CRISPR-Cas mediated base editing approaches for CRB1 related retinal dystrophy.

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PURPOSE

Pathogenic variants in Crumbs homolog 1 (CRB1) gene lead to severe, childhood-onset retinal degeneration leading to blindness in early adulthood. There are no approved therapies and traditional adeno-associated viral vector-based gene therapy approaches are challenged by the size of the CRB1 which exceeds the vector carrying capacity and the existence of multiple CRB1 isoforms.

SETTING / VENUE

Here we describe 3 CRB1 variants, including a novel previously unreported variant, which led to retinal degeneration and offer a CRISPR-Cas mediated DNA base editing strategy as a potential future therapeutic approach.

METHODS

Retrospective case series. Clinical and genetic assessments were performed, including deep phenotyping by retinal imaging. In silico analyses were used to predict the pathogenicity of the novel variant and determine whether the variants are amenable to DNA base editing strategies.

RESULTS

Case 1 was a 24-year-old male with cone-rod dystrophy and retinal thickening typical of CRB1 retinopathy. He had relatively preserved central outer retinal structure and best corrected visual acuity (BCVA) of 60 ETDRS letters in both eyes. Genetic testing revealed compound heterozygous variants in exon 9: c.2843G>A, p.(Cys948Tyr) and a novel variant c.2833G>A, p.(Gly945Arg) predicted to be pathogenic by in-silico analysis. Cases 2 and 3 were two brothers, aged 20 and 24, who presented with severe cone-rod dystrophy and significant disruption of outer nuclear layers. BCVA was reduced to hand movements in Case 2 and 42 ETDRS letters in Case 3. Case 2 was also affected with marked intraretinal fluid, common in CRB1 retinopathy, but responded well to treatment with oral acetazolamide. Genetic testing revealed two c.2234C>T, p.(Thr745Met) variants in both brothers. As G-to-A and C-to-T variants, these all three variants are amenable to adenine base editors (ABEs) by targeting the forward strand in Case 1 variants, and the reverse strand in Cases 2 and 3. Available PAM-sites were detected for KKH-nSaCas9-ABE8e for the c.2843G>A variant, nSaCas9-ABE8e and KKH-nSaCas9-ABE8e for the c.2833G>A variant and nSpCas9-ABE8e for the c.2234C>T variant.

CONCLUSIONS

In this case series, we report three pathogenic CRB1 variants, including a novel c.2833G>A variant, associated with early-onset cone-rod dystrophy. We highlight the severity and rapid disease progression and offer ABEs as a potential future therapeutic approach for this devastating blinding condition.

FINANCIAL DISCLOSURES