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Course of visual decline in relation to the Best1 genotype in vitelliform macular dystrophy

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Abstract

Purpose: To describe the disease course in vitelliform macular dystrophy (VMD) patients with a Best1 mutation, and to determine the association between Best1 genotype and visual prognosis.

Design: Consecutive case series. Participants: Fifty-three VMD patients with Best1 mutations from 27 Dutch families, aged 11 to 87 years.

Methods: Best-corrected visual acuity (VA), fundus appearance, and Arden ratio on the electro-oculogram (EOG) during clinical follow up were assessed from medical records. Mutation analysis of the Best1 gene was performed on DNA samples using denaturing high pressure liquid chromatography (dHPLC) and direct sequencing.

Main Outcome Measures: Cumulative life-time risk of visual decline below 0.5, 0.3, and 0.1, for the entire group and stratified for genotype.

Results: Median age of onset of visual complaints was 33 years (range: 2-78). The cumulative risk of VA below 0.5 (20/40) was 50% at 55 years and 75% at 66 years. The cumulative risk of decline <0.3 (20/63) was 50% by age 66 and 75% by age 74. Two patients progressed to VA<0.1 (20/200). Fourteen different mutations were found. Most patients (96%) had missense mutations; the Thr6Pro, Ala10Val and Tyr227Asn mutations were most common. Visual decline was significantly faster in patients with an Ala10Val mutation than either the Thr6Pro or the Tyr227Asn mutation (p=0.001).

Conclusions: Age of onset of visual complaints varies greatly among VMD patients. All patients show a gradual decrease in VA and most progress to visual impairment at a relatively late age. Our data suggest a phenotype-genotype correlation, as the Ala10Val mutation has a more rapid disease progression than other common mutations.

Introduction

Vitelliform macular dystrophy (VMD) is an autosomal dominant macular disease with an estimated prevalence of 1 in 10,000[1]. The diagnosis is generally established when a vitelliform or egg yolk-like lesion is present in the macula. This lesion consists of lipofuscin located within and below the retinal pigment epithelium (RPE). The egg yolk stage is usually followed by a scrambled egg appearance, which is caused by break down of the deposits. In later stages, the macula may become atrophic or show scar formation as a result of cell death of the RPE and photoreceptors. The clinical diagnosis of VMD is generally confirmed by a decreased light peak-dark trough ratio (Arden ratio) on the electro-oculogram (EOG).

VMD is caused by mutations in a single gene, *Best1* (NM_004183), located on chromosome 11q13[2-5]. This gene, formerly known as the *VMD2* gene, is transcribed into a 585 amino acid protein, bestrophin-1, and belongs to a family of four bestrophins that contain a highly conserved tripeptide motif of arginine (R), phenylalanine (F), and proline (P)[4-8]. The bestrophin-1 protein is located in the basolateral membrane of RPE cells, has four transmembrane domains in the N-terminal part of the protein[9], and forms tetrameric or pentameric Cl^- channels that are activated by alterations in the intracellular Ca^{2+} concentration[7,10-15]. Studies in mice show drastically altered Cl^- conductances in mice with *mBest2* mutations[7]. The *bestrophin-1* protein is expressed at high levels in the RPE[7]. The number of disease-causing mutations is high; approximately 100 mutations at over 80 different positions have been reported[17]. The majority of these are missense mutations that cluster near the transmembrane portions and the NH_2 terminus. The specific functional consequences of these mutations remain to be elucidated, but the current notion is that they alter Cl^- conductance and/or the Ca^{2+} flux through voltage-dependent channels in the RPE[18]. As a consequence, the electrical current in the RPE is reduced, and epithelial transport is disrupted, ultimately resulting in accumulation of lipofuscin[10].

Previously, it was thought that congenital Best disease, adult-onset vitelliform macular dystrophy, and autosomal dominant vitreoretinochoroidopathy (ADVIRC) were different disease entities. The identification of mutations in the *Best1* gene altered this notion, and revealed that most individuals with these phenotypes linked to the same gene[8]. Since then, it is clear that the expression and clinical consequences of a mutation in *Best1* are highly variable[19,20]. Whether the onset and course of disease is determined by the type and location of the mutation, however, is still unresolved.

In the current study, we investigated the genotype-phenotype correlation in VMD patients who were examined in three specialized centers in the Netherlands between 2003 and 2007. Here we report the distribution of *Best1* mutations within this group, and the association between onset and course of the macular dystrophy and visual acuity (VA) over time.

Patients and methods

Study population

Patients with a clinical and molecular diagnosis of VMD who visited three specialized ophthalmogenetic clinics in the Netherlands between 2003 and 2007 were considered eligible for inclusion in the study. The clinical and molecular diagnosis included a VMD lesion in the

retina (see below) and the presence of a disease-causing mutation in the *Best1* gene. Exclusion criteria were: uncertainty with regard to the clinical diagnosis or the absence of a disease causing mutation. Twenty-nine probands and 24 affected relatives met these criteria and were subsequently invited to participate in the study. The study was approved by the Medical Ethical Committee of Erasmus University, and conformed to the tenets of the declaration of Helsinki. All subjects signed a written informed consent prior to inclusion.

Clinical parameters

Age of onset, fundus appearance, best-corrected visual acuity (VA), and results of electrophysiological testing were retrieved from medical records. We evaluated all available parameters at first presentation, and at all subsequent visits. In addition, subjects were invited for an additional clinical examination including fundus photography.

The VMD lesions were evaluated on fundus photographs and classified based on a six stage grading scheme developed by Mohler and Fine[1]. In short, VMD stage 1 represents no changes or subtle RPE pigment changes; stage 2 is an egg yolk-like or vitelliform lesion; stage three a pseudohypopyon; stage 4 a scrambled egg appearance; stage 5 atrophy of the RPE; and stage 6 a fibrous scar with or without choroidal neovascularisation.

We recorded the first occurrence of VA<0.5. This level is the cut-off point for visual disability according to US definition, and reflects the legal inability to drive a car[21]. We also recorded the first measurement of VA<0.3. This is the cut-off point for low vision according to WHO criteria, and is associated with increasing difficulties in reading without visual aids[22].

EOG recordings were generally carried out at initial diagnosis, and performed according to standard ISCEV criteria[23]. Arden ratio's were registered and stratified in normal (≥ 1.85), mildly reduced (< 1.85 and ≥ 1.3), and severely reduced (<1.3).

Mutation analysis

DNA was extracted from peripheral blood leukocytes by standard methods[24]. DNA was amplified using intronic primers described by Petrukhin et al[5]. For exon 4, we developed the following alternative primers: forward strand cgctcgcagcagaaagct, reverse strand ctccacccatcttccattc[17]. After heteroduplex formation, we screened samples for mutations using denaturing high pressure liquid chromatography (dHPLC, Transgenomic). Variations were analyzed by direct sequencing (ABI-310, Applied Biosystems). Sequence changes were annotated according to Genbank accession NM_004183.1. The first A of the ATG was chosen as nucleotide +1, and the ATG as codon +1.

Statistical Analysis

Correlation of VMD stage and VA between right and left eye was calculated with Kappa statistics. Cumulative risks of fundus abnormalities and visual loss were studied with Kaplan-Meier product-limit survival analysis. For fundus abnormalities, first appearance of abnormalities (stage 1 or greater vs. stage 0) and first appearance of atrophy or scar formation (stage 5 or 6 vs. stage 0-4) were analyzed. For visual function, onset of mild (VA<0.5 vs. VA≥0.5) and severe visual impairment (VA< 0.3 vs. VA≥0.3) were determined. These analyses were also performed stratified for the three most frequent mutations and stratified for age

of onset in childhood (<10 years) or in adulthood (>18 years). Differences in cumulative risks between Best1 genotypes were compared using the Breslow test[25].

Table 1. Demographic and clinical characteristics of vitelliform macular dystrophy at first presentation and during follow up.

Variables	At presentation (all patients)		At presentation (pts with follow up)		Follow up	
patients, n	53		40		40	
pedigrees, n	29		23		23	
Gender males, n females, n	 29 25		22 18		 22 18	
Age at first presentation (range), yrs	34 (2-78)		35 (2-78)			
VMD stage n (%) 0 1 2 3 4 5 6	better eye 7 (13) 10 (19) 9 (17) 7 (13) 7 (13) 10 (19) 3 (6)	worse 6 (11) 5 (9) 9 (17) 8 (15) 8 (15) 12 (23) 5 (9)	better eye 5 (13) 6 (15) 8 (20) 4 (10) 6 (15) 8 (20) 3 (8)	worse 4 (10) 4 (10) 7 (18) 5 (13) 7 (18) 9 (23) 4 (10)	better eye 4 (11) 1 (3) 3 (8) 1 (3) 5 (14) 17 (46) 6 (16)	worse 3 (8) 0 (0) 3 (8) 0 (0) 3 (8) 14 (38) 14 (38)
EOG recordings n (%) normal (≥1.85) reduction mild (1.3-1.85) reduction severe (<1.3)	41 (76) 0 (0) 9 (22) 32 (78)					
VA n (%) ≥0.5 0.5-0.3 0.3-0.1 <0.1	better eye 38 (72) 13 (25) 2 (4) 0 (0)	worse 22 (42) 10 (19) 18 (35) 2 (4)	better eye 26 (65) 12 (30) 2 (5) 0 (0)	worse 16 (40) 7 (18) 14 (35) 3 (8)	better eye 13 (33) 13 (33) 13 (33) 0 (0)	worse 4 (10) 5 (13) 23 (59) 7 (18)

VMD: vitelliform macular dystrophy, EOG: electro oculogram, VA: visual acuity.

Results

Clinical characteristics at first presentation

Demographic data of all 53 patients are presented in Table 1. The patients were genealogically linked to 27 pedigrees; 16 individuals were sporadic patients and 37 had at least one relative with a VMD phenotype. The study population consisted of 29 males and 24 females. The age at the first visit to an ophthalmologist ranged from age 2 to 78 years, with a median of 33 years. Seventeen patients had an early onset (<age 10 yrs), 18 patients an onset during age 10-18 yrs, and 18 patients had an adult onset (>age 18 yrs). At first presentation, all stages were observed (Table 1); the correlation between the left and right eye was high (Kappa 0.76). The VA was below 0.5 in the better eye in 15 (29%) patients and in the worse eye in 30

(59%) patients. VA at first presentation showed little correlation between the two eyes (VA left vs. right eye: Kappa 0.24).
The majority of patients (78%) had a severely reduced EOG, with an Arden ratio < 1.3. The remainder of the patients had a mildly reduced Arden ratio (< 1.85).

Mutations in the Best1 gene

We identified 14 different mutations in the 27 pedigrees (Table 2). Single missense mutations were most common (11/14). The most frequent mutation was the Tyr227Asn in seven members of six pedigrees (22% of probands), followed by Thr6Pro mutation in 18 members of five different pedigrees (19%), and Ala10Val in seven members of two pedigrees (7%). Two patients from one pedigree carried a compound heterozygous missense mutation (Ala195Val and Leu134Val). In addition, we identified an in-frame deletion and an in-frame insertion in two probands.

Table 2. Mutations identified in the 53 patients with vitelliform macular dystrophy in the *Best1* gene.

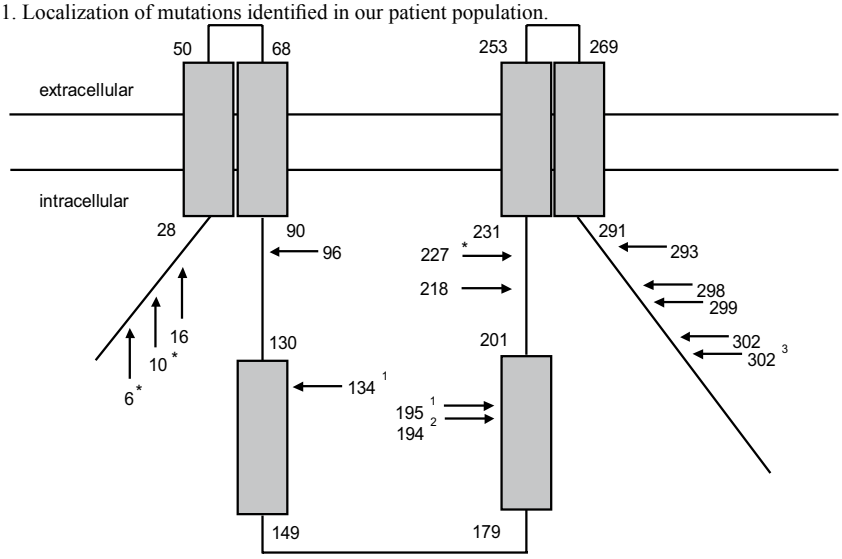
mutation	effect	no. of patients	no. of pedigrees	ref
[c.16A>C]	[p.Thr6Pro]	18	5	5
[c.29C>T]	[p.Ala10Val]	7	2	27
[c.47C>A]	[p.Ser16Tyr]	1	1	28
[c.288G>C]	[p.Gln96His]	5	1	27
[c.583_584insTGG]	[p.Lys194_Ala195insVal]	1	1	28
[c.584C>T] [c. 400C>G]	[p.Ala195Val] [p.Leu134Val]	2	1	28
[c.584C>T]	[p.Ala195Val]	1	1	29
[c.653G>A]	[p.Arg218His]	3	3	29
[c.679 T>A]	[p.Tyr227Asn]	7	6	5
[c.877 C>A]	[p.Gln293Lys]	3	2	27
[c.893T>C]	[p.Phe298Ser]	1	1	30
[c.896G>C]	[p.Gly299Ala]	1	1	31
[c.905A>C]	[p.Asp302Ala]	2	1	32
[c.904_912delGATGATGAT]	[p.Asp302_Asp304del]	1	1	28
	total	53	27	

The A of the ATG-translation initiation codon is described as nucleotide 1, according to NM_004183.2.

Figure 1 shows the location of the mutations in the protein[26]. Three mutations were located in the N-terminal intracellular part of the protein (Thr6Pro, Ala10Val and Ser16Tyr); seven mutations were located in the intracellular loop (Gln96His, Leu134Val, Lys194_Ala195ins-Val, Ala195Val, Arg218His, Tyr227Asn and Gly229Ala); and four mutations were located in the C-terminal intracellular part (Gln293Lys, Phe298Ser, Asp302Ala and Asp302_Asp-304del). The Leu134Val mutation, the Lys194_Ala195insVal mutation and the Ala195Val

mutation were located in the third and fourth putative transmembrane domain. No mutations were found in the extracellular loops.

Figure 1. Localization of mutations identified in our patient population.



Model adapted from Milenkovic et al.[18]. * indicates the three most common mutations in our study group, used for further analysis, 1 indicates these two mutations occured together, 2 indicates an insertion, 3 indicates a deletion.

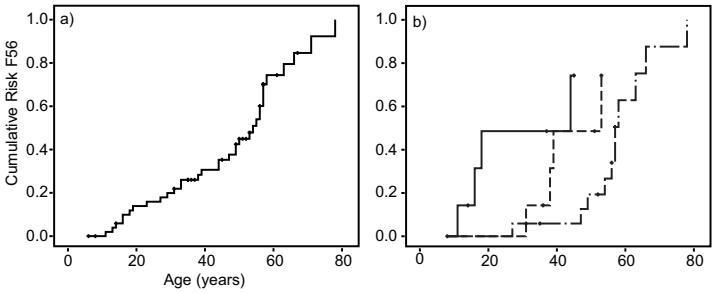
Clinical course and genotype-phenotype correlation

Data on multiple visits were available in 40 patients with a mean follow up of 15.3 years. All but two patients showed progression in stage of disease. Fourteen patients progressed to VMD stage 5 (atrophy of the RPE; 38%); 14 other patients progressed to stage 6 (fibrous scar, 38%). Figure 2a shows the manifestation of VMD stage 5 or 6 as a function of age. Half of the patients who developed atrophy or a scar did so by age 54 years, 75% by 63 years.
We assessed the cumulative risk of VMD stage 5 or 6 for the three most common mutations in our population, and found statistically significant differences (P=0.002) (Thr6Pro, Ala10Val and Tyr227Asn; Fig 2b). Of the patients with the Ala10Val mutation, 50% reached stage 5 or 6 at age 30 years compared to 50% of the patients with the Thr6Pro and Tyr227Asn mutations at age 45 and 58 respectively.

Visual decline was present in all but three patients. One of these patients showed no deterioration during a follow up of only one year, and two patients from a single pedigree remained stable over a 2 and 5 year follow up, respectively, despite subtle changes in the macula, a severely decreased EOG and the presence of a disease-causing mutation. Fifty percent of patients had VA<0.5 at age 55; 75% at age 66 years. For VA<0.3, these ages were 66 years and 74 years, respectively (Figure 3a and 3c). Two patients (5%) progressed to legal blindness (VA $\leq$ 0.1) after age 60 years. The cumulative risk of visual decline stratified for Thr6Pro, Ala10Val, and Tyr227Asn is shown in Figure 3b and 3d. Patients with the Ala10Val mutation

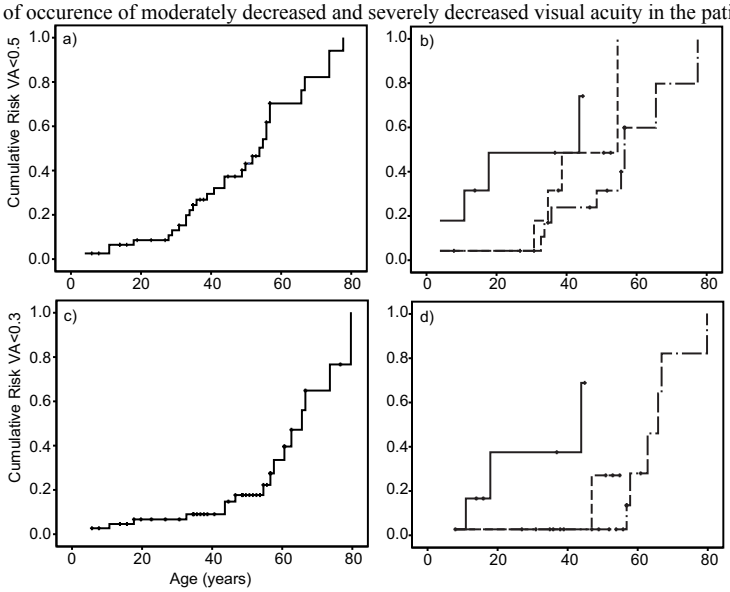
had a significantly faster visual decline than patients with either Thr6Pro or Tyr227Asn. Fifty percent of patients with Ala10Val deteriorated to VA <0.5 at age 29 years, while 50% patients with Thr6Pro and Tyr227Asn did so at ages 58 years and 46 years, respectively (P<0.03). For deterioration to VA<0.3, these ages were 34 years for Ala10Val, and 53 years for Tyr227Asn (P=0.001). For Thr6Pro the majority did not decline below VA 0.3; only 30% did so beyond age 44.

Figure 2. Age of occurence of fundus stage 5 or 6 in the patient group.



Occurrence of fundus stage 5 or 6 (F56) (atrophy or a fibrous scar with or without choroidal neovascularisation) a) in all subjects, b) in the three most common mutations Thr6Pro (dashed), Ala10Val (solid) and Tyr227Asn (dash-dot). Progression is significantly faster in patients with the Ala10Val mutation (p=0.002) Squares indicate censored case.

Figure 3. Age of occurence of moderately decreased and severely decreased visual acuity in the patient group.

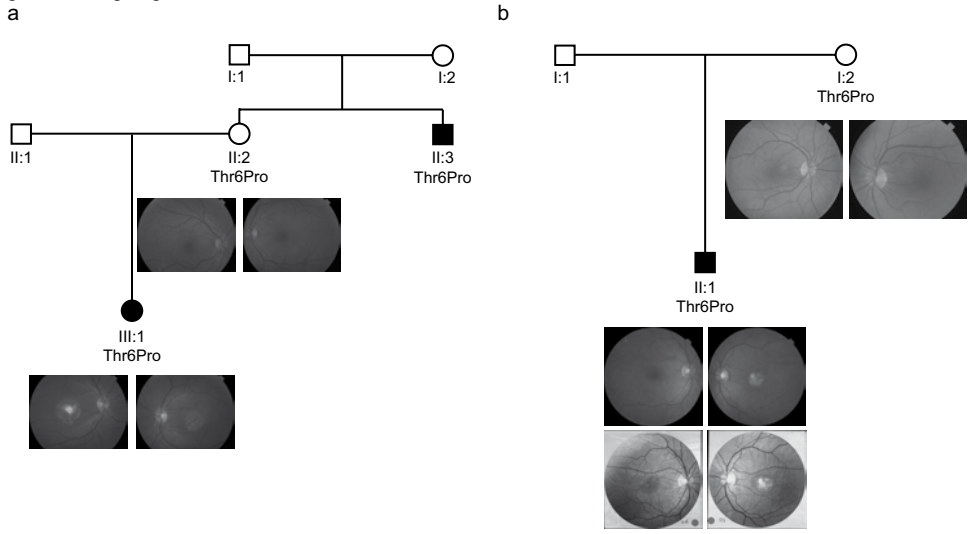


Age of occurrence of a) moderately decreased visual acuity (VA<0.5) in all patients; and b) stratified for the three most common mutations Thr6Pro (dashed), Ala10Val (solid) and Tyr227Asn (dash-dot); c) severely decreased visual acuity (VA<0.3) in all patients; and d) stratified for the three most common mutations. Visual decline is significantly faster in patients with the Ala10Val mutation (VA<0.5 p<0.03; VA<0.3 p=0.001). Squares indicate censored cases.

Case presentation of phenotypic variability

Two pedigrees with the Thr6Pro mutation showed remarkable variability in the manifestation of the disease. The first pedigree included three relatives with the Thr6Pro mutation (Figure 4a). A female proband presented with visual loss in both eyes at age 27 years. Her right eye had VA 0.2 and a macular scar (VMD stage 6); her left eye had VA 0.6 and showed only subtle macular changes (stage 1). Her EOG showed an Arden ratio of 1.5 in both eyes. Strikingly, her 55 year old mother had no visual complaints but showed very subtle accumulation of lipofuscin near the temporal vascular arcades upon examination (VA 1.0 OU; no macular changes; Arden ratio 2.0) despite the presence of the mutation. The maternal uncle had been diagnosed with VMD at age 51 years (OD: VA 0.8, VMD stage 2; OS: VA 1.2, VMD stage 1; OU: Arden ratio 1.0). His right eye progressed to a severe VMD phenotype in 6 years (VA <0.01, stage 5), while his left eye remained stable.

Figure 4. Two pedigrees with the Thr6Pro mutation.



a) Presentation of the first pedigree. All three individuals (III:1, II:2, and II:3) carried the Thr6Pro mutation. At age 27, the proband (arrow; III:1) had bilateral visual complaints (visual acuity (VA) right eye (OD) 0.2, left eye (OS) 0.6; fundoscopically OD macular scar (stage 6), OS subtle changes (stage 1)) with an Arden ratio of 1.5 in both eyes. Her 55 year old mother (II:2) had no visual complaints (VA 1.0 both eyes (OU); no macular changes; Arden ratio 2.0). The maternal uncle (II:3) was diagnosed at age 51 and progressed to a severe bilateral phenotype (VA<0.01, stage 5) in 6 years. Fundoscopic pictures of individual III:1 at age 27 years, and of individual II:2 at age 55 years. b) Presentation of the second pedigree. Both individuals (II:1 and I:2) carried the Thr6Pro mutation. At age 31, the proband (arrow, II:1) had VA 0.8 and vitelliform macular dystrophy (VMD) stage 2 in the left eye; his right eye had VA 1.0 and no macular changes, Arden ratio's OU were 1.0. See lower two black-and-white fundus photographs. At age 47 years (upper two fundus photographs), his left eye had progressed to VA 0.13 and VMD stage 6, while his right eye remained completely normal. The patients' mother had no clinical signs of VMD apart from an Arden ratio of 1.5 at the age of 57 years (see fundus photographs). Squares indicate males, circles indicate females, filled symbols indicate affected individuals. A color version of this figure can be found in chapter 15.

The second pedigree consisted of a male proband with a unilateral presentation and his mother, both carrying the Thr6Pro mutation (Figure 4b). At age 31 years, the proband's left eye had VA 0.8 and VMD stage 2; his right eye had VA 1.0 and no macular changes, while the Arden ratio's OU were 1.0. At age 47 years, his left eye had progressed to VA 0.13 and VMD stage 6, whereas his right eye remained completely normal. The patients' mother had no clinical signs of VMD apart from an Arden ratio of 1.5 at the age of 57 years.

Statistical considerations

Our study design may have led to ascertainment bias. Therefore we performed multiple analyses to exclude this effect in our data (data not shown). In summary, we found no differences between the age when an end stage phenotype was reached for patients with an age of onset in childhood, compared to an age of onset in adulthood. Moreover, our data do not show any differences in follow up for patients with relatively good visual acuity at the last visit compared to patients with poor VA. Finally, we show that the group of people with follow up and the group without follow up do not differ significantly.

Discussion

We show that VMD, caused by mutations in the *Best1* gene, is a disease with a variable age of onset and clinical course, ultimately leading to visual decline. Our survival analyses indicate that the variability may in part be explained by differences in genotype. The majority of patients deteriorated to such extent that driving was prohibited at middle age, and that reading was generally compromized after age 70. However, development of legal blindness was rare, even at higher ages. The Ala10Val mutation showed a significantly faster decline causing severe visual impairment in the majority of patients by 44 years of age.

Some methodological considerations should be addressed before we elaborate on our findings. Although we are not aware of larger studies describing the course of VMD, our study was still limited in sample size. This hampered precise estimates in our subgroup analyses. Nevertheless, we were able to detect significant differences between the genotypes over time. Another limitation was our retrospective study design. We were obliged to rely on clinical observations from various ophthalmologists and clinics without data acquisition according to standard protocols and time intervals. We do not think this distorted our results significantly, since our participants underwent regular standard ophthalmologic examinations, and our outcome parameters were derived from standard clinical care. Finally, as specified in the results section, our data was not subject to high levels of ascertainment bias.

Thus far, descriptions of the clinical course of VMD were mainly based on reports of small families. In the current study, we calculated the mean rate of visual decline in a large series of VMD cases. On average, a VA<0.5, was reached by age 55 years. A VA<0.3 was reached by age 66. In our study, the functional endpoint was most often visual impairment; only two (5%) patients progressed to legal blindness.

Despite the general visual decline observed in VMD patients, clinicians are well aware of the high variability of the phenotype. This is illustrated by our study, as left and right eyes of individual patients showed little correlation at any given time point, and even unilateral expression occurred. Moreover, severely affected probands who initially appeared to be spo-

radic, revealed, upon family screening, relatives with subtle manifestations of the phenotype. Mutation analysis in these family members showed the presence of the same disease-causing *Best1* mutation as the proband. Therefore we advocate genetic testing in first degree relatives of 'sporadic' probands even in the absence of signs or symptoms of disease. This may reveal unknown carriers, confirm the dominant inheritance pattern, and inevitably improve genetic counseling.

Congenital Best disease and adult-onset vitelliform macular dystrophy were previously thought to be two distinct phenotypes. The former was considered to have an early age of onset and mildly to severely decreased EOG while the latter supposedly had an adult onset, a lack of distinct VMD stages and a mildly decreased to normal EOG[15,27]. Our study suggests that a continuous spectrum of the disease is more likely, since our patients showed high variability in age of onset, VMD stages and EOG values. We hypothesize that congenital Best disease and adult-onset vitelliform macular dystrophy caused by mutations in the *Best1* gene are two extremes of a single etiology.

Why is the clinical course for Ala10Val more severe than for Thr6Pro or Tyr227Asn? This is not immediately clear for a number of reasons: first, both Ala10Val and Thr6Pro lie in the first intracellular domain. Second, in the Ala10Val mutation both amino acids are hydrophobic, whereas Thr6Pro changes the amino acid from hydrophilic to hydrophobic. Third, Tyr227Asn is located closer to one of the transmembrane domains that form the channel than Ala10Val, which would suggest that the former mutation has the greatest effect on the protein. Finally, the Tyr227Asn mutation is located in a more conserved region than the Ala10Val. Hence, we can not explain the faster progression for patients with an Ala10Val mutation, and the underlying biological mechanism still remains to be elucidated.

Apart from the dominant negative effect, either single nucleotide polymorphisms or expression variability in the wild type allele may influence the severity of the disease. This is not uncommon in ophthalmic disorders and has also been shown in autosomal dominant retinitis pigmentosa (RP11 and RP1)[28].

Two contradictory publications exist on the protein structure and the location of the transmembrane domains and intra- or extracellular loops of the Best1 protein[26,29]. Both models describe six hydrophobic domains and an intracellular loop containing either the third[29] or the third and fourth[26] hydrophobic loop. In 2003, Tsunenari et al. described a model in which all but the third hydrophobic domain are transmembrane domains. More recently however, Milenkovic et al. (2007) showed that only the first two and the last two hydrophobic domains are transmembrane domains. In the current publication we based our conclusions of the new theory of Milenkovic et al[26]. Nevertheless, the actual folding of the protein into a three dimensional structure could be different from these models and may explain the differences in the clinical course observed in this study.

In conclusion, our study shows that the highly variable clinical course in the VMD phenotype may be explained, at least in part, by variation in genotype. However, virtually all patients show a gradual decline in VA without reaching legal blindness. It is intriguing that mutations do not necessarily lead to a phenotype. Insight into these mechanisms may provide clues for future clinical care.

References

1. Mohler CW, Fine SL. Long-term evaluation of patients with Best's vitelliform dystrophy. Ophthalmology 1981;88:688-92.
2. Forsman K, Graff C, Nordstrom S, et al. The gene for Best's macular dystrophy is located at 11q13 in a Swedish family. Clin Genet 1992;42:156-9.
3. Stone EM, Nichols BE, Streb LM, et al. Genetic linkage of vitelliform macular degeneration (Best's disease) to chromosome 11q13. Nat Genet 1992;1:246-50.
4. Marquardt A, Stohr H, Passmore LA, et al. Mutations in a novel gene, VMD2, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). Hum Mol Genet 1998;7:1517-25.
5. Petrukhin K, Koisti MJ, Bakall B, et al. Identification of the gene responsible for Best macular dystrophy. Nat Genet 1998;19:241-7.
6. Stohr H, Milenkowic V, Weber BH. VMD2 and its role in Best's disease and other retinopathies [in German]. Ophthalmologe 2005;102:116-21.
7. Hartzell HC, Qu Z, Yu K, et al. Molecular physiology of bestrophins: multifunctional membrane proteins linked to Best disease and other retinopathies. Physiol Rev 2008;88:639-72.
8. Boon CJ, Klevering BJ, Leroy BP, et al. The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2009;28:187-205.
9. Marmorstein AD, Marmorstein LY, Rayborn M, et al. Bestrophin, the product of the Best vitelliform macular dystrophy gene (VMD2), localizes to the basolateral plasma membrane of the retinal pigment epithelium. Proc Natl Acad Sci U S A 2000;97:12758-63.
10. Sun H, Tsunenari T, Yau KW, Nathans J. The vitelliform macular dystrophy protein defines a new family of chloride channels. Proc Natl Acad Sci U S A 2002;99:4008-13.
11. Xiao Q, Prussia A, Yu K, et al. Regulation of bestrophin Cl channels by calcium: role of the C terminus. J Gen Physiol 2008;132:681-92.
12. Qu Z, Chien LT, Cui Y, Hartzell HC. The anion-selective pore of the bestrophins, a family of chloride channels associated with retinal degeneration. J Neurosci 2006;26:5411-9.
13. Pusch M. Ca(2+)-activated chloride channels go molecular. J Gen Physiol 2004;123:323-5.
14. Qu Z, Fischmeister R, Hartzell C. Mouse bestrophin-2 is a bona fide Cl(-) channel: identification of a residue important in anion binding and conduction. J Gen Physiol 2004;123:327-40.
15. Kramer F, White K, Pauleikhoff D, et al. Mutations in the VMD2 gene are associated with juvenile-onset vitelliform macular dystrophy (Best disease) and adult vitelliform macular dystrophy but not age-related macular degeneration. Eur J Hum Genet 2000;8:286-92.
16. Booij JC, van Soest S, Swagemakers SM, et al. Functional annotation of the human retinal pigment epithelium transcriptome. BMC Genomics [serial online] 2009;10:164. Available at: http://www.biomedcentral.com/1471-2164/10/164. Accessed November 8, 2009.

17. Retina International Mutation Databases [database online]. Updated September 18, 2009. Available at: http://www.retina-international.org/sci-news/mutation.htm. Accessed November 8, 2009.
18. Milenkovic VM, Soria RB, Aldehni F, et al. Functional assembly and purinergic activation of bestrophins. Pflugers Arch 2009;458:431-41.
19. Seddon JM, Sharma S, Chong S, et al. Phenotype and genotype correlations in two Best families. Ophthalmology 2003;110:1724-31.
20. Renner AB, Tillack H, Kraus H, et al. Late onset is common in Best macular dystrophy associated with VMD2 gene mutations. Ophthalmology 2005;112:586-92.
21. Leat SJ, Legge GE, Bullimore MA. What is low vision? A re-evaluation of definitions. Optom Vis Sci 1999;76:198-211.
22. World Health Organization. Prevention of Blindness and Visual Impairment: Change the definition of blindness. Available at: http://www.who.int/blindness/Change%20the%20Definition%20of%20Blindness.pdf. Accessed November 8, 2009.
23. Brown M, Marmor M, Vaegan, et al. ISCEV Standard for Clinical Electro-oculography (EOG) 2006. Doc Ophthalmol 2006;113:205-12.
24. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
25. Crowley J, Breslow N. Statistical analysis of survival data. Annu Rev Public Health 1984;5:385-411.
26. Milenkovic VM, Rivera A, Horling F, Weber BH. Insertion and topology of normal and mutant bestrophin-1 in the endoplasmic reticulum membrane. J Biol Chem 2007;282:1313-21.
27. Musarella MA. Molecular genetics of macular degeneration. Doc Ophthalmol 2001;102:165-77.
28. Daiger SP, Shankar SP, Schindler AB, et al. Genetic factors modifying clinical expression of autosomal dominant RP. Adv Exp Med Biol 2006;572:3-8.
29. Tsunenari T, Sun H, Williams J, et al. Structure-function analysis of the bestrophin family of anion channels. J Biol Chem 2003;278:41114-25.
30. Kramer F, Mohr N, Kellner U, et al. Ten novel mutations in VMD2 associated with Best macular dystrophy (BMD). Hum Mutat 2003;22:418.
31. Boon CJ, Jeroen KB, Keunen JE, et al. Fundus autofluorescence imaging of retinal dystrophies. Vision Res 2008;48:2569-77.
32. Chung MM, Oh KT, Streb LM, et al. Visual outcome following subretinal hemorrhage in Best disease. Retina 2001;21:575-80.

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