

In another aspect, the invention provides a method for determining a relative amount of full length exon 3 L-opsin gene and/or M-opsin gene mRNA compared to exon 3-skipped (EX3(-)) L-opsin gene and/or M-opsin gene mRNA in a subject, said method comprising:

- (a) isolating genomic DNA from a biological sample obtained from the subject;
- 5 (b) expressing exon 3 and introns flanking exon 3 of the L-opsin gene and/or M-opsin gene from the genomic DNA to generate opsin gene mRNA from said subject;
- (c) isolating the L-opsin gene and/or M-opsin gene mRNA;
- (d) reverse transcribing the L-opsin gene and/or M-opsin gene mRNA to generate L-opsin gene and/or M-opsin gene cDNA;
- 10 (e) generating amplification products from the L-opsin gene and/or M-opsin gene cDNA using PCR primers that span exon 3 of the L-opsin gene mRNA and/or M-opsin gene, wherein the amplification products comprise a first population of amplification products representing full length L-opsin gene mRNA and/or M-opsin gene mRNA, and a second population of amplification products representing Ex3(-) L-opsin gene mRNA and/or M-
- 15 opsin gene mRNA; and
- (f) detecting an amount of the first population of amplification products representing full length L-opsin gene and/or M-opsin gene mRNA and an amount of the second population of amplification products representing Ex3(-) L-opsin gene and/or M-opsin gene mRNA.

20 In another aspect, the invention provides a method for determining a subject's predisposition for refractive error comprising:

- (a) testing a biological sample obtained from the subject to identify a L-opsin gene and/or a M-opsin gene variant comprising LIVVA (SEQ ID NO: 1), designated as such for the amino acids encoded at positions 153, 171, 174, 178 and 180 of the L or M opsin
- 25 gene; and
- (b) correlating the opsin gene variant with a predisposition for refractive error.

In another aspect, the invention provides a composition, comprising or consisting of a primer pair capable of selectively amplifying a detectable portion of a human L-opsin gene and/or a human M-opsin gene, wherein the detectable portion includes exon 3, and wherein

30 the primer pair comprises:

- (a) a first primer comprising 12 or more contiguous nucleotides of SEQ ID NO: 14 (exon 2) or a full complement thereof; and
- (b) a second primer pair comprising 12 or more contiguous nucleotides of SEQ ID NO: 15 (exon 4) or a full complement thereof.

We claim

1. A method for determining a relative amount of full length exon 3 L-opsin gene and/or M-opsin gene mRNA compared to exon 3-skipped (EX3(-)) L-opsin gene and/or M-opsin gene mRNA in a subject, said method comprising:

- (a) isolating genomic DNA from a biological sample obtained from the subject;
- (b) expressing exon 3 and introns flanking exon 3 of the L-opsin gene and/or M-opsin gene from the genomic DNA to generate opsin gene mRNA from said subject;
- (c) isolating the L-opsin gene and/or M-opsin gene mRNA;
- (d) reverse transcribing the L-opsin gene and/or M-opsin gene mRNA to generate L-opsin gene and/or M-opsin gene cDNA;
- (e) generating amplification products from the L-opsin gene and/or M-opsin gene cDNA using PCR primers that span exon 3 of the L-opsin gene mRNA and/or M-opsin gene, wherein the amplification products comprise a first population of amplification products representing full length L-opsin gene mRNA and/or M-opsin gene mRNA, and a second population of amplification products representing Ex3(-) L-opsin gene mRNA and/or M-opsin gene mRNA; and
- (f) detecting an amount of the first population of amplification products representing full length L-opsin gene and/or M-opsin gene mRNA and an amount of the second population of amplification products representing Ex3(-) L-opsin gene and/or M-opsin gene mRNA.

2. The method of claim 1, further comprising correlating the relative amount of full length opsin gene mRNA to EX3(-) mRNA with a predisposition for refractive error.

3. The method of claim 1, wherein the biological sample is saliva or blood.

4. The method of claim 1, wherein said detecting comprises a primer extension assay, and wherein detecting the amplification products comprises generating a primer extension product from the amplification products using a primer that binds to the amplification products adjacent to an end of exon 3.

5. The method of claim 4, wherein the primer extension product is detected by MALDI-TOF Mass Spectrometry.
6. The method of claim 1, further comprising, prior to said expressing exon 3 and introns flanking exon 3 of the L-opsin gene and/or M-opsin gene from the genomic DNA to generate opsin gene mRNA, cloning exon 3 and introns flanking exon 3 of the L-opsin gene and/or M-opsin gene from the genomic DNA into an expression vector.
7. The method of claim 1, wherein the generating amplification products comprises
 - (i) contacting the cDNA with a primer pair that spans exon 3 and is capable of selectively amplifying the cDNA of L-opsin gene mRNA and/or cDNA of M-opsin gene mRNA, under conditions suitable for amplification of the cDNA, and
 - (ii) amplifying the cDNA to produce a first population of amplification products comprising full length cDNA amplification products, and a second population of amplification products comprising EX3(-) amplification products.
8. The method of claim 1, wherein said detecting comprises real time qPCR, and wherein the detecting comprises contacting the amplification products with a probe having full sequence complementarity to at least a portion of both the first population of amplification products representing full length L-opsin gene and/or M-opsin gene mRNA and the second population of amplification products representing Ex3(-) L-opsin gene and/or M-opsin mRNA, wherein the contacting occurs under conditions suitable for hybridization of the probe to the amplification products.
9. The method of any one of claims 1-8, wherein the L-opsin gene and/or M-opsin gene comprises an L-opsin gene.
10. The method of any one of claims 1-8, wherein the L-opsin gene and/or M-opsin gene comprises an M-opsin gene.
11. A method for determining a subject's predisposition for refractive error comprising:

- (a) testing a biological sample obtained from the subject to identify a L-opsin gene and/or a M-opsin gene variant comprising LIVVA (SEQ ID NO: 1), designated as such for the amino acids encoded at positions 153, 171, 174, 178 and 180 of the L or M opsin gene; and
 - (b) correlating the opsin gene variant with a predisposition for refractive error.
- 12. The method of any one of claims 1-11, wherein the refractive error is blue cone monochromacy.
- 13. The method of any one of claims 1-11, wherein the refractive error is myopia.
- 14. The method of any one of claims 1-11, wherein the refractive error is high-grade myopia.
- 15. The method of any one of claims 1-11, wherein the refractive error is juvenile onset myopia.
- 16. A composition, comprising or consisting of a primer pair capable of selectively amplifying a detectable portion of a human L-opsin gene and/or a human M-opsin gene, wherein the detectable portion includes exon 3, and wherein the primer pair comprises:
 - (a) a first primer comprising 12 or more contiguous nucleotides of SEQ ID NO: 14 (exon 2) or a full complement thereof; and
 - (b) a second primer pair comprising 12 or more contiguous nucleotides of SEQ ID NO: 15 (exon 4) or a full complement thereof.
- 17. The composition of claim 16, wherein the primer pair comprises:
 - (a) a first primer comprising 12 or more contiguous nucleotides of SEQ ID NO: 11 (exon 1), or a full complement thereof; and
 - (b) a second primer pair comprising 12 or more contiguous nucleotides of SEQ ID NO: 12 (exon 6), or a full complement thereof.
- 18. The composition of claims 16 or 17, wherein at least one primer in the primer pair is detectably labeled.

19. The method of claim 8, where the probe is detectably labeled.