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TLR4多态性与NAFLD易感性的相关性

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摘 要

目的：探究中国青岛地区人群Toll样受体4 (Toll-like receptors 4, TLR4) rs1927914位点多态性与非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)易感性的相关性。方法：纳入2020年12月~2022年06月青岛市市立医院收入院的NAFLD患者218例，健康对照者101例。提取受试者血液中的DNA，使用多聚酶链反应(polymerase chain reaction, PCR)的方法扩增DNA，并检测TLR4基因rs1927914位点的基因型。收集并分析患者的与脂质代谢及肝脏代谢状态相关的指标。使用χ^2检验分析基因型及等位基因频率。符合正态分布的计量资料采用t检验，不符合正态分布的计量资料采用Wilcoxon秩和检验进行组间比较。结果：NAFLD组和对照组TLR4 rs1927914位点的基因型与等位基因分布差异均无统计学意义。结论：在青岛地区人群中，TLR4 rs1927914位点多态性与NAFLD的无明显相关性。

关键词

非酒精性脂肪性肝病，Toll样受体4，单核苷酸多态性

Association between TLR4 Polymorphism and NAFLD Susceptibility

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Abstract

Objective: To explore the polymorphism of Toll-like receptors 4 (TLR4) rs1927914 locus and non-

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alcoholic fatty liver disease in Qingdao population of China association with susceptibility to NAFLD. Methods: 218 patients with NAFLD and 101 healthy controls admitted to Qingdao Municipal Hospital from December 2020 to June 2022 were included. DNA was extracted from the subjects' blood, amplified using polymerase chain reaction (PCR), and genotypes at rs1927914 locus of the TLR4 gene were detected. Indicators related to lipid metabolism and liver metabolic status were collected and analyzed. Genotype and allele frequency were analyzed using χ^2 test. T-test was used for measurement data conforming to normal distribution, and Wilcoxon rank sum test was used for comparison between groups for measurement data not conforming to normal distribution. Results: There were no significant differences in genotype and allele distribution of TLR4 rs1927914 between NAFLD group and control group. Conclusion: There is no significant correlation between TLR4 rs1927914 polymorphism and NAFLD in Qingdao population.

Keywords

Nonalcoholic Fatty Liver Disease, Toll-Like Receptor 4, Single Nucleotide Polymorphism

1. 引言

非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)和非酒精性脂肪性肝炎(nonalcoholic steatohepatitis, NASH)在逐渐成为肝硬化和肝癌的最重要病因，同时也是肝脏相关疾病中发病率增长最快的疾病[1] [2]。预计在 2016~2030 年期间，NAFLD 病例总数仍将继续增长，而我国正处于经济发展阶段，将会是 NAFLD 病例数量增长的重点国家。疾病造成的负担也会进一步增加[3]。遗传因素在 NAFLD 的发生发展中有着重要作用，找出与 NAFLD 相关的基因位点可以对 NAFLD 进行预测。因此，对普通人群进行 NAFLD 预测及预后评估，选出高危人群，进而有针对性的对高危人群进行疾病的一级预防，将会大大减少 NAFLD 对社会及个人的负担。临床和基础研究已经发的可能与 NAFLD 发生发展相关的基因包括 PNPLA3、TM6SF2，MBOAT7，HSD17B13，MARC1 [4] [5] [6] [7]，所以进一步寻找促进 NAFLD 发生及发展基因位点具有重要意义。

Toll 样受体(Toll-like receptors, TLR)家族包括 13 个成员，其中 TLR4 是研究最广泛的一个[8]。主要表达于胎盘，外周血血细胞，脾脏等组织器官，接收来自脂多糖(Lipopolysaccharide, LPS)的信号刺激[9]，在炎症反应及免疫反应[10]中发挥重要作用。其 rs1927914 位点的多态性影响着多系统疾病的发生发展，有研究表明，rs1927914 位点 C 等位基因的突变与其在血清中表达水平提高有关，进而导致血管重构及动脉瘤的生成[11]；另有研究表明，TLR4 rs1927914 位点 C 等位基因与 2 型糖尿病存在相关性[12]。2 型糖尿病与 NAFLD 同为代谢综合征[13]的重要组成部分，为探究 TLR4 rs1927914 位点与 NAFLD 的发生的可能影响，本研究对 TLR4 rs1927914 位点多态性与 NAFLD 的相关性进行探讨。

2. 资料与方法

2.1. 研究对象

纳入 2020 年 12 月~2022 年 06 月就诊于青岛市市立医院消化科及肝病科以及健康查体的，经过超声的影像学手段确诊的 NAFLD 人群及与患者性别，年龄等其他因素相匹配的对照组人群。NAFLD 的定义

参考 2010 年修订版《非酒精性脂肪性肝病诊疗指南》[14]。本研究已通过医院伦理委员会审核，且所有纳入的被研究者皆已签署知情同意书。

2.2. 研究方法

受试者肝脏代谢相关指标及基因位点的测定

所有受试者均抽取禁食 12h 后的全血，然后送血样到检验科，检测指标包括总胆红素(TBiL)、谷丙转氨酶(ALT)、谷草转氨酶(AST)、γ-谷氨酰转移酶(GGT)、甘油三酯(TG)、总胆固醇(TC)、低密度脂蛋白(LDL)、高密度脂蛋白(HDL)。采用聚合酶链式反应对目的基因进行扩增，并对 TLR4 rs1927914 位点多态性分析。PCR 引物序列：上游引物：ACGTTGGATGACAGTAGAACTATCTAGGAC；下游引物：ACGTTGGATGGGAAAGTAGCAAGTGCAATG。提取 DNA 后，由博淼生物科技(北京)有限公司采用基于 Massarray 技术进行位点核苷酸多态性测序。

2.3. 统计学处理

使用 SPSS 20.0 软件进行统计学处理。使用平均值±标准差用来表示符合正态分布的数据，使用中位数，(四分位数)用来表示不符合正态分布的数据，两种数据组间比较分别采用 t 检验及 Wilcoxon 秩和检验。计数资料组间比较采用 χ^2 检验。$P < 0.05$ 为差异有统计学意义。

3. 结果

3.1. 一般资料结果的比较

NAFLD 组与对照组相比较，血清 BMI、ALT、AST、GGT、ALP、TG 水平均较对照组高，血清 HDL 水平较低，差异均有统计学意义($P < 0.05$)，其余指标无明显统计学差异($P > 0.05$) (表 1)。

Table 1. Comparison of general clinical data between the control group and the NAFLD group
表 1. 对照组与 NAFLD 组一般临床资料比较

指标	对照组	NAFLD 组	t/z 值	*P* 值
BMI (kg/m^2)	24.22 (21.8, 27.455)	26.84 (24.40, 29.30)	z = −4.864	<0.001
TBi L(ummol/L)	13.35 (10.53, 17.50)	12.80 (10.40, 16.80)	z = −0.606	0.545
ALT (U/L)	17.36 (12.98, 25.68)	28.38 (17.90, 41.96)	z = −5.438	<0.001
AST (U/L)	19.10 (16.19, 24.00)	24.26 (19.74, 32.94)	z = −5.214	<0.001
GGT (U/L)	18.00 (12.00, 23.87)	30.75 (21.35, 54.18)	z = −7.253	<0.001
ALP (U/L)	74.76 (59.53, 87.4)	87.53 (72.32, 104.40)	z = −3.562	0.026
TG (mmol/L)	1.02 (0.77, 1.41)	1.72 (1.14, 2.41)	z = −7.092	<0.001
TC (mmol/L)	4.91 (4.26, 5.53)	5.09 (4.36, 5.78)	z = −1.291	0.197
LDL (mmol/L)	3.00 (2.43, 3.46)	3.13 (2.63, 3.55)	z = −1.356	0.175
HDL (mmol/L)	1.31 (1.14, 1.50)	1.16 (1.00, 1.32)	z = −4.409	<0.001

BMI：身体质量指数；TBIL：总胆红素；ALT：谷丙转氨酶；AST：谷草转氨酶；GGT：γ-谷氨酰转移酶；ALP：碱性磷酸酶；TC：总胆固醇；TG：三酰甘油；LDL：低密度脂蛋白；HDL：高密度脂蛋白。

3.2. TLR4 rs1927914 基因型分析

经检验，NAFLD 组与对照组的 TLR4 rs1927914 多态性分布情况均符合 Hardy-Weinberg 平衡。TLR4

rs1927914 携带等位基因 C 与未携带等位基因 C 在 NAFLD 组与对照组无组间差异(P 值均>0.05) (表 2)。

Table 2. Distribution of TLR4 rs1927914 genotypes and alleles in NAFLD group and control group
表 2. NAFLD 组和对照组 TLR4 rs1927914 基因型和等位基因的分布

	对照组	NAFLD 组	χ^2 值	P 值
基因型			0.857	0.651
TT	38	78		
CT	45	108		
CC	18	32		
等位基因			0.024	0.876
C	121	264		
T	81	172		

3.3. 不同基因型组间一般生化指标的比较

对 TLR4 rs1927914 携带等位基因 C 与未携带等位基因 C 组间进行一般生化指标的比较，携带等位基因 C 组的 TG 水平低于未携带等位基因 C 组，差异存在统计学意义(P 值 < 0.05)，其余指标在两组间未见差异(P 值均>0.05) (表 3)。

Table 3. Comparison of general biochemical ratios between C alleles and non-C alleles
表 3. 携带 C 等位基因及未携带 C 等位基因组一般生化比指标的比较

指标	TT	CT + CC	t/z 值	P 值
BMI (kg/m^2)	26.52 (23.88, 28.72)	25.88 (23.41, 28.91)	t = −0.899	0.369
TBiL (ummol/L)	12.50 (10.00, 16.80)	13.35 (10.88, 17.20)	z = −0.942	0.346
ALT (U/L)	23.75 (15.19, 35.83)	23.00 (15.00, 32.78)	z = −0.120	0.905
AST (U/L)	22.04 (18.38, 29.03)	22.13 (18.17, 29.77)	z = −0.260	0.795
GGT (U/L)	25.82 (17.00, 48.02)	24.79 (16.88, 44.19)	z = −0.509	0.509
ALP (U/L)	85.75 (69.04, 97.28)	85.86 (70.46, 103.00)	z = −0.593	0.593
TG (mmol/L)	1.55 (1.06, 2.17)	1.32 (0.88, 2.02)	t = −0.038	0.038
TC (mmol/L)	5.04 (4.26, 5.51)	4.99 (4.33, 5.77)	z = −0.493	0.493
LDL (mmol/L)	3.04 (2.41, 3.50)	3.13 (2.61, 3.55)	z = −0.246	0.246
HDL (mmol/L)	1.19 (1.03, 1.37)	1.22 (1.06, 1.42)	z = −0.345	0.345

4. 讨论

NAFLD 的发生需要遗传因素与后天环境因素共同作用，现已发现多个基因多态性能通过影响肝脏脂质代谢影响 NAFLD 的发生发展[15] [16]。TLR4 rs1927914 位点多态性能够影响心血管系统代谢[17]，血管瘤[11]的生成，糖尿病[12]及糖尿病并发症[18]的发生发展。本研究首次探究青岛地区人群 TLR4 rs1927914 位点多态性与 NAFLD 发生的相关性，进一步完善 NAFLD 易感性的相关研究。

TLR4 rs1927914 位点多态性变化在全身各系统代谢中都起着重要作用，针对其对肺癌[19]，食管癌[20]，特应性皮炎[19]，银屑病[21]，动脉瘤[11]，脑血管疾病[22]等均有研究。特别是近 3 年来，相关研

究倾向于探索 TRL4 rs1927914 对免疫功能紊乱相关疾病的影响，比如对过敏性鼻炎[23]，肾病综合征[24]的研究。另外，在在一项来自巴西的研究表明，TLR4 rs1927914 的多态性[24]可能对麻风结节性红斑治疗反应有影响，试图将该位点多态性应用于治疗效果及疾病发病等的预测上。在近些年针对该位点与代谢综合征的关系研究表明，该位点可能影响代谢综合中的发生发展。在 2015 年的一项研究显示，TLR4 rs1927914 位点多态性与糖尿病存在相关性，其 C 等位基因组在糖尿病组和对照组有着明显的分布差异($P < 0.01$，OR 值为 1.46) [12]；这提示我们 TLR4 rs1927917 位点多态性可能影响全身代谢，糖尿病及 NAFLD 都是代谢综合征的重要组成部分，是全身代谢紊乱的不同表现形式[25] [26]。在针对 TLR4 rs1927914 位点多态性与肝脏疾病的研究中，有人发现 T 等位基因携带与肝移植后肝癌复发有相关性[27]，而该位点纯合子与更低水平的肝癌发生率有关[28]，这些研究提示我们 TLR4 rs1927914 位点多态性在肝脏代谢中起着一定比作用；然而，我们的研究未发现 TLR4 rs1927914 与 NAFLD 存在相关性，这与 Xu [12]等人研究中，该基因 C 等位基因位点对代谢综合征的影响不完全一致。但在携带等位基因 C 人群与不携带等位基因 C 人群的生化相关指标比较中，携带等位基因 C 的人群有着更低的甘油三酯水平。而在另一项对 TRL4 rs1927914 位点的研究中，未见 C 等位基因对血脂代谢紊乱的影响[17]，这可能与我们的统计的变量类型不同有关。血清甘油三酯水平的差异提示 TLR4 rs1927914 位点多态性可能影响了甘油三酯的代谢，而甘油三酯水平的波动往往是包括肝脏在内的多器官共同作用的结果[29]，结合既往研究中 C 等位基因的存在与糖尿病存在相关性，我们推测，TLR4 rs1927914 位点 C 等位基因携带可能减少了肝脏甘油三酯的分泌，进而导致了肝脏的脂质蓄积，这种作用机制同样发生于膜蛋白基因点突变导致的肝脏脂质蓄积[30] [31]。另外，既往研究中，TLR4 rs1927914 位点多态性在肝移植后肝癌复发中起作用[27]，这启示我们 TRL4 rs1927914 对肝脏器官的代谢有着特异性影响，而 NAFLD 与 HCC 又存在于同一疾病谱中[32] [33] [34]。因此，尽管此次我们的研究未发现 TLR4 rs1927914 位点多态性与 NAFLD 存在相关性，但仍不能忽视该位点对全身代谢的影响。

综上所述，在本次研究，我们未发现 TLR4 rs1927914 位点多态性与 NAFLD 有相关性。但是在本次研究中，我们发现，C 等位基因携带组甘油三酯水平低于未携带 C 等位基因组。本研究纳入的人群样本数量少，地区单一，针对 TLR4 rs1927914 对 NAFLD 多态性的影响还需要更大样本量，多中心的研究进行研究。

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