Supplemental Figure Titles, Table Titles, and Table Legends

Supplemental Figure S1. Schematic representation of the midigene assay workflow

Supplemental Figure S2. RT-PCR analysis for the non-canonical splice site variants in *ABCA4*

Supplemental Figure S3. Sequence analysis of RT-PCR products from *in vitro* splice assays

Supplemental Figure S4. Splice defect due to c.768G>T in photoreceptor precursor cells

Supplemental Figure S5. Capillary fragment analyzer results for RT-PCR products of wild-type and mutant midigenes

Supplemental Figure S6. Capillary fragment analyzer results for human retina RT-PCR products

Supplemental Table S1. Sequence variants in wild-type BA clones

Supplemental Table S2. *In-silico* predictions for the non-canonical splice site variants
AF = Allele Frequency; n.a. = not applicable

Supplemental Table S3. Capillary electrophoresis-based quantitative analysis of splice products from midigenes. For each fragment, measurement was performed in duplicate, corrected for fragment size and expressed as area under the curve (Corr.peak area). Fragment sizes assessed by capillary system (size) did not always match with the ones calculated after agarose gel running (Fragment; Detected on agarose gel) and Sanger sequence validation (Actual size). For fragments marked with an asterisk, sequence results were not available. Fragment quantities were expressed as percentage over the entire fragment mix.

Supplemental Table S4. Correlation between splice site variants and clinical features in STGD1 patients. All variants were previously found in a compound heterozygous manner (Cornelis et al. 2017 and references therein), except for eight variants, i.e. c.160+5G>C, c.302+4A>C, c.768G>T, c.4253+4C>T, c.4667G>C, c.5461-10T>C, c.5461-8T>G and c.6729+5_6729+19del. When clinical information was available for the homozygous cases, this was used to assess the severity of the corresponding variant. If more than one patient carried a variant, we assessed the clinical phenotype

of the case(s) for which the second variant was known, and if this variant could be classified either as severe variant (stop mutation, frameshift mutation, canonical splice site mutation) or as mild variant based on statistics (p.[Gly863Ala, Gly863del], p.(Ala1038Val), p.(Gly1961Glu), c.5714+5G>A, and p.(Arg2030Gln)(Cornelis et al. 2017). A non-canonical splice variant was deemed mild if the second allele was severe and the patient showed an intermediate STGD1 phenotype. It was considered severe if the second allele was severe and the patient showed early-onset STGD1 (onset ≤ 10 yrs). The variant was also considered severe if the second allele was mild and the patient showed an intermediate STGD1 phenotype. In 25/47 variants, we observed concordance between the *in vitro* splice assay result and the clinical assessment in one or more patients; in 22/47 variants a conclusion was not possible.

Supplemental Table S5. Gateway-tagged *ABCA4* primers to amplify genomic segments spanning exon 40 and 39-41

Supplemental Table S6. Gateway-tagged *ABCA4* primers to amplify genomic segments from bacterial artificial chromosome clone DNA

Supplemental Table S7. Primers used to sequence wild-type *ABCA4* genomic segments in wild-type entry clones *= entry clone vector forward primer; **= entry clone vector reverse primer

Supplemental Table S8. Oligonucleotides to introduce mutations in wild-type entry clones

Supplemental Table S9. Primers to analyze mutations introduced in wild-type entry clones

Supplemental Table S10. Primers for reverse transcription-PCR analysis. #= Rhodopsin exonic primer; *53= Rhodopsin 5' UTR

Supplemental Table S11. Capillary electrophoresis-based quantitative analysis of human retina RT-PCR products

Supplemental Table S12. Densitometric analysis of splice defects due to selected *ABCA4* variants. For each fragment, intensity measurement was performed in duplicate (Raw intensity 1 and 2) and corrected for fragment size (corrected intensity 1 and 2). For fragments marked with an asterisk (*), sequence results were not available. Highlighted in red are the fragment sizes that were arbitrarily assessed in the absence of sequenced fragments of the same size. (#) indicates the fragments for which sequencing results were obtained from larger amplicons (Supplemental Fig. S3).