The crystallin proteins contribute to the transparency and refractive properties of the lens by establishing a uniform concentration gradient in the lens. The α-crystallins, which are composed αA and αB, not only perform an important role in preserving lens transparency, they also serve as molecular chaperones by preventing aberrant protein interactions (Andley 2007). The targeted disruption of αA-crystallin in mice as well as several spontaneous mutations in the gene result in cataracts (Chang et al. 1996; Brady et al. 1997; Xia et al. 2006). There is no clear relationship between disruption of αB-crystallin and cataracts. In mice, *Cryab*$^{-/-}$ show general loss of body weight, muscle degeneration, and abnormal skeletal development, but do not exhibit cataracts (Brady et al. 2001). In contrast, there are a number of known mutations in human αB-crystallin that result in dominant cataracts (Graw 2009b), in addition to desmin-related myopathy or dilated cardiomyopathy (Vicart et al. 1998).

The β/γ-crystallins compose a large family of genes (>14 genes) that are organized as individual genes (*Cryba1*, *Cryba2*, *Crygf*, *Crygs*, *CrygN*), duplicates (*Cryba4–Crybb1* and *Crybb2–Crybb3*), and one major cluster (*Crygb*, *-c*, *-d*, *-e*) (Graw 2009a). A key feature of the β/γ-crystallins is the Greek key motif that allows for the dense packing of proteins in the lens to minimize light scatter and provide transparency (Graw 2009a). A second feature is the motif's Ca^{2+} binding properties (Suman et al. 2011). The role of Ca^{2+} in cataract development has been investigated by several groups (Duncan and Wormstone 1999; Sanderson et al. 2000; Nagai et al. 2008). The relationship between Ca^{2+} and crystallin proteins could play a role in the calcification observed in the SS/Jr-Ctr lens.

Mutations in most β/γ-crystallins genes result in cataract formation in mice and humans (Graw 2009b). Mice that

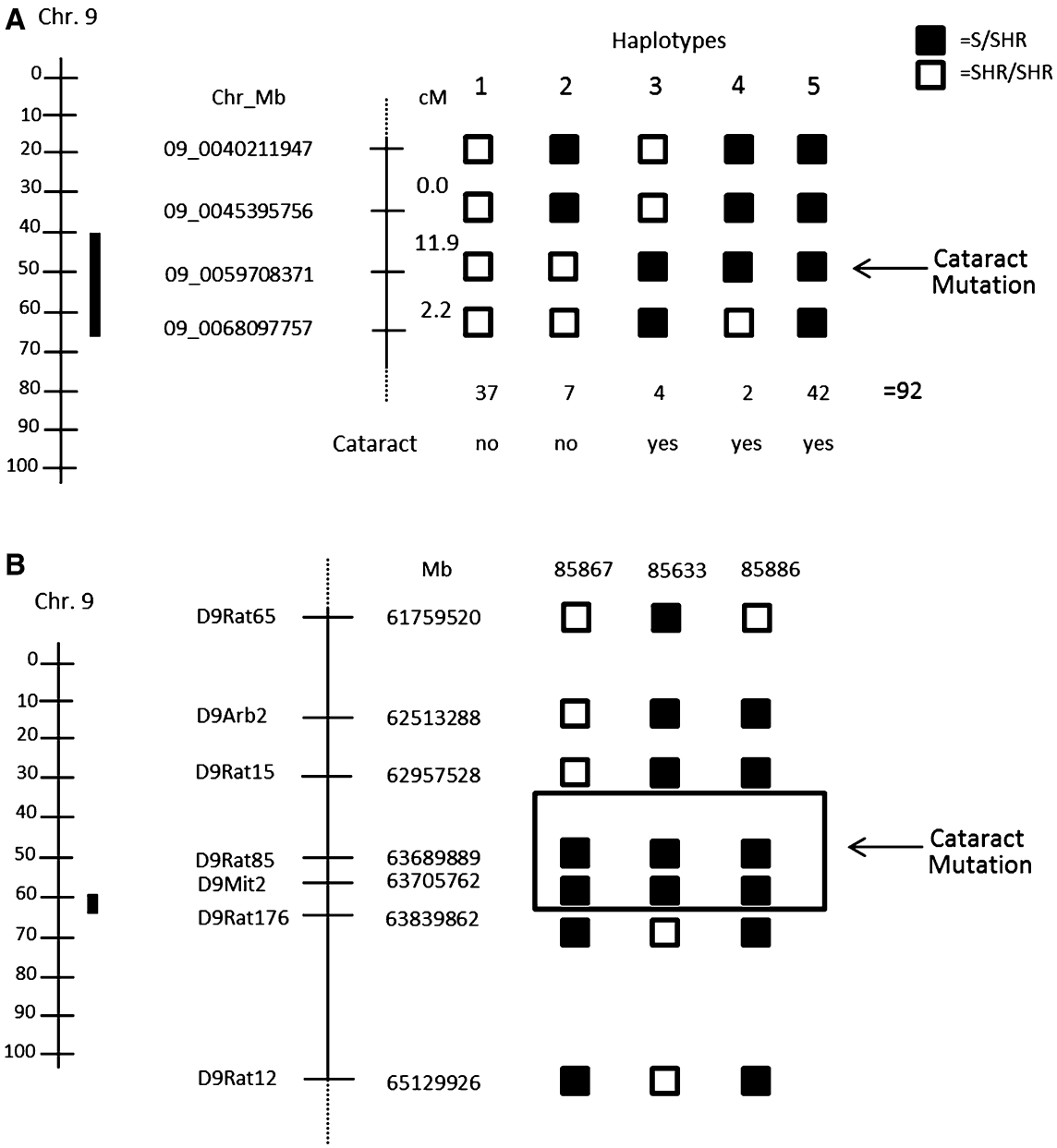

Fig. 5 Haplotype analysis and refinement of cataract mutation to rat chromosome 9. **a** Location of the 95 % confidence interval (CI) of the genomic interval linked to the cataract mutation (*solid black bar*) next to the physical map (*left*), and haplotype analysis of the F_1(SS/Jr-Ctr × SHR) × SHR population (*right*). The haplotype analysis provides a refinement of the QTL based on genotype and absence of cataract (haplotypes 1–2) or cataract status (haplotypes 3–5). Haplotypes 3–5 exhibit cataracts and share the SS/Jr-Ctr/SHR genotype at 09_0059708371 (denoted by the arrow). **b** Refinement of the cataract QTL using key recombinant animals from BC. All three animals exhibited cataracts and shared the SS/Jr-Ctr allele at only microsatellite markers *D9Rat85* (63,689,889 Mb) to *D9Mit2* (63,839,862 Mb). The genomic interval containing the cataract mutation is denoted by the boxed area

exhibit mutations in *Crygd* (such as observed in SS/Jr-Ctr) demonstrate variability in onset, severity, and type of cataracts (Smith et al. 2000; Graw et al. 2002; Wang et al. 2007). This is likely related to the location of the mutation in the gene and its functional consequence (i.e., protein domain). In general, the loss of solubility of mutated crystallin proteins has been frequently suggested as the cause of cataract formation, as most *Crygd* mouse mutants exhibit only single amino acid changes (Andley 2007). This is unlikely the case for SS/Jr-Ctr as there appears to be the complete absence of protein expression. The development of some cataracts in *Cryg* mutants has been attributed to the formation of large amyloid-like intranuclear inclusions and/or termination of the secondary lens fiber differentiation, as indicated by the presence of remnants of cell nuclei (Sandilands et al. 2002).

Table 2 Genes located in refined cataract locus between 62.9 and 63.8 Mb on chromosome 9

Ensembl ID	Gene name	Location (bp)	Description
ENSRNOG00000013412	Creb1	63170785	Cyclic AMP-responsive element-binding protein 1
ENSRNOG00000031069	D4A9A7_RAT[a]	63237141	Uncharacterized protein
ENSRNOG00000014212	Fam119a	63246137	Uncharacterized/similar to methyltransferase like 21A (Mettl21A)
ENSRNOG00000023807	F1M4U0_RAT	63351853	Uncharacterized/similar to cyclin Y-like 1 (Ccnyl1)
ENSRNOG00000014678	Fzd5	63386990	Frizzled-5
ENSRNOG00000023760	Plekhm3	63441846	Peckstrin homology domain containing, family M, member 3
ENSRNOG00000042709	LOC100363697	63661748	Uncharacterized/similar to aldo–keto reductase family 1
ENSRNOG00000014790	Cryge	63698609	Gamma-crystallin E
ENSRNOG00000032219	Crygd	63711051	Gamma-crystallin D
ENSRNOG00000014950	Crygc	63720587	Gamma-crystallin C
ENSRNOG00000032926	Crygb	63730906	Gamma-crystallin B
ENSRNOG00000015020	Idh1	63769401	Isocitrate dehydrogenase
ENSRNOG00000015158	Pikfyve	63798657	Similar to phosphatidylinositol 5-kinase, type III isoform 2

[a] No orthologs (predicted only in rat)

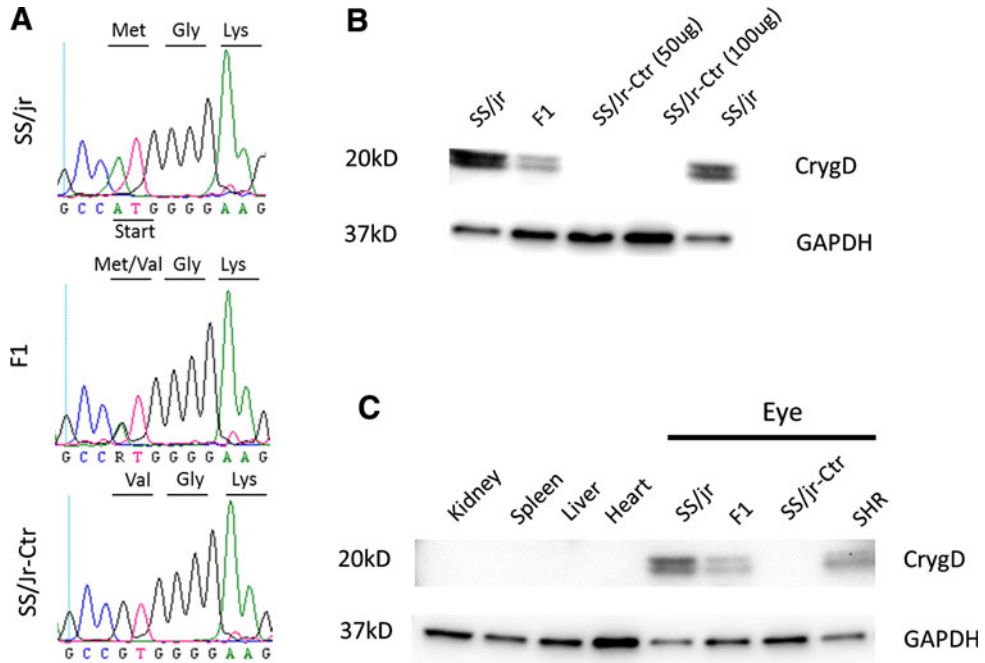

Fig. 6 Sequence and Western blot analyses of whole eye and other organs from SS/Jr and SS/Jr-Ctr. **a** DNA sequencing chromatographs show a point mutation in the start codon (ATG → GTG) in SS/Jr-Ctr and F_1 DNA compared to wild-type SS/Jr. The start codon (ATG), which encodes a methionine, is predicted to be a valine in the SS/Jr-Ctr and F_1. **b** Western blot analysis of eye. No CRYGD protein is evident in the SS/Jr-Ctr eye but is observed in wild-type SS/Jr animals. In eyes from F_1 animals, there is an approximately 50 % reduction in the amount of CRYGD protein observed. **c** Western blot analysis of homogenates from various organs and eye. No CRYGD protein is evident in tissues related to cardiovascular health and disease (heart or kidney)

One motivation in elucidating the gene mutation that causes cataracts in the SS/Jr-Ctr strain was the possibility that the gene could be linked to kidney and/or cardiovascular disease that is exhibited by the strain. The kidney and eye share similar developmental pathways, including PAX2 and WT1 (Dahl et al. 1997; Izzedine et al. 2003). WT1 is necessary for ureteric bud formation (Mrowka and Schedl 2000) as well as retinal ganglion cell differentiation (Wagner et al. 2002). There are a number of retinal abnormalities found in inherited renal diseases (Savige et al. 2011). However, γ-crystallins appear to be expressed only in the lens as opposed to α-crystallins and other eye proteins, which are also expressed in other tissues and organs (Graw 1997). Thus, it is unlikely that the *Crygd*

mutation would have any impact on cardiovascular or kidney disease and no further studies were performed to explore this possible association.

There are only a couple spontaneous cataract models in the rat with either mapped or identified genetic cause, including an unidentified gene on chromosome 15 in the Ihara epileptic rat (Yokoyama et al. 2001), connexin 46 (*Gja3*) (Yoshida et al. 2005), and connexin 50 (*Gja8*) (Liska et al. 2008). The *Gja8* point mutation (L7Q) in the SHR-*Dca* is different than that observed in the UPL rat (R340W) and appears to have a significant effect on the onset and severity of cataracts (Liska et al. 2008). Like the SS/Jr-Ctr model, the SHR-*Dca* is a model of hypertension, and while there appears to be no clear link between γ-crystallins and hypertension in the SS/Jr-Ctr model, there have been some studies that suggest a role of gap junctions in hypertension (Figueroa et al. 2006; Lübkemeier et al. 2011).

In summary, we have identified a novel mutation in the start codon of the γ-crystallin D gene that leads to a complete knockout and the most likely cause of cataract formation in the SS/Jr-Ctr rat. This finding could provide new use for the model and allow investigation into eye development, cataract formation, and the function of crystallin proteins.

Acknowledgments MRG is supported by NIH/NHLBI HL094446 and the Robert M. Hearin Foundation. ACH is supported by 1T32HL105324. EGS is supported by Medical Research funds from the Department of Veterans Affairs and NIH Grants HL27255 and HL75321.

Disclosures The authors have no conflicts of interest to disclose.

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