Functional Characterization of Bestrophin-1 Missense Mutations Associated with Autosomal Recessive Bestrophinopathy

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PURPOSE. Autosomal recessive bestrophinopathy (ARB) is a retinal dystrophy affecting macular and retinal pigmented epithelium function resulting from homozygous or compound heterozygous mutations in BEST1. In this study we characterize the functional implications of missense bestrophin-1 mutations that cause ARB by investigating their effect on bestrophin-1’s chloride conductance, cellular localization, and stability.

METHODS. The chloride conductance of wild-type bestrophin-1 and a series of ARB mutants were determined by whole-cell patch-clamping of transiently transfected HEK cells. The effect of ARB mutations on the cellular localization of bestrophin-1 was determined by confocal immunofluorescence on transiently transfected MDCK II cells that had been polarized on Transwell filters. Protein stability of wild-type and ARB mutant forms of bestrophin-1 was determined by the addition of proteasomal or lysosomal inhibitors to transiently transfected MDCK II cells. Lysates were then analyzed by Western blot analysis.

RESULTS. All ARB mutants investigated produced significantly smaller chloride currents compared to wild-type bestrophin-1. Additionally, co-transfection of compound heterozygous mutants abolished chloride conductance in contrast to co-transfections of a single mutant with wild-type bestrophin-1, reflecting the recessive nature of the condition. In control experiments, expression of two dominant vitelliform macular dystrophy mutants was shown to inhibit wild-type currents. Cellular localization of ARB mutants demonstrated that the majority did not traffic correctly to the plasma membrane and that five of these seven mutants were rapidly degraded by the proteasome. Two ARB-associated mutants (p.D312N and p.V317M) that were not trafficked correctly nor targeted to the proteasome had a distinctive appearance, possibly indicative of aggresome or aggresome-like inclusion bodies.

CONCLUSIONS. Differences in cellular processing mechanisms for different ARB associated mutants lead to the same disease phenotype. The existence of distinct pathogenic disease mechanisms has important ramifications for potential gene replacement therapies since we show that missense mutations associated with an autosomal recessive disease have a pathogenic influence beyond simple loss of function. (Invest Ophthalmol Vis Sci. 2011;52:3730–3736) DOI:10.1167/iovs.10-6707

Bestrophin-1 is a membrane protein primarily expressed in the basolateral membrane of the retinal pigment epithelium (RPE).1–5 During embryogenesis the RPE is essential for normal ocular development6–8 and throughout life performs critical roles in retinal homeostasis.9–10 The exogenous expression of bestrophin-1 generates a chloride ion conductance, suggesting that it functions as a Ca2+-activated Cl− channel.11 Subsequent postulated bestrophin-1 functions include a regulator of voltage-gated Ca2+ channels,12 a volume-regulated Cl− channel,13 and a HCO3− channel.14 However, the precise physiological role of bestrophin-1 in ion transport is still uncertain.

The RPE location and Cl− channel activity of bestrophin-1 led to the suggestion that it may be responsible for generating the light peak of the electrooculogram (EOG) that is used clinically to assess RPE health.11,15,16 Consistent with this is the observation that abnormal EOGs are characteristic of retinopathies caused by BEST1 mutations. Vitelliform macular dystrophy (VMD [MIM 153,700]) was the first “bestrophinopathy” to be described.3,17 We have since defined other diseases including autosomal dominant vitreoretinochoroidopathy (ADVIRC [MIM 193,220])18 and autosomal recessive Bestrophinopathy (ARB [MIM 611,809]).19

Clinically ARB is associated with central visual loss, an absent EOG, and abnormal electroretinograms (ERGs). Patients have a characteristic retinopathy that includes macular dysfunction with scattered punctuate flecks and subretinal fluid.19 Canine multifocal retinopathy is likely to be the equivalent in dogs.2 We have previously proposed that ARB represents the null bestrophin-1 phenotype in humans based on the identification of a family with a homozygous nonsense mutation in BEST1.19 Phenotypically similar patients with compound heterozygous missense mutations in BEST1 are hypothesized to have a critically reduced bestrophin-1 activity.

The functional consequences of mutations in bestrophin-1 on trafficking and localization have been investigated using
nonpolarized human embryonic kidney 293 (HEK 293) cells. However, in this model system, exogenous bestrophin-1 has a primarily intracellular localization. We have recently demonstrated that Madin-Darby canine kidney II (MDCK II) cells act as a more appropriate in vitro system to study the trafficking and localization of bestrophin-1. MDCK II cells form a polarized epithelial sheet allowing transiently expressed bestrophin-1 to localize to the basolateral membrane, mimicking the in vivo RPE localization. In this study we describe the functional consequences of ARB missense mutations on bestrophin-1’s chloride conductance, cellular localization, and stability.

METHODS

Cloning

Wild-type BEST1 in pAdlox was a kind gift from Alan Marmorstein (University of Arizona). Additional mutant constructs for p.L41P, p.L140V, p.R141H, p.P152A, p.A195V, p.R202W, p.D312N, p.V317M, and p.M325T were generated by site-directed mutagenesis (Quick-Change II kit; Stratagene, Stockport, Cheshire, UK) according to the manufacturer’s protocol. The same methods were used to generate two control VMD mutant constructs used for electrophysiological experiments, p.Y85H and p.T237R. All generated constructs were confirmed by direct sequencing.

In Vitro Studies

Cell Culture. MDCK II cells were cultured in Dulbecco’s Modified Eagle Medium containing L-glutamine, D-Glucose (4500 mg/L), and sodium pyruvate (110 mg/L) supplemented with 10% (v/v) fetal calf serum, 5% (v/v) penicillin/streptomycin, and 1% (v/v) nonessential amino acids, gentamicin (0.2 mg/mL), and penicillin G (100 U/mL) (all from Invitrogen, Paisley, Renfrewshire, UK) at 37°C and 5% CO2. HEK 293 cells were cultured in minimum essential medium with Earle’s salt and L-glutamate containing 9% fetal calf serum, 1% (v/v) nonessential amino acids, gentamicin (0.2 mg/mL), and penicillin G (100 U/mL) (all from Invitrogen, Paisley, Renfrewshire, UK) at 37°C and 5% CO2.

Transient Transfection. MDCK II cells and HEK 293 cells were seeded and transiently transfected as previously described. For whole-cell patch clamping experiments HEK293 cells were co-transfected with pAdlox vector containing wild-type or mutant BEST1 and the empty vector pEGFP-C1 in a 5:1 ratio (Exgene500; Fermentas, Marylebone, London, UK) according to the manufacturer’s instructions. Transfected cells were visualized by GFP fluorescence, and recordings were made from these cells.

Whole-Cell Patch-Clamp. Bestrophin-1 Cl− channel activity was measured as previously described. Briefly, conventional whole-cell recordings were made using an Axon Multiclamp 700B amplifier, with command potentials generated by a computer using pClamp 9 software and a Digidata 1320 interface (all Molecular Devices, Los Angeles, CA) with command potentials generated by a computer using the pClamp 9 software and a Digidata 1320 interface (all Molecular Devices, Los Angeles, CA). Transient whole-cell patch clamp experiments were performed using an Axon Multiclamp 700B amplifier, with command potentials generated by a computer using pClamp 9 software and a Digidata 1320 interface (all Molecular Devices, Los Angeles, CA). Transient whole-cell patch clamp experiments were performed using an Axon Multiclamp 700B amplifier, with command potentials generated by a computer using pClamp 9 software and a Digidata 1320 interface (all Molecular Devices, Los Angeles, CA).

Immunofluorescence

Immunofluorescence experiments were performed using transiently transfected MDCK II cells cultured on Transwell filters, at room temperature 48 hours post-transfection, as previously described. Mouse monoclonal anti-bestrophin-1 antibody (NB300-164; Stratex Scientific, Newmarket, Suffolk, UK) and secondary antibody, Alexa Fluor 488 goat anti-mouse IgG1 (Invitrogen), were used to detect bestrophin-1. The Transwell filters were then cut from the inserts and mounted onto a microscope slide with AF1 anti-fadent solution (Citi-fluor, London, UK) ± DAPI stain and sealed with nail varnish. Images were collected using a confocal (CI) on an upright microscope (90i) with a 60×/1.40 plan apochromatic objective and 3× confocal zoom (all Nikon, Kingston on Thames, Surrey, UK). The confocal settings were as follows: pinhole 30 μm, scan speed 400 Hz unidirectional, format 1024 × 1024. Images for DAPI and FITC were excited with the 405 and 488 nm laser lines, respectively. When it was not possible to eliminate cross-talk between channels, the images were collected sequentially. When acquiring three-dimensional optical stacks the confocal software was used to determine the optimal number of Z sections.

Inhibitor Experiments to Determine the Route of Bestrophin-1 Degradation

Transiently transfected MDCK II cells were incubated with either proteasomal inhibitor benzyloxy-carbonyl-Leu-Leu-phenylalanylalinal (Z-LLF-CHO) (10 μM; Calbiochem, Nottingham, UK) or a combination of lysosomal protease inhibitors leupeptin (100 μM; Enzo Life Sciences, Exeter, UK), and pepstatin A (1 μg/mL; Sigma, Poole, Dorset, UK) 24 hours post-transfection for 5 hours. Cells were then lysed in RIPA buffer. Each experiment was independently repeated on three or more occasions.

Western Blot Analysis

Protein samples for inhibitor experiments were resolved on 12% SDS-PAGE gels and transferred onto transfer membrane (GE Health Care, Amersham, Buckinghamshire, UK) using standard procedures. Primary antibodies were used at the following dilutions: mouse monoclonal anti-human bestrophin1 at 1:2000 (Stratex Scientific, NB300-164) and mouse monoclonal anti-GAPDH at 1:20,000 (Santa Cruz, Heidelberg, Germany). Bands were visualized with an anti-mouse IgG HRP conjugated secondary antibody (Dako, Ely, Cambridgeshire, UK) with the use of ECL detection reagent (GE Health Care). Band intensities were quantified by scanning densitometry with ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). The density of the untreated sample band was compared to those after treatment with either lysosomal or proteasomal inhibitors. All the bands were normalized against the appropriate GAPDH bands. The values for three sets of repeat data were then averaged.

RESULTS

### TABLE 1.

Summary of the Effects of Bestrophin-1 ARB Mutations on Protein Function.

<table>
<thead>
<tr>
<th>Mutation (Previously Reported by)</th>
<th>Occurs In Biallelic State with</th>
<th>Degraded via Proteasome?</th>
<th>Cell Membrane Localization?</th>
<th>Significantly Smaller Chloride Conductance Compared to Wild- Type (Average Chloride Current Measured at 0 mV)</th>
<th>Fold Increase in Protein Type? (Average)</th>
<th>Not Applicable.</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>NA</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(1.1)</td>
<td>N/A</td>
</tr>
<tr>
<td>p.A195V</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>(1.8)</td>
<td>Yes*</td>
</tr>
<tr>
<td>p.V317M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>(3.0)</td>
<td>Yes†</td>
</tr>
<tr>
<td>p.D312N</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>(1.3)</td>
<td>Yes‡</td>
</tr>
<tr>
<td>p.R202W</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(2.7)</td>
<td>No</td>
</tr>
<tr>
<td>p.D312N + p.A195V</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>(2.0)</td>
<td>Yes‡</td>
</tr>
<tr>
<td>p.M325T</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(3.1)</td>
<td>No</td>
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<tr>
<td>p.M325T + p.A195V</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(1.9)</td>
<td>Yes‡</td>
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<tr>
<td>p.A195V + p.D312N</td>
<td>Yes</td>
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<td>No</td>
<td>No</td>
<td>(1.3)</td>
<td>Yes‡</td>
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<tr>
<td>p.A195V + p.M325T</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(2.0)</td>
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<tr>
<td>p.D312N + p.M325T</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(1.3)</td>
<td>Yes‡</td>
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<tr>
<td>p.A195V + p.M325T + p.D312N</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>(2.0)</td>
<td>No</td>
</tr>
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### FIGURE 1.

Current-voltage (I-V) relationships from HEK 293 cells transfected with wild-type or ARB-causing mutant bestrophin-1. (A) Currents (mean ± SE) from 14 cells transfected with WT bestrophin-1 (●), and 14 cells transfected with eGFP (○). (B) Cells transfected with p.A195V alone (■, n = 11) or cotransfected with p.A195V + WT (●, n = 12). (C) Cells transfected with p.D312N alone (■, n = 11) or cotransfected with p.D312N + WT (●, n = 13).

### Whole-Cell Patch-Clamp Analysis of ARB-Causing Bestrophin-1 Mutants

The chloride channel activity of six uncharacterized ARB mutations (p.L41P, p.A195V, p.R202W, p.D312N, p.V317M, and p.M325T) was investigated by transfecting HEK 293 cells with either wild-type bestrophin-1, with one ARB mutant, or cotransfected with wild-type bestrophin-1 plus one ARB mutant, or with two ARB mutants. Figure 1A shows the mean current-voltage relationship obtained from cells transfected with wild-type bestrophin-1 or in control cells transfected only with WT bestrophin-1 (●) and eGFP alone (○). Figure 2 shows that the chloride conductance for these GFP cells was significantly less than for wild-type bestrophin-1 (P < 0.005 by ANOVA). Currents in cells transfected with either the p.A195V or p.D312N ARB mutants alone (■) were greatly reduced compared to wild-type (Figs. 1B and 1C); the mean conductance was also significantly different to wild-type (P < 0.05) (Fig. 3). In contrast, more robust currents were observed in cells cotransfected with wild-type and ARB mutants (Figs. 1B and 2C; ●): the conductance for p.A195V plus wild-type was not significantly different from wild-type alone (P > 0.1; Fig. 2). The reversal Vm for the p.A195V with wild-type was −19.0 ± 1.8 mV (n = 14) and for p.D312N plus wild-type was −17.7 ± 1.4 mV (n = 13). Neither value was significantly different from...
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The proteasomal and lysosomal-dependent degradation of wild-type and ARB-causing bestrophin-1 isoforms was investigated in transiently transfected MDCK II cells (Fig. 5). The addition of proteasomal and lysosomal inhibitors for 5 hours did not affect the amount of wild-type protein detected by Western blot compared to untreated cells (Fig. 5; Table 1). However, the addition of proteasomal inhibitors to cells transfected with ARB-causing isoforms p.L41P, p.L140V, p.R141H, p.A195V, p.R202W, and p.M325T increased the total amount of bestrophin-1 detected compared to untreated cells by between 1.8- and 3.1-fold (Fig. 5; Table 1). The addition of proteasomal inhibitors did not alter the amount of protein detected by Western blot for wild-type or ARB-causing bestrophin-1 isoforms compared to untreated cells (Fig. 5; Table 1).

Discussion

Here we present the first comprehensive functional characterization of bestrophin-1 missense mutations associated with ARB in both homozygous and compound heterozygous states (Table 1). Bestrophin-1 acts as a Cl− channel when expressed heterologously, and VMD-causing mutants, including p.Y85H and p.T237R, exhibit a greatly reduced channel activity and also suppress wild-type channel activity. This behavior is consistent with the autosomal dominant inheritance of the disease. In contrast, we have previously shown that three ARB-causing mutants (p.R141H, p.P152A, and p.L140V) exhibit reduced channel activity when expressed alone but do not suppress wild-type channel activity. This was proposed to be consistent with the autosomal recessive inheritance of ARB. In the present study we have characterized the Cl− channel phenotypes of six additional ARB-causing bestrophin-1 missense mutations (p.L41P, p.A195V, p.R202W, p.D312N, p.V317M, and p.M325T) and have shown that each of these mutations severely reduces Cl− channel activity compared to wild-type.
pared to wild-type protein when expressed alone in HEK 293 cells. Furthermore, when the six previously untested mutants were co-expressed with wild-type bestrophin-1 none suppressed wild-type Cl\textsuperscript{−}/H11002 channel activity. The conductances observed with two ARB-causing mutants (p.A195V and p.V317M) plus wild-type bestrophin-1 were not significantly different to wild-type alone. For the first time we additionally demonstrated that the co-transfection of two pairs of mutations observed in the compound heterozygous state in ARB patients essentially abolished Cl\textsuperscript{−} channel activity. These data reflect the autosomal recessive inheritance pattern of ARB and further support our hypothesis that the ARB disease phenotype results when bestrophin-1 activity is abolished or drops below a certain functional threshold.\textsuperscript{19}

The Cl\textsuperscript{−} channel activity of heterologously-expressed bestrophin-1 provides a useful tool in characterizing the properties of disease mutations associated with dominant and recessive phenotypes. However, the role of bestrophin-1 in the RPE is controversial, and it remains unclear whether bestrophin-1 functions as a Cl\textsuperscript{−} channel in vivo.\textsuperscript{28} Therefore differences in Cl\textsuperscript{−} channel properties alone are difficult to interpret in the context of the disease process, and it proves difficult to model the etiology of bestrophinopathies simply in terms of a reduction of Cl\textsuperscript{−} channel activity. For instance, the Cl\textsuperscript{−} channel properties of the ARB-causing mutants p.A195V and p.V317M appeared different to those of other ARB-causing mutants because they generated significantly greater Cl\textsuperscript{−} currents when expressed with wild-type bestrophin-1. This suggests that these mutants may affect RPE cell function differently from the other ARB-causing mutants investigated. Interestingly, as discussed below, p.V317M was subsequently found to display a different intracellular localization when expressed in MDCK II cells, and both p.A195V and p.V317M have different intracellular fates: p.A195V is catabolized rapidly by the proteasome whereas p.V317M is not. The difficulty in describing the disease process in terms of changes in Cl\textsuperscript{−} activity has also been recognized in bestrophin-1 knockout mice, in which the light peak of the EOG (thought to be generated by Cl\textsuperscript{−} channel


FIGURE 5. Investigating the proteasomal- and lysosomal-dependent degradation of wild-type (WT) and ARB-causing bestrophin-1 mutants. (A) MDCK II cells were transiently transfected with WT and ARB-associated bestrophin-1. Twenty-four hours post-transfection proteasomal inhibitor (10 \( \mu \)M Z-LLF-CHO; +P) or lysosomal inhibitors (100 \( \mu \)M leupeptin and 1 \( \mu \)g/mL pepstatin A; +L) were added to the cells and incubated for a further 5 hours. Nontreated (NT) cells were included for control purposes. Whole-cell lysate samples were separated by SDS-PAGE and analyzed by Western blot. Bestrophin-1 was detected using an anti-bestrophin-1 antibody at approximately 68 kDa. GAPDH was detected at 37 kDa. Band intensities were quantified by scanning densitometry, and the density of the untreated sample band was compared to those after treatment with either lysosomal or proteasomal inhibitors. All the bands were normalized against the appropriate GAPDH bands. The values for three sets of repeat data were then averaged. The addition of proteasomal inhibitors did not affect the amount of WT, p.P152A, p.D312N, or p.V317M protein in comparison to untreated cells. The addition of proteasomal inhibitors to cells transiently transfected with mutant isoforms p.L41P, p.L140V, p.R141H, p.A195V, p.R202W, and p.M325T increased the total amount of bestrophin-1 detected compared to NT cells. Densitometry data are presented in Table 1. The addition of lysosomal inhibitors did not notably alter the amount of protein detected for WT or ARB-causing mutant bestrophin-1 in comparison to NT cells.
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ARB, is caused by an altered bestrophin-1 activity. A further complication is that there is considerable evidence to suggest that bestrophin-1 has other functions such as regulating voltage-gated Ca\textsuperscript{2+} channels, and that these functions may or may not be affected by disease-causing mutations.

We have previously demonstrated that polarized MDCK II epithelial cells provide a useful system to study the localization and trafficking of bestrophin-1. Wild-type bestrophin-1 correctly localizes to the basolateral membrane, reflective of its in vivo localization in the RPE. Additionally we demonstrated that the ARB-causing mutants p.L140V and p.D312N mislocalize in polarized MDCK II cells. HEK 293 cells were used for the electrophysiological experiments, because MDCK II cells endogenously express a number of anion channels. In preliminary experiments these channels were found to contribute to the whole-cell conductance in the presence and absence of bestrophin-1, making data interpretation problematic (data not shown). In the present study we determined that the majority (5/7) of previously untested ARB-causing mutants also mislocalize in MDCK II cells (Fig. 4). This mislocalization suggests a mechanism indicative of their pathogenicity in vivo. We have also investigated whether ARB-causing bestrophin-1 mutants were turned over in the cell at different rates compared to wild-type protein by treating transiently transfected MDCK II cells with proteasomal and lysosomal inhibitors. The expression of six out of the nine ARB-causing mutants was increased by the additional of a proteasomal inhibitor (Fig. 5; Table 1) suggesting that these mutants are misfolded and targeted for degradation via an ubiquitin-proteasome dependent mechanism. Interestingly the expression of two mutants with a similar cell membrane localization to wild-type bestrophin-1, p.L141P and p.A195V, is increased in the presence of proteasome inhibitors. This suggests that although these two mutants are correctly trafficked they are nonetheless misfolded and targeted for proteasomal degradation.

If ARB is the null bestrophin-1 phenotype in humans, we predict that ARB patients with compound heterozygous or homozygous missense mutations have an equivalent reduction in bestrophin-1 activity. Our data demonstrate that such mutations can cause (1) reduced chloride channel activity, (2) mislocalization of protein away from the basolateral membrane, (3) protein misfolding leading to an increased rate of protein degradation by the ubiquitin-proteasome dependent pathway. It is also evident from these data that ARB-causing missense mutations do not all have similar functional consequences on bestrophin-1. For example, we suggest that the pathogenic mechanism associated with p.L140V, p.R141H, p.R202W, and p.M525T is a consequence of both mislocalization and protein misfolding. These mutants did not traffic correctly to the cell membrane and showed increased levels of proteasomal degradation compared to wild-type protein. Conversely ARB-causing mutants p.P152A, p.D312N, and p.V317M were the only ones of the nine investigated that were not rapidly degraded by the ubiquitin-proteasome dependent pathway. Although a significantly reduced chloride conductance was detected from all these mutants (indicating at least some reached the cell membrane), trafficking for p.D312N and p.V317M is clearly abnormal and is distinct in appearance from the other ARB-causing mutants investigated, having an aggregated appearance in the cytoplasm (Fig. 5, and Fig. 5 in Ref. 24). Notably these mutations are only five residues apart and occur in a group of four ARB mutations (along with p.M325T and p.W338X) in the putative cytoplasmic C-terminal and after a long run residues that, when mutated, cause VMD. Soluble protein aggregates are cytotoxic and are targets for lysosomal degradation. Since we saw no evidence of lysosomal degradation for either p.D312N or p.V317M, it may be that the distinct cytoplasmic appearance of these two mutants indicates that they are forming aggresomes or aggresome-like inclusion bodies. These bodies are microtubule dependent and are transported to perinuclear compartments for sequestration. Such aggregates are thought to be cytotoxic against the toxicity of misfolded proteins and can be considered as part of another protein quality control system for cell survival.

Pulse-chase experiments would allow a detailed quantitative analysis of the stability and degradation rates of ARB-causing mutants. However, low expression levels of bestrophin-1 in transiently transfected MDCK II cells meant that this cell line was inappropriate for such studies. Although a higher transfection efficiency was obtained in HeLa cells, the predominately cytoplasmic localization of bestrophin-1 in these cells meant that they too were inappropriate to use as the data would not be physiologically relevant (data not shown).

As there is increasing interest in gene replacement therapy, and the fact that most humans have missense rather than nonsense mutations, we believe the proper functional characterization of mutations is a necessary prerequisite for potential target autosomal recessive conditions, such as ARB. Mutations in ABCA4 cause a range of autosomal recessive retinopathies including Stargardt disease (STGD1 [MIM248200]). Gene replacement therapy has been proposed for these conditions, but a study looking at establishing inclusion/exclusion criteria for appropriate candidates found that one-third of nontruncating ABCA4 alleles caused a more severe phenotype than premature truncations. The authors of the study highlight, as we do here, that missense isoforms associated with autosomal recessive disease may have a pathogenic component beyond a simple loss of function. The efficacy of gene replacement therapies may be different for such patients. This study highlights the observation that different pathologic mechanisms can lead to identical disease phenotypes. Continued study of bestrophin-1 structure and function will provide further clues to the pathogenic mechanisms for the bestrophinopathies.

Acknowledgments

The authors thank Lisa Swanton and Peri Roboti (Faculty of Life Sciences, The University of Manchester) for their help and advice with the lysosomal and proteasomal inhibitor work.

References


