

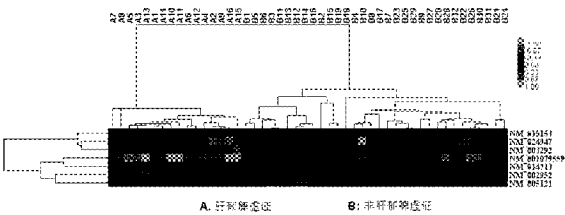
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权利要求书 1 页 说明书 27 页 附图 3 页

(54) 发明名称
与慢性乙型肝炎中医肝郁脾虚证相关的基因
序列的应用
(57) 摘要
本发明公开了一组与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用,用于制备辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品。利用本发明的一组基因序列,可从分子水平对慢性乙型肝炎中医肝郁脾虚证进行准确辨证,并可作为治疗慢性乙型肝炎中医肝郁脾虚证的药物靶标。



1. 一组基因序列的用途,其特征在于,用于制备辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品,该组基因序列为 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

2. 根据权利要求 1 所述的用途,其特征在于,所述辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品包括:用基因芯片检测、实时定量 PCR 或免疫检测诊断慢性乙型肝炎中医肝郁脾虚证的产品。

3. 一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的基因芯片,其特征在于,所述基因芯片包含:与 SEQ ID No. 1 ~ SEQ ID No. 7 所示各基因序列和 / 或其互补序列特异性杂交的探针。

4. 一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的试剂盒,其特征在于,所述试剂盒包含:特异性针对 SEQ ID No. 1 ~ SEQ ID No. 7 所示各基因序列的引物或探针。

5. 一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的试剂盒,其特征在于,所述试剂盒包含:SEQ ID No. 1 ~ SEQ ID No. 7 所示基因序列各表达蛋白的特异性抗体。

6. 一种权利要求 3 所述基因芯片的使用方法,其特征在于,包括如下步骤:

从人外周静脉血白细胞中抽提总 mRNA;

反转录标记 mRNA;

选取权利要求 3 所述的基因芯片,在适于与该芯片进行杂交的条件下,加入经标记的 mRNA,并使其反应足够时间;

洗涤后检测杂交反应的结果。

7. 一种基因序列的用途,其特征在于,用于制备治疗慢性乙型肝炎中医肝郁脾虚证的药物,所述基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

8. 一种基因序列的用途,其特征在于,用于筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物,所述基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

9. 一种蛋白质的用途,其特征在于,用于筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物,所述蛋白质的基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用

技术领域

[0001] 本发明涉及分子诊断领域,尤其涉及一组与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用。

背景技术

[0002] 慢性乙型肝炎 (chronic hepatitis type B, CHB) 患者是指既往有乙型病毒性肝炎或 HBV 携带史,或乙型急性肝炎病程超过半年,而目前仍有肝炎症状、体征及肝功能异常者。我国是慢性乙型肝炎高发地区,据统计,有 7.5 亿人曾感染过 HBV (占全国人口 57.6%),其中慢性 HBV 感染者约 1.2 亿,慢性感染者中 15%~25% 最终也将死于与 HBV 相关的肝病,如肝硬化和肝癌。我国每年约 30 万例因慢性乙型肝炎或相关疾病死亡,占传染病死亡第一位。由于西医缺乏有效治疗措施,在我国,中医及中西医结合治疗是目前的主要方法,且取得了较好的临床效果。但目前仍有许多关键问题亟待解决,尤其是对慢性乙型肝炎的中医证候及辨证体系缺乏规范化研究。

[0003] 肝郁脾虚证在慢性乙型肝炎中占有较大比例,对该证型的深入研究有着重要意义。按照《中药新药临床研究指导原则(试行)》规定的诊断标准,慢性乙型肝炎中医肝郁脾虚证的辨证有以下若干要素组成:脾虚:面色萎黄,身体困重,神疲体倦乏力,纳差,腹胀,少气懒言,大便稀烂或溏薄,舌淡胖有齿痕、苔腻,脉缓;肝郁:情志不遂,胁肋刺痛胀闷或少腹胀满窜痛,善太息,心慌,舌淡红、苔白腻,脉弦细。但是,通过“望、闻、问、切”四诊对以上信息很难量化,导致对于肝郁脾虚证的判断很大程度上依赖于医生的诊疗水平与经验,这在一定意义上客观地制约了临床医生对疾病本质的把握,进一步影响中医及中西医结合疗法对于慢性乙型肝炎中医肝郁脾虚证患者的治疗效果。因此,对于慢性乙型肝炎中医肝郁脾虚证的诊断迫切需要一种准确的、快捷的与可定量的诊断方法。

[0004] 表达谱基因芯片为从分子水平进行疾病诊断提供了有力工具,通过平行考察上万种基因的表达情况,得到每个患者基因表达的分子图谱。然后利用多种聚类分析,如层次聚类、自组织分析、主成分分析等方法将具有不同基因表达谱的疾病进行区分归类,进而为疾病诊断提供可靠的分子水平依据。目前,国际上已有文献报道,通过基因表达谱的研究可以显著区分疾病及疾病分型。如 Golub 等阐述了将基因芯片技术应用于白血病分型,显示出了快速、简便和高特异性等优点。他们设计出的 DNA 芯片成功地分析了急性髓细胞性白血病 (AML) 和急性淋巴细胞性白血病 (ALL),为人类的慢性乙型肝炎中医肝郁脾虚证疾病分型及早期诊断奠定了基础 (Science. 286 :531-7)。在国内研究中,马晓娟等通过对比冠心病血瘀证和非血瘀证患者的静脉血白细胞表达谱找到 48 条基因,为血瘀证的诊断提供了基因层面的依据 (中西医结合学报,6(4):355-360)。但是,目前仍没有关于辅助诊断慢性乙型肝炎中医肝郁脾虚证的基因序列的任何报道。

发明内容

[0005] 本发明要解决缺乏客观准确的辅助诊断慢性乙型肝炎中医肝郁脾虚证的分子依

据的技术问题,提供一组与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用,该组基因序列为诊断慢性乙型肝炎中医肝郁脾虚证提供可靠的分子水平依据。

[0006] 为了解决上述技术问题,本发明通过如下技术方案实现:

[0007] 在本发明的一个方面,提供了一组基因序列的用途,用于制备辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品,该组基因序列为 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

[0008] 优选的,所述辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品包括:用基因芯片检测、实时定量 PCR 或免疫检测诊断慢性乙型肝炎中医肝郁脾虚证的产品。

[0009] 在本发明的另一方面,还提供了一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的基因芯片,所述基因芯片包含:与 SEQ ID No. 1 ~ SEQ ID No. 7 所示各基因序列和/或其互补序列特异性杂交的探针。

[0010] 在本发明中,所述探针可以是 DNA、RNA、DNA-RNA 嵌合体、PNA 或其它衍生物。所述探针的长度没有限制,只要完成特异性杂交、与目的核苷酸序列特异性结合即可。

[0011] 在本发明中,利用基因芯片或实时定量 PCR 诊断慢性乙型肝炎中医肝郁脾虚证的方法是通过检测 SEQ ID No. 1 ~ SEQ ID No. 7 基因在同一样本中相对于其内参基因 NM 001101 (ACTB) 的表达变化进行辨证,其参考相对表达值如下表 1:

[0012] 表 1 SEQ ID No. 1 ~ SEQ ID No. 7 基因相对于内参基因的表达值

	基因转录号	肝郁脾虚证	非肝郁脾虚证
	NM_001101(ACTB)	1	1
[0013]	NM_015153 (SEQ ID No. 1)	0.029~0.094	0.029~0.061
	NM_024947 (SEQ ID No. 2)	0.011~0.036	0.009~0.019
	NM_001079559 (SEQ ID No. 3)	0.063~0.208	0.129~0.275
	NM_003292 (SEQ ID No. 4)	0.024~0.079	0.023~0.05
	NM_014713 (SEQ ID No. 5)	0.138~0.454	0.157~0.335
	NM_002952 (SEQ ID No. 6)	0.087~0.285	0.086~0.184
	NM_005121 (SEQ ID No. 7)	0.047~0.155	0.05~0.107

[0014] 在本发明的另一方面,还提供了一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的试剂盒,所述试剂盒包含:特异性针对 SEQ ID No. 1 ~ SEQ ID No. 7 所示各基因序列的引物或探针。

[0015] 在本发明的另一方面,还提供了一种用于辅助诊断慢性乙型肝炎中医肝郁脾虚证的试剂盒,所述试剂盒包含:SEQ ID No. 1 ~ SEQ ID No. 7 所示基因序列各表达蛋白的特异性抗体。

[0016] 利用本发明的试剂盒,可以检测慢性乙型肝炎中医肝郁脾虚证患者 SEQ ID No. 1 ~ SEQ ID No. 7 基因的转录及蛋白表达情况,从而判断慢性乙型肝炎患者是否为中医肝郁脾虚证。

[0017] 在本发明的另一方面,还提供了一种上述基因芯片的使用方法,包括如下步骤:

[0018] 从人外周静脉血白细胞中抽提总 mRNA;

[0019] 反转录标记 mRNA;

[0020] 选取上述基因芯片,在适于与该芯片进行杂交的条件下,加入经标记的 mRNA,并使其反应足够时间;

[0021] 洗涤后检测杂交反应的结果。

[0022] 在本发明的另一方面,还提供了一种基因序列的用途,用于制备治疗慢性乙型肝炎中医肝郁脾虚证的药物,该基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

[0023] 优选的,所述治疗慢性乙型肝炎中医肝郁脾虚证患者的药物包括:对于 SEQ ID No. 1 ~ SEQ ID No. 7 中表达上调的基因,所述药物为抑制该基因表达或抑制其表达蛋白活性的物质;对于 SEQ ID No. 1 ~ SEQ ID No. 7 中表达下调的基因,所述药物为增加该基因表达或增强其表达蛋白活性的物质。

[0024] 在本发明的另一方面,还提供了一种基因序列的用途,用于筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物,所述基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

[0025] 在本发明的另一方面,还提供了一种蛋白质的用途,用于筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物,所述蛋白质的基因序列选自 SEQ ID No. 1 ~ SEQ ID No. 7 所示的序列。

[0026] 本发明与慢性乙型肝炎中医肝郁脾虚证相关的基因序列,可作为慢性乙型肝炎中医肝郁脾虚证的诊断标志物,从分子水平对慢性乙型肝炎中医肝郁脾虚证进行准确辨证,进而能够为慢性乙型肝炎临床中医及中西医结合治疗提供可靠依据。同时,这些基因也可作为控制慢性乙型肝炎中医肝郁脾虚证的药物治疗靶标,为设计和筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物提供新的靶点。

附图说明

[0027] 下面结合附图和具体实施方式对本发明作进一步详细的说明。

[0028] 图 1 是本发明实施例 2 中 RNA 提取的质量检测 Agilent2100 峰图;

[0029] 图 2 是本发明利用 6 例慢性乙型肝炎中医肝郁脾虚证患者与 6 例慢性乙型肝炎非中医肝郁脾虚证患者样本外周静脉血白细胞表达谱数据进行偏最小二乘法 (PLS) 建立的判别模型示意图;

[0030] 图 3 是本发明利用 36 例慢性乙型肝炎患者外周静脉血白细胞表达谱数据进行盲法验证的主成分分析 (PCA) 图;

[0031] 图 4 是本发明的 48 例慢性乙型肝炎患者外周静脉血白细胞 SEQ ID No. 1 ~ SEQ ID No. 7 基因转录信号值的无监督基因聚类分析图;

[0032] 图 5 是本发明的 48 例慢性乙型肝炎患者外周静脉血白细胞 SEQ ID No. 1 ~ SEQ ID No. 7 基因与相应的 ACTB 基因转录信号值比较值的无监督基因聚类分析图。

具体实施方式

[0033] 下列实施例中,未注明具体条件的实验方法,通常按常规条件,如《精编分子生物学实验指南》(F.M. 奥斯伯, R.E. 金斯顿, J.G. 塞德曼等主编,马学军,舒跃龙的译. 北京:科学出版社,2004) 中所述的方法进行。

[0034] 实施例 1 临床样品的准备

[0035] (a) 模型建立样品准备:经由三位中医临床专家辨证一致的 6 例慢性乙型肝炎中医肝郁脾虚证患者,6 例慢性乙型肝炎非中医肝郁脾虚证患者于上午 (9-10 点) 空腹抽取外周静脉血 3ml, -20℃ 储存于 Applied Biosystems 公司的血液 RNA 采集管中。此步骤由上海中医药大学附属曙光医院肝病所及检验科协助完成。

[0036] (b) 模型验证样品准备 : 收集 36 例用于扩大验证的慢性乙型肝炎外周静脉血样本, 处理方法同上。

[0037] 实施例 2 全血白细胞总 RNA 的提取、纯化和质量检测

[0038] (1) 全血白细胞总 RNA 的提取、纯化

[0039] 采用 AppliedBiosystems 公司的全血 RNA 抽提试剂盒 (Tempus Spin RNA Isolation Reagent Kit) 进行白细胞总 RNA 的提取与纯化, 操作步骤按照该试剂盒标准操作说明书进行。

[0040] (2) 总 RNA 的质量检测

[0041] 用 Agilent 2100bioanalyzer 分析提取的 RNA, 清晰的峰形和最低的背景荧光显示完整、未降解的 RNA (见图 1)。

[0042] 实施例 3 基因表达谱实验

[0043] (1) 芯片实验过程

[0044] 采用 Affymetrix U-219 表达谱芯片, 实验过程严格按照 Affymetrix 表达谱芯片操作手册进行。

[0045] (2) 芯片扫描与数据读取

[0046] 芯片采用 GeneChip® Scanner 3000 (P/N00-00212, Affymetrix) 进行扫描, 用 Command Console Software 3.1 (Affymetrix) 读取原始数据。

[0047] (3) 数据标准化

[0048] 质控合格的数据采用 Gene Spring Software 11.0 (Agilent) 进行归一化处理, 所用的算法为 MAS5.0。

[0049] (4) 差异筛选和统计分析

[0050] 差异基因筛选, 以 80 % 样本中有检测信号为标准, Gene Spring Software 11.0 (Agilent) 作慢性乙型肝炎中医肝郁脾虚证和慢性乙型肝炎非中医肝郁脾虚证的组间差异基因 T-test 结合 SAM 分析, p 值取 0.05, FDR 值取 0.05。

[0051] 实施例 4 判别模型建立

[0052] (1) 判别模型建立

[0053] 以经三位中医临床专家确诊的 6 例慢性乙型肝炎中医肝郁脾虚证患者全血表达谱芯片实验数据为实验组, 以 6 例慢性乙型肝炎非中医肝郁脾虚证全血表达谱芯片实验数据为对照组。经标准化处理和统计分析后, 将实验组与对照组比较 $p < 0.05$, $FDR < 0.05$ 的所有差异基因用 SIMCA-P11.5 进行偏最小二乘法 (PLS) 建立判别模型。

[0054] (2) 结果

[0055] 找到 SEQ ID No. 1 ~ SEQ ID No. 7 共 7 条基因可以有效鉴别出慢性乙型肝炎中医肝郁脾虚证与非肝郁脾虚证, 如图 2 所示。图 2 为偏最小二乘法 (PLS) 判别模型示意图, 其中 $R^2Y = 0.997$, $Q^2 = 0.917$, 说明模型判别度较高。

[0056] 实施例 5 模型验证

[0057] (1) 模型验证

[0058] 进一步收集临床慢性乙型肝炎病人外周静脉血样本 36 例, 运用本发明的含有针对上述 SEQ ID No. 1 ~ SEQ ID No. 7 基因特异探针的自制芯片进行该 7 条基因的特异检测。将此 36 例样本数据结合模型建立时的 12 例样本共同进行主成分分析 (PCA)。

[0059] (2) 结果

[0060] 如图 3 所示,发现 10 例病人的表达谱数据与模型建立时 12 例数据中 6 例慢性乙型肝炎中医肝郁脾虚证十分接近,26 例与模型建立时 12 例数据中 6 例慢性乙型肝炎非中医肝郁脾虚证十分接近。结合临床资料,经中医临床专家辨证,该 10 和 26 例患者分别为慢性乙型肝炎中医肝郁脾虚证和非肝郁脾虚证患者。说明通过此 SEQ ID No. 1 ~ SEQ ID No. 7 基因可以对慢性乙型肝炎中医肝郁脾虚证与非中医肝郁脾虚证进行有效区分和辨证。无监督基因聚类图 (gene cluster) 亦可有效区分慢性乙型肝炎中医肝郁脾虚证与非肝郁脾虚证患者 (见图 4)。如图 4 所示,上方是样本编号,样本编号下方是表达图谱,红色表示基因上调 (相对于该基因的平均数),绿色表示下调,黑色表示未变化,右边为该 SEQ ID No. 1 ~ SEQ ID No. 7 共 7 条基因转录号。A 组:慢性乙型肝炎中医肝郁脾虚证患者;B 组:慢性乙型肝炎非中医肝郁脾虚证患者。

[0061] 实施例 6 诊断应用

[0062] (1) 数据处理

[0063] 将 16 例慢性乙型肝炎中医肝郁脾虚证与 32 例慢性乙型肝炎非中医肝郁脾虚证患者 SEQ ID No. 1 ~ SEQ ID No. 7 共 7 条基因的转录信号值与对应患者的 ACTB 基因的转录信号值相除后得到各 SEQ ID No. 1 ~ SEQ ID No. 7 基因相对于 ACTB 基因的参考相对表达值,并做无监督基因聚类图 (gene cluster)。

[0064] (2) 结果

[0065] 如图 5 所示,用 SEQ ID No. 1 ~ SEQ ID No. 7 基因相对于 ACTB 基因的参考相对表达值做出无监督基因聚类图 (gene cluster) 仍可较好区分慢性乙型肝炎中医肝郁脾虚证与慢性乙型肝炎非中医肝郁脾虚证患者。

[0066] 实施例 7 治疗慢性乙型肝炎中医肝郁脾虚证药物的筛选

[0067] 以 SEQ ID No. 1 ~ SEQ ID No. 7 基因中任意一个基因作为靶点,设计和筛选治疗慢性乙型肝炎中医肝郁脾虚证的药物抗肿瘤转移药物。具体方法如下。

[0068] 对于 SEQ ID No. 1 ~ SEQ ID No. 7 中表达上调的基因,若候选药物可降低该基因的表达或降低过表达蛋白的活性,则表明该候选药物是能控制慢性乙型肝炎中医肝郁脾虚证的潜在物质;对于 SEQ ID No. 1 ~ SEQ ID No. 7 中表达下调的基因,若候选药物可增加该基因的表达或提高该表达蛋白的活性,则表明该候选药物是能控制慢性乙型肝炎中医肝郁脾虚证的潜在物质。

[0069] 以上所述实施例仅表达了本发明的实施方式,其描述较为具体和详细,但并不能因此而理解为本发明专利范围的限制。应当指出的是,对于本领域的普通技术人员来说,在不脱离本发明构思的前提下,还可以做出若干变形和改进,这些都属于本发明的保护范围。因此,本发明的保护范围应以所附权利要求为准。

[0070] 序列表

[0071] <110> 上海生物芯片有限公司

[0072] <120> 与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用

[0073] <160>7

[0074] <170>PatentIn version 3.3

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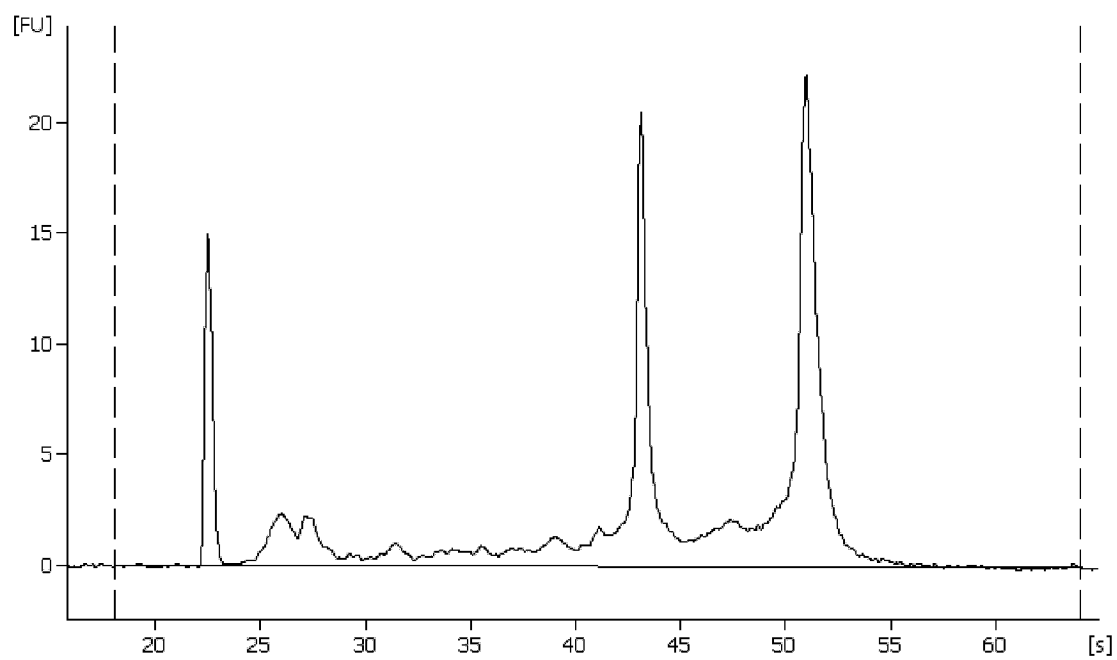


图 1

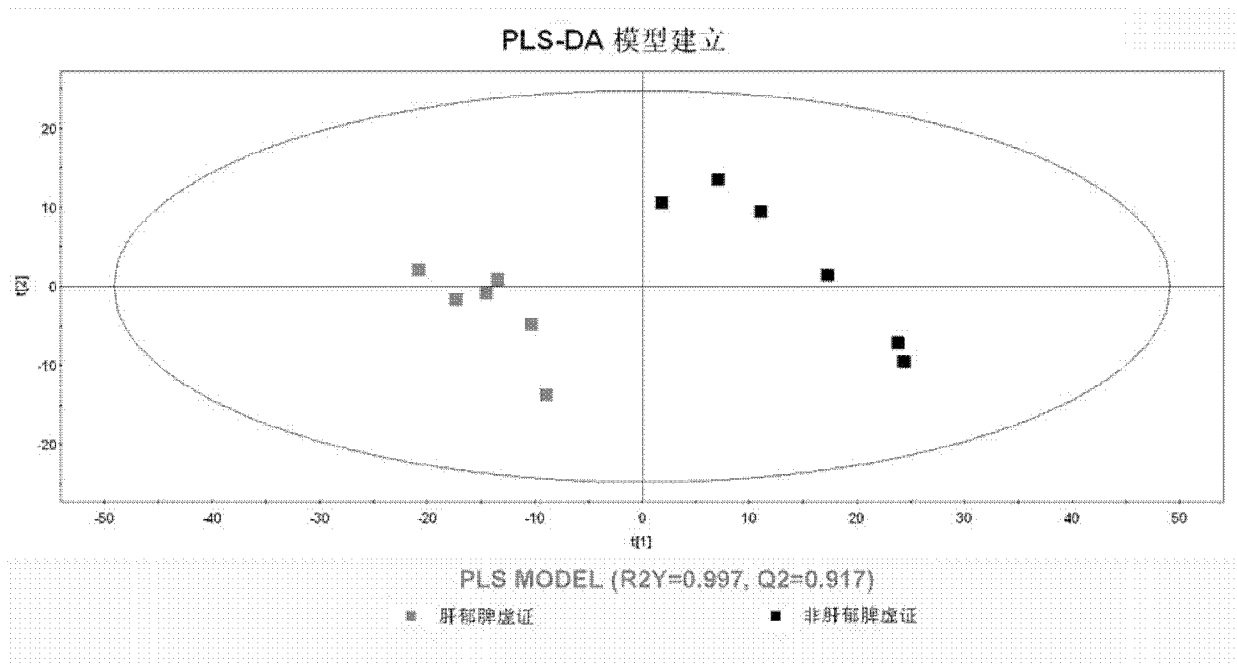


图 2

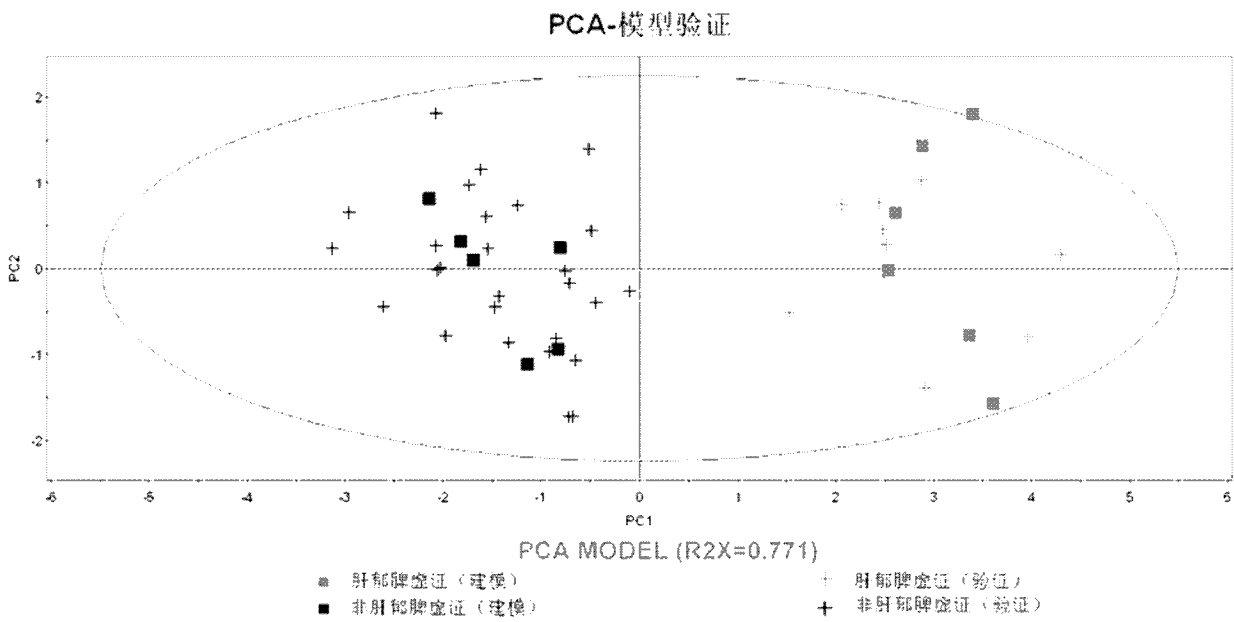


图 3

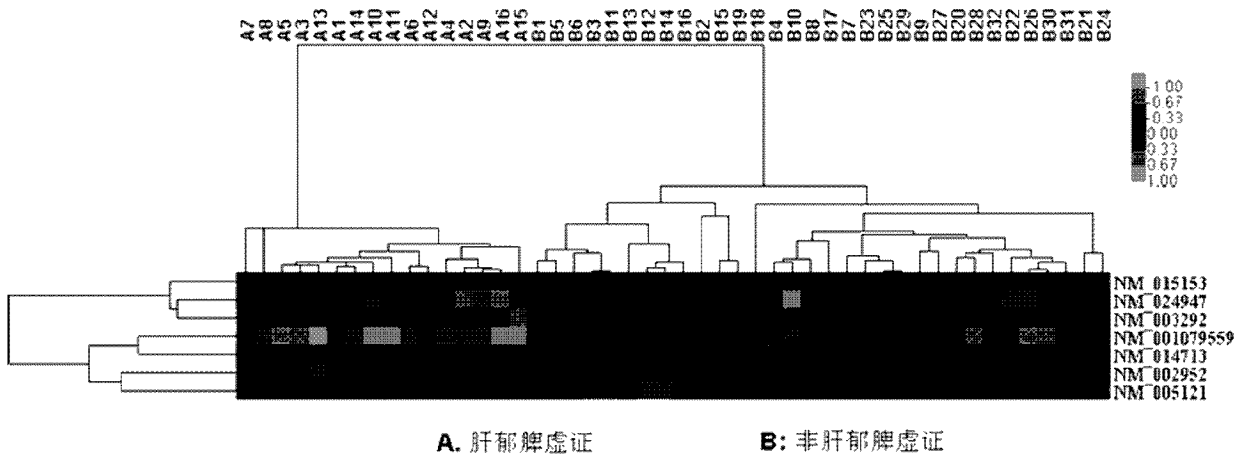


图 4

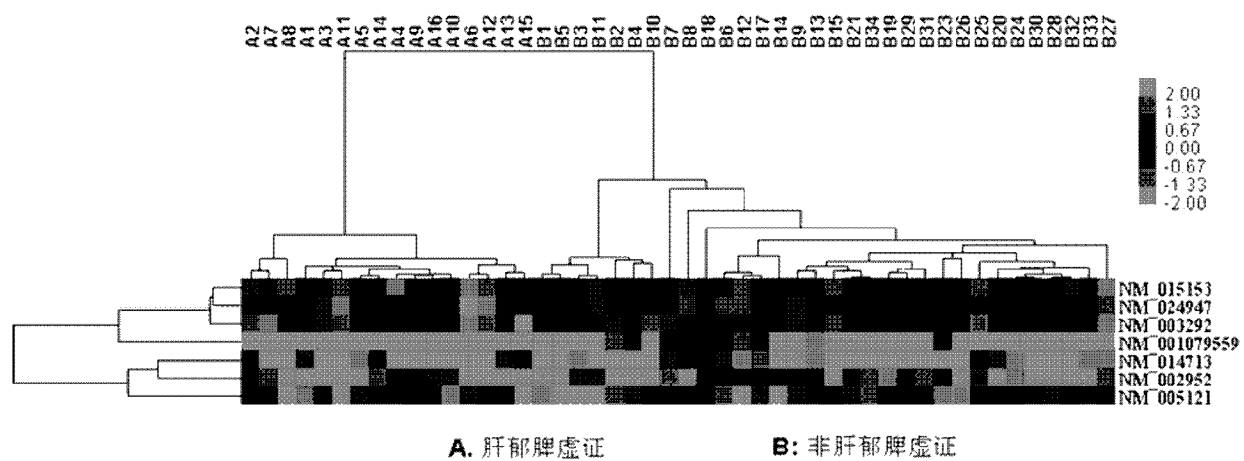


图 5

摘要(译)

本发明公开了一组与慢性乙型肝炎中医肝郁脾虚证相关的基因序列的应用，用于制备辅助诊断慢性乙型肝炎中医肝郁脾虚证的产品。利用本发明的一组基因序列，可从分子水平对慢性乙型肝炎中医肝郁脾虚证进行准确辨证，并可作为治疗慢性乙型肝炎中医肝郁脾虚证的药物靶标。

