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Resolving the dark matter of ABCA4 for 1054 Stargardt disease probands through integrated genomics and transcriptomics.

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PURPOSE: Missing heritability in human diseases represents a major challenge, and this is particularly true for ABCA4-associated Stargardt disease (STGD1). We aimed to elucidate the genomic and transcriptomic variation in 1054 unsolved STGD and STGD-like probands. METHODS: Sequencing of the complete 128-kb ABCA4 gene was performed using single-molecule molecular inversion probes (smMIPs), based on a semiautomated and cost-effective method. Structural variants (SVs) were identified using relative read coverage analyses and putative splice defects were studied using in vitro assays. RESULTS: In 448 biallelic probands 14 known and 13 novel deep-intronic variants were found, resulting in pseudoexon (PE) insertions or exon elongations in 105 alleles. Intriguingly, intron 13 variants c.1938-621G>A and c.1938-514G>A resulted in dual PE insertions consisting of the same upstream, but different downstream PEs. The intron 44 variant c.6148-84A>T resulted in two

PE insertions and flanking exon deletions. Eleven distinct large deletions were found, two of which contained small inverted segments. Uniparental isodisomy of chromosome 1 was identified in one proband. CONCLUSION: Deep sequencing of ABCA4 and midigene-based splice assays allowed the identification of SVs and causal deep-intronic variants in 25% of biallelic STGD1 cases, which represents a model study that can be applied to other inherited diseases.

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