Clinical and Genetic Heterogeneity in Multifocal Vitelliform Dystrophy

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Objective: To describe the clinical and genetic findings in 15 patients with multifocal vitelliform lesions.

Methods: All patients and, if possible, affected family members underwent an ophthalmic examination and their genomic DNA was analyzed for mutations in the vitelliform macular dystrophy 2 (VMD2) gene. Patients who did not have a mutation in the VMD2 gene were screened for mutations in the peripherin/RDS gene.

Results: Patient age at onset of the disease was highly variable, ranging from 5 to 59 years. The peripheral lesions varied in number, size, and overall appearance but showed similar characteristics at autofluorescence imaging and optical coherence tomography compared with the central vitelliform lesion. Mutations in the VMD2 gene were identified in 9 of 15 patients. One patient without a VMD2 mutation carried a sequence variant in the 5’ untranslated region of the peripherin/RDS gene.

Conclusions: Multifocal vitelliform dystrophy is a clinically and genetically heterogeneous retinal disease that can be caused by mutations in the VMD2 gene. Other genes associated with this phenotype remain to be identified.

Clinical Relevance: Clinical and molecular genetic characterization of multifocal vitelliform dystrophy may lead to better understanding of the pathophysiological mechanisms underlying this phenotype and may enable a more accurate prognosis in individual patients.

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MULTIFOCAL VITELLIFORM lesions have been described in several articles.1-16 Lesions typically are manifested as sharply demarcated yellowish cysts in the macula, near the retinal vascular arcades and surrounding the optic disc. A variation in size and number (as many as 20) can be observed. Lesions may grow and merge with neighboring lesions. The evolution of the peripheral lesions may parallel the evolution of the central lesion but may also have a different time course.1,7,9,10,12 The previously reported cases of multifocal vitelliform lesions were either familial or sporadic. Most lesions showed at least partial staining on fluorescein angiograms.3,8,10 Although the electro-oculogram (EOG) often demonstrated reduced responses, some patients had normal EOG responses.3,8 Results of electroretinography were in the normal range in most patients with multifocal vitelliform lesions.8 Some authors consider this phenotype an atypical variant of Best vitelliform macular dystrophy (BVMD), especially in combination with an autosomal dominant mode of inheritance and abnormal EOG findings. Best vitelliform macular dystrophy is associated with mutations in the vitelliform macular dystrophy 2 (VMD2) gene, which can be found in virtually all patients with a positive family history and in 28% to 69% of sporadic cases.17,19 Typically, EOG findings are moderately to severely abnormal, although normal EOG findings have been reported in patients with BVMD carrying a mutation in VMD2.5,17,20,21 In adult-onset foveomacular vitelliform dystrophy (AFVD), vitelliform lesions are generally unifocal and located in the macula, although, rarely, a multifocal distribution may be seen.21 Contrary to typical BVMD, the lesions in AFVD are usually 0.33 to 1 disc diameter in greatest diameter, with a central pigmented spot, although lesions may also mimic those seen in BVMD.23 In asymptomatic patients, age at onset of visual disturbance is usually
between 30 and 50 years. The EOG results are normal or only slightly abnormal. Adult-onset foveomacular vitelliform dystrophy shows genetic heterogeneity; mutations in the peripherin/RDS gene were found in as many as one-third of patients with AFVD and mutations in the VMD2 gene were identified in approximately 25% of such patients.17,24,25

To date, 2 studies have been published describing 2 patients with multifocal vitelliform lesions carrying mutations in the VMD2 gene.11,14 The purpose of this study was to clinically characterize findings in 15 patients with multifocal vitelliform lesions and to investigate the possible role of the VMD2 and peripherin/RDS genes in the pathogenesis of this peculiar phenotype.

**CLINICAL STUDIES**

This study conformed to the tenets of the Declaration of Helsinki and was approved by the Committee on Research Involving Human Subjects at the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. Informed consent was obtained from all participants. Fifteen probands, who had a diagnosis of multifocal vitelliform dystrophy on the basis of ophthalmoscopic findings, were examined. Whenever possible, affected family members and their parents also were examined. Their medical histories were obtained, and subsequent clinical examination included best-corrected Snellen visual acuity, Amsler grid testing, fundus examination with indirect ophthalmoscopy, and fundus photography. In addition, autofluorescence imaging (Heidelberg Retina Angiograph [HRA2]; Heidelberg Engineering, Dossenheim, Germany) and optical coherence tomography (Stratus OCT 3; Carl Zeiss Meditec Inc, Dublin, California) were performed. Fluorescein angiography had been performed previously in 11 of 15 patients. All patients underwent EOG, and 8 patients were tested according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards. In 7 patients, the EOGs were recorded before ISCEV regulations according to an older protocol described by Thijsen et al.26 Six patients gave permission for full-field electroretinography, which was carried out using ISCEV guidelines.

**MOLECULAR GENETIC STUDIES**

Blood samples were obtained from all probands and, whenever possible, from their parents and other affected family members. The genomic DNA was isolated according to previously described protocols.27 Sequence analysis of the 10 coding exons and splice junctions of the VMD2 gene was performed in all patients.28 In the patients who did not have mutations in VMD2, the 3 exons and splice junctions of peripherin/RDS were amplified with the polymerase chain reaction and analyzed using direct sequencing.29

**RESULTS**

Fifteen patients with multifocal vitelliform dystrophy were included in this study. Six were males and 9 were females, aged 5 to 59 years (mean age±SD, 40.6±18.1 years; median age, 49.0 years) when first seen. Nine patients reported additional family members with comparable reports of visual disturbances. All patients experienced decreased visual acuity as the initial symptom.

**ANALYSIS OF VMD2 AND PERIPHERIN/RDS GENES**

Sequence analysis of the VMD2 gene revealed mutations in 9 of 15 patients (60%), whereas in the other 6 patients, no mutations in the VMD2 gene were detected. The VMD2 gene mutations in these 9 patients included 5 different missense mutations, 1 in-frame deletion, and 1 in-frame insertion. Four mutations (p.Asn296Lys, p.Lys194_Ala195insVal, p.Asp302_Asp304del, and p.Ser16Tyr) are novel, and 3 mutations (p.Ala195Val, p.Thr6Pro, and p.Phe298Ser) have been previously described in patients with BVMD and AFVD.17,19,30,31 The 4 novel mutations were not identified in 185 control individuals. The missense mutation p.Ala195Val was found in 2 unrelated probands (patients 1 and 8). Two other patients with multifocal vitelliform lesions (patients 3 and 9), who were not related, both carried the missense mutation p.Thr6Pro. Two amino acid changes, p.Ala195Val and p.Leu134Val, were detected in patient 1. Segregation analysis in the patient’s family members indicated that both changes reside on the same allele. The p.Ala195Val mutation has been described previously in patients with BVMD,28,31 whereas the pathogenic nature of p.Leu134Val is unknown. This p.Leu134Val variant was not identified in 185 controls. Sequence analysis of the peripherin/RDS gene in the remaining 6 patients identified 1 sequence variant (c.-11A>C) in the 5’ untranslated region in patient 15. This change is not a known polymorphism, is not present in the single nucleotide polymorphism databases, and was not found in 92 controls. The variant is not predicted to affect regulatory elements or transcription factor binding sites. However, pathogenicity of this variant cannot be excluded.

**CLINICAL FEATURES IN PATIENTS WITH VMD2 MUTATIONS**

Three male and 6 female patients carried a mutation in the VMD2 gene. Age at onset of disease was highly variable (mean age±SD 29.8±17.7 years; median age, 33 years), as was visual acuity (Table 1). Visual acuity ranged from 20/20 to 20/800. The appearance of the central lesions varied from typical vitelliform round to oval lesions (Figure 1B) to large and diffuse lesions with multiple yellowish deposits of vitelliform material (Figure 2A). Peripheral lesions varied not only in number but also in size and appearance, from small and well-circumscribed yellow-white spots to large and diffuse lesions with irregular yellow-white deposits, comparable to the central lesion. These eccentric lesions were located superonasal to the optic disc and adjacent to the temporal vascular arcades. Like the central lesions, the extramacular lesions varied in number, size, and appearance during a follow-up period of several years (Figure 1A and B). At autofluorescence imaging, areas of highly increased autofluorescence within lesions corresponded with the yellow-white material as seen at opthalmoscopy (Figure 1C and Figure 2B). Fluorescein angiographic characteristics were variable, but all lesions had a predominantly hyperfluorescent appearance in the early and late phases. Optical coherence tomographic (OCT)
images of the central lesion showed hyporeflective material under the macula that elevated the central retina to a variable degree. In a number of cases, areas of increased reflectivity could be seen within this hyporeflective space, corresponding to the yellowish material seen at ophthalmoscopy. Areas within the lesion with a cica-

Table 1. Clinical and Molecular Findings in Patients With VMD2 Mutations

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Family History</th>
<th>Age at Onset, y</th>
<th>Visual Acuity</th>
<th>No. of Lesions, OD/OS</th>
<th>EDG Findings, OD/OS</th>
<th>ERG Findings, Mutation Effect</th>
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Abbreviations: EOG, electro-oculogram; ERG, electroretinogram; ISCEV, International Society for Clinical Electrophysiology of Vision; ellipses, data not available. aValues are given as Arden ratios: ISCEV, normal if 2.0 or higher; non-ISCEV, normal if 1.8 or higher. bResults not recorded according to ISCEV standards and had been obtained previously in our research laboratory.

Figure 1. Left eye in patient 5, who carries the p.Asp302, Asp304del mutation, at age 18 years. A, Fundus image shows a central cicatricial lesion. In addition, a lesion superior to the optic disc with characteristics of a pseudohypopyon is evident (arrowhead). B, At age 44 years, the lesion superior to the optic disc shows a “scrambled egg” appearance. Black arrow indicates the suprapapillary lesion; white arrow, vertical optical coherence tomographic section through the central lesion. C, At autofluorescence imaging, the central lesion shows markedly decreased autofluorescence of the area corresponding to the scar noted at funduscopy, surrounded by a zone of mottled alterations in autofluorescence intensity. The suprapapillary lesion shows spots of increased autofluorescence at the edges of the lesion that correspond to the remnants of vitelliform material seen at ophthalmoscopy. D, A vertical optical coherence tomographic section through the central lesion (white arrow in B) reveals a prominent hyporeflective structure corresponding to the central scar seen at funduscopy. This structure is surrounded by a hyporeflective subretinal space, suggesting the presence of subretinal fluid. E, A vertical optical coherence tomographic section through the suprapapillary lesion (black arrow in B) shows a dome-shaped structure with an optically clear center and a hyporeflective band above it, possibly the elevated photoreceptor layer.
tricial appearance at ophthalmoscopy were prominent and hyperreflective on OCT images and were often in contact with the overlying retina. The OCT characteristics of the larger extramacular lesions were similar to those of the central lesions (Figure 1D and E). The size of the extramacular lesions ranged from smaller than 1 optic disc area (25 of 38 lesions) to larger than 5 optic disc areas (7 of 38 lesions). The larger extramacular lesions were found superior to the optic disc. Demarcation of individual lesions was better with autofluorescence imaging (Figure 2) and OCT. The EOG findings were abnormal in all patients. Electroretinography was performed in 3 patients and revealed normal cone and rod responses. Eight patients reported family members with similar visual disturbances. Mutation screening in 9 of these family members revealed the same mutation in the VMD2 gene as in the proband. Ophthalmic examination was performed in these 9 family members and revealed multifocal lesions in 5 individuals and a unifocal lesion in 4 individuals. Three of the 4 family members who had unifocal BVMD were 15 years or younger. The 44-year-old father of patient 4, who, like his daughter, carried a p.Lys194_Ala195insVal mutation in the VMD2 gene, displayed no abnormalities at elaborate eye examination and had normal EOG findings (Arden ratio: OD 2.0; OS 2.2).

CLINICAL FEATURES IN PATIENTS WITHOUT VMD2 MUTATIONS

This group, consisting of 2 male and 4 female patients, also had variable ages at onset of disease (mean age ± SD, 37.8 ± 14.0 years; median age, 38.5 years) and visual acuity (Table 2). Decreased visual acuity was the initial symptom in all patients. One patient had a family history of similar visual disturbances. Funduscopic, autofluorescence, and fluorescein angiographic characteristics in this group were comparable to those described in the group of patients who carried a mutation in the VMD2 gene (Figure 3A and B and Figure 4A and B). The extramacular lesions also had the same appearance at OCT as the central lesions, which resembled the OCT appearance of the lesions in the patients with multifocal vitelliform dystrophy who carried the VMD2 mutation (Figure 3C and D). The number of lesions was higher in this group, and all lesions were smaller than 1 optic disc area except for those in patient 15. Electro-oculographic findings were abnormal in 2 of 6 patients. Electoretino-
grams, recorded in 3 patients, showed normal findings. The father of patient 10 had multifocal pigmentary changes in both eyes in combination with normal EOG findings when he was examined at age 65 years. The 64-year-old brother of patient 10 had nonspecific multifocal pigmentary changes in both eyes. His EOG findings were within the normal range. Patient 15 experienced further loss of visual acuity at age 65 years, when neovascularization developed in the foveal area in both eyes.

Multifocal vitelliform dystrophy shows characteristic lesions that are also seen in BVMD and AFVD. In this retinal dystrophy, multiple yellow-white lesions are seen, often in the posterior pole and adjacent to the vascular arcades. This phenotype should be differentiated from several other disorders including acute exudative polymorphous vitelliform maculopathy syndrome, viti-
liginous (bird-shot) chorioretinitis, central serous chorioretinopathy, multifocal serous detachments of the retinal pigment epithelium (RPE), and ocular manifestations of large-cell non-Hodgkin lymphoma.\textsuperscript{32,33}

In the current study, we identified 7 different VMD2 mutations in 9 of 15 unrelated patients with multifocal vitelliform dystrophy. Comparison of clinical data between patients with and without confirmed mutations in VMD2 revealed differences in age at onset, visual acuity, and electrophysiological findings. Age at onset was highly variable but seems to be younger in the group of patients with a VMD2 mutation compared with those without this mutation (mean age, 29.8 vs 37.8 years, respectively). The loss of visual acuity was more pronounced in patients carrying mutations in VMD2. All patients with VMD2 mutations had abnormal EOG findings compared with 2 of 6 patients without VMD2 mutations. The widespread dysfunction of the RPE in patients with VMD2 mutations, as indicated by the abnormal EOG findings, is not unexpected since the bestrophin protein encoded by the VMD2 gene is located in the basolateral membrane of the RPE, where it is assumed to function as a calcium-sensitive chloride channel.\textsuperscript{34}

The appearance of the extramacular lesions varied in both groups, from small vitelliform spots to large and diffuse lesions. These extramacular lesions seemed to have the same characteristics as the central lesion at ophthalmoscopy, autofluorescence imaging, and OCT, although the stages between the lesions could differ.\textsuperscript{33} The appearance on fluorescein angiograms varied but was usually hyperfluorescent in both the early and late stages, probably dependent on the amount of vitelliform material in the lesions and the degree of atrophy.

Best vitelliform macular dystrophy generally causes lesions in the posterior pole, but at angiography using indocyanine green angiography, small peripheral hyperfluorescent spots may be observed.\textsuperscript{30} Histopathologic analysis has also shown abnormalities of the RPE, Bruch membrane, and the choroid throughout the fundus.\textsuperscript{11,37-39} It is possible that certain mutations in the VMD2 gene lead to more severely affected RPE, which may lead to the formation of extramacular vitelliform lesions. This is supported by the findings of 5 affected family members who also had multifocal lesions. Four affected family members had a unifocal central lesion. Three of these 4 patients were younger than 15 years. It is possible that extramacular lesions are more likely to develop with older age. Contradictory to this hypothesis is the finding that the father of patient 4 had the same mutation as his daughter despite having a normal-appearing fundus and even normal EOG findings, which is a rare exception to the rule that heterozygous carriers of a VMD2 mutation have abnormal EOG findings.\textsuperscript{17,20,60}

Additional genetic and environmental factors may also influence the development of multifocal vitelliform lesions in patients with mutations in the VMD2 gene. Analysis of the VMD2 and peripherin/RDS genes did not reveal a mutation in 6 patients. A disease-related alteration in these genes cannot be ruled out completely because heterozygous deletions of 1 exon or more are missed at polymerase chain reaction–based mutation analysis, and the introns and promoter were not analyzed for mutations. An alternative explanation could be the involvement of a thus far unknown gene in the pathogenesis of the multifocal vitelliform phenotype in these patients. The EOG findings were normal in 4 of 6 patients without a mutation in the VMD2 gene, indicating that there was no generalized disturbance of the electrical activity of the RPE in this subgroup. In patient 15, an alteration in the 5′ untranslated region of the peripherin/RDS gene was found, for which the pathogenic nature is unclear.

The clinical and genetic heterogeneity of retinal disorders with multifocal vitelliform lesions suggests that the clinical diagnosis is inaccurate and overlap between the phenotypes may occur. In our opinion, a patient who has multifocal vitelliform lesions in combination with an autosomal dominant inheritance pattern, abnormal EOG findings, and a mutation in the VMD2 gene should be diagnosed as having multifocal BVMD.

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REFERENCES

Until within recent years, the ophthalmologist in this country either was largely self taught so far as his specialty was concerned or obtained his first special training abroad, notable in Vienna, Berlin or London. For years Vienna was the Mecca for American would-be ophthalmologists. Those who studied there could be divided into three classes; those who had failed to obtain an internship in America, those who sought the prestige connected with foreign study and, those who were chiefly motivated by the desire to obtain the best possible training. Those in the third class were fewest, but generally remained abroad the longest.