Hum Genet (1999) 104 : 449–453

RAPID COMMUNICATION

Rando Allikmets · Johanna M. Seddon
Paul S. Bernstein · Amy Hutchinson · Andrew Atkinson
Sanjay Sharma · Bernard Gerrard · Wen Li
Michael L. Metzker · Claes Wadelius · C. Thomas Caskey
Michael Dean · Konstantin Petrukhin

Evaluation of the Best disease gene in patients with age-related macular degeneration and other maculopathies

Received: 11 March 1999 / Accepted: 6 April 1999

Abstract Vitelliform macular dystrophy (VMD2, Best disease, MIM153700) is an early onset, autosomal, dominant macular degeneration characterized by the deposition of lipofuscin-like material within and below the retinal pigment epithelium (RPE); it is associated with degeneration of the RPE and overlying photoreceptors. Recently, we cloned the gene bestrophin, which is responsible for the disease, and identified a number of causative mutations in families with VMD2. Here, we report that the analysis of bestrophin in a collection of 259 age-related macular degeneration (AMD) patients provides evidence that mutations in the Best disease gene do not play a significant role in the predisposition of individuals to AMD. However, our results suggest that, in addition to Best disease, mutations within the bestrophin gene could be responsible for other forms of maculopathy with phenotypic characteristics similar to Best disease and for other diseases not included in the VMD category.

R. Allikmets (✉) · A. Hutchinson · B. Gerrard
Intramural Research Support Program, SAIC-Frederick,
National Cancer Institute-Frederick Cancer Research
and Development Center, Frederick, MD 21702-1201, USA

J. M. Seddon · S. Sharma
Department of Ophthalmology,
Massachusetts Eye and Ear Infirmary, Harvard Medical School,
Boston, MA 02114, USA

P. S. Bernstein,
Department of Ophthalmology, Moran Eye Center,
University of Utah, Salt Lake City, UT 84132, USA

W. Li · M. L. Metzker · C. T. Caskey · K. Petrukhin
Department of Human Genetics, Merck Research Laboratories,
West Point, PA 19486, USA

C. Wadelius
Department of Genetics and Pathology, Unit of Clinical Genetics,
University Hospital, S-751 85 Uppsala, Sweden

A. Atkinson · M. Dean
Laboratory of Genomic Diversity,
National Cancer Institute-Frederick Cancer Research
and Development Center, Frederick, MD 21702-1201, USA

Present addresses:
R. Allikmets, Department of Ophthalmology,
Columbia University, Eye Research Addition, 2nd Floor,
630 West 168th Street, New York, NY 10032, USA
(e-mail: rla22@columbia.edu,
Tel.: +1 212 305 8989, Fax: +1 212 305 7014)

A. Hutchinson, Department of Ophthalmology,
Columbia University, New York, USA

R. Allikmets, J. M. Seddon, and P. S. Bernstein contributed equally to this study

Introduction

Vitelliform macular dystrophy (VMD2, Best disease, MIM153700) represents an autosomal dominant form of macular degeneration characterized by the deposition of lipofuscin-like material within and below the retinal pigment epithelium (RPE). This accumulation is associated with the degeneration of RPE and overlying photoreceptors and may culminate in geographic atrophy of the macula and/or choroidal neovascularization (Frangieh et al. 1982; Weingeist et al. 1982; O'Gorman et al. 1988). Recently, we and others cloned the gene involved in the disease, and causative mutations have been described in a number of families (Petrukhin et al. 1998; Marquardt et al. 1998). The disease gene encodes an RPE-specific protein called bestrophin, which is closely related to a group of *C. elegans* proteins with unknown function (RFP family; Petrukhin et al. 1998). Isolation of the gene for Best disease provides the opportunity to study the mechanisms of age-related macular degeneration (AMD), as the two conditions share some common phenotypic features. AMD is the leading cause of vision loss among the elderly, with about 30% of the human population over the age of 75 manifesting some form of maculopathy (Klein et al. 1992). Clinically, AMD is divided into two forms: 80%–90% of patients are diagnosed as having the "dry" form of the disease, and the remaining 10%–20% manifest the "wet" subtype (Bressler et al. 1988). Both environmental and genetic factors have been implicated in the pathogenesis of AMD (Heiba et al. 1994; Seddon et al. 1996, 1997 a). Statistically significant association between

heterozygous sequence variants in the Stargardt disease gene, *ABCR*, and the dry form of AMD has been reported in one study (Allikmets et al. 1997 a). These results have recently been under intense scrutiny, with different groups providing contradictory evidence on this matter (Dryja et al. 1998; Dean et al. 1998; Stone et al. 1998; Lewis et al. 1999; Maugeri et al. 1999; Allikmets et al. 1999). In addition, mutations in the *ABCR* gene have been shown to cause autosomal recessive Stargardt disease (STGD1; Allikmets et al. 1997 b), some forms of retinitis pigmentosa (RP19; Martinez-Mir et al. 1998), and cone-rod dystrophy (Cremers et al. 1998). Assuming that different mutations in a single gene can result in a wide variety of phenotypes and taking into account some phenotypic similarities between AMD and Best macular dystrophy (lipofuscin accumulation, atrophic changes in the macula, and subretinal neovascularization), we have performed mutation analysis of bestrophin in patients with AMD and several other maculopathies, including adult vitelliform macular degeneration.

Materials and methods

The selection of patients and diagnosis has been described previously (Allikmets et al. 1997 a). Additional patients, diagnosed with AMD and other clinical phenotypes, and controls, matched by age and ethnicity, were collected at the Massachusetts Eye and Ear Infirmary, Boston, Mass., USA, and at the Moran Eye Center, Uni-

Table 1 Sequence variants in the Best disease gene (*ND* frequency of polymorphism not determined)

Base pair	Amino acid	Exon/ Intron	Allele frequency in the AMD sample	Allele frequency in the "other maculopathies" samples	Allele frequency in the control sample	Human/mouse conservation
Amino acid substitutions in conserved regions of the protein						
1. 459G→C	E119Q	Exon 4	0/518	1/60 ("bull's eye" maculopathy)	0/392	Conserved
2. 540GC→AA	A146K	Exon 4	0/518	1/60 (Adult vitelliform maculopathy)	0/392	Non-conserved (in highly conserved region)
3. 751C→T	T216I	Exon 6	1/518	0/60	0/392	Conserved
4. 993C→T	P297S	Exon 8	0/518	1/60 (Best disease)	0/392	Conserved
5. 1004G→C	E300D	Exon 8	0/518	1/60 (Best disease)	0/392	Conserved
Amino acid substitutions in non-conserved regions of the protein						
6. 1803C→T	L567F	Exon 10	2/518	0/60	0/392	Non-conserved
7. 1678A→C	E525A	Exon 10	0/518	0/60	1/392	Non-conserved
8. 1773G→A	E557K	Exon 10	0/518	0/60	2/392[a]	Non-conserved
9. 1785A→G	T561A	Exon 10	0/518	0/60	1/392	Non-conserved
Nucleotide substitutions, not resulting in amino acid change						
1. –118(C→T)	None	Promoter	154/518 (29.5%)	ND	116/392 (29.3%)	
2. 305G→C	L67L	Exon 3	8/464 (1.8%)	0/60	10/392 (2.5%)	
3. 323C→A	I73I	Exon 3	35/464 (7.5%)	6/60 (10%)	34/392 (8.5%)	
4. 326G→A	Q74Q	Exon 3	1/518	1/60	0/392	
5. IVS4–24C→T	None	Intron 4	86/518 (16.6%)	9/60 (15%)	68/392 (17.1%)	
6. 800C→A	I232I	Exon 6	1/518	2/60 (3.3%)	0/392	
7. IVS6–9 (delTCCTCC)	None	Intron 6	49/518 (9.4%)	6/60 (10%)	35/392 (8.9%)	
8. IVS6–9 (insTCC)	None	Intron 6	1/518	0/60	0/392	
9. 1127C→T	P341P	Exon 9	12/518 (2.3%)	2/60 (3.3%)	5/392 (1.3%)	
10. 1130G→A	E342E	Exon 9	1/518	0/60	0/392	
11. 1514G→A	T470T	Exon 10	126/404 (31%)	ND	84/304 (28%)	
12. 1661C→T	S519S	Exon 10	90/414 (22%)	ND	72/298 (24%)	
13. 1712T→C	T536T	Exon 10	146/414 (35%)	ND	103/294 (35%)	
14. IVS10–27 T→C	None	Intron 10	1/518	0/60	0/392	
15. 1951insG	None	Exon 11, 3′UTR	1/518	0/60	0/392	
16. 1995T→C	None	Exon 11, 3′UTR	18/518 (3.5%)	1/60 (1.7%)	16/392 (4%)	
17. 2078T→C	None	Exon 11,3′UTR	5/518 (1%)	2/60 (3.3%)	4/392 (1%)	

[a] Both chromosomes were found in a single individual homozygous for this rare E_{527}-to-K substitution. Homozygosity may be explained by consanguineous marriage, as this individual is also homozygous for all other common polymorphisms in the bestrophin gene

versity of Utah, Salt Lake City, Utah, USA, following the same guidelines. DNA isolation, sequencing, and single-strand conformation polymorphism (SSCP) analysis were performed as described earlier (Allikmets et al. 1997b; Petrukhin et al. 1998).

Results and discussion

Previously, we characterized variants in the *ABCR* gene in a cohort of 167 AMD patients, collected at two different clinics (Allikmets et al. 1997a). Here, we screened the same patients, plus an additional 92 cases with large drusen or geographic atrophy from the Massachusetts Eye and Ear Infirmary ($\Sigma = 259$), for variants in the Best disease gene. To model actual disease frequencies, 33 of the 259 patients (13%) in our collection had wet AMD; the remainder (87%) manifested the dry form of the disease. In addition, 30 patients with other clinical phenotypes were analyzed for variants in the bestrophin gene. Clinical entities of these patients included: myopic maculopathy ($n = 1$), cone dystrophy ($n = 4$), multifocal Best disease ($n = 1$), young onset of RPE changes ($n = 1$), atypical retinitis pigmentosa ($n = 1$), Stargardt disease ($n = 2$), "bull's eye" maculopathy of uncertain etiology ($n = 5$), pattern dystrophy ($n = 5$), early onset of drusen ($n = 1$), early onset of RPE detachment ($n = 1$), RPE changes with a family history of pattern dystrophy ($n = 1$), annular pigment dystrophy ($n = 2$), adult vitelliform ($n = 2$), adult vitelliform with family history of Best disease ($n = 1$), and Best disease ($n = 2$) as an internal control for the screening method. The same screening methods, viz., SSCP analysis and direct sequencing, were utilized as described in our previous studies (Allikmets et al. 1997a, 1997b). The control group included 196 individuals of the same age range (over 65 years of age) and racial background, collected and examined at the same clinical centers and declared free of any maculopathy by the same physicians who examined the patients (J.M.S., P.S.B.). All 11 bestrophin exons and 5′- and 3′-untranslated regions were screened for sequence variants in all patients and controls. The results are summarized in Table 1.

A total of six amino acid changes were detected in the bestrophin gene sequence in 7 out of 289 patients. None of these changes were found in a matched control group of 196 individuals (392 chromosomes) and or were detected in patients previously found to possess *ABCR* mutations (Allikmets et al. 997a). As expected, mutations were detected (P297 S and E300D) in one allele of both patients with Best disease (Table 2). Both mutations affect the residues that are highly conserved among members of the RFP family from *C. elegans* and in the mouse ortholog of human bestrophin. A change of proline in position 297 to a different amino acid (P297 A) has been previously described in one family segregating Best disease (Marquardt et al. 1998). Both mutations segregated with the disease in respective families, further suggesting that they represent disease-causing bestrophin variants (data not shown).

Two more sequence variants, E119Q and A146K, were found in two sporadic cases of "bull's eye" maculopathy and adult vitelliform maculopathy, respectively (Table 2). These sequence variants represent non-conservative substitutions resulting in a gain or loss of charge and are located in evolutionarily conserved regions of the protein, indicating a disease-specific mutation (Table 1). This is the first description of mutations in bestrophin in patients with adult vitelliform or "bull's eye" macular degeneration. Given the small number of patients with either form of the disease screened in this study, we cannot draw conclusions regarding the extent of bestrophin involvement in these maculopathies. It is very likely, however, that mutations in bestrophin can cause a variety of disease phenotypes.

Two variants, T216I and L567F, were present in three patients diagnosed with the dry form of AMD. All three

Table 2 Clinical data of seven patients with bestrophin variants (*od* right eye, *os* left eye, *ou* both eyes, *RPE* retinal pigment epithelium, *RPED* retinal pigment epithelial detachment)

Patient ID/age	Age of diagnosis	Visual acuity at ascertainment	Phenotype	Exon	Amino acid	Nucleotide
73-year-old Hispanic male	20	20/60, 20/300	Best disease	8	P297S	993C→T
70-year-old Caucasian female	50	20/200, 20/60	Best disease	8	E300D	1004G→C
61-year-old Caucasian female	57	20/50, 20/640	RPE mottling od, bull's-eye maculopathy os	4	E119Q	459G→C
60-year-old Caucasian female	54	20/32, 20/20	Adult vitelliform od, RPE atrophy os	4	A146K	540GC→AA
67-year-old Caucasian male	57	20/25, 20/25	Drusenoid RPEDs ou; grade 3b	6	T216I	751C→T
61-year-old Caucasian female	57	20/25, 20/32	Hard drusen od, soft os; grade 3	10	L567F	1803C→T
75-year-old Caucasian male	66	20/40, 20/200	Soft drusen and geographic atrophy; grade 4	10	L567F	1803C→T

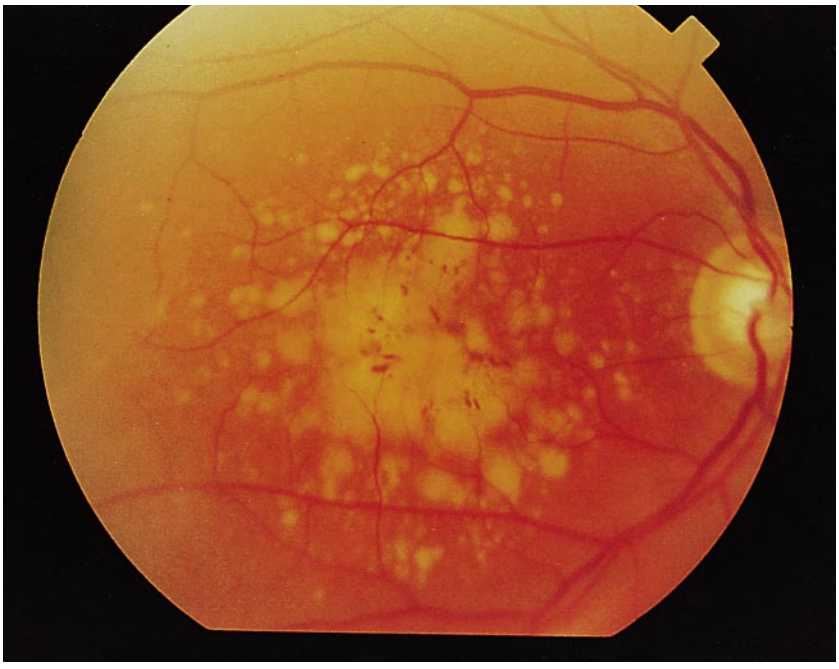

Fig. 1 Macula of the right eye of the patient with the T216I mutation showing multiple soft drusen and a drusenoid retinal pigment epithelial detachment

AMD patients represented sporadic cases with no known family history of the disease. One of the two sequence variants identified in two AMD patients (L567F, 2/259, 0.8%) was found in the region of high dissimilarity between the mouse and human proteins (25% mouse/human identity in the protein region encoded by exons 10–11 versus 75% identity in the region encoded by exons 2–9). None of the Best disease mutations reported in the literature is localized to this region of the protein (Petrukhin et al. 1998; Marquardt et al. 1998). Multiple sequence alignment of human bestrophin and members of the RFP families from *C. elegans* confirmed the lack of conservation in this part of the homologous proteins. Additionally, we identified three missense substitutions in exon 10 in individuals from the control sample (E525A, E557K, and T561A), which indicates the functional redundancy of this part of the protein. Although we failed to detect the AMD-specific L567F alteration in control individuals, we cannot unequivocally state that this change is pathogenic, since Leu_{567} is not conserved in evolution, and since the L567F substitution does not result in change of charge or polarity. The second AMD variant (T216I, 1/259, 0.4%) was found in the 67-year-old patient with soft drusen and drusenoid RPE detachment (Fig. 1), consistent with AMD, stage 3b, according to an established grading system (Seddon et al. 1997b; Table 2). Thr_{216} is conserved in evolution from mouse to man, is located very close to the cluster of the known Best disease mutations (R218C, R218S, R218Q, Y227N, Y227C), results in a change in polarity, and has not been found in 196 disease-free individuals. Therefore, it is highly likely that this variant represents a disease-causing alteration.

In addition, nine synonymous substitutions were detected, and a number of single nucleotide polymorphisms was found in introns and in 5′- or 3′-untranslated regions (Table 1). Variants ranged from common polymorphisms (up to 35% of alleles examined) to rare changes, seen in only one individual (Table 1). Allelic frequencies of common variants were very close or almost identical in both patients and controls, indicating that our control group was appropriately matched with the patient population. We did not observe any statistically significant difference in frequencies of silent variants or those from noncoding regions between the patient and control groups (Table 1, data not shown).

In conclusion, our data provide evidence that mutations in the Best disease gene do not play a significant role in the predisposition of individuals to AMD. However, it cannot be ruled out that, in rare cases, bestrophin variants may increase susceptibility to AMD. Best macular dystrophy is a progressive autosomal dominant disorder with juvenile onset. It would be expected that mutations that lie in one allele of the bestrophin gene and that affect the protein function would cause an individual to develop VMD at an early age. We hypothesize that, on rare occasions, some variants that do not have a drastic effect on the protein function could still predispose an individual to AMD later in life. In addition, mutations in bestrophin may lead to other phenotypic characteristics that are not included in the Best disease category. However, distinguishing between the diagnosis of late onset Best disease, adult vitelliform macular degeneration, and some forms of AMD can be difficult, complicating the analysis of genotype/phenotype correlation. Further genetic studies will help to improve the existing classification of retinal diseases. Allelic series, i.e., different mutations in one gene causing different diseases, are well-documented phenomena in ophthalmology (ABCR, reviewed in van Driel et al. 1998; peripherin/RDS, reviewed in Keen and Inglehearn 1996). Our data indicate that this may also be the case with the

bestrophin gene, in which different mutations in the coding region cause a variety of phenotypic manifestations.

Acknowledgements We appreciate the participation of the patients, their families, and their physicians, without whose cooperation this work would not have been possible. We thank Mark Leppert, Andy Peiffer, Karen Clements, and Sandy Cheung for assistance in enrolling patients. This work was supported in part by Federal funds from the National Cancer Institute, National Institutes of Health, under contract no. NO1-CO-56000 (R.A.), and in part by National Eye Institute grants EY11309 (J.M.S.) and EY11600 (P.S.B.). This work was also supported in part by a career development award to P.S.B. and by an unrestricted grant to the Department of Ophthalmology of the University of Utah by Research to Prevent Blindness. J.M.S. is a Lew R. Wasserman Merit Award recipient from Research to Prevent Blindness. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, et al (1997a) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 277 : 1805–1807

Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, et al (1997b) A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 15 : 236–246

Allikmets R, International ABCR Screening Consortium (1999) Association of G1961E and D2177N variants in the *ABCR* gene with age-related macular degeneration (ARVO abstract). Invest Ophthalmol Vis Sci 40 : S 775 (abstract no. 4089)

Bressler NM, Bressler SB, Fine SL (1988) Age-related macular degeneration. Surv Ophthalmol 32 : 375–413

Cremers FP, Pol DJ van de, Driel M van, Hollander AI den, Haren FJ van, Knoers NV, Tijmes N, et al (1998) Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene *ABCR*. Hum Mol Genet 7 : 355–362

Dean M, Allikmets R, Shroyer NF, et al (1998) Science [online] http://sciencemag.org/cgi/content/full/279/5354/1107a

Driel MA van, Maugeri A, Klevering BJ, Hoyng CB, Cremers FP (1998) ABCR unites what ophthalmologists divide. Ophthalmic Genet 19 : 117–122

Dryja T, Briggs CE, Berson EL, et al (1998) Science [online] http://sciencemag.org/cgi/content/full/279/5354/1107a

Frangieh GT, Green WR, Fine SL (1982) A histopathologic study of Best's macular dystrophy. Arch Ophthalmol 100 : 1115–1121

Heiba IM, Elston RC, Klein BE, Klein R (1994) Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. Genet Epidemiol 11: 51–67

Keen TJ, Inglehearn CF (1996) Mutations and polymorphisms in the human peripherin-RDS gene and their involvement in inherited retinal degeneration. Hum Mutat 8 : 297–303

Klein R, Klein BE, Linton KL (1992) Prevalence of age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 99 : 933–943

Lewis RA, Shroyer NF, Singh N, Allikmets R, Hutchinson A, Li Y, Lupski JR, Leppert M, Dean M (1999) Genotype/phenotype analysis of a photoreceptor specific ABC transporter gene, ABCR, in Stargardt disease. Am J Hum Genet 64 : 422–434

Marquardt A, Stohr H, Passmore LA, Kramer F, Rivera A, Weber BH (1998) Mutations in a novel gene, VMD2, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). Hum Mol Genet 7 : 1517–1525

Martinez-Mir A, Paloma E, Allikmets R, Ayuso C, Rio T del, Dean M, Vilageliu L, et al (1998) Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. Nat Genet 18 : 11–12

Maugeri A, Driel MA van, Pol DJ van de, Klevering BJ, Haren FJ van, Tijmes N, Bergen AA, et al (1999) The 2588G → C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 64 : 1024–1035

O'Gorman S, Flaherty WA, Fishman GA, Berson EL (1988) Histopathologic findings in Best's vitelliform macular dystrophy. Arch Ophthalmol 106 : 1261–1268

Petrukhin K, Koisti MJ, Bakall B, Li W, Xie G, Marknell T, Sandgren O, et al (1998) Identification of the gene responsible for Best macular dystrophy. Nat Genet 19 : 241–247

Seddon JM, Willett WC, Speizer FE, Hankinson SE (1996) A prospective study of cigarette smoking and age-related macular degeneration in women. JAMA 276 : 1141–1146

Seddon JM, Ajani UA, Mitchell BD (1997a) Familial aggregation of age-related maculopathy. Am J Ophthalmol 123 : 199–206

Seddon JM, Samelson LJ, Page WF, Neale MC (1997b) Twin study of macular degeneration: methodology and application to genetic epidemiologic studies. Invest Ophthalmol Vis Sci 38 : S 676

Stone EM, Webster AR, Vandenburgh K, et al. (1998) Allelic variation in ABCR associated with Stargardt disease but not age-related macular degeneration. Nat Genet 20 : 328–329

Weingeist TA, Kobrin JL, Watzke RC (1982) Histopathology of Best's macular dystrophy. Arch Ophthalmol 100 : 1108–1114