Title: Characterizing genetic and structural variants in opsin genes associated with color vision deficiency using ultra-long nanopore sequencing

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Abstract

Color vision deficiencies are caused by variants in the highly homologous opsin genes *OPN1LW* and *OPN1MW*. The repetitive structure and large size of this gene cluster make it challenging to resolve with standard sequencing approaches. This study aimed to investigate single-nucleotide polymorphisms (SNPs) and structural variants (SVs) in opsin genes underlying color vision deficiency.

Nineteen individuals underwent anomaloscope testing to diagnose trichromatic, dichromatic, or anomalous trichromatic phenotypes. Variants in *OPN1LW* and *OPN1MW* were first analyzed using long-range PCR and Sanger sequencing. To assess structural variation, we performed ultra-long Nanopore sequencing on five individuals: one trichromatic control and four diagnosed with red-green deficiencies.

Sanger sequencing confirmed genetic diagnoses for 17/19 individuals, while two showed discrepancies between clinical phenotype and genotype. In two individuals diagnosed with protanopia, long-range PCR and Sanger sequencing suggested absence of *OPN1LW*. Nanopore sequencing supported this by revealing decreased read coverage and sequence misalignment in the *OPN1MW* region, suggesting a possible *OPN1LW/MW* fusion gene.

These findings demonstrate the limitations of conventional methods in studying the opsin gene cluster and highlight the value of ultra-long Nanopore sequencing in resolving complex genomic variation. This approach enhances the detection of pathogenic SVs, provides a more accurate genetic basis for color vision deficiencies, and has potential to improve diagnostics in inherited vision disorders.