Patatin样磷酯酶结构域蛋白3基因rs738409位点多态性与非酒精性脂肪肝易感性关系的Meta分析

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【摘要】　目的　探讨Patatin样磷酯酶结构域蛋白3(PNPLA3)基因rs738409位点多态性与非酒精性脂肪肝易感性关系。方法　检索PubMed以及中文数据库(中国生物医学文献数据库、中国知网、万方数据库、维普数据库),收集有关PNPLA3基因rs738409位点多态性与脂肪肝易感性的病例对照研究,提取文献相关数据进行Meta分析,以病例组与对照组PNPLA3基因rs738409位点各种基因模型的OR值及其95%CI为效应指标,并根据研究人群种族进行亚组分析。结果　共28篇研究符合纳入标准,累计病例数6 216例,对照组8 218例。Meta分析显示,PNPLA3基因rs738409多态性与非酒精性脂肪肝易感性有关联[纯合子比较模型(GG vs. CC):OR=2.42,95%CI:1.83 ~ 3.21,P<0.001;杂合子比较模型(CG vs. CC):OR=1.28,95%CI:1.15 ~ 1.43,P<0.001;显性遗传模型(CG+GG vs. CC):OR=1.31,95%CI:1.17 ~ 1.46,P<0.001;隐性遗传模型(GG vs. CC+GC):OR=2.26,95%CI:1.76 ~ 2.90,P<0.001];亚组分析显示,亚洲以及高加索人群PNPLA3基因rs738409位点多态性与非酒精性脂肪肝易感性有关联。敏感性分析显示Meta分析结果稳定。结论　PNPLA3基因I148M多态性与非酒精性脂肪肝易感性明显相关。
【关键词】　非酒精性脂肪肝;Patatin样磷酯酶结构域蛋白3;基因多态性;易感性;Meta分析

Association between patatin-like phospholipase domain-containing protein 3 gene rs738409 polymorphism and non-alcoholic fatty liver disease susceptibility: a Meta-analysis　Wu Pengbo[1], Shu Yongxiang[1], Guo Fang[1], Luo Hesheng[1], Zhang Guo[2], Tan Shiyun[1]. 1 Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan 430060, China; 2 Department of Gastroenterology, the People's Hospital of Guangxi Zhuang Autonomous Region
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【Abstract】　Objective　To explore the association between patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene rs738409 polymorphism and the susceptibility of non-alcoholic fatty liver disease (NAFLD). Methods　Data bases were comprehensively searched to retrace all the related studies on the association between PNPLA3 gene rs738409 polymorphism and susceptibility. Of NAFLD, the pooled OR with 95% CI of the association between PNPLA3 gene rs738409 polymorphism and NAFLD susceptibility were performed using different genetic models. Subgroup analysis based on the source of population and sensitivity analysis was performed to detect the stability of results. Results　28 original studies with 6 216 patients and 8 218 controls were involved in the final combination of data. Findings from the meta-analyses showed that there were strong associations between PNPLA3 gene rs738409 polymorphism and the susceptibility of NAFLD, under different genetic model comparisons [GG vs. CC: OR=2.42, 95%CI: 1.83–3.21, P<0.001; CG vs. CC: OR=1.28, 95%CI: 1.15–1.43, P<0.001; CG+GG vs. CC: OR=1.31, 95%CI: 1.17–1.46, P<0.001; GG vs. CC+GC: OR=2.26, 95%CI: 1.76–2.90, P<0.001]. Similar results were found in both Asian and Caucasian populations. Conclusion　Results from the Meta-analysis strongly suggested that there appeared significant association between PNPLA3 gene rs738409 polymorphism and the susceptibility of NAFLD.
【Key words】　Non-alcoholic fatty liver; Patatin-like phospholipase domain-containing protein 3; Gene polymorphism; Susceptibility; Meta-analysis

非酒精性脂肪肝病（NAFLD）是以肝脂肪变性和脂肪异常蓄积为主要病理特征的临床代谢综合征[1]。近年来NAFLD发病率明显上升[1-3]。Patatin样磷脂酶结构域蛋白3（PNPLA3）基因多态性与NAFLD的易感性近年来成为流行病学研究的焦点。但由于单个研究结论不一，现对已发表的相关研究进行Meta分析，以分析PNPLA3基因位点多态性与NAFLD之间的相关性。

资料与方法

1. 文献收集：计算机检索PubMed以及中文数据库（中国生物医学文献数据库、中国知网、万方数据库、维普数据库），检索时间为1990年6月至2014年7月。英文检索式："non-alcoholic fatty liver disease OR NAFLD" AND "mutation OR variant OR polymorphism" AND "PNPLA3"；中文检索式："非酒精性脂肪肝"和"基因多态性或突变"和"PNPLA3"。文种限中、英文，手工检索相关学术杂志。

2. 文献纳入和排除标准：纳入标准：①涉及PNPLA3基因rs738409多态性与NAFLD易感性相关的文献；②所有纳入研究均为公开发表的病例对照或巢式病例对照；③全文发表；④文献中数据完整，能直接或间接提供统计指标OR值及其95%CI；⑤病例均为经确诊NAFLD，其诊断符合影像学标准或者病理学标准。排除标准：①研究未设立对照组；②具体数据描述不清；③重复报道；④NAFLD相关的综述以及动物实验。

3. 资料提取和质量评价：由2名评价者独立根据纳入标准，对检索文献进行筛选，若存在意见分歧通过讨论达成一致。2名评阅人独立完成对原始文献信息收集。文献信息包括：第一作者姓名、出版年份、国家/地区、病例组对照组基因型分布及其频率。根据文献对纳入研究进行质量评价[4]。质量指标：①样本量是否充分；②病例诊断是否清楚；③病例组与对照组匹配情况；④对照组基因型分布是否符合Hardy-Weinberg（H-W）遗传平衡定律；⑤基因检测方法是否合理；⑥数据是否充分。6项指标每满足1项记为1分，其中总分≥3分者为质量可靠。

4. 统计学方法：采用Stata 12.0软件进行Meta分析。采用χ^2检验分析各研究结果间的异质性；各研究结果间的异质性检验采用Q检验和P统计量，$P<0.05$为各研究间存在异质性，I^2用以衡量异质性大小程度，若各研究结果间不存在异质性，合并采用固定效应模型计算OR值；反之采用随机效应模型合并效

应OR值，$P<0.05$为差异有统计学意义。敏感性分析为依次排除单个文献后重新进行Meta分析，观察合并效应是否改变，Begger、Egger检测森林图对称性以判断是否存在发表偏倚。

结　果

1. 文献检索：根据中英文检索式初检出文献643篇，经阅读文题和摘要初筛，排除不符合文献433篇，进一步查找和阅读全文复筛，排除文献182篇，最终纳入28篇文献[5-32]（图1）。所有纳入研究均为病例对照研究。纳入研究的基本特征见表1。共纳入14 434例，其中NAFLD患者病例数6 216例，对照组8 218例。CC、CG、GG基因型在病例组和对照组的分布分别为36.19%、42.88%、20.91%和48.70%、40.39%、10.91%。纳入的研究时间2009－2014年。除文献[29]外其他文献质量评分均>5分，提示文献质量较好。文献对照度不符合H-W遗传平衡定律，考虑敏感性分析，最终纳入32项病例对照研究。

图1　文献纳入过程
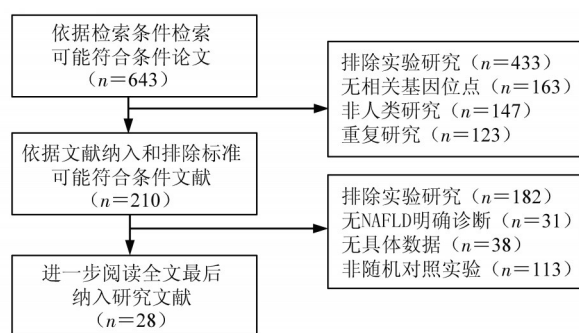
2. Meta分析：PNPLA3基因位点多态性与NAFLD易感性Meta分析结果显示，纯合子比较模型（GG vs. CC）：OR=2.42，95%CI：1.83～3.21，P<0.001；杂合子比较模型（CG vs. CC）：OR=1.28，95%CI：1.15～1.43，P<0.001（图2）；显性遗传模型（CG＋GG vs. CC）：OR=1.31，95%CI：1.17～1.46，P<0.001；隐性遗传模型（GG vs. CC＋GC）：OR=2.26，95%CI：1.76～2.90，P<0.001；亚组分析显示，亚洲以及高加索人群PNPLA3基因rs738409位点多态性与NAFLD易感性亦明显关联（表2）。

3. 发表偏倚分析：在总体人群中4种基因模型Meta分析漏斗图经Begger检验以及Egger检验显示，P>0.5，提示漏斗图对称、无发表偏倚存在。亚组分析显示，在亚洲人群中CG vs. CC以及GG vs. CC＋GC模型Egger检验显示，均P<0.05（图3），提示漏斗图可能不对称、存在发表偏倚。高加索人群分析无发表偏倚。

表1　纳入文献一般情况

第一作者	发表年份	国家/地区	病 例 组			对 照 组			H-W 遗传平衡	文献 质量
			CC	CG	GG	CC	CG	GG		
Hotta[5]	2010	日本	45	111	97	175	296	104	0.28	8
Hyysalo[6]	2014	芬兰	51	39	8	104	82	14	0.69	6
Kantartzis[7]	2009	德国	91	90	20	394	261	43	0.98	7
Kawaguchi[8]	2012	日本	88	236	203	247	468	217	0.87	5
Kolesnikova[9]	2014	乌克兰	295	51	2	66	37	5	0.95	7
Krawczyk[10]	2011	德国	64	39	5	124	72	26	0.00	8
Li[11]	2012	中国	70	84	49	94	90	18	0.59	6
Lin[12]	2011	中国	25	102	26	167	192	59	0.75	6
Liu[13]	2014	欧洲	28	43	29	125	117	33	0.49	5
Niu[14]	2014	中国	48	153	189	183	176	50	0.45	8
Peng[15]	2012	中国	183	276	93	235	259	59	0.32	6
Rametta[16]	2014	意大利	51	67	19	150	95	15	0.85	8
Ravikanth[17]	2013	印度	31	84	57	45	31	6	0.84	6
Romeo[18]	2010	意大利	8	11	5	249	174	28	0.74	5
Rotman[19]	2010	美国	190	388	198	200	118	18	0.91	9
Sookoian[20]	2009	阿根廷	18	40	45	41	42	11	0.96	8
Speliotes[21]	2010	美国	148	296	148	855	482	68	0.99	7
Valenti[22]	2010	意大利	103	114	36	118	56	5	0.59	7
Valenti[23]	2010	意大利	142	140	39	118	56	5	0.59	9
Valenti[23]	2010	日本	65	61	25	118	56	5	0.59	6
Valenti[24]	2012	意大利	55	68	21	146	95	16	0.80	6
Wagenknecht[25]	2011	美国	119	75	12	206	292	140	0.06	7
Wagenknecht[25]	2011	美国	24	5	0	224	106	12	0.90	7
Wang[26]	2011	中国	40	80	36	269	335	119	0.40	8
徐静[27]	2013	中国	45	128	142	155	139	42	0.22	5
Zain[28]	2012	中国	14	27	13	25	23	6	0.84	5
Zain[28]	2012	印度	10	15	6	43	19	2	0.96	6
Zain[28]	2012	马来西亚	20	29	10	46	29	5	0.88	6
Bhatt[29]	2013	印度	112	35	15	149	16	8	0.00	3
Corbin[30]	2013	美国	154	161	46	53	28	4	0.90	5
Goran[31]	2010	美国	7	29	34	38	61	19	0.50	8
Gorden[32]	2013	美国	411	244	47	218	103	5	0.06	7

表2　*PNPLA3*基因位点多态性与NAFLD易感性Meta分析

| 模型比较	关联性检验			异质性检验			发表偏倚检验	
	OR值(95%CI)	Z值	P值	χ^2值	I^2值(%)	P值	Begger检验	Egger检验
GG vs.CC	2.42(1.83~3.21)	6.17	0.000	196.10	84.7	0.000	0.563	0.993
CG vs. CC	1.28(1.15~1.43)	4.38	0.000	85.19	64.8	0.000	0.708	0.367
CG+GG vs. CC	1.31(1.17~1.46)	4.70	0.000	115.71	74.1	0.000	0.892	0.389
GG vs. CC+GC	2.26(1.76~2.90)	6.39	0.000	170.65	82.4	0.000	0.865	0.832
亚洲人群								
GG vs.CC	2.33(1.83~2.97)	6.89	0.000	35.87	66.5	0.000	0.161	0.084
CG vs. CC	1.29(1.18~1.41)	5.62	0.000	15.63	23.2	0.209	0.077	0.023
CG+GG vs. CC	1.31(1.22~1.41)	7.12	0.000	19.12	37.2	0.086	0.059	0.026
GG vs. CC+GC	2.24(1.75~2.86)	6.44	0.000	42.67	71.9	0.000	0.502	0.338
高加索人群								
GG vs.CC	2.29(1.37~3.83)	3.17	0.002	149.29	87.9	0.000	0.726	0.086
CG vs. CC	1.24(1.04~1.48)	2.34	0.019	72.92	75.3	0.000	0.093	0.089
CG+GG vs. CC	1.26(1.05~1.52)	2.43	0.015	98.67	81.8	0.000	0.142	0.064
GG vs. CC+GC	2.14(1.36~3.36)	3.29	0.001	122.59	85.3	0.000	0.484	0.105
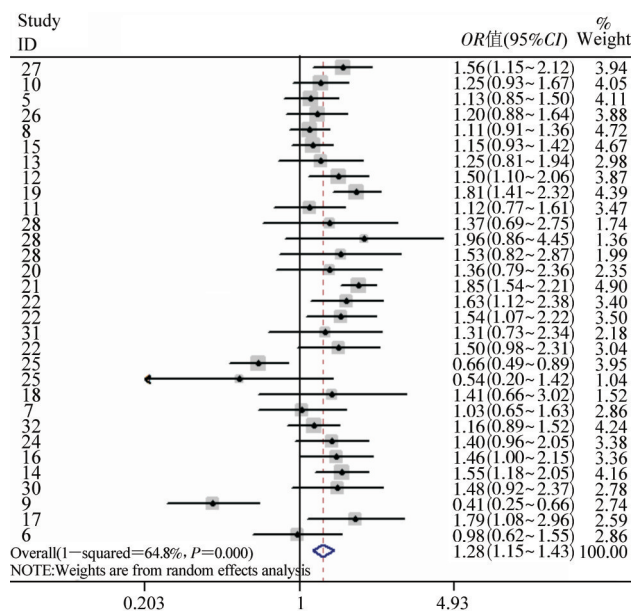
图2　总人群*PNPLA3*基因位点多态性与NAFLD易感性
Meta分析CG *vs.* CC模型森林图

4.敏感性分析：依次单独剔除1篇文献后，各基因模型Meta分析结果未见明显改变，提示结果稳定（图4）。

讨　　论

*PNPLA3*基因位于22q13.31，编码含有481个氨基酸组成的功能未知的非分泌性蛋白，该蛋白属于马铃薯贮藏蛋白样磷脂酶家族[33]。研究表明人类组织中，*PNPLA3*主要表达在肝脏，其次为皮肤和脂肪细胞。但该蛋白的生物学功能尚不十分清楚，与*PNPLA3*最为类似的结构体PNPLA2已被证实具有三酰甘油水解酶和酰基甘油转酰基酶活性[34]。

在发展中国家对NAFLD研究已成为消化内科医生关注的一个热点。近年来，随着人们生活水平的日益提高，NAFLD发病率逐渐升高，少数患者进展成肝硬化甚至肝癌。因而明确NAFLD的发病机制，以期找到NAFLD治疗的切入点和拓宽药物开发思路。目前，国内外很多研究证实*PNPLA3*基因突变与NAFLD发病密切相关，本研究通过Meta分析显示，*PNPLA3*基因rs738409多态性与NAFLD易感性有明显关联性。Meta分析异质性检验显示，*PNPLA3*基因rs738409点的各研究之间异质性较大，异质性的产生原因较多，其中研究人群遗传背景不同是造成基因多态性与疾病关联性分析研究之间异质性的重要来源之一；本研究根据研究人群遗传背景进行亚组分析，发现多数基因模型各研究之间异质性未明显减少，提示遗传背景不同不是异质性的主要来源。此外，Meta分析显示，无明显发表偏倚，敏感性分析结果稳
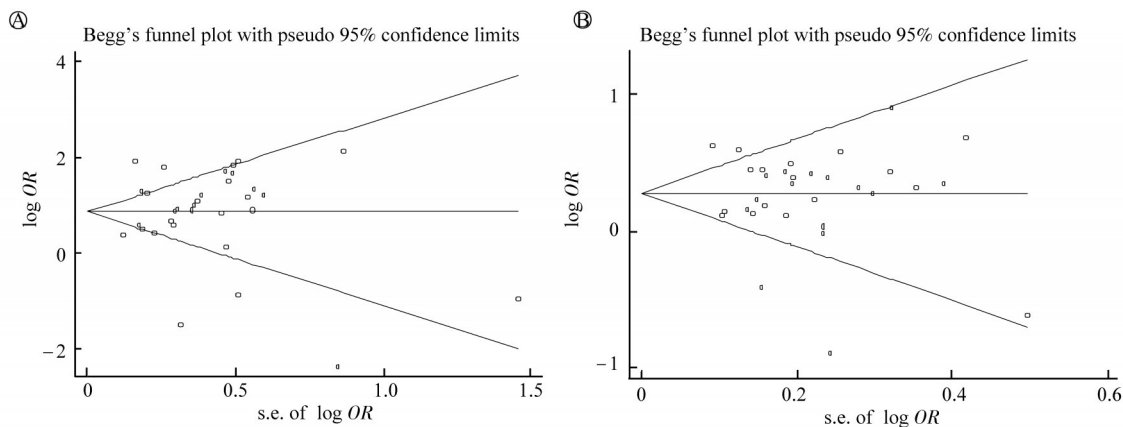

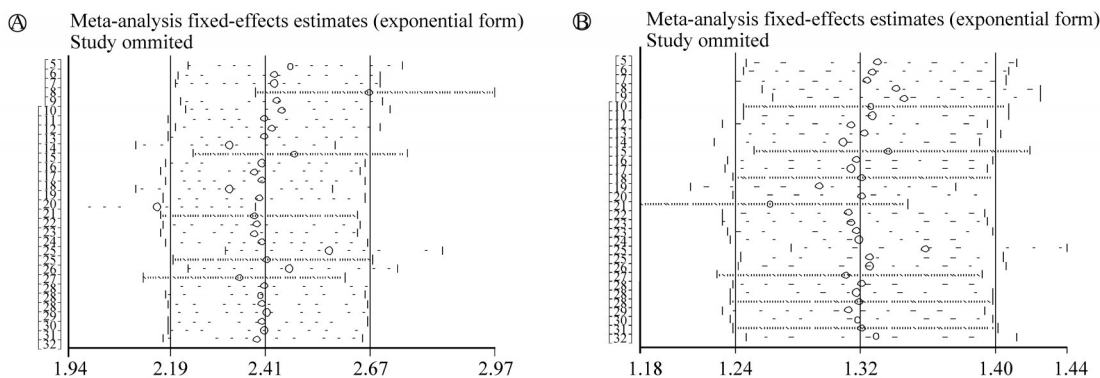**图3**　*PNPLA3*基因位点多态性与NAFLD易感性Meta分析CG *vs.* CC（Ⓐ）以及GG *vs.* CC＋GC（Ⓑ）发表偏倚

图4　*PNPLA3*基因位点多态性与NAFLD易感性Meta分析CG *vs.* CC（Ⓐ）以及GG *vs.* CC＋GC（Ⓑ）敏感性分析

定,提示该Meta分析结果科学可靠。

　　本研究表明*PNPLA3*基因多态性与NAFLD发病密切相关,然而其机制尚不明了,有待相关研究进一步验证[18,23,35]。我国学者徐静等[27]的研究显示,不同rs738409基因型胰岛素抵抗明显有差别,通过分析上述研究发现*PNPLA3*基因rs738409多态性可能是通过影响肝脏炎症以及机体胰岛素抵抗等方面影响NAFLD易感性。

　　本研究存在不足:只检索了国内外几个代表性数据库,检索语言仅为中文和英文,且只限于公开发表的文献,影响了纳入研究的全面性。纳入研究的人群无法分清民族。饮食和社会环境是影响NAFLD易感性的重要因素,本研究纳入病例-健康对照组的研究往往忽略上述因素。为进一步明确*PNPLA3*基因rs738409多态性与NAFLD易感性的内在联系,尚需要开展更大样本量、设计更严谨的研究。

<div align="center">参 考 文 献</div>

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