Phenotypes of 16 Stargardt macular dystrophy/fundus flavimaculatus patients with known ABCA4 mutations and evaluation of genotype–phenotype correlation

Abstract Purpose: To determine the phenotypic variability in patients with compound heterozygous or homozygous ABCA4 mutations, and to correlate the phenotypes with the functional properties of the altered protein. Methods: Sixteen patients from 13 families with signs of Stargardt macular dystrophy/fundus flavimaculatus and known mutations on both alleles of the ABCA4 gene (15 compound heterozygous, one homozygous) were characterized by clinical examination, fundus autofluorescence, psychophysics (color vision, kinetic and two-color dark- and light-adapted static threshold perimetry), and electrophysiology (Ganzfeld, multifocal ERG, EOG). Results: The homozygous 5917delG mutation resulted in the earliest disease manifestation (at 5 years) and a general cone–rod dysfunction, whereas the compound heterozygous mother (5917delG, G1961E) exhibited a very mild phenotype. Compound heterozygotes for the IVS40+5G→A and the C1488Y or Y362X mutation showed also an early age of onset but only a central dysfunction. The effect of the 2588G→C mutation, the G1961E mutation, and the complex mutation L541P-A1038V depended on the mutation in the second allele. Genotype–phenotype correlation appeared possible in most instances. Psychophysics revealed a simultaneous yet not necessarily congruent cone and rod dysfunction. Conclusions: The type and combination of ABCA4 mutations in compound heterozygous patients determined were compatible with the severity of the phenotype as to age of onset and the functional consequences in the majority of patients. Unexplained phenotypic differences indicate the influence of other factors. ABCA4 mutations result in cone and rod dysfunction. Different disease durations limit the power of presently available genotype–phenotype correlations.

Introduction

Stargardt macular dystrophy (STGD) [47] is one of the most common hereditary macular dystrophies [7]. The gene for the frequent autosomal recessive form, i.e., STGD1, has been mapped to the short arm of chromosome 1 [24], and mutations in the ABCA4 (formerly ABCR) gene among patients with STGD/fundus flavimaculatus (FFM) were first identified by Allikmets et al. [3]. Further mutational analyses have shown an average mutation detection rate of 30–70% in STGD1 patients [29, 38, 41, 42, 44, 46, 51]. In addition, ABCA4 mutations have been identified in autosomal-recessive cone–rod dystrophy and retinitis pigmentosa [8, 25, 33, 35, 41, 43]. Heterozygous mutations in the ABCA4 gene in age-related macular degeneration have been interpreted controversially [1, 2, 11, 42]. The functional analysis of mutant ABCA4 protein revealed a range of biochemical defects [49]. Due to its suggested topological organization [21] there is a possi-
bility of variable phenotypes in affected patients depending on the position of the underlying mutations. It is proposed, that the ABCA4 activity inversely correlates with the disease severity [50], from age-related disorders to severe cone–rod and rod–cone dystrophies. An agreement was found for the following grading system [50]: (1) Null mutations cause the most severe phenotype, i.e., autosomal-recessive retinitis pigmentosa (RP). (2) Combinations of a severe mutation with a moderate mutation result in autosomal-recessive cone–rod dystrophy. (3) Missense mutations show a varying protein function impairment depending on the effect of the mutation. The combinations severe/mild or moderate/moderate cause the STGD/FFM phenotype. (4) Single heterozygous mutations result in normal or very mild phenotypes possibly predisposing to age-related macular degeneration (AMD).

Several studies have reported homozygous or compound heterozygous ABCA4 mutations in patients with cone–rod or rod–cone dystrophy [8, 25, 33, 35, 41, 43]. The term “RP” or “RP-like dystrophy”, used to describe the phenotype of these patients, may lead to confusion. For example, in the patients from the Netherlands [10], an early severe maculopathy was evident, whereas other authors used the term “a.r. RP” without detailed fundus-copic description [33, 41].

Fishman et al. [17] identified three different phenotypes among 49 STGD patients associated with single heterozygous (16 patients) or compound heterozygous (13 patients) ABCA4 mutations and suggested that there is an association between the heterozygous ABCA4 mutation (Gly1961Glu change, exon 42) in one allele and a certain phenotype (fundus: small macular lesion; visual acuity: well preserved; angiography: no dark choroid; Ganzfeld ERG: normal). Lewis et al. [29] and Shroyer et al. [45] proposed that selected combinations of specific ABCA4 mutant alleles may determine at what age the onset of visual impairment occurs.

ABCA4 was first reported to be expressed exclusively in rods [2]. This was a surprise as STGD usually presents as a macular disease, and results on rod ERGs have been equivocal [5, 14, 20, 27, 34, 37, 39, 40, 48] although dark adaptation has been documented to be delayed [16, 19, 22]. Recently, Molday et al. [36] have shown that ABCA4 is present in cones as well as in rods, and have suggested that the visual deterioration arises directly from ABCA4-mediated foveal cone degeneration. Sun et al. [49] provided further insight into the transport mechanism of the ABCA4 protein with the functional analysis of human ABCA4 and its variants.

The intent of the present study is to describe the phenotypic variability of both cone and rod function in patients who carry ABCA4 gene mutations on both alleles, and to correlate the phenotypes with the biochemical effects described for the ABCA4 protein [49].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fundus classification by Fishman et al. [17]</th>
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<tr>
<td>Phenotype</td>
<td>Fundus</td>
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<tr>
<td>I</td>
<td>Small atrophic-appearing foveal lesion, localized perifoveal yellowish-white flecks</td>
</tr>
<tr>
<td>II</td>
<td>Numerous yellowish-white fundus lesions throughout posterior pole</td>
</tr>
<tr>
<td>III</td>
<td>Extensive atrophic-appearing RPE changes</td>
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Methods

Sixteen patients with clinical signs of STGD/FFM from 13 families (one mother–daughter pair, two siblings of two families) were included in the analysis due to the fact that two likely disease-causing ABCA4 alleles were identified in a previous study [42]. Informed consent was obtained from all subjects after explanation of the testing procedures. The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Regensburg. All but patient 7a were examined by at least one of the authors (C.G., M.A.D., B.L.). A careful family history was taken with regard to eye problems in the patients and grandparents, in particular with respect to AMD. The age at disease onset was defined as the age at which decreased visual acuity was first noted. The clinical ophthalmic examination performed in all patients included best-corrected visual acuity (VA), slit-lamp examination, and dilated fundus examination by direct and indirect ophthalmoscopy (fundus classification according to Fishman et al. [17]; Table 1).

Psychophysics

Color vision was tested with a Nagel anomaloscope in 11/16 patients and with the Lanthony Panel D15, desaturated, arrangement test in patient 5. Kinetic visual fields were tested on a Goldmann perimeter with targets V-4e and III-4e to I-1e in both eyes of 12 of 16 patients. Static two-color threshold perimetry was performed on the eye with the better VA (except patient 12, in whom both eyes were tested) as follows: dark-adapted state (45 min dark adaptation, 500 nm cut-off and 600 nm cut-on filter; 12/16 patients), light-adapted state (600 nm cut-on filter; 11/16 patients), modified HFA (Humphrey Field Analyzer, by Fitzke, Institute of Ophthalmology, London), 30/2 central field strategy, sensitivity loss expressed according to the STATPAC algorithm [23]. Light- and dark-adapted thresholds were determined using the values with the 600 nm (light-adapted) cut-on and 500 nm (dark-adapted) cut-off filter. The results were displayed as the mean sensitivity losses in rods (RSL, at 500 nm) and cones (CSL, at 600 nm) within 13° (16 test loci) or beyond 13° (58 test loci), and compared to the mean sensitivity of normal subjects (eight healthy subjects aged 20–30 years, 10th percentile).

Electrophysiology

Ganzfeld ERG was recorded with DTL electrodes (Spirit; Nicolet) in 9 of 16 patients according to the ISCEV standard [32] and in 6 of 16 patients in the photopic state only. The amplitudes and implicit times were compared to age-matched normal values. Multifocal ERG (mERG) was recorded with a bipolar Burian–Allen (BA) electrode in 15 of 16 patients in the eye with the better VA (except patient 12: both eyes tested) using a standard set-up [VE-RIS Scientific 3.1; 103 hexagons (except in patient 2; 61 hexagons); luminance: 135 cd/m² (white), 0.1 cd/m² (black); recording...