HHS Public Access

Author manuscript

Adv Exp Med Biol. Author manuscript; available in PMC 2024 October 17.

Published in final edited form as:

Adv Exp Med Biol. 2023 ; 1415: 289–295. doi:10.1007/978-3-031-27681-1_42.

Genotype–Phenotype Association in *ABCA4*-Associated Retinopathy

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Abstract

Stargardt disease (STGD1) is the most common inherited retina degeneration. It is caused by biallelic *ABCA4* variants, and no treatment is available to date. STGD1 shows marked phenotypic variability, especially regarding the age of onset. The underlying genotype can partially explain this variability. Notably, a subset of ABCA4 variants was previously associated with an earlier disease onset than truncating *ABCA4* variants, pointing toward pathogenic mechanisms beyond the loss of gene function in these patients. On the other end of the spectrum, variants such as p. Gly1961Glu were associated with markedly slower extrafoveal disease progression. Given that these drastic differences in phenotype are based on genotype (resulting in important prognostic implications for patients), this chapter reviews previous approaches to genotype–phenotype correlation analyses in STGD1.

Keywords

Stargardt disease; ABCA4-associated retinopathy; Disease Progression; Genotype; Genotype–Phenotype correlation

1 Introduction

Stargardt disease (STGD1, or ABCA4-retinopathy) is caused by biallelic variants in the ATP Binding Cassette Subfamily A Member 4 (ABCA4) gene and represents the most frequent inherited retinal degeneration [1]. Dysfunction of the retinal-specific phospholipid-transporting ATPase ABCA4 results in lipofuscin accumulation in the retinal pigment epithelium (RPE), leading to eventual atrophy of the RPE and neurosensory retina [2, 3].

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Conflict of Interest Statement The authors have declared that no conflict of interest exists.

The clinical course of Stargardt disease [4] is characterized by centrifugally progressing RPE atrophy [5, 6], centrifugally progressing flecks [7, 8], and peripapillary sparing [9]. The typical age of onset (in terms of symptoms) is between the first and the second decade of life, but there is a wide variation in the age of onset [10]. Initially, best-corrected visual acuity may be preserved due to foveal sparing [11].

Accumulation of lipofuscin in the RPE is the histopathological hallmark of STGD1 [12] and can be quantified in vivo using autofluorescence imaging [13]. Importantly, this increase of autofluorescence precedes the loss of function, implying that vision could be preserved if lipofuscin accumulation is slowed [13]. Multiple therapeutic approaches are being evaluated in clinical trials or preclinical models [2, 3]. Therapeutic strategies currently include slowing vitamin A delivery to the retina (e.g., RBP4-antagonist STG-001 [ClinicalTrials.gov Identifier: NCT04489511]), slowing the visual cycle biochemically (e.g., RPE65-inhibitor Emixustat [NCT03772665]), or slowing the rate of lipofuscin accumulation (e.g., ALK-001, NCT04239625). Other currently studied therapeutic approaches include the complement factor C5 inhibitor Zimura (NCT03364153) and oral metformin (NCT04545736).

Despite marked phenotypic differences among patients with STGD1, genotypic characteristics are not considered within inclusion or exclusion criteria in ongoing trials (beyond the confirmation of the diagnosis). Previously, a subset of *ABCA4* variants was shown to be associated with an earlier disease onset than truncating *ABCA4* variants implying pathogenic mechanisms beyond the mere loss of function [14]. Potentially, these variants lead to heterogeneity of treatment effects (hypothetically, patients with these severe variants benefit to a lesser degree from gene-replacement therapy). On the other end of the spectrum, patients with mild variants may lead to underpowered studies due to slow progression rates, diminishing differences in the treatment and control group (in the absence of stratified sampling).

Given the importance of understanding phenotypic variability and its underlying genetic correlates to inform patients of their prognosis and clinical trial design, this chapter reviews previous approaches to genotype–phenotype correlation analyses in STGD1.

2 Genotype–Phenotype Correlation Analyses

Published genotype–phenotype analyses approaches in *ABCA4*-associated retinopathy can be subclassified according to (i) the metric of disease severity and (ii) the scale of the resulting genotype severity estimate. Early genotype–phenotype correlation analyses were based on qualitative, phenotypic descriptions of disease severity and reported variant severity in a qualitative manner (cf. Sect. 2.1 below). More recent studies employed semi-quantitative metrics of disease severity, such as the Lois electrophysiologic (ERG) classification [15] or ultra-widefield fundus autofluorescence imaging [16]. However, these two studies reported the variant severity in a four- or three-step ordinal scaled manner (cf. Sect. 2.2 below). Only one study so far applied a quantitative, interval-scaled metric of disease severity and reported variant severity in an interval- scaled manner (cf. Sect. 2.3) [14].

This review focuses only on clinical genotype–phenotype analyses. Besides these clinical approaches, complemental laboratory studies have investigated the effect of *ABCA4* variants, including biochemical assays of ATPase activity [17–19], of protein processing [20], in vitro splice assays [21], and rodent models [22–24].

2.1 Qualitative Genotype–Phenotype Correlation Analyses

Shortly after the identification of *ABCA4* as the causal gene for STGD1 [25–28], qualitative genotype–phenotype correlations were reported. These were based on either self-reported age of onset or descriptive characterizations of the fundus.

For example, Rozet et al. reported in 1998 that they could identify truncating and missense variants in patients with a classical STGD1 phenotype and only missense variants in patients with a lateonset STGD1 phenotype (termed FFM in their publication) [28]. Lewis et al. subsequently provided the first granular genotype–phenotype analysis highlighting that variants 5′ to codon 863 tend to be associated with an earlier onset [29].

2.1.1 Qualitative Descriptions of Mild Variants—

In another early study, Fishman et al. 1999 [30] reported that the typical phenotype associated with *ABCA4* p.Gly1961Glu was characterized by central atrophy but preserved retina-wide cone and rod function as measured by full-field electroretinogram (ERG). This association of p. Gly1961Glu with the absence of severe retina-wide cone and rod sensitivity loss was also confirmed by Gerth et al. using dark-adapted two-color perimetry [31]. *ABCA4* p.Gly1961Glu has also been linked to the “optical gap phenotype,” characterized by disruption of the ellipsoid zone at the fovea, suggesting that the p. Gly1961Glu variant selectively affects foveal cones. This variant was also shown to lead to a lesser degree of lipofuscin accumulation using quantitative autofluorescence imaging [32, 33].

Further, the *ABCA4* variant p.Asn1868Ile, which has a minor allele frequency of ~7% in the general population, was linked to a mild phenotype [34]. It is characterized by a late age of onset and foveal sparing, as well as an overall lower degree of lipofuscin accumulation [32–34].

2.1.2 Qualitative Descriptions of Severe Variants—

Fukui et al. further extended the spectrum of STGD1 with the description of two brothers homozygous for c.1760 + 2 T > G with severe pan-retinal degeneration and bone-spicule-like hyperpigmentary changes [35]. Further variants have been linked to a severe STGD1 manifestation with an early onset, including the complex allele p.[Leu541Pro;Ala1038Val] and the intronic variant c.5461–10 T > C [36].

More recently, more severe forms of early-onset STGD1 have also been referred to as “Generalized Choriocapillaris Dystrophy,” [37] and “Rapid-Onset Chorioretinopathy” [36]. However, clear-cut genetic or phenotypic characteristics that differentiate these severe forms of STGD1 from the overall spectrum of STGD1 are lacking to date.

2.2 Semi-quantitative Genotype–Phenotype Correlation Analyses

Few studies have summarized the severity of *ABCA4* variants based on interval-scaled metrics of disease severity but reported the results as an ordinal-scaled, four-step ranking of severity [38].

Fakin et al. reported in 2016 a genotype–phenotype correlation analysis based on 82 patients with one of 15 missense variants of interest that were in trans with an established null variant (i.e., compound heterozygous for a null and missense variant; referred to "hemizygous" by Fakin et al.) [38]. Further patients homozygous for a subset of these missense variants (N = 10) and patients with biallelic null variants (N = 10) served as comparators. In a first step, the Lois ERG classification was applied to the data of the "hemizygous" patients. If a given variant in trans with a null variant was always associated with a Lois ERG group 1 phenotype (pattern ERG abnormality, but normal full-field ERG), it was classified as mild (e.g., p.Gly1961Glu and p.R2030Q). Variants in trans with a null variant, which were predominantly associated with a Lois ERG group 3 phenotype (abnormal pattern ERG, abnormal photopic, and scotopic full-field ERG), were classified as "null-like." Variants in trans with null variants associated with a range of ERG phenotypes were classified as intermediate. In a second step, the phenotype of the "hemizygous" patients was compared to the linear slope of the dark-adapted A-wave amplitude in patients with biallelic null variants. Specifically, the number of eyes that fell within or outside of the 95% prediction interval for patients with biallelic null variants was evaluated. Based on this ERG analysis, the intermediate variants were stratified as "intermediate +" (variants in trans with null that were mostly associated with a dark-adapted A-wave amplitude outside of the 95% prediction interval for biallelic null variants) and "intermediate-" (dark-adapted A-wave amplitude within of the 95% prediction interval for biallelic null variants) [38]. Last, the authors compared patients with variants that led in trans with null variants to a severe ERG phenotype, to patients that were homozygous for these missense variants. Patients homozygous for some of these variants (p.Arg212Cys, p.Arg1108Cys, and p.Pro1380Leu) had significantly better amplitudes compared to their "hemizygous" counterparts. Accordingly, these variants were classified as "intermediate -" [38]. In a later publication, deep-intronic mutation c.5196 + 1137G > A variant was added to the classification as "intermediate" based on the same approach [39].

This approach separated previously considered mild variants (e.g., p.Gly1961Glu) from those previously reported as severe (e.g., p. Cys2150Tyr, or p.[Leu541Pro;Ala1038Val]). However, the proposed classification approach has several limitations: (i) the approach always necessitates data from patients with a known null or "null-like" variant in trans, (ii) does not allow to identify variants that are more severe than null-variants, and (iii) provides only ordinal-scaled estimates of variant severity [38].

Recently, Heath Jeffery and coworkers expanded on this classification based on a large cohort of patients imaged using the total lesion size (defined by the outer boundary of flecks) measured from ultra-widefield fundus autofluorescence imaging. Specifically, the authors evaluated 81 STGD1 patients from 65 families. Patients were either classified based on prior estimates for variant severity (group A: biallelic null variants; group B1: with a mild variant [p. Gly1961Glu, p.Asn1868Ile]; group B2: with an intermediate

variant [p.Leu2027Phe, or the complex allele p.[Gly863Ala,Gly863del;Asn18 68Ile]), or an uncertain severity (group C). Then, patients in group C with a self-reported age of onset <14 years were assigned into the null/severe variant cohort and patients with a total lesion size of <200 mm^2 as group B1 and with a total lesion size >200 mm^2 as group B2. Based on this approach, Heath Jeffery and coworkers estimated the severity for 32 variants.

Like the previous approach, the analysis did not fully account for the age-dependent nature of the applied severity metric (total lesion size). These ordinal-scaled estimates of variant severity result in a significant loss of information.

2.3 Quantitative Genotype–Phenotype Correlation Analyses

Only one previous study employed an interval-scaled metric as the criterion for genotype–phenotype correlation analysis and reported an interval-scaled metric for the variant severity [14].

Cideciyan and coworkers evaluated 66 patients with STGD1 using light-adapted and dark-adapted perimetry [14]. According to the authors, sensitivity loss could be described by a central, centrifugally progressing component and a retina-wide component (mean retina-wide sensitivity loss for loci at ≥30° eccentricity from the fovea). In 36 patients, abnormal extramacular rod or cone sensitivity was evident at one or more visits. It progressed at an average rate of 1.1 log/decade (rod sensitivity loss) and 0.45 log/decade (cone sensitivity loss). Based on these slope estimates, the patient's age, and the respective retinal sensitivity, the authors computed for each patient the age of retina-wide disease initiation (ADI). For patients with two truncating variants, the ADI was 10.6 years. Subsequently, the authors estimated for each variant the severity (in terms of the delay of retina-wide disease initiation) assuming an additive contribution of each variant. Notably, one-third of the nontruncating variants were found to cause earlier onset disease than truncating variants. This was considered as an evidence for pathogenic pathways beyond the mere loss of biallelic function [14]. In a later report by the same group, it was quantitatively shown using this approach that the complex variant p.[Leu541Pro;Ala1038Val] is associated with an earlier disease onset than the p.Ala1038Val variant [22].

The disadvantages of their approach were that all analyses were based on the assumption of an invariant slope of sensitivity loss across patients [14]. However, the raw data are suggestive of some between-patient variability in the slope (Figure 3 in Cideciyan et al.) [14]. Further, the model of an additive association of variant severity with the ADI is most likely an oversimplification of the underlying biology.

3 Future Directions

Severity estimates based on clinical characteristics are available for many *ABCA4* variants. Importantly, these estimates of variant severity are mostly congruent. However, four gaps in the literature are evident that could be addressed.

3.1 Interval-Scaled Severity Measures as Input

Previous studies have summarized continuous data as an ordinal-scaled measure of severity. However, dichotomizing (or discretizing) a continuous variable will result in a considerable loss of power [40]. The one study that applied an interval-scaled measure of disease severity in terms of the ADI (based on the sensitivity loss and patient's age) did so assuming an invariant slope of sensitivity loss across subjects [14]. Using linear mixed model analysis, it would be possible to derive slope estimates for patients with multiple measurements as well as for patients with only a single measurement (through "pooling") [41].

3.2 Interval-Scaled Reporting of Variant Severity

Second, for most *ABCA4* variants, only ordinal-scaled (four- or three-step) data have been published, even though interval-scaled measures of disease severity constituted the starting point for these analyses. This drastic reduction in granularity complicates the comparison of estimates of allele severity across studies. It also hinders the comparison to in vitro splice assay data, which is typically reported in an interval-scaled manner [21]. Moreover, *ABCA4* variants more severe than truncating and frameshift variants have been described (e.g., the complex variant p. [Leu541Pro;Ala1038Val]) [14, 22]. However, the above-mentioned ordinal-scaled classification does not provide a distinct class for more severe variants than "null-like" variants. From a clinical trial perspective, identifying variants more severe than "null-like" variants is a priority since disease mechanisms beyond the mere loss of function could contribute to treatment effect heterogeneity.

3.3 Differentiating Age-Dependent Severity from Genuine Phenotypic Differences

Third, the age-dependent nature of disease severity metrics has not always been (explicitly) accounted for in the genotype–phenotype analyses. The framework outlined by Cideciyan and coworkers, which defined a fixed criterion age for each patient based on the (estimated) age of retina- wide disease initiation, provides a severity metric independent of the patient's age of presentation [14].

3.4 Latent Variable Methods

Last, measures for disease severity for STGD1 have not been assessed systematically using latent variable methods. Outside of "stationary" dystrophies (e.g., congenital stationary night blindness or achromatopsia), the degree of rod and cone degeneration tends to be correlated in most IRDs. While the commonly applied Lois ERG classification for STGD1 treats photopic and scotopic B-wave amplitudes as differential information [15], these measures are partially redundant in STGD1. On the other hand, BCVA appears to be reflective of an unrelated latent process in STGD1. As a result, the common *ABCA4* p.Gly1961Glu variant has been reported to be "mild" (based on full-field ERG, peripheral perimetric sensitivity) [38], but as "moderate" (based on the age of onset [i.e., BCVA]) [42]. Thus, the application of latent variable methods (e.g., principal component analysis or factor analysis) is warranted to pool redundant visual function metrics (improving the signal-to-noise ratio) and identify nonredundant (low covariance) metrics of disease severity.

4 Summary

Previous studies have reported qualitative phenotype–genotype correlations at the level of individual variants and few in a semi-quantitative or quantitative manner [14, 16, 38]. Only one previous publication has accounted for the time-dependent nature of disease severity metrics and reported a genotype–phenotype analysis with interval-scaled estimates of variant severity [14]. Besides larger sample sizes, future genotype–phenotype analyses in STGD1 could be improved by employing interval-scaled measures of disease severity, reporting interval-scaled measures of allele severity, using age-independent disease severity metrics (instead of disease severity at patient's age of presentation) and applying latent variable methods to separate redundant information from genuine phenotypic characteristics.

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