

# **Unique point mutations in OPN1LW associated with protanopia.**

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## **ABSTRACT**

The OPN1LW gene, located on the q-arm of the X-chromosome at location Xq28, is commonly mutated in individuals with protanopia, a moderately severe color vision defect that renders dichromatic vision. The product of OPN1LW translation is a normally functioning photosensitive pigment named opsin located exclusively in the cone cells of the retina. Point mutations, deletions, etc. to OPN1LW disrupt the cone cell's spectral sensitivity to light, especially at wavelength 492 nm, dimming the brightness of red, orange, and yellows to a level where reds can be confused with black or dark gray, and red traffic lights may not appear be illuminated. Recently published studies indicate protanopia affects 7 percent of the US male population (approximately 10 million men) [1]. Primers were designed to anneal the six exons of OPN1LW, and subsequent PCR allowed for the comparison of sequences from an individual with protanopia (mutant), an individual without protanopia (wild type) and the published National Center for Biotechnology Information sequence for the OPN1LW gene.

## **INTRODUCTION**

Protanopia, or dichromatic color vision, is a moderately severe color vision defect that is sex-linked. Human color vision is trichromatic, meaning that normal eyes can capture the blend of red, blue and green that comprise natural light. The response to light by the eye is governed

by retinal photo receptor cells containing different photopigments with spectral sensitivities and absorption spectra: short (S), middle (M), and long (L)-sensitivities [2]. S-cones absorb light in the 400 nm - 500 nm range, M-cones absorb light between 450 nm - 630 nm and L-cones absorb in the 500 nm – 700 nm range. Absorbance ranges vary due to the differences in protein structure (by different amino acid composition) of L, M or S-cones.

Physiologically, S-cones capture luminance contrast, M-cones are responsible for the yellow-blue color subsystem, and L-cones are responsible for the red-green color subsystem. The loss or mutation of any of these cone photopigments leads to reduction in color vision to two dimensions (i.e. dichromacy). In humans and higher primates, the amount of light captured by the M and L cones confer information about color contrast providing discrimination of all colors [2]. While rod cells in the eye are solely responsible for night vision, the seven million cone cells recognize all colors of the visible spectrum.

The opsin genes that encode L and M-cone pigments (red-green and yellow-blue, respectively) are located on the q-arm of the X-chromosome at location Xq28 existing in a head-to-tail tandem array with as many as six gene copies [3]. The common arrangement of the gene array is assumed to be a single L-cone opsin gene followed by one, two, or three M-cone opsin genes. The research detailed herein focuses on the red-sensitive opsin gene (red cone photoreceptor pigment) referred to as OPN1LW.

While several articles have explored differing mutations (point, single-base, random, etc.) implicated in protanopia, it is unclear if all mutations causing protanopia have been detected. Due to this, the focus of this project was to capture the entire OPN1LW gene in a wild-type subject and mutant-type. It was believed that sequencing of the exonic regions would provide

evidence for the mutations that generated protanopia in the mutant by comparison with wild-type.

#### PROTANOPIA DEFECTS

Protanopes, possessing mutated/non-functional (or missing) long-wavelength sensitive retinal cones (L-cones), are unable to distinguish between colors in the green-yellow-red section of the spectrum. Most debilitating is their neutral point for light at wavelength 492 nm, which prevents the discrimination of light of this wavelength from white. Protanopia, more broadly referred to as dichromacy, reduces the brightness of red, orange, and yellow. This dimming can be so pronounced that reds can be confused with black or dark gray, and red traffic lights may not appear to be illuminated. Usually, protanopes can not perceive any hue difference for reds, yellows or greens. Colors such as violet, lavender, and purple are generally indistinguishable from most shades of blue because their reddish components are so dimmed as to be invisible.

Protanopia is also associated with the formation of a 5'-red-green hybrid gene. This gross structural rearrangement of the visual pigment gene array is more likely to lead to protanopia than point mutations [4]. Ueyama et. al. [5] found a critical missense mutation causing protanopia in exon 6 of the 5'-red-green gene array. Specifically, they discovered a Gly338Glu mutation that reduced the absorbance of light when the mRNA transcript was translated into functional retinal cells.

## MATERIALS AND METHODS

### GENOMIC DNA EXTRACTION/PURIFICATION

Genomic DNA was extracted from mutant-type subject's whole blood with the *DNeasy Blood & Tissue Kit* and genomic DNA from wild-type subject was generously supplied by Dr. Berish Rubin.

### PRIMER DESIGN

Due to the uncertainty of rearrangements or mutations and their culpability in causing protanopia, techniques for isolating specific mutations in genomic DNA could not be relied upon. Instead, the 6 exons of OPN1LW were targeted with primers designed to anneal at the proximal and distal intron flanking each exon, ensuring the entire exon was successfully amplified in PCR. Primers were manufactured by Integrated DNA Technologies, Inc.

| Name                  | Exon | Sequence                   |
|-----------------------|------|----------------------------|
| 1 <sub>forward</sub>  | 1    | 5'-CTGATCCCACAGGCCAGTA-3'  |
| 2 <sub>reverse</sub>  | 1    | 5'-AGCCTGTGTGGCTTGAGAAT-3' |
| 3 <sub>forward</sub>  | 2    | 5'-GACAAAGCTGGAGGGAAACA-3' |
| 4 <sub>reverse</sub>  | 2    | 5'-TGTTTGACTCAAGGGAGCTG-3' |
| 5 <sub>forward</sub>  | 3    | 5'-CCCTCATCTGTCTGCTCTCC-3' |
| 6 <sub>reverse</sub>  | 3    | 5'-GGACCACAGAGCCTTTCCTA-3' |
| 7 <sub>forward</sub>  | 4    | 5'-TTCTGGAAGCAGAGGTGGT-3'  |
| 8 <sub>reverse</sub>  | 4    | 5'-TCACAGAGCTGAGGACAACG-3' |
| 9 <sub>forward</sub>  | 5    | 5'-TAGGCCACACCTAGCTGCAT-3' |
| 10 <sub>reverse</sub> | 5    | 5'-AGGGCACACCTAAGCCTTCT-3' |
| 11 <sub>forward</sub> | 6    | 5'-GGCACGTACATTCAGCACAG-3' |
| 12 <sub>reverse</sub> | 6    | 5'-GATGGGTAGGAGGCAGACCT-3' |

**Figure 1** Primers for OPN1LW gene. Designed primers were generated by Integrated DNA Technologies, Inc.

Primers were diluted based upon the manufactured concentration as a solute for 100pmol/uL concentration (i.e. if original concentration was 35.5 nmol, 355 uL dH<sub>2</sub>O was added

to achieve a 100 pmol concentration). From stock solution, 1:10 dilutions were generated (volume = 100 uL) and split into 2 50uL aliquots (final concentration of 10 pmol/uL).

### POLYMERASE CHAIN REACTION

PCR was prepared so that sample template DNA was paired with both primers for a specific exon (a negative control with dH<sub>2</sub>O instead of template DNA was prepared for each reaction). Separate PCR reactions were performed using both mutant and wild type template DNA samples. PCR parameters were as follows: 94°C for 5 min., 45 cycles of 94°C 30 sec., 58°C 30 sec., 72°C 1 min., followed by a final extension at 72°C for 7 min. and stored at 4°C for ∞, with a reaction volume of 25 uL.

| Tube #     | Primer <sub>forward</sub> | Primer <sub>reverse</sub> | DNA  | dH <sub>2</sub> O | GoTaq | Fragment length (bp) |
|------------|---------------------------|---------------------------|------|-------------------|-------|----------------------|
| 1          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 409                  |
| 1 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |
| 2          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 631                  |
| 2 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |
| 3          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 341                  |
| 3 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |
| 4          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 574                  |
| 4 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |
| 5          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 660                  |
| 5 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |
| 6          | 0.5                       | 0.5                       | 0.40 | 11.1              | 12.5  | 198                  |
| 6 (-) ctrl | 0.5                       | 0.5                       | 0    | 11.5              | 12.5  | 0                    |

**Figure 2** Protocol for PCR used to amplify the 6 exons of OPN1LW. All volumes expressed in microliters (uL).

### GEL ELECTROPHORESIS, PCR PURIFICATION & SEQUENCING

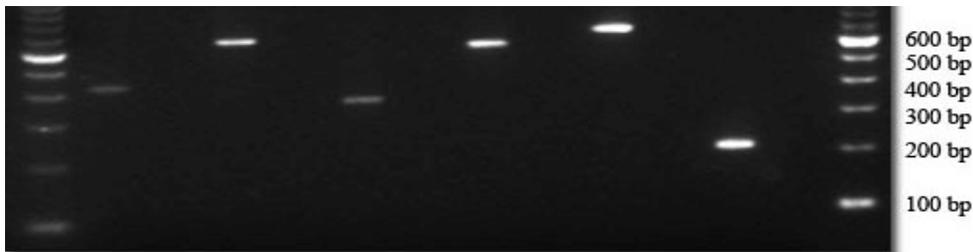
Gel electrophoresis of all PCR products was performed using a 1.5% agarose gel at 134 mV for 40 minutes. Exposure to UV light detected bands representing exons 1 – 6 with respective base pair lengths of 409, 631, 341, 574, 660, and 198.

PCR purification for exons 1-6 for wild type and mutant DNA yielded sufficiently pure product. These exons were prepared for sequencing, performed by Genewiz, Inc. Sequencing data was analyzed using Geneious Pro 2.5.4 alignment and translation software.

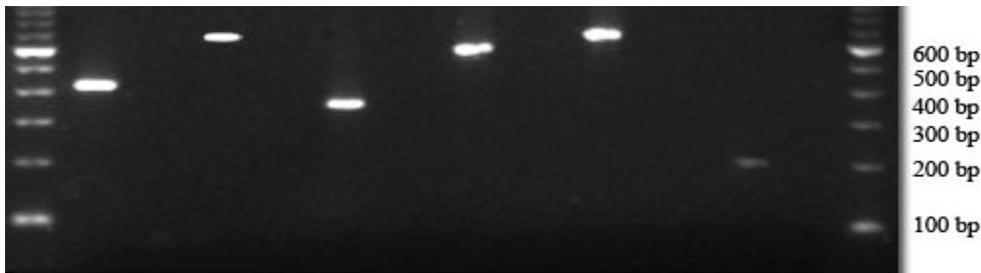
## RESULTS

The six exons of OPN1LW were successfully amplified by PCR. PCR products were run on a gel with negative control lanes in separating each exon. From left to right, we can visualize exons one through six for mutant and wild type (figure 3, figure 4). PCR products, and gel bands, were of predicted base pair length.

Sequencing results for the six exons (figures 5 – 10) demonstrate strong similarity for exons 1, 2 and 4, while there is considerable disparity in exons 3, 5 and 6. Protein products produced from the sequence results indicate that exons 1, 2 and 4 produce nearly identical proteins. However, as expected, proteins produced from exons 3, 5 and 6 vary considerably for wild type, mutant and NCBI sequence samples.



**Figure 3** Gel for mutant DNA with 6 bands (l to r. exon 1 = 409, exon 2 = 631, exon 3 = 341, exon 4 = 574, exon 5 = 660, exon 6 = 198)



**Figure 4** Gel for wild type DNA with 6 bands (l to r. exon 1 = 409, exon 2 = 631, exon 3 = 341, exon 4 = 574, exon 5 = 660, exon 6 = 198)

## EXON 1 - NUCLEOTIDE SEQUENCE & PROTEIN ALIGNMENT

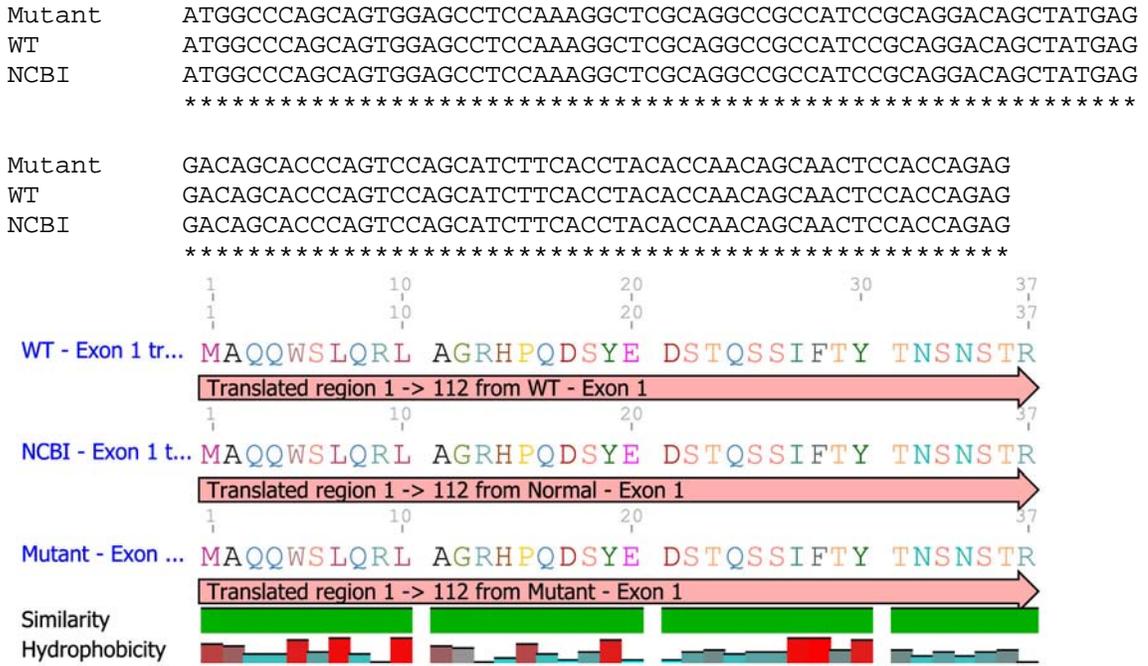


Figure 5 Nucleotide sequence and protein alignment for exon 1.

## EXON 2 - NUCLEOTIDE SEQUENCE & PROTEIN ALIGNMENT

Mutant GCCCCTTCGAAGGCCCGAATTACCACATCGCTCCCAGATGGGTGTACCACCTCACCAGTG  
 NCBI GCCCCTTCGAAGGCCCGAATTACCACATCGCTCCCAGATGGGTGTACCACCTCACCAGTG  
 WT GCCCCTTCGAAGGCCCGAATTACCACATCGCTCCCAGATGGGTGTACCACCTCACCAGTG  
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Mutant TCTGGATGATCTTTGTGGTCACTGCATCCGTCTTCACAAATGGGCTTGTGCTGGCGGCCA  
 NCBI TCTGGATGATCTTTGTGGTCACTGCATCCGTCTTCACAAATGGGCTTGTGCTGGCGGCCA  
 WT TCTGGATGATCTTTGTGGTCATTGCATCCGTCTTCACAAATGGGCTTGTGCTGGCGGCCA  
 \*\*\*\*\*

Mutant CCATGAAGTTCAAGAAGCTGCGCCACCCGCTGAACTGGATCCTGGTGAACCTGGCGGTCTG  
 NCBI CCATGAAGTTCAAGAAGCTGCGCCACCCGCTGAACTGGATCCTGGTGAACCTGGCGGTCTG  
 WT CCATGAAGTTCAAGAAGCTGCGCCACCCGCTGAACTGGATCCTGGTGAACCTGGCGGTCTG  
 \*\*\*\*\*

Mutant CTGACCTAGCAGAGACCGTCATCGCCAGCACTATCAGCATTGTGAACCAGGTCTCTGGCT  
 NCBI CTGACCTAGCAGAGACCGTCATCGCCAGCACTATCAGCATTGTGAACCAGGTCTCTGGCT  
 WT CTGACCTGGCAGAGACCGTCATCGCCAGCACTATCAGCNTTGTGAACCAGGTCTATGGCT  
 \*\*\*\*\*

Mutant ACTTCGTGCTGGGCCACCCTATGTGTGTCCTGGAGGGCTACACCGTCTCCCTGTGTG  
 NCBI ACTTCGTGCTGGGCCACCCTATGTGTGTCCTGGAGGGCTACACCGTCTCCCTGTGTG  
 WT ACTTCGTGCTGGGCCACCCTATGTGTGTCCTGGAGGGCTACACCGTCTCCCTGTGTG  
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## DISCUSSION

In this study, exons 1 – 6 of the OPN1LW gene were amplified and analyzed via PCR and sequencing technologies. The full sequence of the exon (protein coding portion) of the gene was aligned using multiple sequence aligning software (Geneious Pro 2.5.4). Aligned exonic regions were also translated into their protein sequence (Geneious Pro 2.5.4) to confirm similarity in eventual gene products and to assess the effect individual mutations had on amino acid selection. Similarity for both nucleotide sequence and protein product were analyzed. Mutant type sequence (DNA from Anthony Paratore) and wild type sequence (DNA from Berish Rubin) were compared to the published sequence (NCBI NM\_000513) for OPN1LW gene. Alignment of mutant and wild type to the published sequence was performed to show the variability of sequence that can occur without generating protanopia.

Analysis of exon 1, 2 and 4 demonstrated that the mutation causing protanopia is not located in these coding regions.

Analysis of exon 3, 5, 6 was inconclusive because reverse sequencing data was not available to verify sequencing results. Further sequencing is required to rectify this problem.

Ironically, exon 6, despite numerous mutations, did not exhibit the characteristic Gly→Glu substitution, well established to cause some forms of protanopia. This may indicate that protanopia is more likely caused by whole gene rearrangement rather than by individual point mutations.

In theory, red-green dichromacy (protanopia) can arise from a single point mutation occurring in the coding sequence of the L- or M-cone pigment genes (i.e. OPN1LW) which vitiates and destabilizes the opsin protein or impairs its quantum efficiency (ability to capture wavelengths of differing value that correspond to different colors). Alternatively, protanopia

may arise from major deletions/insertions in the coding sequences, such as those exhibited in exon 6 of the mutant sequence.

Furthermore, 4 – 8% percent of Caucasian males with normal vision have a 5'M 3'L hybrid gene in addition to normal L- and M-cone pigment genes. However, it is evident that the expression level of this hybrid gene is insufficient to disrupt normal color vision in these individuals [6].

### **CONCLUSION**

Protanopia disrupts an individual's ability to select and/or differentiate colors in daily tasks. This disease provides the opportunity to assay mutations in humans that cause harmless, easily testable disability. In the context of this study, we can conclude that there are multiple mutations causing protanopia in mutants with altered opsin gene arrays. Further study should see the design of a technique to detect if protanopia is usually caused by more complicated problems, such as the formation of hybrid genes. Such a technique will allow molecular biologists to distinguish between protanopes with a singular point mutation, or those with gene rearrangements that are presently difficult to detect.

## REFERENCES

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