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Differential role of arginine mutations on the structure and functions of α -crystallin[☆]

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Abstract

Background— α -Crystallin is a major protein of the eye lens in vertebrates. It is composed of two subunits, α A- and α B-crystallin. α -Crystallin is an oligomeric protein having these two subunits in 3:1 ratio. It belongs to small heat shock protein family and exhibits molecular chaperone function, which plays an important role in maintaining the lens transparency. Apart from chaperone function, both subunits also exhibit anti-apoptotic property. Comparison of their primary sequences reveals that α A- and α B-crystallin possess 13 and 14 arginine residues, respectively. Several of them undergo mutations which eventually lead to various eye diseases such as congenital cataract, juvenile cataract, and retinal degeneration. Interestingly, many arginine residues of these subunits are modified during glycation and even some are truncated during aging. All these facts indicate the importance of arginine residues in α -crystallin.

Scope of review—In this review, we will emphasize the recent *in vitro* and *in vivo* findings related to congenital cataract causing arginine mutations in α -crystallin.

Major conclusions—Congenital cataract causing arginine mutations alters the structure and decreases the chaperone function of α -crystallin. These mutations also affect the lens morphology and phenotypes. Interestingly, non-natural arginine mutations (generated for mimicking the glycation and truncation environment) improve the chaperone function of α -crystallin which may play an important role in maintaining the eye lens transparency during aging.

General significance—The neutralization of positive charge on the guanidino group of arginine residues is not always detrimental to the functionality of α -crystallin.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Keywords

Congenital cataract; α -Crystallin; Arginine mutations; Chaperone; Post-translational modifications

1. Introduction

The eye is a remarkable sensory organ which imparts vision to perceive the world around us. Vision impairment takes place when there is a loss in the transparency of the vertebrate eye lens. The human lens is a transparent tissue in the eye with a refractive index of 1.40 [1]. The loss in transparency and the development of opacity of the ocular lens is termed as cataract. It is mainly classified into four categories on the basis of age. First is the congenital or infantile cataract which takes place in the first year of life; followed by juvenile cataract which happens within the first decade of life. The third category is the pre-senile cataract which happens before 45 years and the last is the senile cataract which occurs beyond 45 years [2]. As per World Health Organisation (WHO) report of 2012, cataract is a global health burden. According to this report nearly 39 million people around the world are blind among which ~18 million are affected by cataract. Therefore reasons behind cataract formation need to be understood properly.

Lens development is a vital event in the organogenesis of the eye [3]. The ocular lens is a transparent avascular tissue which is formed by the proliferation and differentiation of the lens epithelial cells. Lens development is a complex process and this development is broadly categorized into four phases [4] (Fig. 1). The initial phase of development leads to the association of head ectoderm with the optic vesicles. Usually, primary and secondary lens fiber cells are generated in phases 2 and 3, respectively. In the final phase, these fiber cells continue to accumulate over the old fiber cells. In this phase, when the fiber cells begin to elongate, a massive increase in the concentration of crystallin proteins takes place [5–7]. These crystallin proteins provide the structural integrity to the lens fiber cells and they continue to do so for the rest of the life span of the organism. Crystallin proteins as well as secondary fiber cells persist for the entire life span without significant turnover in the lens [8].

The term “Crystallin” denotes to a set of soluble proteins which are mainly responsible for the refractive properties of eye lens. Lens consists of three major crystallin proteins (α -, β - and γ -crystallins) and the concentration of these three crystallin proteins together is 400 mg/ml [9]. Among them, α -crystallin is the major one. It exists as a highly heterogeneous aggregate with an average molecular weight of 650–800 kDa [10]. It has two subunits (α A- and α B-crystallin) and these two subunits generally associate in 3:1 ratio to form a large oligomeric protein (~40 mer) [11]. α A-crystallin comprises of 173 amino acid residues and α B-crystallin consists of 175 amino acid residues. They share ~60% sequence similarity (Fig. 2) and belong to small heat shock protein (sHSP) family. Both of them are major β -sheet protein and possess highly conserved “ α -crystallin domain” (~80–100 amino acid residues). This domain is flanked by an N-terminal domain and preceded by a C-terminal tail [10]. Besides lens, these two subunits are also found in other non-lenticular tissues like retina [12]. In retina, they are expressed mostly in the ganglion cell, inner synaptic layers,

and in photoreceptors [13–15]. α A- and α B-crystallin knockout studies revealed that they play a critical role in lens development [16–18].

Besides, their contribution to the lens development, both α A- and α B-crystallin exhibit molecular chaperone function. In 1992, it was Horwitz who for the first time demonstrated that α -crystallin exhibits molecular chaperone function [19]. Since then, several studies revealed that α -crystallin can prevent the thermally and chemically induced aggregation of different client proteins including β - and γ -crystallins [20–22]. More importantly, α -crystallin drew more attention when it was found that it can prevent aggregation of client proteins including γ -crystallin against UV-irradiation [23,24]. It can also assist the refolding of different denatured enzymes in an ATP-independent manner [25]. All these findings are more relevant to the *in vivo* chaperone function of α -crystallin, where lens proteins are often exposed to several external insults including UV-irradiation, thermal and chemical stresses which can induce protein aggregation and thereby cause cataract. It is believed that the molecular chaperone function of α -crystallin is indispensable for eye lens transparency and protects retina and retinal pigment epithelium (RPE) from stress induced degeneration [15,26].

Several point mutations in α -crystallin are found in various eye diseases such as cataract, and retinal degeneration [27,28]. For example, G98R in α A-crystallin gives rise to pre-senile cataract in an Indian family [27]. Similarly an autosomal recessive congenital cataract mutation in α A-crystallin is found at the 9th position where the tryptophan residue gets converted into a termination codon (W9X) [28]. Point mutations in α B-crystallin gene are also reported in the literature [29,30]. A point mutation in α B-crystallin (P20S) causes autosomal dominant congenital cataract [29]. Mutation in the α B-crystallin gene is also associated with retinal degeneration [30]. Interestingly, when we carefully looked into the different mutations associated with the four categories of cataract, we observed that the frequency of arginine mutations both in α A- and α B-crystallin is more in congenital cataract. Apart from these mutations, the modification of several arginine residues also takes place in this long lived α -crystallin protein due to glycation which is an important post-translational modification in the eye lens [31]. Even, truncation of some arginine residues in α -crystallin takes place during aging [32]. All these facts suggest that arginine residue may be playing a crucial role in the structure, stability, and molecular chaperone function of α -crystallin. The purpose of this review is to emphasize the recent findings on congenital cataract causing arginine mutations both in α A- and α B-crystallin in various families and how such mutations affect their structure and function and promote their aggregation inside eye lens during cataractogenesis. Another purpose of this review is to highlight the impact of several non-natural arginine mutations (created to imitate glycation and truncation atmosphere) on the structure and chaperone function of α -crystallin and its possible implications on eye lens transparency.

2. Arginine mutations and congenital cataract

The total number of arginine in human α A-crystallin is 13 and that in human α B-crystallin is 14 (Fig. 2). Some of these arginine residues are hot spots for mutation which leads to congenital cataract (Table 1). In this section, we discuss a number of congenital cataract

causing arginine mutations both in α A- and α B-crystallin in various families and the effect of these mutations in generating different phenotypes.

2.1. Arginine mutations in α A-crystallin in different families

The N-terminal domain of α A-crystallin mainly comprises of six congenital cataract mutations (R12C, R21W, R21L, R21Q, R49C and R54C) which span over different families from different parts of the world (Table 1). Among them, the occurrence of R12C mutation has been found to be most across the globe (Table 1). This mutation was observed in several Danish families that had a posterior polar cataract with the involvement of the anterior and the posterior poles as per the reports of Hansen et al. [34]. This inherited cataract was also associated with microcornea as observed in several Chinese families [36-37]. Reports revealed that R12C mutation in Chinese families was connected with axial elongation in the lenses which was accompanied with myopia [42]. This mutation was also found in several Brazilian families that were associated with ADCC [35]. Apart from R12C mutation, several Danish families were reported with R21W mutation that had a central and laminar cataract with variable opacities of the anterior and the posterior poles [34]. Khan et al. reported an autosomal recessive cataract mutation at R54C in the families in Saudi Arabia, where the family members were homozygous and had total congenital cataract with abnormalities of microcornea as well as bilateral punctuate cataract [41]. All these three mutations namely, R12C, R21W, and R54C, were also observed in several Indian families that involved pediatric ADCC with microcornea [33]. In addition to these mutations, three other mutations in the N-terminal domain of α A-crystallin are so far reported namely, R21L, R21Q, and R49C, in several German, South Australian families, and Caucasian family, respectively (Table 1) [38-40]. While R21L mutation led to congenital cataract which was also associated with macular hypoplasia [38], R21Q mutation resulted in ADCC with phenotype ranging from very mild lamellar opacity to dense nuclear cataract [39].

The next amino acid stretch following the N-terminal domain is the “ α -crystallin domain” (ACD) which consists of the amino acid residues from 64 to 144 (Fig. 2). This domain contains seven arginine residues out of which the arginine residues at 116th and 117th positions are involved in the mutation related to congenital cataract. Litt et al. by performing a linkage analysis on a family of the Western countries found a missense mutation which substituted the arginine at 116th position with cysteine (R116C). This mutation segregated in the disease phenotype in the family and caused ADCC [43]. This mutation was also found in Indian, French, and Chinese families [44-46]. All the three families were associated with ADCC but the complications related to the congenital cataract with each of the families were different. The Indian families were associated with microcornea along with a unique fan shaped cataract [46]. The R116C mutation in the French family resulted in nuclear cataract and iris coloboma [44]. While the phenotype in the Chinese families associated with this mutation resulted in nuclear cataract with some members showing nystagmus or small blepharophimosis [45]. Apart from the R116C mutation, the conversion of arginine to histidine at 116th position (R116H) in α A-crystallin gene also resulted in ADCC. The R116H mutation in α A-crystallin was mainly observed in three families, *i.e.* Danish, Chilean, and Chinese families [34, 47-48]. All the families were linked with ADCC with diverse complications associated with congenital cataract. Both the Danish and the Chilean

families were associated with microcornea. In addition to microcornea, the cataract in Chilean families was even more complicated with corneal opacity [47]. In the Chinese families, this mutation resulted in autosomal congenital anterior polar cataract [48]. Another mutation at arginine 117, adjacent to R116 was also observed. Sun et al. while analyzing twenty five Chinese families with ADCC found a novel substitution of arginine at 117th position to histidine (R117H), along with the deletion of the adjacent tyrosine [42]. Thus the ACD of α A-crystallin majorly consists of three arginine mutations (R116C, R116H and R117H) which are predominantly associated with congenital cataract.

The C-terminal domain of α A-crystallin contains two arginine residues. However, no report with congenital cataract mutation is known which involves these two residues. The arginine mutation causing congenital cataract mainly takes place in the N-terminal domain of α A-crystallin. It is quite well known that the N-terminal domain (NTD) of the small heat shock proteins is involved in the binding of different aggregation prone client proteins [49-50]. Therefore, these arginine mutations in the N-terminal domain possibly may play a role in the structure and chaperone function of α -crystallin.

2.2. Arginine mutations in α B-crystallin in different families

Several arginine residues in α B-crystallin are also associated with congenital cataract. The N-terminal domain of α B-crystallin contains five arginine residues and two of them undergo mutation which leads to congenital cataract formation (Fig. 2). Chen et al. found a missense mutation at arginine 11 in α B-crystallin gene associated with autosomal dominant nuclear cataract in a Chinese family [51]. The arginine 11 was converted to histidine (R11H) and the cataract opacities were observed in the nuclear as well as in the embryonal and fetal areas [51].

The ACD of α B-crystallin comprises of two congenital cataract causing arginine mutations. The first arginine mutation occurs at position 69. Previously the group that identified an autosomal dominant congenital cataract R117H in α A-crystallin, also obtained a novel mutation in the Chinese families which converted the arginine 69 to cysteine (R69C) in α B-crystallin [42]. The second arginine mutation in ACD was reported by Vicart et al. at arginine 120. This is a missense mutation in the gene where arginine 120 was replaced with glycine (R120G) [52]. Along with cataract, this mutation was also associated with numerous other diseases [52]. The C-terminal domain of α B-crystallin consisting of three arginine residues is not involved in any congenital cataract mutation. Thus three arginine mutations (R11H, R69C and R120G) were found in α B-crystallin gene, which were associated with congenital cataract. Furthermore a plethora of *in vitro* and *in vivo* studies on some congenital cataract causing arginine mutation has been carried out in order to understand the mechanism of the cataract formation and the role of the chaperone function of α -crystallin in preventing the formation of cataract.

3. *In vitro* studies associated with arginine mutations in human α -crystallin

The arginine mutations in α -crystallin which are associated with congenital cataract are mostly found in the N-terminal domain and in the “ α -crystallin domain” (ACD) of α -crystallin (Fig. 2, Table 1). Among them, only the effect of few arginine mutations on the structure and chaperone function of α -crystallin has been explored *in vitro*. Recent studies have revealed that such mutations trigger structural perturbations and decreased functionality of α A- and α B-crystallin. This causes increased aggregation of other crystallin proteins, thereby resulting in lens opacification [53-54].

Among the several arginine mutations as stated in Table 1, the most extensively studied mutation is R116C which lies in the ACD of α A-crystallin. As reported by Litt et al. R116C mutation in α A-crystallin [43] resulted in an appreciable increase in its oligomeric mass, perturbation in its tertiary structure, and significant reduction in β -sheet content of the protein [54-55]. Altered structure of α A-crystallin due to this point mutation reduced the availability of hydrophobic patches at the protein surface [54]. The decreased surface hydrophobicity and altered conformations perhaps reduced the chaperone function of α A-crystallin [54]. Arginine residues are known to be involved in the formation of salt bridges and hydrogen bonds [56]. R116 possibly helps in maintaining the proper structural integrity of α A-crystallin by forming salt bridges through the positive charge of its guanidino group [55-57]. Mutation of this arginine residue to cysteine (R116C), could disrupt the formation of such salt bridges which may be another possible cause for the enhanced oligomeric mass of α A-crystallin [55-57]. Abraham and coworkers [57] replaced R116 of α A-crystallin with lysine (R116K), glycine (R116G), cysteine (R116C), and aspartate (R116D). The mutants, R116C and R116G, showed similar alterations in structure, oligomerization, and chaperone function of α A-crystallin. This implied that the presence of cysteine at 116th position of α A-crystallin is not the sole cause for the structural and functional alterations of this protein. While replacement of R116 with a positively charged amino acid, lysine (R116K), preserved the structure and function, substitution of the R116 with a negatively charged amino acid, aspartate (R116D), caused a drastic alteration in its structure and decreased chaperone function of α A-crystallin. All these observations suggest that the presence of positive charge at 116th position of α A-crystallin is important for its structure and chaperone function [58].

Moreover, decreased chaperone function may alter the binding affinity of α A-crystallin towards aggregation prone client proteins which is a potential factor for the development of autosomal dominant congenital cataract. Additionally, individuals suffering from this disease and possessing R116C mutation are heterozygous in nature *i.e.* the coexistence of α A-R116C and α A-wild-type (α A-wt) along with α B-crystallin in the lens [43]. Therefore, study of the heterozygous system is more relevant to understand the cataract formation. Interestingly the reconstituted α -crystallin containing α A-R116C and α B-crystallin exhibited higher oligomeric assembly, altered tryptophan microenvironment, decreased surface hydrophobicity and lower chaperone activity than that of α -crystallin containing α A-wt and α B-crystallin [59]. Arginine 116 is located at the subunit interface and is most likely to take part in subunit-subunit interactions. Subunit exchange study revealed that α A-R116C

forms heteroaggregates with α B-crystallin faster than wild-type α A-crystallin [59]. This observation suggests that removal of this arginine residue (R116) reduces the net positive charge leading to stronger electrostatic interaction between the heteroaggregates, and hence faster subunit exchange. The lowered subunit exchange rate of R116C α A-crystallin homoaggregates compared to wild-type α A-crystallin further supports this proposition [60]. Petrash and coworker also showed that R116C mutation enhanced the membrane binding capacity of α A-crystallin [60]. It is interesting to note that the membrane association of α -crystallin was found to increase with aging and cataract formation [61]. Therefore from all these structure–function studies, it can be concluded that R116C mutation perturbed the structure, enhanced the oligomeric mass, and lowered the chaperone function of α A-crystallin. Even this point mutation increased the formation of heteroaggregates and its high binding affinity to membrane. Altogether, these factors may cause the insolubility and development of congenital cataract.

Another congenital cataract mutation at 116th position of α A-crystallin is the conversion of arginine to histidine (α A-R116H) [34]. This point mutation perturbed the secondary and tertiary structures of α A-crystallin. α A-R116H exhibits anti-chaperone activity which may be due to the enhanced surface hydrophobicity shown by the mutant protein [53]. Equilibrium GdnHCl unfolding experiments clearly revealed that this mutation stabilized the unfolding prone intermediate possessing larger surface hydrophobicity [53]. This additional exposure of hydrophobic sites led the mutant protein to interact with each other and form nonnative large oligomers or aggregates. It was also observed that, at low concentrations of GdnHCl *i.e.* under mild or moderate chemical stress, the self-aggregation propensity of R116H α A-crystallin was enhanced greatly. Altogether, aggregation prone property of R116H α A-crystallin (either self-aggregation and/or co-aggregation with aggregation prone client protein) may overload the appearance of aggregates and contribute to the onset of the congenital cataract. A proposed model which describes the effect of R116H mutation on the structure and chaperone function of α A-crystallin is shown in Fig. 3 [53].

Several other α A-crystallin mutants known to cause congenital cataract in human are as follows: R12C [34], R21W [33], R21L [38], R49C [40], and R54C [62]. All of these mutations are found to occur in the N-terminal domain of α A-crystallin (Fig. 2). Chaperone activity assay revealed that among all these mutations, R12C and R21W mutations caused decrease in chaperone function of α A-crystallin (Fig. 4) [63]. This was accompanied by an increase in the α -helical content, perturbation in tertiary structure, and enhancement in oligomeric size of α A-crystallin.

Secondary structural elements of the mutants were found to be associated with increased α -helical content with R21W having the maximum α -helical content, followed by R21L, R12C, R54C, and R49C [63]. Near UV-CD spectra of mutant proteins revealed that all these mutations perturbed the tertiary structure of α A-crystallin. Analysis of quaternary structure of the mutants revealed that R21L and R49C had intact oligomeric assembly as that of wild-type protein, while R12C, R21W, and R54C mutations moderately increased the oligomeric size of α A-crystallin. Assessment of the available surface hydrophobic patches of these mutant proteins showed a drastic increase in the surface hydrophobicity for R21W and R21L, while other mutants had lowered surface hydrophobicity as compared to the wild-

type protein [63]. Another independent study revealed that the subunit exchange rates of these α A-crystallin mutants, R21W and R116C, with wild-type α B-crystallin decreased considerably as compared to when wild-type α A-crystallin interacted with wild-type α B-crystallin [64]. In summary, the drastic decrease in the chaperone function of R12C and R21W may be the consequence of: 1) reduction in subunit exchange rate with α B-crystallin; 2) alterations in the secondary, tertiary, and quaternary structures of the mutant proteins, which may be the critical factors behind the formation of congenital cataract. Subunit exchange studies by Abraham's group also revealed that both R54C and R49C mutations lowered the subunit exchange kinetics between α A- and α B-crystallin [64]. This decrease in subunit exchange rate and altered secondary structure due to these two mutations, may be a possible cause for cataract induction in the lens.

Alternative mechanism by which these two mutations can cause the cataract formation may involve the formation of inter and intra-molecular disulfide linkages due to the presence of cysteine residues at the 49th and 54th positions of α A-crystallin. Mchaourab and coworkers showed that R49C mutation led to the formation of intermolecular disulfide linkage in α A-crystallin *via* two step mechanism [65] (Fig. 5). Covalent protein cross-links including formation of disulfide bonds are characteristic features of age-related cataract [66]. Thus formation of disulfide linkages may be responsible for the onset of congenital cataract.

The consequences of arginine mutation in human α B-crystallin (R120G), which is associated with congenital cataract on the structure and chaperone function of this protein have been extensively studied [52]. Three dimensional structural analysis revealed that arginine at the 120th (R120) position of α B-crystallin forms an important part of the inter-subunit contact of the protein [9,67,68]. In fact, R120 of one monomer forms an ion-pair with D109 of other monomers within the isolated dimer of " α -crystallin domain" of α B-crystallin. Formation of such salt bridges resulted in the formation of a deep groove between β 6 and β 7 strands of neighboring monomers which stabilized the structure of α B-crystallin. However with the evolution of R120G mutation in α B-crystallin, disruption of this ion-pair network has been observed (Fig. 6). In consequence to such a change, H83 and D80, located in the β 3-strands of neighboring monomers have been found to be involved in formation of two alternative salt bridges (Fig. 6) [9]. The β 3-strands of the two neighboring monomers form a lid to the deep groove between β 6 and β 7 strands. Formation of salt bridge between H83 and D80 led to the closure of such lid.

Such significant changes in the structure of α B-crystallin due to R120G mutation have major influence on the conformations of the protein. Circular dichroism measurements, gel filtration chromatography and dynamic light scattering measurements revealed that the secondary and tertiary structures of α B-crystallin due to R120G mutation were perturbed with an increase in oligomeric mass [55,69,70]. The surface hydrophobicity of α B-crystallin increased due to this mutation with a slight decrease in the subunit exchange rate [71]. Cryo EM studies revealed that wild-type α B-crystallin assemblies are spherical in shape with a central cavity, whereas the R120G mutant had an irregular shape with no defined central cavity [69]. Altered conformations of α B-crystallin due to R120G mutation also reduced the overall structural stability of the protein [61]. As a consequence of all the abovementioned structural alterations and stability, the chaperone function of α B-crystallin is compromised

[69]. R120G mutant protein is more polydispersed with decreased thermal stability than wild-type α B-crystallin and prone to aggregation. As a result of these structural instabilities, the mutant variant (R120G) failed to protect the aggregation of unfolded client proteins (Fig. 7) [69]. Moreover, in the presence of R120G α B-crystallin, the kinetics of aggregation process increased and aggravated the aggregation of prone client protein. The R120G mutation led α B-crystallin to evolve as a defective chaperone and co-aggregate with other client proteins in chaperone assays. This mutation significantly altered the interaction of α B-crystallin with intermediate filaments. The mutant protein has been found to possess higher affinity for intermediate filaments in comparison to the wild-type protein, thereby reducing the ability of α B-crystallin to prevent those filament–filament interactions required to induce gel formation [61]. Thus, this mutation induces increased protein–protein interaction between R120G α B-crystallin and intermediate filaments which triggers the aggregation of intermediate filaments and thus the formation of congenital cataract [61,72]. *In vitro* experiments revealed that replacement of arginine with lysine, did not disturb the protein structure and function, whereas replacement of arginine with asparagine, had a major impact on the chaperone activity of the protein [73]. Replacement of arginine with a neutral molecule, *i.e.* glycine, led to decreased structural stability. This revealed that R120 is crucial for the structural and functional integrity of the protein. R120G mutation led to the altered structure and chaperone function of α B-crystallin which is likely to be the molecular basis for the onset of congenital cataract in human lenses. Apart from these *in vitro* studies, a great number of *in vivo* studies are also carried out, some of which are discussed in the subsequent section.

4. *In vivo* studies associated with arginine mutations in α -crystallin

To understand the mechanism of congenital cataract formation, several *in vivo* studies are carried out with many of these congenital cataract causing arginine mutants of α -crystallin. Different cell-lines and knock-in mouse model have been used for this purpose. To understand the pathogenesis of cataract, Abraham and coworkers transfected seven congenital cataract causing mutants (R12C, R21L, R21W, R49C, R54C, R116C and R116H) in HeLa cells and found that mutant α A-crystallins formed aggregates and aggresomes [74]. Aggregation of these mutants increased significantly when they were coexpressed with wild-type α A-crystallin. *In vivo* study further provided an indication of the involvement of these mutants (especially R21W, R116C and R116H) in ubiquitination pathway which may be one of the possible causes for the formation of congenital cataract in humans [74].

Apart from the formation of aggresomes, the experiments executed in knock-in mouse model by Andley's group revealed that R49C mutation in α A-crystallin aggravates the protein insolubility and cell death *in vivo* [75]. Extensive *in vivo* studies were done with R49C mutant of α A-crystallin to understand the molecular basis behind the congenital cataract formation. Using different knock-in mouse models it was shown that both heterozygous and homozygous mutant mice exhibited abnormalities in lens [76-77]. The extent of lens abnormality in heterozygous mutant mice is small and accompanied with several other detrimental factors such as decreased protein solubility and irregular interactions with β -crystallin [76]. In contrast, homozygous mutant mice experienced severe damage in lens including abnormal protein folding, failure in proper development of

equatorial bow region and damage in fiber cells [77, 78]. In both cases, the formation of nuclear opacity was observed, but in homozygous mutant mice the opacity was found to develop at an early age compared to heterozygous mutant mice [77,78]. All these adverse effects induced by R49C mutation in α A-crystallin may collectively cause congenital cataract.

Several *in vivo* studies were also performed with another congenital cataract causing mutant of α A-crystallin *i.e.* R116C- α A-crystallin. In earlier section, we already discussed that this mutation increased the oligomeric size and decreased the chaperone function of α A-crystallin [55]. *In vivo* studies corroborate these *in vitro* findings. Andley and coworkers expressed this mutant in human lens epithelial cell line (HLE B-3) and observed growth of α A-crystallin aggregates. Compared to the wild-type α A-crystallin, this mutant had less protective ability against UV-A induced apoptosis of lens epithelial cells [79]. All these facts further reinforce that chaperone function of α A-crystallin is critical for the eye lens transparency. In order to understand the effect of this mutation on the lens morphology, a series of transgenic mouse was raised [80]. The expression of the mutant protein resulted in posterior cortical cataract which was also associated with posterior suture. The presence of this mutant protein (even in very low amount) was sufficient enough to induce lens opacity [80]. In another independent study, Fu et al. investigated the effect of this mutation on the protein–protein interaction between the α A-R116C mutant and other crystallins using HeLa cell line [81]. They found that this mutation decreased the interaction between α A-crystallin and other lens crystallin proteins such as β B2 and γ C-crystallins. This decreased protein–protein interaction may induce protein insolubility and cataract formation [81].

The same study also indicated that the pattern of protein–protein interaction for the mutant R120G α B-crystallin is different from that of R116C α A-crystallin [81]. This difference may be attributed to the differential conformation of these two mutant proteins as evidenced by CD experiments [81]. Using cell culture experiments and knock-in mouse model, different groups demonstrated that co-aggregation of R120G α B-crystallin with intermediate filament proteins may be the molecular mechanism behind the development of congenital cataract in R120G α B-crystallin [72,76,82,83]. Simon et al. also explored the cellular fate of HeLa cells expressing wild-type and R120G α B-crystallin [83]. This mutation altered the cell morphology grossly and lowered the protective ability of α B-crystallin against the oxidative stress induced cell death. These findings again reflect the significance of chaperone function of α B-crystallin in context of lens clarity. In addition to these studies, knock-in mouse model of this mutant protein (R120G α B-crystallin) demonstrated that the abundance of β - and γ -crystallins decreased significantly in mutant mice lenses [76]. They also found faster degradation of α -crystallins in these lenses. Therefore, it can be concluded that alterations in the abundance of the β - and γ -crystallins and degradation of the α -crystallins may play an important role in the development of the lens opacity [76]. Unfortunately, *in vivo* studies with several congenital cataract causing arginine mutants of both α A-(R21Q and R117H) and α B-crystallin (R11H and R69C) are still absent in the literature. We strongly feel that *in vivo* studies with these mutants will provide additional insights into the molecular mechanism of congenital cataract formation.

5. Role of some non-natural arginine mutations in α -crystallin on its chaperone function

Apart from these arginine mutations, several arginine residues are usually modified during different post-translational modifications such as, glycation and truncation. Glycation is a non-enzymatic reaction where carbohydrates react with amino groups of proteins. Numerous sugars, lipid peroxidation products, and sugar metabolites can initiate this reaction, which then produces a variety of adducts with proteins that are collectively known as, advanced glycation end products (AGEs) [12]. Formation of AGEs in large amounts has been evidenced in aged lenses [84–86]. Several investigations revealed that α -dicarbonyl compounds, such as 3-deoxyglucosone, methylglyoxal (MGO), and glyoxal are the major sources for AGEs formation in tissues including lens [87]. Human lenses are found to have higher concentrations of MGO in comparison to plasma [87]. MGO reacts with lysine, arginine, and histidine residues in proteins to produce stable AGEs such as, lysine modified adducts [*e.g.* methylglyoxal lysine dimer (MOLD), a lysine–lysine cross-linking structure, N^{ϵ} -(carboxyethyl)lysine [88–90] and arginine modified adducts [*e.g.* hydroimidazolone and argpyrimidine] [31–91]. Since, the kinetics of the reaction between lens proteins and MGO is very fast, it can be presumed that MGO modification may accumulate in lens which may be detrimental for eye lens transparency. But one interesting report came out in 2003 which showed that the chaperone function of α A-crystallin increased upon a mild modification with MGO [92]. Mass spectroscopic analysis of MGO modified α A-crystallin revealed that three arginine residues, R21, R49, and R103 were modified to argpyrimidine [92]. To find out whether neutralization of positive charge on the guanidino group of these arginine residues by argpyrimidine modification, is the molecular basis behind the improved chaperone function of α A-crystallin, a series of single and double mutants and one triple mutant of α A-crystallin was raised, in which MGO-modifiable arginine residues (R21, R49, and R103) were replaced with neutral alanine [93]. Conformational studies revealed that the mutant proteins exhibited significantly altered tertiary structure. Surface hydrophobicity experiments showed that while R21A and R103A mutant had enhanced surface hydrophobicity, R49A exhibited lower surface hydrophobicity compared to that of wild-type α A-crystallin [93]. Altered tertiary structure and availability of hydrophobic patches at the surface of the mutant proteins was accompanied by distinct alteration in the chaperone activity of mutant proteins as compared to the wild-type protein. R21A and R103A exhibited enhanced chaperone function, while R49A lost most of its chaperone function as compared to the wild-type α A-crystallin. Indeed, the double mutant, R21A/R103A and the triple mutant, R21A/R49A/R103A had better chaperone function than the wild-type α A-crystallin [93]. So as to find out the efficacy of these mutants *in vivo*, apoptotic studies were performed with R21A and R49A mutant of α A-crystallin. α A-crystallin is a well known anti-apoptotic protein. The R21A mutant showed enhanced anti-apoptotic activity as compared to the wild-type protein, which coincides well with its increase chaperone function. On the contrary, the R49A mutant which had less chaperone activity than the wild-type protein, exhibited less anti-apoptotic function than α A-crystallin [94]. Therefore, from these studies it can be concluded that chaperone activity of α A-crystallin is directly related to its anti-apoptotic functions. In another separate study, mild MGO modification of R12 residue to hydroimidazolone in α A-crystallin enhanced its chaperone function. So as to find

out whether neutralization of the positive charge of arginine residue is the basis for such an enhancement, R12A mutant of α A-crystallin was raised to mimic the hydroimidazolone modification [95]. The R12A mutation perturbed the tertiary structure of α A-crystallin which was associated with an increase in thermodynamic stability of the protein. Equilibrium binding studies revealed that R12A mutation brought about additional exposure of client protein binding sites on α A-crystallin. All these alterations due to R12A mutation improved the chaperone function of α A-crystallin [95]. In summary, the positive charge neutralizations on R12, R21, and R103 are important determinants for the chaperone function of α A-crystallin and may play a crucial role in reducing protein aggregation during lens aging, thereby preventing cataract formation.

Truncation of amino acid residues from the C-terminal region of α A-crystallin is another major post-translation modification which α A-crystallin experiences during aging. Abraham and co-workers found several C-terminal truncated α A-crystallin in rat lens [32]. Those are α A₁₋₁₆₈, α A₁₋₁₆₃, α A₁₋₁₆₂, α A₁₋₁₅₇, and α A₁₋₁₅₁. These truncated α A-crystallin proteins accumulated more in cataractous lenses than normal clear lenses [32]. Both the arginine residues in the C-terminal region (R157 and R163) are cleaved from α A-crystallin during aging which might indicate an important role of these arginine residues on its structure and chaperone function. *In vitro* structure–function studies related to these truncated proteins also support this possibility. Deletion of 10 and 11 amino acid residues from C-terminal domain forming α A₁₋₁₆₃ and α A₁₋₁₆₂ respectively resulted in an oligomeric size of 520 kDa and 150 kDa respectively for α A-crystallin in comparison to 600 kDa for the uncleaved wild-type protein. Significant alterations in the tertiary structure and decrease in chaperone function of α A₁₋₁₆₂ mutant were observed, while that of α A₁₋₁₆₃ remained almost similar to that of wild-type protein. In summary, deletion of 10 amino acid residues from the C-terminal end of α A-crystallin had negligible influence on its structure and chaperone function, whereas deletion of one more amino acid residue (R163) from C-terminal end of α A-crystallin significantly altered its structure and chaperone function [96]. Therefore, this study particularly demonstrated that R163 may be critical for the oligomerization and chaperone function of α A-crystallin. Mutation of R163 to glycine (R163G) did not alter the oligomeric size in the full-length α A-crystallin [97]. However, R163G mutants of the truncated proteins lacking E164, E165, and K166 residues exhibited a major decrease in its oligomeric size. Additionally, R163G mutants of other truncated α A-crystallin proteins which were devoid of P167, S167, and S168, exhibited a mild alteration in its oligomeric size. Therefore, the above findings revealed that R163 itself as well as its neighboring residues play a critical role in influencing the structure and chaperone function of α A-crystallin [97]. For full length α A(1–173) crystallin, R163G mutation did not alter the oligomeric size and chaperone function of full length wild-type α A-crystallin. However, the same mutation significantly reduced the oligomeric size and chaperone function of the truncated α A(1–163) [97]. These observations suggest that apart from the positive charge, the neighboring residues of R163 also play an important role in inter-subunit electrostatic interactions in the C-terminal domain of α A-crystallin.

Another arginine residue at the 56th position of α B-crystallin is associated with autosomal recessive juvenile cataract. This mutation replaces the highly conserved arginine 56 to tryptophan (R56W) [98]. This is the first mutation in α B-crystallin gene with recessive

causing cataract mutation. This mutation of α B-crystallin was also found in retina, which gave rise to a significant rod-cone degeneration in homozygous individuals with aphakia since childhood [30]. All these studies indicated that the presence of arginine residue at the 56th position in α B-crystallin may be critical for its structure and chaperone function. Several indirect and direct *in vitro* studies revealed the importance of this arginine residue on the chaperone function of α B-crystallin. Sharma and coworkers demonstrated that deletion of ⁵⁴FLRAPSWF⁶¹ (harboring R56) from the N-terminal domain of human α B-crystallin, improves its chaperone function [99]. This particular sequence is thought to participate in substrate interaction and subunit exchange with α A-crystallin. Nagaraj and coworkers did an extensive study to understand whether the positive charge of the R56 residue influences these functions of α B-crystallin [100]. For this study, they raised several mutants of this crystallin protein in which R56 was deleted (R56 Δ) or replaced by alanine (R56A). Although the oligomeric size of α B-crystallin remained unaltered due to these mutations, secondary and tertiary structures of the protein were perturbed. This was associated with increased surface hydrophobicity and chaperone function in R56 mutant, while the same in R56A mutant was found to decrease. The deleted mutant (R56 Δ) showed an enhanced exposure of surface hydrophobic patches while the reverse was observed for substituted mutant (R56A). Altogether, these changes led to the substantial increase in the chaperone function of α B-crystallin due to R56 mutation. In contrast, R56A mutant lost most of its chaperone function [100]. Therefore, it can be concluded from these results that R56 may be important for the structure and chaperone function of α B-crystallin. But the effect of the juvenile cataract causing arginine mutation (R56W) on the structure and chaperone function of α B-crystallin is still to be investigated. These investigations will further unfold the molecular basis behind the formation of autosomal recessive juvenile cataract.

From the preceding sections, it can be evidenced from both *in vitro* and *in vivo* studies that congenital cataract causing arginine mutations as well as non-natural arginine mutations have immense effect on the conformation, chaperone function, solubility, and anti-apoptotic properties of α A- and α B-crystallin. Different effects caused by these mutations are summarized in Tables 2 and 3, respectively.

6. Concluding remarks

This review strongly indicates that arginine residue, especially the positive charge on its guanidino group is key for the structure and function of α -crystallin. Neutralization of this positive charge by mutation perturbs the structure of this crystallin protein grossly and as a result of which its chaperone function is impaired. Due to this impairment in the chaperone function, α -crystallin possibly fails to hold aggregation prone client proteins in the eye lens and thus different types of cataract are formed. This review also reflects that the statement. "Removal of positive charge from the arginine residue is always detrimental for α -crystallin and eye lens" is not always true [92-93]. In fact, modifications of several arginine residues where its positive charge are being neutralized, improves the chaperone and anti-apoptotic properties of α -crystallin. These improved chaperone and anti-apoptotic properties may play a role in protein aggregation during lens aging and cataract formation. Overall, this review explicitly indicates that the chaperone function α -crystallin is not only governed by the

positive charge of arginine residues, but is also dependent on the conformational changes which this residue imparts to the protein.

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Fig. 1.

General phases of lens development. Phase-1. The vertebrate lens initially develops a surface ectoderm which is exposed to multiple inducing factors during the gastrulation stage.

Association of the presumptive lens ectoderm (PLE) with the optic vesicle (OV) leads to the formation of lens placode. Phase-2. Invagination of the lens placode forms the lens pit and consequently forming the lens vesicle. Cells in the anterior of the lens vesicle lead to the formation of lens epithelium while those in the posterior give rise to primary lens fiber cells.

Phase-3. Differentiation of the lens epithelial cells near the equator gives rise to the secondary fiber cells. Phase-4. The lens then continues the addition of the lens fiber cells

over the old fiber cells. It acquires the correct mechanical stiffness required for focussing light. These secondary fiber cells persist for the whole life span without any turnover in the lens cells. (Reproduced with permission from The International Journal of Biochemistry & Cell Biology 40 (2008) 317–323).

Fig. 2.

Sequence alignment of α A- and α B-crystallin. Sequences of α A- and α B-crystallin were aligned using the program ClustalW. The N-terminal domain (NTD) is shown in turquoise, the “ α -crystallin domain” (ACD) is shown in light gray and the C-terminal domain (CTD) is shown in yellow. The symbols (*, : and .) represent identical residue, conserved substitution and semi-conserved substitution respectively. The arginine residues which are involved in juvenile and congenital cataract are marked in green and red respectively.

Fig. 3.

A proposed model of the effects of the R116H mutation on the structural stability and function of α A-crystallin. [Reprinted from *Biochim Biophys Acta* 1804, 948–956, Pang et al., Effects of congenital cataract mutation R116H on α A-crystallin structure, function and stability. (Copyright 2010), with permission from Elsevier].

Fig. 4.

Chaperone activity assays of human αA -wt and the mutants using ADH as the target protein. The αA /ADH ratios being (A) 1:2 and (B) 1:5 (w/w). [Reprinted from *Mol Cell Biochem* 362, 93–102, Kore et al., Quaternary structural parameters of the congenital cataract causing mutants of alphaA-crystallin. (Copyright 2012), with permission from Springer].

Fig. 5.

Scheme describing the disulfide bond rearrangement in the interaction of αA-R49C and βB1-crystallin. A nucleophilic attack on the disulfide bond linking βB1-crystallin and the bimane leads to the formation of a transient disulfide link between βB1- and αA-R49C. Another disulfide exchange occurs leading to the formation of a covalently linked αA-crystallin dimer. [Reprinted from *FEBS Lett* 583, 175–179, Kumar et al., Disulfide cross-links in the interaction of a cataract-linked αA-crystallin mutant with βB1-crystallin. (Copyright 2009), with permission from Elsevier].

Fig. 6. Structural consequences of missense mutations in the “ α -crystallin domain” of α B-crystallin. [Reprinted from *Int J Biochem Cell Biol* 44, 1687–1697, Clark et al., sHSP in the eye lens: crystallin mutations, cataract and proteostasis. (Copyright 2012), with permission from Elsevier].

Fig. 7.

R120G mutation in α B-crystallin results in its decreased chaperone-like function. (A) Aggregation of α -lactalbumin after the addition of 20 mM DTT at 37 °C and pH 6.7. Curve 1, 0.25 mg of α -lactalbumin; curve 2, 0.25 mg of α -lactalbumin plus 0.25 mg of wild-type α B-crystallin; curve 3, 0.25 mg of α -lactalbumin plus 0.25 mg of R120G α B-crystallin. (B) Aggregation of alcohol dehydrogenase induced by heat (42 °C) and 2 mM EDTA. Curve 1, 0.2 mg of alcohol dehydrogenase; curve 2, 0.2 mg of alcohol dehydrogenase plus 0.2 mg of wild-type α B-crystallin; curve 3, 0.2 mg of alcohol dehydrogenase plus 0.2 mg of R120G α B-crystallin; curve 4, 0.2 mg of alcohol dehydrogenase plus 0.4 mg of R120G α B-crystallin. [Reprinted from *Proc Natl Acad Sci U S A* 96, 6137–6142, Bova et al., Mutation R120G in α B-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. (Copyright 1999), with permission from National Academy of Sciences, USA].

Table 1

Mapped arginine mutations of α -crystallin in congenital cataract.

Protein (gene)	Chromosome (locus)	Nucleotide change	Amino acid change	Inheritance	Families	Cataract phenotype	References
α A-Crystallin (CRYAA)	21q22.3	c34C > T	R12C	AD	Danish, Indian, Chinese, and Brazilian	Posterior polar, microcornea	[33-34-36-37]
		c61C > T	R21W	AD	Danish and Indian	Central and laminar, microcornea	[33-34]
		c62C > G	R21L	AD	German	Bilateral, macular hypoplasia	[38]
		c62G > A	R21Q	AD	South Australian	Lamellar, nuclear	[39]
		c145C > T	R49C	AD	Caucasian	Nuclear	[40]
		c160C > T	R54C	AR, AD	Indian and Saudi Arabian	Bilateral, microcornea, microphthalmus	[33-41]
		c346C > T	R116C	AD	Western	Nuclear, microcornea, fan shape, iris coloboma	[43-46]
		c346G > A c347G > A	R116H	AD	Danish, Chilean, and Chinese	Anterior polar, microcornea	[34-47-48]
		c.350_352delGCT	R117H, Y118Del	AD	Chinese	N/A	[42]
		c32G > A	R11H	AD	Chinese	Nuclear	[51]
α B-Crystallin (CRYAB)	11q22.3	c205C > T	R69C	AD	Chinese	N/A	[42]
		c358A > G	R120G	AD	French	Discrete lens opacities	[52]

Abbreviations: AD: autosomal dominant; AR: autosomal recessive; N/A: not applicable.

Table 2

Summarized results obtained from different *in vitro* and *in vivo* studies associated with congenital cataract causing arginine mutations.

Protein	Amino acid change	Study type		In vivo							References			
		In vitro		CF	SH	OA	TS	SS	SE	Other studies	In vivo		Other studies	References
		CF	SH								Cell lines	Mouse models		
αA-Crystallin	R12C	Decreased	Decreased	Increased	Altered	Altered	Decreased	–	–	Aggresome formation	–	–	[37-63-64-74]	
	R21W	Decreased	Decreased	Increased	Altered	Altered	Decreased	–	–	Aggresome formation	–	Involved in ubiquitination pathway	[63-64-74]	
	R21L	Decreased	Increased	Unaltered	Altered	Altered	Unaltered	–	–	Aggresome formation	–	–	[63-64-74]	
	R21Q	–	–	–	–	–	–	–	–	–	–	–	–	
	R49C	Unaltered	Decreased	Unaltered	Altered	Altered	Decreased	Leads to disulfide linkage	Aggresome formation, failed to prevent apoptosis	Protein insolubility, cell death and lens damage	–	–	[40-63–65-74–78]	
R54C	Unaltered	Decreased	Increased	Altered	Altered	Decreased	–	–	Aggresome formation	–	–	[63-64-74]		
R116C	Decreased	Decreased	Increased	Altered	Altered	Decreased	–	–	Aggresome formation, reduced anti-apoptotic function	Resulted in posterior cortical cataract.	Enhanced membrane binding, capacity	[43-54-55-58–60-63-64-74-79-80]		
R116H	Anti-chaperone	Increased	Increased (non-native)	Slightly altered	Slightly altered	–	Self aggregation	Aggresome formation	–	Involved in ubiquitination pathway	[53-63-74]			
αB-Crystallin	R117H, Y118 Del	–	–	–	–	–	–	–	–	–	–	–	–	
	R11H	–	–	–	–	–	–	–	–	–	–	–	–	
	R69C	–	–	–	–	–	–	–	–	–	–	–	–	
	R120G	Decreased	Increased	Increased	Altered	Altered	Slightly decreased	Degradation of α-crystallin in lenses	Altered cell morphology, lower protective ability against cell death	–	–	[9-55-66–70-73-76-83]		

Abbreviations: chaperone function (CF), surface hydrophobicity (SH), oligomeric assembly (OA), tertiary structure (TS), secondary structure (SS), subunit exchange (SE).

Table 3
Summarized results obtained from different *in vitro* and *in vivo* studies associated with non-natural arginine mutations.

Protein	Amino acid change	Study type	References									
			<i>In vitro</i>					<i>In vivo</i>			Mouse models	
			PTM	CF	SH	OA	TS	SS	SE	Other studies	Cell lines	
αA-Crystallin	R12A	Modified to hydroimidazolone (glycation)	Increased	Unaltered	Unaltered	Slightly increased	Altered	Slightly altered	–	Increased stability and more exposed client binding sites		[95]
	R21A	Modified to argpyrimidine (Glycation)	Increased	Increased	Increased	Slightly decreased	Altered	Unaltered	–	Less polydisperse	Increased anti-apoptotic ability	[92–94]
	R49A	Modified to argpyrimidine (glycation)	Decreased	Decreased	Decreased	Slightly increased	Altered	Unaltered	–	More polydisperse	Decreased anti-apoptotic ability	–
	R103A	Modified to argpyrimidine (Glycation)	Increased	Increased	Increased	Increased	Altered	Unaltered	–	More polydisperse		[92,93]
αB-Crystallin	R56A/ R56	–	Decreased/increased	Decreased/increased	Decreased/increased	Unaltered/unaltered	Altered/altered	Altered/altered	Decreased	–	–	[100]

Abbreviations: chaperone function (CF), surface hydrophobicity (SH), oligomeric assembly (OA), tertiary structure (TS), secondary structure (SS), subunit exchange (SE), posttranslational modification (PTM).