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MÉDICAMENTS ANTIGLAUCOMATEUX
 (54) Title: METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS

(57) **Abrégé/Abstract:**

Methods for diagnosing glaucoma and for screening therapeutic agents for their usefulness in treating glaucoma based on the detection of aberrant expression of beta glucocorticoid receptor (GRbeta).

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(21) International Application Number: PCT/US97/21054 (22) International Filing Date: 14 November 1997 (14.11.97) (30) Priority Data: 60/033,227 5 December 1996 (05.12.96) US (71)(72) Applicants and Inventors: CLARK, Abbot, F. [US/US]; 5603 Rachel Court, Arlington, TX 76017 (US). WORDINGER, Robert, J. [US/US]; 411 Summit Ridge Drive, Euless, TX 76039 (US). (74) Agents: YEAGER, Sally, S. et al.; Alcon Laboratories, Inc., Patent Dept., Q-148, 6201 South Freeway, Fort Worth, TX 76134-2099 (US).		(81) Designated States: AU, CA, JP, MX, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS		
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METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS

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Background of the Invention

10 Glaucoma is usually diagnosed by monitoring a patient's visual field loss, changes
in the appearance of their optic disc, and their intraocular pressure. Glaucoma is currently
treated using one or more of three strategies to lower the elevated intraocular pressure
associated with the disease: with pharmaceuticals (such as beta-blockers, carbonic
anhydrase inhibitors, and miotics), with laser trabeculoplasty, and/or with glaucoma
filtration surgery. All of these therapies indirectly lower intraocular pressure but do not
15 address the underlying disease process occurring in the trabecular meshwork. It would be
advantageous to be able to diagnose glaucoma before a patient begins experiencing a loss
in their visual field and deterioration of their optic disc.

20 There is a large body of evidence suggesting that glucocorticoids are involved in
the generation of ocular hypertension and glaucoma. See Clark, A. F., *Journal of
Glaucoma*, "Steroids, Ocular Hypertension, and Glaucoma," 4:354-369, 1995. Several
investigators have shown that the human trabecular meshwork (TM) contains the classical
glucocorticoid receptor (GR α). See Weinreb, et al., *Invest. Ophthalmol. Vis. Sci.*,
"Detection of Glucocorticoid Receptors in Cultured Human Trabecular Cells," 21:3, 403-
25 407, 1981, and Hernandez, et al., *Invest. Ophthalmol. Vis. Sci.*, "Glucocorticoid Target
Cells in Human Outflow Pathway: Autopsy and Surgical Specimens," 24:1612-1616,
1983. Recently, the expression of an alternatively spliced form of the human
glucocorticoid receptor (GR β) was discovered in non-ocular tissues and cells. See
Bamberger, et al., *The Journal of Clinical Investigation*, "Glucocorticoid Receptor β , a
30 Potential Endogenous Inhibitor of Glucocorticoid Action in Humans," 95:2435-2441,
1995, and Oakley, et al., *The Journal of Biological Chemistry*, "The Human
Glucocorticoid Receptor β Isoform," 271:16, 9550-9559, 1996. This alternatively spliced
form of the glucocorticoid receptor (GR) is expressed as a protein which no longer binds
glucocorticoids, but is able to interfere with the activated form of the normal
35 glucocorticoid receptor and block or alter physiological functions of the glucocorticoid
receptor.

WO 96/14411 discloses a method for diagnosing glaucoma in a patient which comprises determining whether the amount of a trabecular meshwork induced glucocorticoid response protein present in the trabecular meshwork of an eye of a patient exceeds the amount of that trabecular meshwork induced glucocorticoid response protein present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the trabecular meshwork induced glucocorticoid response protein is indicative of Glaucoma.

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Summary of the Invention

The present invention is directed to methods for diagnosing glaucoma by testing a person for aberrant GR β expression. Also set forth are methods for screening for therapeutic agents useful for treating glaucoma.

Description of Preferred Embodiments

Surprisingly, it has been found that cultured human trabecular meshwork cell lines derived from glaucomatous donors express mRNA for both an alternate splice form of the human glucocorticoid receptor (GR β), as well as the normal glucocorticoid receptor (GR α), whereas normal TM cell lines only express mRNA for GR α . It is believed that the elevated intraocular pressure associated with primary open-angle glaucoma may be due to the aberrant expression of GR β in the trabecular meshwork. Therefore, determining that an individual abnormally expresses GR β in their trabecular meshwork or other tissues can lead to a diagnosis of glaucoma. Also, this discovery can be used to determine whether agents have therapeutic value in treating glaucoma by determining whether they interact with GR β or alter the expression of GR β . This can be done using ligand binding assays or GR β functional assays.

Diagnosing aberrant GR β expression or defects in the GR gene which encodes GR β can be done by using procedures well known to those skilled in the art. See Caskey, C. T., *J.A.M.A.*, "Molecular Medicine. A Spin-off From the Helix," 269:15, 1986-1992, 1993. For example, subjects could be screened for the presence of a genetic defect in GR β by analyzing the DNA derived from peripheral blood leukocytes. Types of DNA analyses could include, but would not be limited to: restriction fragment length polymorphisms (RFLP), single-stranded conformation polymorphisms (SSCP), polymerase chain reaction (PCR), denaturing gradient gels, allele specific oligonucleotide ligation assay, and allele specific hybridization assay. In addition, trabecular meshwork, or other relevant cells from subjects could be analyzed for GR β expression by a number of techniques such as reverse-transcription polymerase chain reaction (RT-PCR), immunoassays, GR functional assays, etc.

We Claim :

1. A method for diagnosing glaucoma which comprises detecting the expression of an alternate splice form of the human glucorticoid receptor, termed GR β , in a sample from a
5 subject, wherein said detection of GR β in said sample is indicative of glaucoma in said subject.
2. The method of claim 1, wherein GR β expression is analyzed by a method selected from the group of assays consisting of: reverse-transcription polymerase chain reaction
10 (RT-PCR) and immunoassay.
3. The method of claim 2, wherein said expression is analyzed by RT-PCR.
4. The method of claim 2, wherein said expression is analyzed by an immunoassay.
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5. A method for identifying an agent suitable for treating glaucoma comprising determining whether said agent alters the expression of GR β , wherein a lower expression of GR β in the presence versus the absence of said agent, is indicative that said agent is suitable for treating glaucoma.