b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative of a negative clinical outcome.

In another aspect, the invention relates to an *in vitro* method for selecting a subject to be treated with a therapy for an ectatic disease of the cornea which comprises:

a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and

b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that said subject is candidate to be treated with a therapy for an ectatic disease of the cornea.

In another aspect the invention relates to the use of TLR2 and/or TLR4 as a marker for determining the diagnosis of an ectatic disease of the cornea in a subject.

In another aspect, the invention relates to the use of TLR2 and/or TLR4 as a marker for determining the risk of developing an ectatic disease of the cornea in a subject.

In another aspect, the invention relates to the use of TLR2 and/or TLR4 as a marker for determining the clinical outcome of a subject suffering from an ectatic disease of the cornea.

Finally, in another aspect, the invention relates to the use of TLR2 and/or TLR4 as a marker for selecting a subject to be treated with a therapy for an ectatic disease of the cornea.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Median [quartiles] of TLR2 expression levels in corneal cells by study groups in cohort A. A gradual increase in the expression of TLR2 is observed in a corneal ectasia as keratoconus compared to control subjects: Control < Subclinical keratoconus < keratoconus.

Figure 2: Median [quartiles] of TLR4 expression levels in corneal cells by study groups in cohort A. A gradual increase in the expression of TLR4 is observed in a corneal ectasia as keratoconus compared to control subjects: Control < Subclinical keratoconus < keratoconus.

superior dioptric asymmetry greater than 1.9 D can be classified as "clinical keratoconus"; 2) The distance visual acuity (DVA) of the subclinical KC eye with value of 1.0 (without correction or with spherical and /or cylindrical compensation < 1.50D).

The term "pellucid marginal degeneration" or "PMD" as used herein refers to a degenerative corneal condition characterized by a clear bilateral thinning in the inferior and peripheral region of the cornea although some cases affect only one eye. Unlike keratoconus pain is not typically present in pellucid marginal degeneration, and aside from vision loss, no symptoms accompany the condition. However, in rare cases, PMD may present with sudden onset vision loss and excruciating eye pain, which occurs if the thinning of the cornea leads to perforation. Normally, PMD does not present with vascularization of the cornea, scarring, or any deposits of lipid.

The term "keratoglobus" as used herein refers to a degenerative non-inflammatory disorder of the eye in which structural changes within the cornea cause it to become extremely thin and change to a more globular shape than its normal gradual curve. It causes corneal thinning, primarily at the margins, resulting in a spherical, slightly enlarged eye.

The term "ectasia post refractive corneal surgery" as used herein refers to a condition due to the weakening of the inner layer of the cornea which occurs during surgery. It results in blurred and distorted vision due to increased myopia and agtismatism.

The term "TLR2" or "Toll-like receptor 2" as used herein, refers to a protein that in humans is encoded by the *TLR2* gene. TLR2 has also been designated as CD282 (cluster of differentiation 282). TLR2 is one of the toll-like receptors and plays a role in the immune system. In particular, the sequence of human TLR2 protein is provided in the NCBI database entry NP_003255 (version of 25 May 2014).

The term "TLR4" or Toll-like receptor 4"as used herein, refers to a protein that in humans is encoded by the *TLR4* gene. TLR 4 has also been designated as CD284 (cluster of differentiation 284). The molecular weight of TLR 4 is approximately 95 kDa. In particular, the sequence of human TLR4 protein is provided in the NCBI database entry NP_003257 (version of 25 May 2014).

The term "subject", as used herein, refers to all animals classified as mammals and includes, but is not restricted to, domestic and farm animals, primates and humans,

CLAIMS

1. An *in vitro* method for diagnosing an ectatic disease of the cornea in a subject which comprises:
   a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and
   b) comparing said expression level with a reference value
   wherein, if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that the subject suffers from an ectatic disease of the cornea.

2. The *in vitro* method according to claim 1 wherein said ectatic disease of the cornea is selected from subclinical keratoconus, clinical keratoconus, pellucid marginal degeneration, keratoglobus and ectasia post refractive corneal surgery.

3. The *in vitro* method according to claim 2, wherein said ectatic disease of the cornea is subclinical keratoconus, in which case the reference value corresponds to the expression levels of TLR2 and/or TLR4 measured in a sample from a healthy subject.

4. The *in vitro* method according to claim 2, wherein said ectatic disease of the cornea is clinical keratoconus, in which case the reference value corresponds to the expression levels of TLR2 and/or TLR4 measured in a sample from a subject suffering from subclinical keratoconus.

5. The *in vitro* method according to claim 2, wherein said ectatic disease of the cornea is pellucid marginal degeneration, in which case the reference value corresponds to the expression levels of TLR2 and/or TLR4 measured in a sample from a healthy subject .

6. The *in vitro* method according to claims 1 to 5, wherein the sample from said subject is selected from conjunctival and corneal tissue.

7. The *in vitro* method according to claims 1 to 6, wherein said expression level comprises determining the level of mRNA encoded from the TLR2 and/or TLR4 gene or determining the level of the TLR2 and/or TLR4 protein.

8. The *in vitro* method according to claim 7, the protein level is determined by a method selected from immunohistochemistry, Western blot, flow cytometry or by ELISA.

9. The *in vitro* method according to anyone of claims 1 to 8 which also comprises determining at least one parameter selected from: diopters, corneal thickness and corneal elevation.

10. An *in vitro* method for determining the risk of developing an ectatic disease of the cornea in a subject which comprises:

    a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and
    b) comparing said expression level with a reference value.

wherein, if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that the subject has high risk of developing an ectatic disease of the cornea.

11. The *in vitro* method according to claim 10, wherein the subject has been previously diagnosed as having a refractive defect.

12. The *in vitro* method according to any of claims 10 or 11, wherein said subject has developed a previous ocular pathology selected from: ocular itching, eye rubbing, biomicroscopic signs and conjunctival hyperemia.

13. An *in vitro* method for determining the clinical outcome of a subject suffering from an ectatic disease of the cornea, comprising:

    a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and

b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative of a negative clinical outcome.

14. An *in vitro* method for selecting a subject to be treated with a therapy for an ectatic disease of the cornea which comprises:

a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and

b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that said subject is candidate to be treated with a therapy for an ectatic disease of the cornea.

15. The *in vitro* method according to claim 14, wherein said therapy is selected from corneal crosslinking, intracorneal rings and refractive surgery.

16. The *in vitro* method according to any of claims 10 to 14, wherein said ectatic disease of the cornea is selected from subclinical keratoconus, clinical keratoconus, pellucid marginal degeneration, keratoglobus and ectasia post refractive corneal surgery.

17. The *in vitro* method according to claims 10 to 16, wherein the sample from said subject is selected from conjunctival and corneal tissue.

18. The *in vitro* method according to claims 10 to 17, wherein said expression level comprises determining the level of mRNA encoded from the TLR2 and/or TLR4 gene or determining the level of the TLR2 and/or TLR4 protein.

19. The *in vitro* method according to claim 18, wherein the protein level is determined by a method selected from immunohistochemistry, Western blot, flow cytometry or by ELISA.

20. An *in vitro* method for determining whether a subject suffering from a refractive defect is a candidate for a refractive surgery therapy which comprises:
    a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and
    b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that said subject is not candidate to be treated with a refractive surgery for said refractive defect.

21. *In vitro* use of TLR2 and/or TLR4 as a marker for determining the diagnosis of an ectatic disease of the cornea in a subject, for determining the risk of developing an ectatic disease of the cornea in a subject, as a marker for determining the clinical outcome of a subject suffering from an ectatic disease of the cornea or as a marker for selecting a subject to be treated with a therapy for an ectatic disease of the cornea.

22. The use of a kit comprising an antibody, a polypeptide, a primer and/or a probe which specifically bonds to TLR2 and/or TLR4 for determining the diagnosis of an ectatic disease of the cornea in a subject, for determining the risk of developing an ectatic disease of the cornea in a subject, for determining the clinical outcome of a subject suffering from an ectatic disease of the cornea or for selecting a subject to be treated with a therapy for an ectatic disease of the cornea.

23. The *in vitro* method according to claim 20, wherein the sample from said subject is selected from conjunctival and corneal tissue.

24. The *in vitro* method according to claims 20 or 23, wherein said expression level comprises determining the level of mRNA encoded from the TLR2 and/or TLR4 gene or determining the level of the TLR2 and/or TLR4 protein.

25. The *in vitro* method according to claim 24 wherein the protein level is determined by a method selected from immunohistochemistry, Western blot, flow cytometry or by ELISA.

26. An *in vitro* method for determining the risk that a subject suffers ectasia following refractive surgery which comprises:

    a) determining the expression level of TLR2 and/or TLR4 in a sample from said subject; and

    b) comparing said expression level with a reference value

wherein if the expression level of TLR2 and/or TLR4 is higher than said reference value is indicative that said subject shows high risk of suffering ectasia following refractive surgery.

27. The *in vitro* method according to claim 26, wherein the sample from said subject is selected from conjunctival and corneal tissue.

28. The *in vitro* method according to claims 26 or 27, wherein said expression level comprises determining the level of mRNA encoded from the TLR2 and/or TLR4 gene or determining the level of the TLR2 and/or TLR4 protein.

29. The *in vitro* method according to claim 28 wherein the protein level is determined by a method selected from immunohistochemistry, Western blot, flow cytometry or by ELISA.