A novel mutation in the \textit{VMD2} gene in an Italian family with Best maculopathy

A. Sodi (1), I. Passerini (2), F. Simonelli (3), F. Testa (3), U. Menchini (1), F. Torricelli (2)

(1) Department of Oto-Neuro-Ophthalmological Surgical Sciences, University of Florence, Italy.
(2) Eye Clinic, Second University of Naples, Italy.
(3) Department of Oto-Neuro-Ophthalmological Surgical Sciences, University of Florence, Italy; Viale Morgagni 85, 50134 Firenze, Italia. E-mail : asodi@tin.it

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INTRODUCTION

Vitelliform macular dystrophy (Best disease) is an autosomal dominant, early-onset form of macular degeneration in which the primary defect is thought to occur at the level of the retinal pigment epithelium (RPE) [1-3]. In the early stages, the disease is characterized by abnormal deposition of lipofuscin-like material at the posterior pole of the eye, with the presence of the typical vitelliform disc; later, disintegration of the central yellow lesions progressively leads to a scrambled egg appearance and then to a cyst with fluid (pseudo-hypopyon stage). Ultimately, visual acuity drops considerably, with the development of a central area of retinal atrophy. The course of the disease can be complicated by choroidal neovascularization, macular hole formation, and retinal detachment [4-9]. Because of RPE alterations, the electroculogram (EOG) is severely subnormal during all stages of the disease as well as in most of the carriers with normal fundus [10].

The \textit{VMD2} gene considered responsible for the disease [3] mapped to the long arm (q13) of chromosome 11. \textit{VMD2} encodes a 585-amino acid protein with an approximate mass of 68 kDa, which has been designated bestrophin. Immunochemical studies suggest that bestrophin is a plasma membrane protein localized to the...
basolateral surface of RPE cells; recently it was proposed that bestrophin is a chloride channel responsible for generating the standing transmembrane potential [11-19]. This hypothesis is in agreement with the abnormalities of EOG responses currently reported in Best disease patients.

In the present study, we screened the VMD2 gene for mutations in a group of Italian patients affected by autosomal dominant Best disease.

**MATERIALS AND METHODS**

Five Italian families, some members of which were affected by autosomal dominant Best disease, were recruited through the Hereditary Retinal Degenerations Referring Center of the Eye Clinic, University of Florence, Italy.

Criteria for the Best phenotype included the following: (1) juvenile-to-adult onset, (2) bilateral macular dystrophy with the typical round lipofuscin deposits at the posterior pole (including patients with different stages of the disease), (3) normal electroretinogram, (4) abnormal EOG with Arden ratio always below 160, and (5) autosomal dominant inheritance.

Research procedures were in agreement with institutional guidelines and the Declaration of Helsinki. Informed consent was obtained from all patients after the nature of the procedures to be performed was fully explained. All subjects included in the study underwent a complete eye examination including personal and family medical history, fluorescein angiography, electroretinogram (ERG), and EOG. The electrophysiological examinations were recorded according to ISCEV Guidelines.

In the same session, 10 ml of peripheral blood were obtained from the antecubital vein using EDTA-containing vials. After DNA extraction, PCR amplification of 11 exons and flanking intronic regions of VMD2 from DNA of Best subjects and relatives was undertaken by means of a multiblock MWG PCR System; cycling parameters for the reactions were optimized for each exon. The samples were examined using standard DHPLC techniques (WAVE-MAKER™ Software (Transgenomics, San Jose, CA, USA). Samples showing heteroduplex by DHPLC were then sequenced on an ABI sequencer (ABI Prism 3100) using Big Dye Terminator chemistry (Applied Biosystems, Foster City, KA, USA). PCR products were purified using the QIAquick PCR Purification Kit (Quiagen GmbH, Hilden, Germany). Finally, data obtained from the Sequence Analysis Software (Applied Biosystems) were aligned with the wild-type VMD2 gene sequence (Gen-Bank Database; http://www.ebi.ac.uk/queries/bq.html). According to standard existing guidelines, a sequence mismatch was considered as a disease-causing mutation only if absent in 150 healthy controls, associated with amino acid change, and confirmed by a new independent PCR, and, whenever possible, by restriction enzyme digestion. Finally, the degree of evolutionary conservation of the affected residue, directly related to its importance in the functional protein structure, was assessed by interspecies amino acid alignment analysis [20-23].

**RESULTS**

Fourteen Italian patients from five independent families, six members of which were affected by Best disease, were clinically examined. The pedigrees of the five families are shown in figure 1. DNA samples of all the individuals were analyzed for mutations in all 11 exons of the VMD2 gene using the DHPLC approach and direct sequencing techniques. The most significant clinical and genetic features of our series are summarized in table I.

Three missense mutations (R25W, R218C, R218G) were identified in the six affected patients (V4, V53, V183, V217, V310, V373) and in six clinically healthy relatives (V186, V187, V219, V280, V374, V375). In two unaffected patients (V52, V54), no mutation was detected.

The R218G mutation (fig. 2) had not been previously described, and was not detected in 150 unaffected control individuals (300 chromosomes) of Italian origin.

**Key-words:** Best disease, VMD2 gene, mutation analysis, choroidal neovascularisation.
Table I
Clinical and genetic results. Patient V55 (mother of patients V52 and V54) was not available for the genetic test. Patients V52, V54, V219, and V374 were not available for the electrophysiological examinations.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Fundoscopy</th>
<th>EOG (Arden test)</th>
<th>ERG</th>
<th>VMD2</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM (V373)</td>
<td>6</td>
<td>♀</td>
<td>OU: vitelliform disc</td>
<td>RE: 122 LE: 140</td>
<td>Normal</td>
<td>R218G</td>
<td></td>
</tr>
<tr>
<td>MM (V374)</td>
<td>3</td>
<td>♂</td>
<td>OU: normal</td>
<td>not performed</td>
<td>Not performed</td>
<td>R218G</td>
<td></td>
</tr>
<tr>
<td>AM (V375)</td>
<td>38</td>
<td>♂</td>
<td>OU: normal</td>
<td>RE: 140 LE: 145</td>
<td>Normal</td>
<td>R218G</td>
<td></td>
</tr>
<tr>
<td>IG (V217)</td>
<td>56</td>
<td>♂</td>
<td>OU: vitelliform disc (partially atrophic)</td>
<td>RE: 130 LE: 145</td>
<td>Normal</td>
<td>R25W</td>
<td></td>
</tr>
<tr>
<td>StG (V280)</td>
<td>23</td>
<td>♂</td>
<td>OU: normal</td>
<td>RE: 128 LE: 133</td>
<td>Normal</td>
<td>R25W</td>
<td></td>
</tr>
<tr>
<td>SaG (V219)</td>
<td>37</td>
<td>♀</td>
<td>OU: normal</td>
<td>Not performed</td>
<td>Not performed</td>
<td>R25W</td>
<td></td>
</tr>
<tr>
<td>MS (V310)</td>
<td>37</td>
<td>♂</td>
<td>OU: vitelliform disc (scrambled egg)</td>
<td>RE: 130 LE: 109</td>
<td>Normal</td>
<td>R218C</td>
<td></td>
</tr>
<tr>
<td>DP (V4)</td>
<td>41</td>
<td>♀</td>
<td>RE: advanced CNV, fibrous scar LE: vitelliform disc, (scrambled egg)</td>
<td>RE: 114 LE: 102</td>
<td>Normal</td>
<td>R218C</td>
<td></td>
</tr>
<tr>
<td>ALB (V53)</td>
<td>12</td>
<td>♂</td>
<td>RE: advanced CNV, fibrous scar LE: vitelliform disc (scrambled egg)</td>
<td>RE: 120 LE: 115</td>
<td>Normal</td>
<td>R218C</td>
<td></td>
</tr>
<tr>
<td>SM (V54)</td>
<td>20</td>
<td>♀</td>
<td>OU: normal</td>
<td>Not performed</td>
<td>Not performed</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>FM (V52)</td>
<td>23</td>
<td>♂</td>
<td>OU: normal</td>
<td>Not performed</td>
<td>Not performed</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>MF (V183)</td>
<td>59</td>
<td>♂</td>
<td>RE: vitelliform disc (scrambled egg) LE: multiple vitelliform discs</td>
<td>RE: 150 LE: 145</td>
<td>Normal</td>
<td>R25W</td>
<td></td>
</tr>
<tr>
<td>CF (V186)</td>
<td>22</td>
<td>♀</td>
<td>OU: normal</td>
<td>RE: 150 LE: 155</td>
<td>Normal</td>
<td>R25W</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: R218G mutation (652C→G) screened by DHPLC and corresponding sequence chromatogram: nucleotide substitution is indicated by an arrow in the chromatogram.

Three patients showed a similar egg yolk-like appearance in both eyes (V217, V310, V373), but three different mutations (R25W, R218C, R218G).

Patient V373, with the novel mutation (R218G), was a 6-year-old child whose macular distrophy was discovered during a school ophthalmological screening. Her visual acuity was only minimally affected. Unfortunately, in this case it was not possible to take a picture of the eye fundus because of the poor collaboration on the part of the very young patient. The father (V375) and the very young brother (V374) of patient V373 did show no fundus abnormality. The EOG was abnormal in the father (V375), while it could not be performed in the brother (V374).

In the other two cases (patient V217 with R25W and patient V310 with the R218C mutation), the typical vitelliform structures were found during routine ophthalmoscopy at the age of approximately 30 years; visual acuity was severely reduced in both eyes.

Patient V183 showed the R25W mutation and a multifocal appearance of the disease with the contemporary presence of more than one egg yolk-like deposit at the posterior pole in the left eye.

A mutation was also identified in the offspring (in both cases one daughter and one son, both clinically unaffected) of patients V217 and V183.

Another two patients (mother and son) (V4, V53) showed the R218C mutation and a peculiar clinical
picture: an advanced stage of the disease with disruption of the lipofuscin-like deposit in one eye and the onset of choroidal neovascularization in the other eye. In both cases, the onset of neovascularization was very early (25 years of age for the mother and 9 years of age for the son).

In this family, the clinically healthy sister (V55) of the affected patient (V4) was not available for the genetic test. Her son (V52) and daughter (V54) accepted to undergo the genetic investigation, but the screening of the VMD2 gene did not detect a mutation.

**DISCUSSION**

Mutations of the VMD2 gene were detected in heterozygosis in all the affected patients and in some unaffected relatives. One mutation (R218G) had not been previously reported in the literature, whereas the other ones (R218C, R25W) have already been described [17, 24, 25].

R218G is located in exon 6. It is a missense mutation and it involves an amino acid that is highly conserved across different species, involved in the most common VMD2 mutations. This particular amino acid may represent a mutation hotspot with significant functional relevance; it is probably located in a protein region serving as a site of attachment or an active site for an enzyme. This mutation determines an amino acid change from arginine to glycine, which is potentially effective because the two amino acids show different properties: arginine is a basic polar molecule with hydrophilic properties, while glycine is nonpolar and hydrophobic.

In our series, the same mutation was associated with differences in clinical phenotypes (early choroidal neovascularization, unifocal or multifocal appearance). In fact, in one family the R218C mutation was associated with an early onset of choroidal neovascularization in the affected mother and her 9-year-old son (fig. 3), while no choroidal neovascularization was reported in another family sharing the same mutation.

Another patient with the R25W mutation showed a typical isolated vitelliform disc in the macular area.
(fig. 4), while another family with the same mutation showed a multifocal location of the vitelliform deposits (fig. 5). Finally, a mutation was also identified in the apparently normal daughter and son of two affected patients; they showed no visual loss or abnormalities of the eye fundus, but an abnormally reduced Arden ratio on the EOG examination was always noted. These data confirm that Best disease might present variable expressivity, with some patients only showing electrophysiological alterations without the overt expression of the disease and others showing significant abnormalities of the posterior pole with sometimes variable clinical features.

In conclusion, three mutations of the VMD2 gene were identified in a group of six Italian patients with a clinical diagnosis of Best macular dystrophy and in some of their clinically unaffected relatives. One of these mutations, R218G, had never been reported in the literature. In our series, the genotype-phenotype correlation was very poor and patients sharing the same mutation might present very different clinical pictures. On the other hand, similar fundus appearances were reported in patients with different genotypes.

Further efforts to identify the complete mutations spectrum of the VMD2 gene in Best maculopathy are required for a better understanding of the physiopathology of the disease and a better evaluation of possible correlations between specific genotypes and the natural history of the macular dystrophy.

REFERENCES