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HSF4 Mutation p.Arg116His Found in Age-related Cataracts and in Normal Populations Produces Childhood Lamellar Cataract in Transgenic Mice

Zhe Jing[1,*], **Rajendra K. Gangalum**[1,*], **Ankur M. Bhat**[1], **Yoshiko Nagaoka**[3], **Meisheng Jiang**[3], and **Suraj P. Bhat**[1,2]

[1]Jules Stein Eye Institute, Geffen School of Medicine at UCLA, University of California, Los Angeles, CA 90095

[2]Geffen School of Medicine, Molecular Biology Institute and Brain Research Institute, Geffen School of Medicine at UCLA, University of California, Los Angeles, CA 90095

[3]Department of Molecular and Medical Pharmacology, Geffen School of Medicine at UCLA, University of California, Los Angeles, CA 90095

Abstract

The p.Arg116His mutation in the heat shock transcription factor-4 (*HSF4*) has been associated with age-related cataracts, but it is also seen in 2% of the normal population indicating either reduced penetrance or that the normal subjects were not old enough to express the phenotype. Based on the proximity of p.Arg116His to two known mutations in the DNA binding domain of HSF4, namely p.Leu114Pro and p.Arg119Cys, which segregate with childhood lamellar cataract, we tested the possibility that this phenotype may have been missed by the ophthalmologist and/or that it did not spread to the visual axis so as to affect vision significantly. Here we demonstrate via BAC (bacterial artificial chromosome) transgenesis that p.Arg116His recreates the childhood lamellar cataract in mice suggesting that incomplete penetrance associated with early cataracts may not be an absence but a limitation of the detection of the phenotype.

Keywords

HSF4; Cataract; Ocular lens; BAC recombineering; Transgenic mice

Up to half of all congenital cataracts are inherited and most of these are catalogued as autosomal dominant [Francis and Moore 2004; Shiels and Hejtmancik 2013; Zetterstrom and Kugelberg 2007]. Light deprivation of the developing eye due to the cataracts in the ocular lens, during infancy, impedes the brain development of the child [Blakemore and Cooper 1970; Kalia et al., 2014]. Based on screening of three Chinese and one Danish kindred spanning nine generations, four mutations in the DNA binding domain of HSF4

Address for correspondence: Suraj P. Bhat, Ph.D., 100 Stein Plaza, BH263, Geffen School of Medicine @ UCLA, Los Angeles, CA 90095, Tel. 310 825 9543, bhat@jsei.ucla.edu.
*These authors contributed equally to this work.

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(MIM# 602438) were discovered [Bu et al., 2002] which segregated with early childhood lamellar cataract [Marner et al., 1989], consistent with the predominantly post-natal expression of this transcription factor [Somasundaram and Bhat 2004]. The postnatal appearance of the disease phenotype also agrees with HSF4 null studies, which indicated that the early embryonic development of the lens is normal but the secondary fiber cell morphogenesis is impaired [Fujimoto et al., 2004; Min et al., 2004; Shi et al., 2009].

Interestingly a screening of age-related cataract patients has revealed additional mutations [Shi et al., 2008] that purport a role for HSF4 function in age-related cataracts. Among these mutations, the role of p.Arg116His in age-related cataract could not be established decisively because this mutation was also found in 2 control/normal individuals, who were only, “40 and 46 years old and are likely to suffer age-related cataracts in the future”[Shi et al. 2008] suggesting that this mutation had not reached its age of pathological expression.

The presence of p.Arg116His in ‘normal’ population thus could be explained away by variable expressivity. It could also be a case of what is commonly known as incomplete or reduced penetrance [Sosnay and Cutting 2014]. However, two known mutations in the DNA binding domain of HSF4 namely, p.Leu114Pro and p.Arg119Cys, segregate with childhood lamellar cataract [Bu et al. 2002]. Based on this knowledge, the proximity of p.Arg116His to these mutations would suggests a similar phenotype, yet the observation [Shi et al. 2008] that it is associated with age-related cataracts with incomplete penetrance raises the possibility that the early childhood cataract phenotype may have been missed by the ophthalmologist or that it did not spread to the visual axis so as to impede transmission of light into the eye significantly and therefore did not affect the vision; the phenotype, therefore, was clinically ‘normal’. Such individuals, upon genotypic characterization, would contribute to the unexplained phenomenon of “incomplete penetrance”.

Incomplete penetrance or the presence of p.Arg116His may simply be an inability to assay the phenotype either morphologically or at the molecular level. Alternatively, it is possible that the individual with this mutation had never a need to go to an ophthalmologist because she/he is ‘normal’. It is important to note that opacity in the ocular lens becomes pathological only if and when it interferes with the visual axis; otherwise the effected individual does not go to the ophthalmologist and will be considered ‘normal’. Because cataract is a physical phenotype that can be observed with an ophthalmoscope as opacity in the lens we decided to test the concept of penetrance by expression of the HSF4 gene containing the p.Arg116His mutation in transgenic mice.

The lamellar cataract phenotype (associated with most mutations in the DBD of the HSF4) appears within a morphologically confined space (the lens nucleus, involving only a few fiber cells or lamellae) and it appears in a developmentally dictated fashion. It is therefore important that the expression of the mutation is temporally and spatially akin to the *in vivo* condition. Because BACs contain large stretches of native sequences, the expression of the transgene is usually similar to the expression of the endogenous gene [Heintz 2001]. The p.Arg116His mutation was introduced into the Hsf4 gene exon3 g.115G>A (NC_000074.6) within a BAC (Fig. 1A). The BAC clone, RP023-203H14, was purchased from Children’s Hospital Oakland Research Institute. The rpsL-neo template and plasmid pRed E/T were

purchased from Gene Bridges GmbH (Heidelberg, Germany). The mutated BAC was used to generate transgenic mice [Gangalum et al., 2011] (Fig. 1 A–C). For details of experimental procedures please see online Supp. Methods (Supp. Fig. S1).

Fig. 1D shows the ophthalmoscope images of the lamellar cataract phenotype in the transgenic mouse lens. The pathology is bilateral as is known for the human population [Zetterstrom and Kugelberg 2007]. It is typically punctate and appears as dots or specks, very much confined to the nucleus just like the known early childhood lamellar cataract [Francis et al., 2000; Marner et al. 1989]. Although somewhat enhanced, these cataracts remain restricted to the lens nucleus in older F2 mice (5 month). These morphological phenotypes are corroborated by histological and molecular analyses of the post-natal day 02 lenses (Fig. 2). There is disturbance in the secondary fiber cell differentiation, abnormal location of the fiber cell nuclei in the posterior of the lens (Fig. 2A, B) and e a general decrease in total crystallin expression attended by their abnormal distribution (aggregation) (Fig 2C and Supp. Fig. S2). We also find that there is appreciable absence of the vimentin and Fgf7 gene products (Fig. 2C and Supp. Fig. S3). This is in contrast to the Hsf4 knockout (KO) where an increase in Fgf7 transcripts was reported in six week old lenses; it was unclear if the protein levels were increased [Fujimoto et al. 2004]. It is difficult to compare our data (the restricted lamellar cataract phenotype recreated here by a point mutation p.Arg116His) with the KOs, where the reported phenotype of a severe total cataract, suggests involvement of multiple phenotypes.

It is important to consider that a lot of the changes that we see (e.g. aggregation of proteins and loss of vimentin and Fgf7) may be secondary and causatively not related to the lamellar cataract, which is morphologically restricted to a few fiber cells. Importantly, this data points to the heterogeneity of genetic expression controlled by Hsf4 in different fiber cells of the developing lens. It is this heterogeneity, from fiber to fiber that we believe generates the lamellar cataract phenotype.

Abnormal location of the fiber cell nuclei in the posterior of the lens (Fig. 2B and Supp. Fig. S4) suggests a block in the secondary fiber cell differentiation, which entails degradation of the cellular organelles including the nuclei in the wild type normal lens as part of the terminal differentiation program that minimizes scatter and presents a clear path for the transmission of focused light onto the retina, making vision possible. Some of these phenotypes have been reported to be associated with Hsf4 knockout mice [Fujimoto et al. 2004; Min et al. 2004; Shi et al. 2009]. DNase 2β (DLAD) is known to be involved in lens nuclear degradation [Nishimoto et al., 2003], recently this gene has been suggested to be down stream of Hsf4 [Cui et al., 2013]. Importantly, we do not see any drastic changes in the size of the transgenic eye (Supp. Fig. S5), again confirming what is reported in the humans [Zetterstrom and Kugelberg 2007].

Childhood cataracts are associated with a large number of syndromes including mental retardation, microencephaly and cerebero-oculo-facial–skeletal syndrome. It remains to be established if the onset and/or the severity of the phenotype in the ocular lens (for example a total cataract versus a restricted opacity in a few fiber cells) is indicative of the severity of the associated systemic abnormalities [Chograni et al., 2011].

The data presented above suggests that the presence of p.Arg116His in normal individuals may be due to a lack of the recognition of the incipient opacity in the infant lens by the ophthalmologist or a case of missed ophthalmic exam in early childhood. In either case it was a blessing in disguise for the 'patient'. If the phenotype had been recognized, the ophthalmologist would have surgically removed the lens in order to make sure that the infant eye received light which is important for the development of the normal brain [Kalia et al. 2014]. Unfortunately surgical management of the cataracts in infants entails a lifelong follow up and impaired vision [Chen et al., 2006; Haargaard et al., 2008; Zetterstrom and Kugelberg 2007]. In many instances the surgery may not be warranted because the cataract does not cloud the visual axis [Amaya et al., 2003] as seems to be the case with this mutation (p.Arg116His). Currently, there is no established catalogue of mutations that cloud the visual axis and those that don't. This knowledge, if catalogued could prove extremely useful to the decision-making process for the ophthalmologist whether the lens should be removed from an infant eye. If it was established that p.Arg116His and other similar genetic mutations that produce a cataract but do not impair vision, a large number of infant eye surgeries could be avoided.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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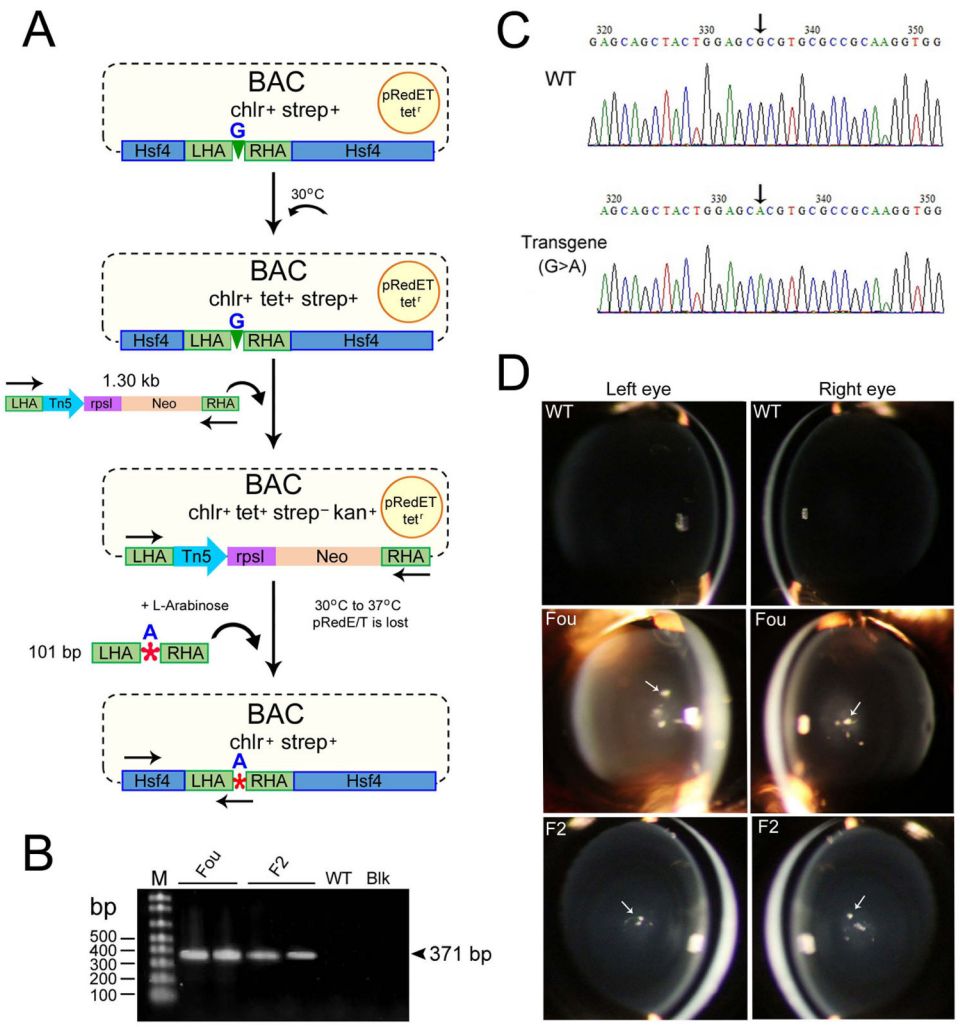

Figure 1.

Recapitulation of the human lamellar cataract phenotype in transgenic mice. **A:** Four recombineering steps are indicated that change the nucleotide G to A in the Hsf4-gene (blue) within the BAC that results in p.Arg116His mutation in the Hsf4 gene product (AF160966.1:c.347G>A) The recombination functions were derived from pRedET plasmid (yellow circled). LHA and RHA (green) are left and right homologous arms surrounding the mutation in exon 3 of the Hsf4 gene. The rpsL-Neo cassette (1.3 kb long) was generated with these homologous arms and introduced into the 220 Kb long BAC containing the Hsf4 gene. Next the LHA and RHA (fragment, 101 bp long, containing the mutation A indicated by red asterisks) was introduced using the Counter-Selection BAC modification Kit (Gen Bridges, Gmbh, Germany) (See Methods and Supp. Fig. S1). **B:** Genotyping was done with stringent PCR. **C:** The PCR products were sequenced to confirm (downward arrows) the change (G>A). **D:** The lamellar cataract phenotypes in the transgenic mouse eyes (arrows) indicate the opacities represented as specks that may represent a cataractous fiber or a group of fiber cells (lamellae) in the transgenic lens. M= marker, Fou = Founders (5 month old), F2 – F2 generation (21 day old), WT = wild type, Blk = reaction blank.

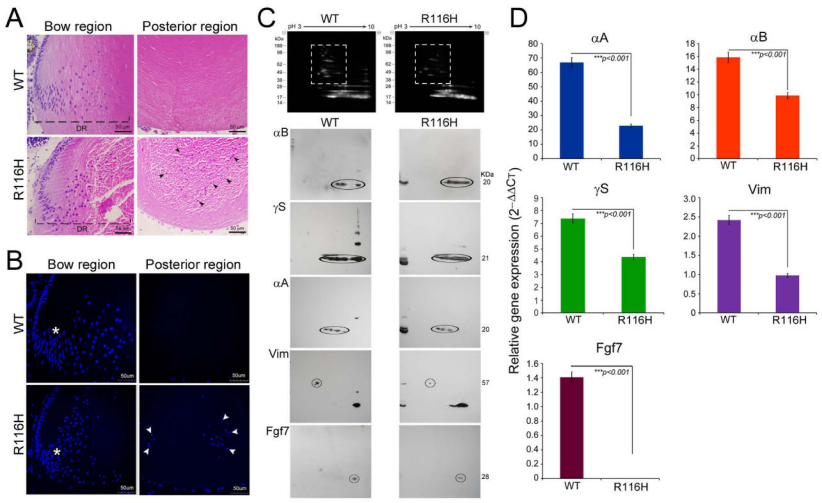

Figure 2.

Histological and biochemical characterization of the Postnatal day 02 p.Arg116His (R116H) transgenic lens. **A:** Bow or the differentiating region (indicated by dotted line DR) and the posterior regions are shown. There are disturbances in the differentiating region such as the shape of the fiber cell nuclei, which seem to be circular in R116H transgenic mice in comparison to the WT. Importantly, we see abnormal location of the fiber cell nuclei in the posterior region in R116H mutant lens (black arrowheads), not seen in the WT. This is seen even more clearly in the DAPI stained sections of this region shown in B**. B:** The nuclei in the bow region are indicated by asterisks, while the nuclei in the posterior region are indicated by white arrowheads. This data is further augmented by the data shown in Supp. Fig. S4. **C:** 2D gel analysis and immunoblotting for various crystallins, vimetin and Fgf7. The top panel shows dey(Flamingo) –stained fluorescent gels. Markers are on the left. Note loss of the higher molecular weight proteins (dotted square) in the R116H transgenic lens. Proteins from only one lens were analyzed (40 μg/gel). Two such gels were made, one was used for staining with Flamingo (shown in the top panel) and the other was immunoblotted and probed with one antibody at a time and stripped (Stripping buffer, Thermo Scientific) and re-probed with the next antibody in the following order (αB, γS, αA, vimentin and Fgf7, reactions circled). While there are discernable changes in expression, we see changes in the distribution αA, αB and γS crystallin (see Supp. Fig. S2). Drastic changes can be seen in vimentin and Fgf7. **D:** Assessment of transcript levels (RT-qPCR) for gene products analyzed in **C.** Appreciable decreases are seen in all of the transcripts employing the $2^{-\Delta\Delta Ct}$ analyses.