Experimental Eye Research 116 (2013) 371–385

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Functional characterization of an AQP0 missense mutation, R33C, that causes dominant congenital lens cataract, reveals impaired cell-to-cell adhesion

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ARTICLE INFO

Article history:
Received 25 July 2013
Accepted in revised form 30 September 2013
Available online 9 October 2013

Keywords:
AQP0
water channel
water permeability
cell-to-cell adhesion
trafficking
congenital cataract
connexin 50

ABSTRACT

Aquaporin 0 (AQP0) performs dual functions in the lens fiber cells, as a water pore and as a cell-to-cell adhesion molecule. Mutations in AQP0 cause severe lens cataract in both humans and mice. An arginine to cysteine missense mutation at amino acid 33 (R33C) produced congenital autosomal dominant cataract in a Chinese family for five generations. We re-created this mutation in wild type human AQP0 (WT-AQP0) cDNA by site-directed mutagenesis, and cloned and expressed the mutant AQP0 (AQP0-R33C) in heterologous expression systems. Mutant AQP0-R33C showed proper trafficking and membrane localization like WT-AQP0. Functional studies conducted in *Xenopus* oocytes showed no significant difference ($P > 0.05$) in water permeability between AQP0-R33C and WT-AQP0. However, the cell-to-cell adhesion property of AQP0-R33C was significantly reduced ($P < 0.001$) compared to that of WT-AQP0, indicated by cell aggregation and cell-to-cell adhesion assays. Scrape-loading assay using Lucifer Yellow dye showed reduction in cell-to-cell adhesion affecting gap junction coupling ($P < 0.001$). The data provided suggest that this mutation might not have caused significant alterations in protein folding since there was no obstruction in protein trafficking or water permeation. Reduction in cell-to-cell adhesion and development of cataract suggest that the conserved positive charge of Extracellular Loop A may play an important role in bringing fiber cells closer. The proposed schematic models illustrate that cell-to-cell adhesion elicited by AQP0 is vital for lens transparency and homeostasis.

1. Introduction

Opacification of the lens or cataract accounts for about half of all cases of blindness worldwide. An estimated 20 million people suffer from bilateral cataracts; this number is estimated to reach 32 million globally by 2020 (World Health Organization website, undated). According to the American Society of Cataract and Refractive Surgery, three million Americans undergo cataract surgery annually. Causes for cataract development include age, defects in lens proteins caused by gene mutation, diseases such as diabetes, exposure to ionizing radiation, oxidative accumulation of free radicals, or a combination of these factors.

In the adult mammalian eye, the ocular lens is a relatively transparent avascular organ that relies mainly on solute- and water channels for the distribution of nutrients and removal of metabolic waste to maintain transparency and homeostasis (Mathias et al., 1997; Robinson and Patterson, 1983; Varadaraj et al., 1999). Aquaporins (AQPs) constitute a superfamily of small integral membrane channel proteins that allow passage of water solely, or water and certain small solutes across the plasma membranes, depending on the osmotic gradient. In mammals, the superfamily comprises 13 isoforms (AQP0-12). Among them, three aquaporin water channels are expressed in the lens, namely AQP0, AQP1 and AQP5. AQP0 is expressed abundantly in the fiber cell membrane (Bassnett et al., 2009; Broekhuyse et al., 1976; Chepelinsky, 2009; Shiels et al., 2001; Varadaraj et al., 2007). AQP1 is expressed in the monolayer of epithelial cells (Hamann et al., 1998; Varadaraj et al., 2005, 2007) and AQP5 is expressed in both epithelial and fiber cells (Bassnett et al., 2009; Grey et al., 2013; Kumari et al., 2012).

Abbreviations: WT-AQP0, Wild type human aquaporin 0; AQP1, Aquaporin 1; AQP0-R33C, Aquaporin 0 R33C mutant; FRET, Forster Resonance Energy Transfer; Pw, Water permeability.

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0014-4835/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2013.09.019

In the lens, AQP0 facilitates water permeation (Shiels, 2012; Shiels et al., 2001; Tong et al., 2013; Varadaraj et al., 1999, 2005, 2010) and cell-to-cell adhesion (Kumari and Varadaraj, 2009; Buzhynskyy et al., 2007; Costello et al., 1985; Kumari et al., 2011; Liu et al., 2011; Michea et al., 1994, 1995; Scheuring et al., 2007; Varadaraj et al., 2010). AQP0 has a molecular mass of ~28 kDa, six putative transmembrane domains, and two half-membrane-spanning domains (Fig. 1A). Three extracellular (A, C, and E) and two intracellular loops (B and D) serve as connectors of transmembrane domains. Highly conserved NPA motifs that line the water pore of aquaporin are located in loops B and E. The amino and carboxyl termini are intracellular with respect to the plasma membrane (Gonen et al., 2004, 2005; Harries et al., 2004). Molecular and structural studies confirmed that aquaporins are functional only in the tetrameric form in the membrane (Cheng et al., 1997; Murata et al., 2000; Ren et al., 2000; Walz et al., 1997). However, each subunit functions as a water channel.

AQP0 mutations identified thus far invariably resulted in autosomal dominant lens cataracts, underlining the importance of this protein for lens transparency. To date, eleven such mutations in human (Table 1a; Fig. 1B) and four in mice have been reported (Table 1b; Fig. 1B). Gu et al. (2007) identified a mutation in a five-

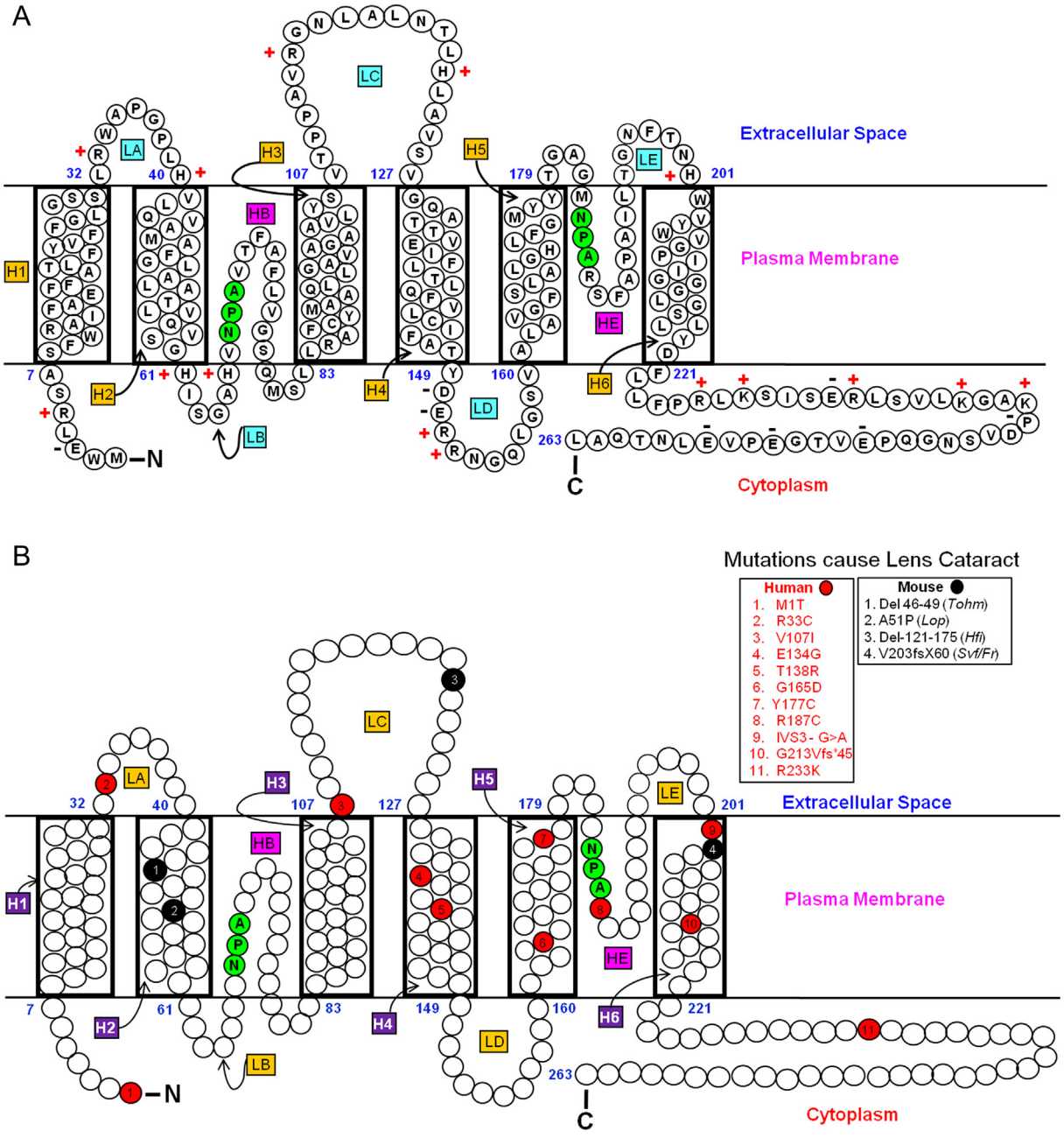

Fig. 1. (A). Schematic representation of human AQP0. Monomeric structure shows folds, helix assignment, and location in the membrane. Membrane-spanning helices are denoted as H1–H6 and loops as LA–LE. The two pore lining helices are shown as HB and HE. Highly conserved NPA motifs in loops B and E (shaded green) that line the water pore of aquaporin are shown. NH_2, amino terminus; COOH, carboxy terminus. '+' and '−' represent amino acid charges in extracellular and cytoplasmic domains. **(B)**. AQP0 mutations in humans and mice. Schematic illustration of the locations of eleven mutations in humans (red) and four mutations in mice (black) that cause inherited lens cataracts. AQP0 secondary structure domain designations are as given in (Fig. 1A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)