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(54) **MARKERS FOR LXR ACTIVATION**

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(57) **ABSTRACT**

The present invention relates to surrogate markers for LXR activation, and methods of diagnosing a disease linked to LXR activation, methods of monitoring the treatment of patients suffering from a disease linked to LXR activation, and methods of identifying compounds which modulate LXR activity.

MARKERS FOR LXR ACTIVATION

[0001] Liver-X-Receptors (LXR) are nuclear hormone receptors that regulate the expression of genes involved in cholesterol and lipid metabolism and bile acid synthesis. LXRs have been implicated in a number of diseases, such as atherosclerosis, dyslipidemia and diabetes. Recent data also suggests a role in inflammation. Several genes have been shown to be regulated by LXR, including ABCA1 (Costet, P., et al., *Sterol-dependent transactivation of the ABC*1 *promoter by the liver X receptor/retinoid X receptor*. J Biol Chem, 2000.275(36): p. 28240-5; Schwartz, K., R. M. Lawn, and D. P. Wade, *ABC*1 *gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR*. Biochem Biophys Res Commun, 2000. 274(3): p. 794-802); WO02/070011 discloses ABCA-1 as a surrogate marker for PPAR activation; ABCG1 (Kennedy, M. A., et al., *Characterization of the human ABCG*1 *Gene; LXR activates an internal promoter that produces a novel transcript encoding an alternative form of the protein*. J Biol Chem, 2001. 10: p. 10.); ApoC1 (Stulnig, T. M., et al., *Novel roles of liver X receptors exposed by gene expression profiling in liver and adipose tissue*. Mol Pharmacol, 2002.62(6): p. 1299-305); ApoE (Laffitte, B. A., et al., *LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes*. Proc Natl Acad Sci USA, 2001. 98(2): p. 507-12); FAS (Joseph, S. B., et al., *Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by LXRs*. J Biol Chem, 2002. 14: p. 14); LDLR (Stulnig, T. M., et al., *Novel roles of liver X receptors exposed by gene expression profiling in liver and adipose tissue*. Mol Pharmacol, 2002. 62(6): p. 1299-305); NR1H3; (Laffitte, B. A., et al., *Autoregulation of the Human Liver X Receptor alpha Promoter*. Mol Cell Biol, 2001. 21(22): p. 7558-68); SREBPF1 (DeBose-Boyd, R. A., et al., *Expression of sterol regulatory element-binding protein* 1c (SREBP-1c) *mRNA in rat hepatoma cells requires endogenous LXR ligands*. Proc Natl Acad Sci USA, 2001. 98(4):p. 1477-82). Also of interest is US2004/0023276, which discloses LXR-ligand induced genes and proteins.

[0002] The present invention relates to surrogate markers for LXR activation, and methods of diagnosing a disease linked to LXR activation, methods of monitoring the treatment of patients suffering from a disease linked to LXR activation, and methods of identifying compounds which modulate LXR activity.

[0003] The present invention provides a marker for detecting or monitoring LXR modulation, comprising at least one nucleic acid selected from the group consisting of the nucleic acids listed in tables 2 and/or 3. The term "modulation" as used herein relates to an activation or inhibition of the transcriptional activity of LXR. Thus, said nucleic acids can serve as surrogate markers for modulation of LXR activity.

[0004] The term "marker" as used herein refers to a single nucleic acid or polypeptide, or a panel of multiple nucleic acids or polypeptides.

[0005] Preferably, said nucleic acids are nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, said nucleic acids are nucleic acids listed in table 2, with the exception of Seq. ID No. 3, 4, 5 and 20. In another more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises at least one nucleic acid of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises at least one nucleic acid selected from the group consisting of the nucleic acids of Seq ID No. 20, 22 or 26, more preferably at least one nucleic acid selected from the group consisting of the nucleic acids of Seq ID No. 22 or 26 and most preferably, the marker comprises the nucleic acid of Seq ID No. 26.

[0006] In another preferred embodiment, the marker comprises at least one nucleic acid listed in table 3. More preferably, said at least one nucleic acid is selected from the group consisting of the nucleic acids of Seq ID No. 16, 17 and 23 to 25. In a most preferred embodiment, said at least one nucleic acid is selected from the group consisting of Seq ID No. 16 or 24.

[0007] The present invention also pertains to a marker for diagnosing a disease involving dysregulation of LXR activity, comprising one or more of the nucleic acids selected from the group consisting of the nucleic acids listed in tables 2 and/or 3. Thus, said nucleic acids can serve as surrogate markers for the modulation of LXR activity.

[0008] Preferably, said nucleic acids are nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, said nucleic acids are nucleic acids listed in table 2, with the exception of Seq. ID No. 3, 4, 5 and 20. In another more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 16, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises at least one nucleic acid of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises at least one nucleic 15 acid selected from the group consisting of the nucleic acids of Seq ID No. 20, 22 or 26, more preferably at least one nucleic acid selected from the group consisting of the nucleic acids of Seq ID No. 22 or 26 and most preferably, the marker comprises the nucleic acid of Seq ID No. 26.

[0009] In another preferred embodiment, said at least one nucleic acids are selected from the group consisting of the nucleic acids listed in table 3. More preferably, said at least one nucleic acids are selected from the group consisting of the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said at least one nucleic acids are selected from the group consisting of Seq ID No. 16 and/or 24.

[0010] The invention also pertains to a marker for diagnosing a disease involving dysregulation of LXR activity, comprising at least one nucleic acid selected from the group consisting of the nucleic acids listed in table 3 and one or more nucleic acids listed selected from the group consisting of the nucleic acids in table 2. Preferably, the marker comprises one nucleic acid listed in table 3 and one nucleic acid listed in table 2.

[0011] The polypeptides encoded by the nucleic acids listed in tables 2 and/or 3 can also be used as markers. Thus, the present invention also provides a marker for detecting or monitoring LXR modulation, comprising one or more polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in tables 2 and/or 3.

[0012] Preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, said polypeptides are the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq. ID No. 3, 4, 5 and 20. In another more preferred embodiment, the marker comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises at least one polypeptide encoded by the nucleic acids selected from the group consisting of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises at least one polypeptide encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, more preferably at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 22 or 26 and most preferably, the marker comprises the polypeptides encoded by the nucleic acid of Seq ID No. 26.

[0013] In another preferred embodiment, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3. More preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0014] In another embodiment, the present invention provides a marker for detecting or monitoring LXR modulation, comprising at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acid listed in table 3 and one or more polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. Preferably, the marker comprises one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3 and one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. More preferably, the marker comprises two polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3 and two polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. Even more preferably, the marker comprises three polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3 and three polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. Most preferably, the marker comprises four polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3 and four polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2.

[0015] Further to this, the present invention also provides a marker for diagnosing a disease involving dysregulation of LXR activity, comprising one or more polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in tables 2 and/or 3.

[0016] Preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, said polypeptides are the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq. ID No. 3, 4, 5 and 20. In another more preferred embodiment, the marker comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises at least one polypeptide encoded by the nucleic acids selected from the group consisting of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises at least one polypeptide encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, more preferably at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 22 or 26 and most preferably, the marker comprises the polypeptides encoded by the nucleic acid of Seq ID No. 26.

[0017] In another preferred embodiment, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3. More preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0018] The invention also pertains to a marker for diagnosing a disease involving dysregulation of LXR activity, comprising at least one polypeptide selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 3 and one or more polypeptides selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. Preferably, the marker comprises one polypeptide selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 3 and one polypeptide selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 2. More preferably, the marker comprises at least two polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 3 and at least two polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 2. Even more preferably, the marker comprises at least three polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 3 and at least three polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 2. Most preferably, the marker comprises at least four polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 3 and at least four polypeptides selected from the group consisting of the polypeptides encoded by a nucleic acid listed in table 2.

[0019] The nucleic acids and polypeptides which constitute the novel markers hereinbefore described are useful for several processes. A method for screening compounds that modulate LXR activity is provided, comprising the steps of contacting said compounds with a host, and measuring the expression of at least one nucleic acid selected from the group consisting of the nucleic acid listed in tables 2 and/or 3.

[0020] Preferably, said nucleic acids are nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, said nucleic acids are nucleic acids listed in table 2, with the exception of Seq. ID No. 3, 4, 5 and 20. In another more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises at least one nucleic acid selected from the group consisting of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises at least one nucleic acid of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises at least one nucleic acid selected from the group constisting of the nucleic acids of Seq ID No. 20, 22 or 26, more preferably at least one nucleic acid selected from the group constisting of the nucleic acids of Seq ID No. 22 or 26 and most preferably, the marker comprises the nucleic acid of Seq ID No. 26.

[0021] In one embodiment of the method hereinbefore described, the expression of at least one nucleic acid selected from the group consisting of the nucleic acids herein before described is compared to the expression of said at least one nucleic acid in a control. The control can either be an untreated host, which may be the same host before the treatment or a different host, and/or the same host after an appropriate period of treatment for normalization to pretreatment levels. In a preferred embodiment of the method hereinbefore described, the compound that modulates LXR activity is either an antagonist or an agonist.

[0022] In another preferred embodiment, said at least one nucleic acids are selected from the group consisting of the nucleic acids listed in table 3. More preferably, said at least one nucleic acids are selected from the group consisting of the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said at least one nucleic acids are selected from the group consisting of Seq ID No. 16 and/or 24.

[0023] Several methods for measuring expression of said nucleic acids can be used. Methods such as Northern Blotting, and quantitation of the bands by densitometry are well known in the art and may be used, although they may not be sufficiently accurate. Other methods include the use of genechips, microarray analysis, dot blotting or different quantitative PCR methodologies. Preferably, Taqman or real time quantitative PCR is used. In a preferred embodiment, expression levels of at least one nucleic acids selected from the group consisting of Seq ID No. 16 to 26 are determined by Taqman quantitative PCR using the forward primers, reverse primers and probes listed in table 1. In a more preferred embodiment, the expression levels of at least one nucleic acid selected from the group consisting of Seq ID No. 20, 21, 22 and 26 are measured by Taqman quantitative PCR. In a most preferred embodiment, the primers and probes used for measuring at least one nucleic acid selected from the group consisting of Seq ID No. 20, 21, 22 and 26 are primers and protes of Seq ID No. 39 to 41 (for measuring expression levels of the nucleic acid of Seq ID No. 20), or Seq ID No. 42 to 44 (for measuring expression levels of the nucleic acid of Seq ID No. 21), or Seq ID No. 45 to 47 (for measuring expression levels of the nucleic acid of Seq ID No. 22), or Seq ID No. 57 to 59 (for measuring expression levels of the nucleic acid of Seq ID No. 26).

[0024] Taqman quantitative PCR is performed as follows:

[0025] Three oligonucleotides are used: a forward primer, a reverse primer, and a probe. All of them are specific for the target and are able to bind to it. The TaqMan assay uses a probe technology that exploits 5'→3'-nuclease activity of an enzyme, the most commonly used being Taq polymerase. The assay efficiency is largely dependent on this 5'→3' nuclease activity. In this regard one should be careful in choosing a suitable polymerase. Indeed, some polymerases available on the market appear not to be suitable for real-time RT-PCR, even though the manufacturers claim they possess 5'-exonuclease activity. The probe is an oligonucleotide with a reporter dye at the 5' end and a quencher dye at the 3' end. The fluorescent reporter dye is attached covalently to the 5' end and can be FAM (6-carboxyfluorescein), TET (tetrachloro-6-carboxyfluorescein), JOE (2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein), HEX (hexacholoro-6-carboxyfluorescein), or VIC. The reporter is quenched by TAMRA (6-carboxytetramethylrhodamine), bound to the 3' end by a linker arm. DABCYL [4-(48-dimethylaminophenylazo)benzoic acid] can also be used as a quencher dye, but its use is much more prevalent in the molecular beacon probes. An advantage of using DABCYL in the TaqMan probes is its reduced autofluorescence compared with TAMRA When the probe is intact the quencher dye absorbs the fluorescence of the reporter dye due to the proximity between both. The proximity between quencher and fluorophore permits FRET, and fluorescence emission does not occur. By the 5'-exonuclease activity of the Taq:polymerase the probe is hydrolyzed and the reporter dye is separated from the quencher, resulting in an increase in fluorescence emission. During PCR amplification, if the target of interest is present, the probe specifically anneals to the target. The Taq polymerase cleaves the probe, allowing an increase in fluorescence emission. This increase in fluorescence is measured cycle by cycle and is a direct consequence of the amplification process (Giulietti, A., et al., *An Overview of eal-Time Quantitative PCR: Applications to Quantify Cytokine Gene Expression*. Methods 25, 386-401 (2001)).

[0026] Another method provided by the present invention is a method for screening compounds that modulate LXR activity, comprising the steps of contacting said compounds with a host, and measuring the expression of at least one polypeptide selected from the group consisting of the polypeptides encoded by the nucleic acids listed in tables 2 and/or 3. Preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, the marker comprises the nucleic acids, or the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq ID No. 3 to 5 and 20. In another more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, and most preferably, the marker comprises the nucleic acid of, or the polypeptide encoded by Seq ID No. 26.

[0027] In another preferred embodiment, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3. More preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0028] In another embodiment of the method hereinbefore described, the expression of at least one of the polypeptides is compared to the expression of said at least one polypeptide in a control. The control can be an untreated host or a host treated with a carrier. The carrier may be the solvent in which the compound is dissolved or resuspended. In a preferred embodiment of the method hereinbefore described, the compound that modulates LXR activity is either an antagonist or an agonist.

[0029] The present invention also provides a method for monitoring treatment of patients suffering from a disease associated with dysregulation of LXR activity, comprising the steps of purifying mRNA or protein from monocytes/macrophages or from total blood isolated from patients treated with a modulator of LXR activity and measuring the expression of at least one of the nucleic acids, or at least one of the polypeptides encoded by one of the nucleic adds listed in tables 2 and/or 3.

[0030] Preferably, said polypeptides are encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, the marker comprises the nucleic acids, or the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq ID No. 3 to 5 and 20. In another more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, and most preferably, the marker comprises the nucleic acid of, or the polypeptide encoded by Seq ID No. 26.

[0031] In another preferred embodiment, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids listed in table 3. More preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said polypeptides are selected from the group consisting of the polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0032] The control can either be an untreated host, which may be the same host before the treatment or a different host, and/or the same host after an appropriate period of treatment for normalization to pretreatment levels.

[0033] The present invention also provides a method for diagnosing a disease involving dysregulation of LXR activity, comprising the steps of extracting mRNA or protein from total blood or from purified monocytes/macrophages, and measuring the expression of at least one of the nucleic acids, or at least one of the polypeptides encoded by one of the nucleic acids listed in tables 2 or 3.

[0034] Preferably, said polypeptides are encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, the marker comprises the nucleic acids, or the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq ID No. 3 to 5 and 20. In another more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, and most preferably, the marker comprises the nucleic acid of, or the polypeptide encoded by Seq ID No. 26.

[0035] In another preferred embodiment, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or the polypeptides encoded by the nucleic acids, listed in table 3. More preferably, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0036] The control can either be an untreated host, which may be the same host before the treatment or a different host, and/or the same host after an appropriate period of treatment for normalization to pretreatment levels.

[0037] Further to the methods and markers hereinbefore described, the present invention provides a use of one or more nucleic acids, or one or more polypeptides encoded by the nucleic acids listed in tables 2 and/or 3 as a marker for LXR modulation. Preferably, said polypeptides are encoded by the nucleic acids listed in table 2. In a more preferred embodiment, the marker comprises the nucleic, acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 4 to 7, 12, 14, 20 to 22 and 26. In another preferred embodiment, the marker comprises the nucleic acids, or the polypeptides encoded by the nucleic acids listed in table 2, with the exception of Seq ID No. 3 to 5 and 20. In another more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 6, 7, 12, 14, 21, 22 and 26. In an even more preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1, 12 or 21. In a most preferred embodiment, the marker comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 1 or 21. In another embodiment, the marker more preferably comprises the nucleic acids of, or the polypeptides encoded by the nucleic acids of Seq ID No. 20, 22 or 26, and most preferably, the marker comprises the nucleic acid of, or the polypeptide encoded by Seq ID No. 26.

[0038] In another preferred embodiment, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or the polypeptides encoded by the nucleic acids, listed in table 3. More preferably, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or the polypeptides encoded by the nucleic acids of Seq ID No. 16, 17 and 23 to 25. Most preferably, said at least one nucleic acids or polypeptides are selected from the group consisting of the nucleic acids, or polypeptides encoded by the nucleic acids of Seq ID No. 16 and/or 24.

[0039] The present invention also pertains to compounds identified by the methods hereinbefore described, and to the use of compounds identified by a method hereinbefore described for the preparation of a medicament for the treatment of a disease involving dysregulation of LXR activity.

EXAMPLES

[0040] The invention will now be illustrated by reference to the following examples which should not be construed as a limitation thereto.

Cell Culture and RNA Preparation

[0041] The myelogenous monocytic leukemia cell line (THP1; ATCC TID-202) was cultured in RPMI 1640 medium with Phenol Red containing 2 g/l glucose and 2 mM Glutamine, 10% Fetal Bovine Serum FBS (v/v), (Gibco), 1% Penicillin-Streptomycin P/S (v/v) (Gibco), 50 □M 2-Mercaptoethanol (Bio-Rad) and 1% 100 mM Sodium Pyruvate (v/v) (Gibco). Cells were incubated at 37° C. and 5.2% CO_2. For experimental treatments, cells were seeded at a density of 1×10^6/ml in 6-well plates in medium containing 100 nM phorbol 12-myristate 13-acetate (PMA) for 72 hours to fully differentiate them into adherent macrophages. The medium was then replaced by culture medium containing PMA with vehicle (DMSO) or test compound for 6 or 24 hours prior to harvesting. Total RNA was isolated using the TriZol reagent (Life Technologies) or the RNeasy mini kit (Qiagen) according to the manufacturer's protocols. To remove contaminating DNA, the samples were treated with RNAse free DNAse (GibCo).

Gene Expression Measurement by DNA Chips

[0042] Synthesis of first and second strand cDNA were performed using the SuperScript Choice Gene Chip Kit (Life Technologies) and reagents from Gibco. The double stranded cDNA, containing an incorporated T7 RNA polymerase binding site, was purified by extraction with a mix of phenol:chloroform:isoamylalcohol (Life Technologies). The organic and aqueous phases were separated by Phase Lock Gel (Eppendorf) and double-stranded cDNA was recovered by precipitation according to the manufacturer's protocol and then resuspended in water.

[0043] The double-stranded cDNA was converted to biotin-labeled cRNA by in vitro transcription (IVT) using a T7 kit (Ambion) and biotin-containing ribonucleotides (Enzo-LOXO GmbH). The IVT-material was purified from unincorporated ribonucleotides using RNeasy spin columns (Qiagen). Following cleanup, the single-stranded biotin-labeled cRNA were chemically hydrolyzed to smaller fragments in 500 mM calcium acetate, 150 mM magnesium acetate, pH 8.1 for 35 min at 95° C. The reaction was terminated by chilling samples on ice.

[0044] Probes were hybridized to the HGU95AV2 A GeneChip Microarray (Affymetrix) which contains features representing ~12,000 genes. All washing, hybridization, detection, and signal amplification steps were performed using a GeneChip Fluidics Station (Affymetrix). Fluorescence intensity data was collected from the hybridized GeneArrays using a GeneArray scanner (Affymetrix). The raw files containing the fluorescence intensity information were transformed into data files using the Affymetrix Microarray Suite (MAS) software. Differentially expressed genes were identified using the Roche Affymetrix Chip Experiment Analysis (RACE-A) software. Differences between vehicle-treated vs test compound-treated wells (n=4 per group) were evaluated by student t-test and expressed as fold change vs control.

Gene Expression Measurement by Taqman Quantitative PCR

[0045] Gene expression levels were measured by Taqman quantitative RT-PCR on an ABI PRISM 7700 sequence detection system (Applied Biosystems). Total RNA was reverse transcribed into cDNA using the Superscript II RT kit (Life Technologies). The primer/probe sequences used are listed in Table I. Primers were designed using PrimerSelect Software (Perkin Elmer). Differences between vehicle-treated vs test compound-treated wells (n=4 per group) were evaluated by student t-test and expressed as fold change vs control. TaqMan quantitative PCR was performed as described (Giulietti, A., et al., *An Overview of eal-Time Quantitative PCR: Applications to Quantify Cytokine Gene Expression*. Methods 25, 386-401 (2001)).

Example 1

[0046] LXRalpha and LXRbeta are nuclear receptors that heterodimerize with RXR and have been shown to regulate expression of several target genes such as ABCA1, ABCG1, SREBF1, FASN, LPL, and CYP7A [1-9]. To identify surrogate genes that could be measured in patients to evaluate efficacy of a treatment regime, THP1 macrophages were treated with a selective LXR ligand. At 200 nM, T0901317 [10] is a selective LXR agonist (EC50=30 nM and 10 nM on human LXRalpha and LXRbeta, respectively). Gene expression changes were evaluated by Gene Chip Microarray analysis. The LXR agonist T0901317 showed strong induction of ABCA1, ABCG1, APOC1, APOE, ASM3A, C3F, CDC42EP4, CXCR4, PASN, LDLR, NR1H3, SREBP1, VLDLR, FADS1, FADS2, CYB561, IL15, PPFIA2, SERPINI1, KIAA0763, PPIC, LASSI, NFATC4, NR4A3, PRODH2, PRKCI (Table 2). Thus, all of these genes are surrogate markers for LXR modulation and their activity can be measured using GeneChip Microarray technology.

Example 2

[0047] To evaluate an alternative method of measuring surrogate marker genes, THP1 macrophages were treated with T0901317 as described above. Gene expression changes were measured by Taqman quantitative PCR for ABCA1, ABCG1, APOC1, APOE, ASM3A, CDC42EP4, CXCR4, FASN, LDLR, NR1H3, VLDLR. The selective LXR agonist produced a robust and significant increase in expression of each of these marker genes (Table 3). These results, taken together, suggest that measurement of mRNA expression of surrogate marker genes by multiple methods can be effectively used to evaluate therapeutic efficacy of an LXR treatment regimen.

TABLE I

List of Taqman primers and probes used in this study

Gene Name	Forward Primer	Seq ID No	Reverse Primer	Seq ID No.
A.				
ABCA1	AACCCACCACAGGCATGG	27	ACACTTAGGGCACAATTCCACA	28
ABCG1	CCCTCCAGTCATGTTCTTCGA	30	ATGATGGAGCGACCCCCT	31
APOC1	CATGAGGCTCTTCCTGTCGC	33	TGGGCCTTCCAAGACGATC	34
APOE	CGTTGCTGGTCACATTCCTG	36	GCTCTGTCTCCACCGCTTG	37
ASM3A	GAATCTAAAGGGAGAGTCCATCTGG	39	TCCGGCTGCAAATCTTCAAT	40
CDC42EP	TCGGGTATGAGCCCCTGAG	42	GGAGGTGGGTCAGGCTGTT	43
CXCR4	GCAGGACCTGTGGCCAAGT	45	CGCTCTGGAATGTTCAGTTCC	46
FASN	TTGCATTGCTGGTAGAGACCC	48	CACACGCTGCCTGAGGAGT	49
LDLR	CCCCAGGGACAAAACACTGT	51	GCTCCGAAACCAGAAAGGCT	52
NR1H3	TGTAACCGGCGCTCCTTTT	54	TGGTGCCATGGGCCA	55
VLDLR	CAAATAATACCCCCGTCGGA	57	CCAGCCGAGAGGAAGAAAAA	58

Gene Name	Probe	Seq ID No.
B.		
ABCA1	TCCCAAAGCCCGGCGGTTC	29
ABCG1	CCCTCCAGTCATGTTCTTCGA	32
APOC1	CCCGGTCCTGGTGGTGGTTCTGT	35
APOE	CAGGATGCCAGGCCAAGGTGGA	38
ASM3A	AGCTGGAGTATATCCTGACCCAGACCTACGA	41
CDC42EP	TTGACTGCCGGTTATTTTTCTGTCCTGG	44
CXCR4	TTAGTTGCTGTATGTCTCGTGGTAGGACTGTAGAAAA	47
FASN	CAGGCCTGTCCACCCTGCCAA	50
LDLR	CCCCCCAGTGCAGGGAACCG	53

TABLE I-continued

List of Taqman primers and probes used in this study

NR1H3	TGACCGGCTTCGAGTCACGCC	56
VLDLR	TGGTAACCGAGCCAGCAGCTGAAGTCT	59

All sequences are depicted in the 5' to 3' direction. The Taqman probes were labeled with 5'-FAM and 3'-TAMRA and purified by HPLC.

[0048]

TABLE II

Identification of surrogate marker genes of LXR activation by Gene Chip Microarrays

Gene Name	Unigene ID	Accession	Fold-change	p-Value	Treatment Time (hrs)	Seq ID No
ASM3A	Hs.42945	NM 006714.1	+2.84	<0.001**	6	20
C3F	Hs.300423	NM 005768.3	+9.5	0.038*	6	1
CDC42EP4	Hs.3903	AF099664.1	+39.62	<0.001**	24	21
CXCR4	Hs.89414	NM 003467.1	+3.05	<0.001**	6	22
VLDLR	Hs.73729	D16493.1	+3.88	0.001**	24	26
FADS1	Hs.132898	NM 013402.3	+2.11	<0.001**	24	3
FADS2	Hs.184641	NM 004265.2	+3.24	0.012*	24	4
CYB561	Hs.355264	BC002976.1	+3.16	0.002**	24	5
IL15	Hs.168132	NM 000585.2	+3.44	0.03*	24	6
PPFIA2	Hs.30881	AF034799.1	+3.1	0.016*	24	7
SERPINI1	Hs.78589	NM 005025.1	+2	<0.001**	24	8
KIAA0763	Hs.4764	AB018306.1	+2.38	<0.001**	24	9
PPIC	Hs.110364	NM 000943.2	+2.99	0.001**	24	10
LASS1	Hs.348258	NM 021267.1	+2.26	<0.001**	24	11
NFATC4	Hs.77810	NM 004554.1	+5.62	0.01*	24	12
NR4A3	Hs.80561	X89894.1	+1.99	<0.001**	24	13
PRODH2	Hs.128834	NM 021232.1	+3.87	0.001**	24	14
PRKCI	Hs.1904	NM002740.1	+2.28	<0.001**	24	15

Fold change in mRNA levels in THP1 macrophages treated with LXR agonist T0901317 as measured by Gene Chip Microarray Analysis.

All values are fold-change in mRNA and represent the difference between the means of 4 treated vs 4 controls.

Time of treatment is 6 or 24 hours.

*significant $p < 0.05$;

**highly significant, $p < 0.01$.

[0049]

TABLE III

Identification of surrogate marker genes of LXR activation by Gene Chip Microarrays

Gene Name	Unigene ID	Accession	Fold-change	p-Value	Treatment Time (hrs)	Seq ID No
ABCA1	Hs.211562	AJ012376.1	+3.95	<0.001**	6	16
ABCG1	Hs.10237	NM 004915.2	+12.85	<0.001**	6	17
APOC1	Hs.268571	NM 001645.2	+2.77	0.003**	24	18
APOE	Hs.169401	NM 000041.1	+1.83	0.04*	24	19
FASN	Hs.83190	NM 004104.3	+2.09	0.009**	24	23
LDLR	Hs.213289	NM 000527.2	+1.78	0.001**	24	24

TABLE III-continued

Identification of surrogate marker genes of LXR activation by Gene Chip Microarrays

Gene Name	Unigene ID	Accession	Fold-change	p-Value	Treatment Time (hrs)	Seq ID No
NR1H3	Hs.347353	NM 005693.1	+4.1	0.001**	6	25
SREBF1	Hs.166	NM 004176.2	+1.92	0.001**	24	2

Fold change in mRNA levels in THP1 macrophages treated with LXR agonist T0901317 as measured by Gene Chip Microarray Analysis.
All values are fold-change in mRNA and represent the difference between the means of 4 treated vs 4 controls.
Time of treatment is 6 or 24 hours.
*significant p < 0.05;
**highly significant, p < 0.01.

[0050]

TABLE IV

Evaluation of surrogate marker genes of LXR activation by Taqman quantitative RT-PCR

Gene Name	Unigene ID	Fold-change	p-Value	Treatment Time (hrs)	Seq ID No
ABCA1	Hs.211562	+9.52	<0.001**	6	16
ABCG1	Hs.10237	+110.02	<0.001**	6	17
APOC1	Hs.268571	+4.31	<0.001**	24	18
APOE	Hs.169401	+2.2	<0.001**	24	19
ASM3A	Hs.42945	+6.9	0.01*	6	20
CDC42EP4	Hs.3903	+1.86	<0.001**	24	21
CXCR4	Hs.89414	+3.29	<0.001**	6	22
FASN	Hs.83190	+1.42	0.02*	24	23
LDLR	Hs.213289	+2.02	0.01*	24	24
NR1H3	Hs.347353	+5.02	0.001**	6	25
VLDLR	Hs.73729	+6.63	0.001**	24	26

Fold change in mRNA levels in THP1 macrophages treated with LXR agonist T0901317 as measured by Taqman Quantitative RT-PCR.

All values are fold-change in mRNA and represent the difference between the means of 4 treated vs 4 controls.

Time of treatment is 6 or 24 hours.

*significant p < 0.05;

**highly significant, p < 0.01.

[0051]

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<210> SEQ ID NO 4
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
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<222> LOCATION: (1)..(3149)
<223> OTHER INFORMATION: representative cDNA sequence (NM_004265.2, as

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<210> SEQ ID NO 5
<211> LENGTH: 2194
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens, Similar to cytochrome b-561, clone MGC:2190
<222> LOCATION: (1)..(2194)
<223> OTHER INFORMATION: representative cDNA sequence (BC002976.1, as of 24 March 2003) for gene under Unigene ID Hs.3555264

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<210> SEQ ID NO 6
<211> LENGTH: 1496
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens interleukin 15 (IL15), transcript variant 3
<222> LOCATION: (1)..(1496)
<223> OTHER INFORMATION: representative cDNA sequence (NM_000585.2, as
of 24 March 2003) for gene under Unigene ID Hs.168132

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<210> SEQ ID NO 7
<211> LENGTH: 4060
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens liprin-alpha2 mRNA, PPFIA2
<222> LOCATION: (1)..(4060)
<223> OTHER INFORMATION:

<400> SEQUENCE: 7

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cgtgttggtg tggagcaatg accgagttat tcgctggata caagcaattg gacttcgaga 3540

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gcagattctt gaaagagaat acaataacct cttggccctg ggaactgaaa ggcgactgga 3720

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<210> SEQ ID NO 8
<211> LENGTH: 1559
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens serine (or cysteine) proteinase inhibitor,
clade Ineuroserpin), member 1 (SERPINI1)
<222> LOCATION: (1)..(1559)
<223> OTHER INFORMATION: representative cDNA sequence (NM_005025.1, as
of 24 March 2003) for gene under Unigene ID Hs.78589

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tcaaacatgg taactgctaa agagagccaa tatgtgatga aaattgccaa ttccttgttt 420

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<210> SEQ ID NO 9
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<222> LOCATION: (1)..(4148)
<223> OTHER INFORMATION: representative cDNA sequence (AB018306.1, as of
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tgctgtgc 4148

<210> SEQ ID NO 10
<211> LENGTH: 1015
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens peptidylprolyl isomerase C (cyclophilin C) PPIC
<222> LOCATION: (1)..(1015)
<223> OTHER INFORMATION: representative cDNA sequence (NM_000943.2, as of 24 March 2003) for gene under Unigene ID Hs.110364

<400> SEQUENCE: 10

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<210> SEQ ID NO 11
<211> LENGTH: 2565
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens LAG1 longevity assurance homolog 1
<222> LOCATION: (1)..(2565)

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<223> OTHER INFORMATION: representative cDNA sequence (NM_021267.1, as
of 24 March 2003) for gene under Unigene ID Hs.348258

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<210> SEQ ID NO 12
<211> LENGTH: 2880
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4 (NFATC4)
<222> LOCATION: (1)..(2880)
<223> OTHER INFORMATION: representative cDNA sequence (NM_004554.1, as of 24 March 2003) for gene under Unigene ID Hs.77810

<400> SEQUENCE: 12

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<210> SEQ ID NO 13
<211> LENGTH: 2546
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: H.sapiens mRNA for nuclear receptor
<222> LOCATION: (1)..(2546)
<223> OTHER INFORMATION: representative cDNA sequence (X89894.1, as of
      24 March 2003)  for gene under Unigene ID Hs.80561

<400> SEQUENCE: 13

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cccaagccag gacgcccgcg gaacctctcg gctgtgctct cccatgagtc gggatcgcag    360

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cgccggacgt ccgctcctcc tacactctca gcctccgctg gagagacccc cagccccacc    660

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<210> SEQ ID NO 14
<211> LENGTH: 1677
<212> TYPE: DNA
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<213> ORGANISM: Homo sapiens
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<222> LOCATION: (1)..(1677)
<223> OTHER INFORMATION: representative cDNA sequence (NM_021232.1, as
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<400> SEQUENCE: 14

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<210> SEQ ID NO 15
<211> LENGTH: 2261
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens protein kinase C, iota (PRKCI)
<222> LOCATION: (1)..(2261)
<223> OTHER INFORMATION: representative cDNA sequence (NM_002740.1, as
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<400> SEQUENCE: 15

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<210> SEQ ID NO 16
<211> LENGTH: 6880
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens mRNA for ATP-binding cassette
transporter-1 (ABC-1)
<222> LOCATION: (1)..(6880)
<223> OTHER INFORMATION: representative cDNA sequence (AJ012376.1, as of
24 March 2003) for gene under Unigene ID Hs.211562

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<210> SEQ ID NO 17
<211> LENGTH: 2930
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens ATP-binding cassette, sub-family G (WHITE),
member 1(ABCG1), transcript variant 1
<222> LOCATION: (1)..(2930)
<223> OTHER INFORMATION: representative cDNA sequence (NM_004915, as of
24 March 2003) for gene under Unigene ID Hs.10237

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<210> SEQ ID NO 18
<211> LENGTH: 417
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens apolipoprotein C-I (APOC1)
<222> LOCATION: (1)..(417)
<223> OTHER INFORMATION: representative cDNA sequence (NM_001645.2, as
of 24 March 2003) for gene under Unigene ID Hs.268571

<400> SEQUENCE: 18

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acactggagg acaaggctcg ggaactcatc agccgcatca aacagagtga actttctgcc 240

aagatgcggg agtggttttc agagacattt cagaaagtga aggagaaact caagattgac 300

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tcatgaggac ctgaagggtg acatccagga ggggcctctg aaatttccca caccccagcg 360

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<210> SEQ ID NO 19
<211> LENGTH: 1156
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens apolipoprotein E (APOE)
<222> LOCATION: (1)..(1156)
<223> OTHER INFORMATION: representative cDNA sequence (NM_000041.1, as
of 24 March 2003) for gene under Unigene ID Hs.169401

<400> SEQUENCE: 19

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tcaccaagtt tcacgc 1156

<210> SEQ ID NO 20
<211> LENGTH: 1768
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens acid sphingomyelinase-like
phosphodiesterase (ASM3A),
<222> LOCATION: (1)..(1768)
<223> OTHER INFORMATION: representative cDNA sequence (NM_006714.1 as of
24 March 2003) for gene under Unigene ID Hs.42945

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tatctctaaa aaaaaaaaaa aaaaaaaa 1768

<210> SEQ ID NO 21
<211> LENGTH: 1170
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens Cdc42 effector protein 4 mRNA
<222> LOCATION: (1)..(1170)
<223> OTHER INFORMATION: representative cDNA sequence (AF099664.1) as of
24 March 2003) for gene under Unigene ID Hs.3903

<400> SEQUENCE: 21

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cgccacacca tgcacgttgg ccgggccgga gacgcctttg ggacacctc cttcctcaat 240

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<210> SEQ ID NO 22
<211> LENGTH: 1679
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens chemokine (C-X-C motif) receptor 4 (CXCR4)
<222> LOCATION: (1)..(1679)
<223> OTHER INFORMATION: representative cDNA sequence (NM_003467) as of
24 March 2003) for gene under Unigene ID Hs.89414

<400> SEQUENCE: 22

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<210> SEQ ID NO 23
<211> LENGTH: 8461
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: Homo sapiens fatty acid synthase (FASN)
<222> LOCATION: (1)..(8461)
<223> OTHER INFORMATION: representative cDNA sequence (NM_004104.3) as
of 24 March 2003) for gene under Unigene ID Hs.213289

<400> SEQUENCE: 23

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tgtcagaaga acagatccgc ctgaagaaac tgaagcggca agaggaggaa caggctcatg 600

ccacatcctt gccccccagg cgttcctcac ccccccaaat cctgccccag ctcagcccgg 660

aacaactggg catgatcgag aagctcgtcg ctgcccagca acagtgtaac cggcgctcct 720

tttctgaccg gcttcgagtc acgccttggc ccatggcacc agatccccat agccgggagg 780

cccgtcagca gcgctttgcc cacttcactg agctggccat cgtctctgtg caggagatag 840

ttgactttgc taaacagcta cccggcttcc tgcagctcag ccgggaggac cagattgccc 900

tgctgaagac ctctgcgatc gaggtgatgc ttctggagac atctcggagg tacaaccctg 960

ggagtgagag tatcaccttc ctcaaggatt tcagttataa ccgggaagac tttgccaaag 1020

cagggctgca agtggaattc atcaacccca tcttcgagtt ctccagggcc atgaatgagc 1080

tgcaactcaa tgatgccgag tttgccttgc tcattgctat cagcatcttc tctgcagacc 1140

ggcccaacgt gcaggaccag ctccaggtgg agaggctgca gcacacatat gtggaagccc 1200

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tgaaactggt gagcctccgg accctgagca gcgtccactc agagcaagtg tttgcactgc 1320

gtctgcagga caaaaagctc ccaccgctgc tctctgagat ctgggatgtg cacgaatgac 1380

tgttctgtcc ccatattttc tgttttcttg gccggatggc tgaggcctgg tggctgcctc 1440

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ccgtggcatt aaaagagagt caaagggt 1528

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
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      receptor
<222> LOCATION: (1)..(3308)
<223> OTHER INFORMATION: representative cDNA sequence (D16493.1) as of
      24 March 2003) for gene under Unigene ID Hs.73729

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ggcgccccgg gagagcggcg ccaccggaac cgggagaaaa gccaaatgtg aaccctccca 180

attccagtgc acaaatggtc gctgtattac gctgttgtgg aaatgtgatg gggatgaaga 240

ctgtgttgac ggcagtgatg aaaagaactg tgtaaagaag acgtgtgctg aatctgactt 300

cgtgtgcaac aatggccagt gtgttcccag ccgatggaag tgtgatggag atcctgactg 360

cgaagatggt tcagatgaaa gcccagaaca gtgccatatg agaacatgcc gcatacatga 420

aatcagctgt ggcgcccatt ctactcagtg tatcccagtg tcctggagat gtgatggtga 480

aaatgattgt gacagtggag aagatgaaga aaactgtggc aatataacat gtagtcccga 540

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cgagttcacc tgctccagtg gccgctgcat ctccaggaac tttgtatgca atggccagga 600

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gttccagtgc agcacctcct cctgcatccc catcagctgg gtatgcgacg atgatgcaga 720

ctgctccgac caatctgatg agtccctgga gcagtgtggc cgtcagccag tcatacacac 780

caagtgtcca gccagcgaaa tccagtgcgg ctctggcgag tgcatccata agaagtggcg 840

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ttgccgacct gaccaatttg aatgtgagga tggcagctgc atccatggca gcaggcagtg 960

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actaaagtct ctggagttcc tagctcatcc tcttgcacta acaatatttg aggatcgtgt 2040

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ttataaacta attttgtacg tatgaatgat atctttgacc tcaatggagg tttgcaaaga 3240

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taaccatg 3308

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<213> ORGANISM: Homo sapiens
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<222> LOCATION: (1)..(18)
<223> OTHER INFORMATION:

<400> SEQUENCE: 27

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<210> SEQ ID NO 28
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ABCA1 reverse primer
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION:

<400> SEQUENCE: 28

acacttaggg cacaattcca ca 22

<210> SEQ ID NO 29
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ABCA1 probe
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 29

tcccaaagcc cggcggttc 19

<210> SEQ ID NO 30
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ABCG1 forward primer
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

<400> SEQUENCE: 30

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<210> SEQ ID NO 31
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<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<223> OTHER INFORMATION:

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<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ABCG1 probe
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOC1 forward primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 33

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<210> SEQ ID NO 34
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOC1 reverse primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 34

tgggccttcc aagacgatc                                               19


<210> SEQ ID NO 35
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOC1 probe
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION:

<400> SEQUENCE: 35

cccggtcctg gtggtggttc tgt                                          23


<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOE forward primer
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<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 36

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<210> SEQ ID NO 37
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOE reverse primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 37

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<210> SEQ ID NO 38
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: APOE probe
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION:

<400> SEQUENCE: 38

caggatgcca ggccaaggtg ga                                                22


<210> SEQ ID NO 39
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ASM3A forward primer
<222> LOCATION: (1)..(25)
<223> OTHER INFORMATION:

<400> SEQUENCE: 39

gaatctaaag ggagagtcca tctgg                                             25


<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ASM3A reverse primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 40

tccggctgca aatcttcaat                                                   20


<210> SEQ ID NO 41
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: ASM3A probe
<222> LOCATION: (1)..(31)
<223> OTHER INFORMATION:

<400> SEQUENCE: 41

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDC42EP forward primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 42

tcgggtatga gcccctgag                                                19


<210> SEQ ID NO 43
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDC42EP reverse primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 43

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<210> SEQ ID NO 44
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDC42EP probe
<222> LOCATION: (1)..(28)
<223> OTHER INFORMATION:

<400> SEQUENCE: 44

ttgactgccg gttatttttc tgtcctgg                                      28


<210> SEQ ID NO 45
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CXCR4 forward primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

<400> SEQUENCE: 45

gcaggacctg tggccaagt                                                19


<210> SEQ ID NO 46
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CXCR4 reverse primer
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

<400> SEQUENCE: 46

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
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<220> FEATURE:
<221> NAME/KEY: CXCR4 probe
<222> LOCATION: (1)..(37)
<223> OTHER INFORMATION:

<400> SEQUENCE: 47

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<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: FASN forward primer
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

<400> SEQUENCE: 48

ttgcattgct ggtagagacc c                                              21


<210> SEQ ID NO 49
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: FASN reverse primer
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION:

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cacacgctgc ctgaggagt                                                 19


<210> SEQ ID NO 50
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: FASN probe
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

<400> SEQUENCE: 50

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<210> SEQ ID NO 51
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: LDLR forward primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 51

ccccagggac aaaacactgt                                                20


<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: LDLR reverse primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 52
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<210> SEQ ID NO 53
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: LDLR probe
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 53

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<210> SEQ ID NO 54
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: NR1H3 forward primer
<222> LOCATION: (1)..(13)
<223> OTHER INFORMATION:

<400> SEQUENCE: 54

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<210> SEQ ID NO 55
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: NR1H3 reverse primer
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION:

<400> SEQUENCE: 55

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<210> SEQ ID NO 56
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: NR1H3 probe
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION:

<400> SEQUENCE: 56

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: VLDLR forward primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

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<210> SEQ ID NO 58
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<212> TYPE: DNA
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<220> FEATURE:
<221> NAME/KEY: VLDLR reverse primer
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION:

<400> SEQUENCE: 58

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<210> SEQ ID NO 59
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: VLDLR probe
<222> LOCATION: (1)..(27)
<223> OTHER INFORMATION:

<400> SEQUENCE: 59

tggtaaccga gccagcagct gaagtct                                      27
```

1-5. (canceled)

6. A method for screening compounds that modulate LXR activity, comprising the steps of contacting said compounds with a host, and measuring the expression of at least one of the nucleic acids listed in tables 2 and/or 3.

7. The method of claim 6, wherein the expression of at least one of the nucleic acids listed in tables 2 and/or 3 is compared to a control.

8. A method for screening compounds that modulate LXR activity, comprising the steps of: contacting said compounds with a host, and measuring the expression of at least one of the polypeptides encoded by the nucleic acids listed in tables 2 and/or 3.

9. The method of claim 8 wherein said nucleic acid or nucleic acids is/are selected from the group consisting of Seq ID No. 1,6,7,12,14,21,22 and 26.

10. The method of claim 9 wherein said nucleic acid or nucleic acids is/are SEQ ID No. 1 and/or 21.

11. The method of claim 8 wherein said nucleic acid or nucleic acids is/are selected from the group consisting of SEQ ID No. 1,4 to 7, 12, 14, 20 to 22 and 26.

12. (canceled)

13. A method for monitoring treatment of patients suffering from a disease associated with dysregulation of LXR activity, comprising the steps of:

purifying mRNA or protein from monocytes/macrophages or from total blood isolated from patients treated with a modulator of LXR activity and measuring the expression of at least one of the nucleic acids, or at least one of the polypeptides encoded by one of the nucleic acids listed in table 2 and/or 3.

14. The method of claim 13, wherein the expression of at least one of the nucleic acids, or at least one protein encoded by the nucleic acids listed in tables 2 and/or 3 is compared to a control.

15. A method for diagnosing a disease involving dysregulation of LXR activity, comprising the steps of extracting mRNA from total blood or from purified monocytes/macrophages, and measuring the expression of at lease one of the nucleic acids, or at least one of the polypeptides encoded by one of the nucleic acids listed in tables 2 and/or 3.

16. The method of claim 15, wherein the expression of at least one of the nucleic acids, or at least one protein encoded by the nucleic acids listed in tables 2 and/or 3 is compared to a control.

17-19. (canceled)

* * * * *

专利名称(译)	用于lxr激活的标记		
公开(公告)号	US20060281088A1	公开(公告)日	2006-12-14
申请号	US10/557720	申请日	2004-05-14
[标]申请(专利权)人(译)	WRIGHT MATTHEW乙		
申请(专利权)人(译)	WRIGHT MATTHEW乙		
当前申请(专利权)人(译)	WRIGHT MATTHEW乙		
[标]发明人	WRIGHT MATTHEW BLAKE		
发明人	WRIGHT, MATTHEW BLAKE		
IPC分类号	C12Q1/68 C07H21/04 C07H21/00 C12N15/10 C12N15/11 G01N33/50 G01N33/53		
CPC分类号	C07H21/00 C12Q2600/156 C12Q1/6883 A61P3/10 A61P29/00		
优先权	2003011091 2003-05-21 EP		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及用于LXR活化的替代标志物，诊断与LXR活化相关的疾病的方法，监测患有与LXR活化相关的疾病的患者的治疗的方法，以及鉴定调节LXR活性的化合物的方法。

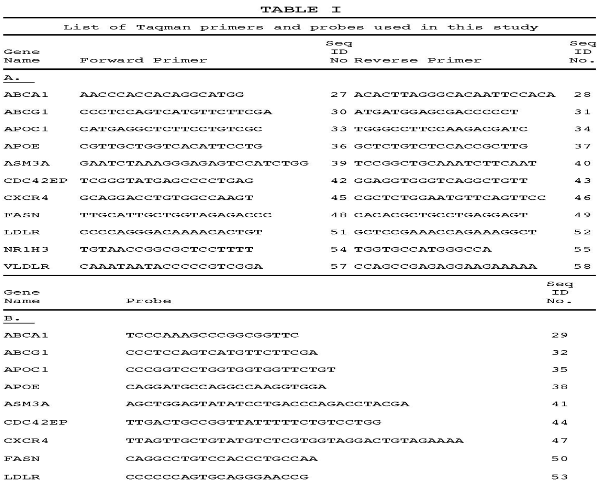

TABLE I

List of Taqman primers and probes used in this study

Gene Name	Forward Primer	Seq ID No	Reverse Primer	Seq ID No.
A.				
ABCA1	AACCCACCACAGGCATGG	27	ACACTTAGGGCACAATTCCACA	28
ABCG1	CCCTCCAGTCATGTTCTTCGA	30	ATGATGGAGCGACCCCCT	31
APOC1	CATGAGGCTCTTCCTGTCGC	33	TGGGCCTTCCAAGACGATC	34
APOE	CGTTGCTGGTCACATTCCTG	36	GCTCTGTCTCCACCGCTTG	37
ASM3A	GAATCTAAAGGGAGAGTCCATCTGG	39	TCCGGCTGCAAATCTTCAAT	40
CDC42EP	TCGGGTATGAGCCCCTGAG	42	GGAGGTGGGTCAGGCTGTT	43
CXCR4	GCAGGACCTGTGGCCAAGT	45	CGCTCTGGAATGTTCAGTTCC	46
FASN	TTGCATTGCTGGTAGAGACCC	48	CACACGCTGCCTGAGGAGT	49
LDLR	CCCCAGGGACAAAACACTGT	51	GCTCCGAAACCAGAAAGGCT	52
NR1H3	TGTAACCGGCGCTCCTTTT	54	TGGTGCCATGGGCCA	55
VLDLR	CAAATAATACCCCCGTCGGA	57	CCAGCCGAGAGGAAGAAAAA	58

Gene Name	Probe	Seq ID No.
B.		
ABCA1	TCCCAAAGCCCGGCGGTTC	29
ABCG1	CCCTCCAGTCATGTTCTTCGA	32
APOC1	CCCGGTCCTGGTGGTGGTTCTGT	35
APOE	CAGGATGCCAGGCCAAGGTGGA	38
ASM3A	AGCTGGAGTATATCCTGACCCAGACCTACGA	41
CDC42EP	TTGACTGCCGGTTATTTTTCTGTCCTGG	44
CXCR4	TTAGTTGCTGTATGTCTCGTGGTAGGACTGTAGAAAA	47
FASN	CAGGCCTGTCCACCCTGCCAA	50
LDLR	CCCCCCAGTGCAGGGAACCG	53