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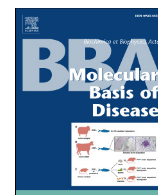

# Increased aquaporin 1 and 5 membrane expression in the lens epithelium of cataract patients

Olatz Barandika BSc [a,1], Maitane Ezquerra-Inchausti BSc [a,1], Ander Anasagasti BPharm [a], Ainara Vallejo-Illarramendi PhD [a], Irantzu Llarena [b], Lucia Bascaran MD [c], Txomin Alberdi MD [c], Giacomo De Benedetti MD [d], Javier Mendicute MD, PhD [c], Javier Ruiz-Ederra PhD [a,*]

[a] *Division of Neuroscience, Biodonostia HRI, San Sebastián, Spain*
[b] *Optical Spectroscopy Platform, CIC biomaGUNE, San Sebastián, Spain*
[c] *Ophthalmology Service, Donostia University Hospital, San Sebastián, Spain*
[d] *Ophthalmology Service, Quirónsalud Donostia Hospital, San Sebastián, Spain*

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ABSTRACT

In this work we have analyzed the expression levels of the main aquaporins (AQPs) expressed in human lens epithelial cells (HLECs) using 112 samples from patients treated with cataract surgery and 36 samples from individuals treated with refractive surgery, with transparent lenses as controls. Aquaporin-1 (AQP1) is the main AQP, representing 64.1% of total AQPs in HLECs, with aquaporin-5 (AQP5) representing 35.9% in controls. A similar proportion of each AQP in cataract was found. Although no differences were found at the mRNA level compared to controls, a significant 1.65-fold increase ($p = 0.001$) in AQP1protein expression was observed in HLECs from cataract patients, with the highest differences being found for nuclear cataracts (2.1-fold increase; $p < 0.001$). A similar trend was found for AQP5 (1.47-fold increase), although the difference was not significant ($p = 0.161$). Moreover we have shown increased membrane AQP5 protein expression in HLECs of patients with cataracts. No association of AQP1 or AQP5 expression levels with age or sex was observed in either group. Our results suggest regulation of AQP1 and AQP5 at the post-translational level and support previous observations on the implication of AQP1 and 5 in maintenance of lens transparency in animal models. Our results likely reflect a compensatory response of the crystalline lens to delay cataract formation by increasing the water removal rate.
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## 1. Introduction

Cataract is a leading cause of blindness, affecting about 18 million people worldwide [1,2]. It is estimated that 1.3 million cataract operations are performed annually in the U.S. In the Spanish National Public Health System, cataract surgery is the most frequent outpatient surgery performed, with over 260,000 cataract operations each year [3]. In order to be able to develop new alternatives to cataract surgery to prevent, or at least delay, cataract, it is necessary to gain a more in-depth understanding of the pathogenic mechanisms involved in this ocular condition.

The lens is an avascular tissue composed of concentric layers of epithelial cells at various stages of differentiation [4,5]. An epithelial cell monolayer extends from the anterior pole of the lens to its equatorial surface, surrounding the elongated lens fibers, which are arranged with the oldest fibers in the lens nucleus. Upon maturation, lens fibers lose their attachment to the capsule, and cellular organelles are degraded in a synchronized manner [6]. Nourishment is provided to the lens by diffusion from the aqueous and vitreous humors. However, it is unlikely that simple diffusion can sustain the metabolic needs of the lens interior [7]. As such, a *circulatory system* in which an asymmetric distribution of ion pumps, transporters, channels and cell junctions drive ion-coupled fluid absorption, thereby facilitating the entry of nutrients and metabolites into the inner lens across the polar regions and exit through the lens equator, has been proposed [7–10]. The lens contains a uniquely high protein concentration and low water content. This tightly packed arrangement of fibers helps maintain an elevated refractive index for transparency, with lens water channels proposed to act by facilitating water removal [11].

The aquaporins (AQPs) are small integral membrane proteins (~30 kDa/monomer) expressed widely in both the animal and plant

*Abbreviations:* AQP, aquaporin; HLECs, human lens epithelial cells.

* Corresponding author at: Department of Neuroscience, Biodonostia HRI, Paseo Dr. Begiristain s/n, E-20014 San Sebastián, Spain.
*E-mail addresses:* javiruizederra@yahoo.es, javier.ruizederra@osakidetza.net (J. Ruiz-Ederra).

[1] Authors contributed equally.

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kingdoms, with 13 members in mammals. AQPs are expressed in epithelia and endothelia, where they are involved in fluid transport, but are also found in other cell types such as skin and fat cells, where they have other functions. In most cell types, the AQPs reside constitutively at the plasma membrane. One exception is kidney AQP2, which undergoes vasopressin-regulated trafficking between endosomes and the cell plasma membrane. Three AQPs are expressed in the lens: AQP0 (major intrinsic protein-MIP) found in the posterior pole and in nuclear fibers; AQP1 at the anterior pole in epithelial cells; and AQP5 recently described in both epithelial and fiber cells. Similar to AQP0, the distribution of AQP5 within lens cells has been reported to change as a function of fiber cell differentiation [12].

Mutations in AQP0 are associated with hereditary cataracts in mice and humans [13,14]. Indeed, cataract-producing AQP0 mutations are thought to produce endoplasmic reticulum-retained and non-functional AQP0 [15,16], although the mechanism linking AQP0 loss-of-function and cataracts remains unclear. Because of its low water permeability, it has been proposed that AQP0 might be involved in regulating the resistance of the paracellular pathway, rather than in cell membrane water permeability [5,17]. Therefore, proposed mechanisms for the implication of mutations in AQP0 in cataract include loss of AQP0-facilitated fiber-fiber adherence [14] and impaired fiber cell dehydration [18].

With respect to AQP1, cataracts were not reported in human subjects with AQP1 deficiency [19], and spontaneous cataracts are not seen grossly in AQP1 null mice [20]. Nevertheless, based on experimental observations of the role of AQP1 in the cornea or in the lens [21,22], we reported a functional implication of lens AQP1 in lens transparency [21], with epithelial cell water permeability being approximately three-fold lower in lenses from AQP1 null mice. Moreover, although AQP1 deletion did not alter baseline lens morphology or transparency, basal water content was significantly higher (by approx. 4%) in AQP1 null mice, and the involvement of AQP1 in cataract development was studied using in vitro and in vivo models, which showed that AQP1 facilitates the maintenance of lens transparency and opposes cataract formation, thereby suggesting the possibility of AQP1 induction to delay cataractogenesis [21].

With regard to AQP5, no cataract phenotype for AQP5 knockout animals has been reported [23], although AQP5 deficiency has recently been linked to cataractogenesis in an ex vivo hyperglycemic mouse model of cataract formation [24]. In humans, AQP5 deficiency has not been associated with cataract. Two recent studies have related AQP5 mutations with non-ocular phenotypes (palmoplantar keratoderma), but with an unclear implication of these mutations in the water permeability of AQP5 [25,26].

In the present work we have shown increased membrane AQP1 and 5 protein expression in the human lens epithelial cells (HLECs) of patients with cataracts, with no changes observed at the mRNA level. Considering the low water content of the lens that is required to maintain a high refractive index for transparency, our results might reflect a compensatory response in an attempt to increase the water removal rate. Furthermore, our results suggest the possibility of increasing AQP1 and/or AQP5 expression levels in the lens epithelial cell membrane by pharmacologic or genetic means as a treatment for cataracts, at least in their early stages, once AQP modulators become available.

## 2. Materials and methods

### 2.1. Study subjects

A total of 148 individual of both sexes from Donostia Teaching Hospital, the Begitek Ophthalmologic Hospital, and the Quironsalud Donostia Hospital were included in the study: 112 cataract samples were obtained from patients submitted to a cataract intervention and 36 samples from clear lens were obtained from patients undergoing refractive lens exchange surgery (these served as controls). The mean age of patients was $67.21 \pm 6.04$ years, ranging from 42 to 82 years for cataract patients, and $57.48 \pm 5.47$ years, ranging from 49 to 70 years, for controls. All patients gave written informed consent, and the research adhered to the tenets of the Declaration of Helsinki. Institutional Ethics Review Committee approval was obtained.

A total of 70/148 samples were used for analysis of mRNA expression levels by quantitative polymerase chain reaction (qPCR), with 64/148 samples being used for protein expression levels by immunoblot analysis, and 14/148 samples being used for immunolocalization analysis.

Cataracts were classified as cortical, nuclear, or posterior subcapsular according to clinical criteria. The cataract samples used included only cataracts with stage NC2-NC4, C2-C3, P2P3 in the LOCS III system [27], whereas all controls had clear lenses. The main clinical information is summarized in Supplementary Table 1.

### 2.2. Tissue collection

Femtosecond laser-assisted lens removal was performed using the Victus femtosecond laser platform (Bausch & Lomb, New York, USA). A 5.0 mm capsulotomy applying 7.0 μJ was performed, and circular sections of anterior lens capsules with attached anterior lens epithelial cells (HLECs) were isolated and stored at −80 °C until RNA/protein extraction.

### 2.3. RNA quantification by real-time quantitative PCR

Total RNA was extracted from single HLEC samples using the RNeasy Micro Kit following DNase Treatment (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total RNA concentration was determined by spectrophotometry using a Nanodrop (Thermo Fisher Scientific) and cDNA was synthesized using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific). Candidate genes were analyzed by qPCR as described previously [28]. Briefly, primers spanning exon-exon junctions were designed using the Primer Express Software (Applied Biosystems; see Supplementary Table 2) and specificity was verified by ePCR (http://www.ncbi.nlm.nih.gov/sutils/e-pcr/reverse.cgi). qPCR was carried out in triplicate using the 7900HT Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions. Gene expression was calculated in cataract versus control samples using the standard curve method. Target genes were normalized by means of a normalization factor, based on the geometric mean of three internal control genes [29]. This normalization factor was calculated using the expression levels of the three best-scoring genes, which were GAPDH, TUBA1B, and ACTB (see Supplementary Table 2). Data are expressed as fold change of gene expression in cataract versus control samples.

### 2.4. Immunoblot analysis

Isolated control and cataract HLECs were homogenized in 100 μL of loading buffer (62.5 mM of 1 M Tris pH 7.5, 5% glycerol, 1% bromophenol blue, 2% SDS and 5% β-mercaptoethanol). Homogenates were boiled for 5 min and supernatants were centrifuged at 5000*g* before loading in SDS-PAGE 4–20% polyacrylamide-gradient gel (Mini-Protean TGX; Bio-Rad, Hercules, CA, USA). One control sample was used in all gels for normalization purposes. Given the limited amount of sample from each patient, proteins were extracted directly with a loading sample buffer, in order to maximize protein extraction. To determine the range of linearity for GAPDH and AQPs, a standard curve was generated with 2-fold dilutions of a control sample that showed high expression levels of AQPs. Only those samples with GAPDH and AQP intensity signals falling within these values were considered for the analysis. Proteins were electrotransferred to PVDF membranes (Amersham Hybond LFP 0.2 PVDF; GE Healthcare Life Science, Little Chalfont, UK), blocked with 5% BSA (Bio-Rad) and 2% horse serum in TBST for 1 h, then incubated with the following primary antibodies: rabbit anti-

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