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血小板膜 P2Y₁₂ 受体基因 T744C 多态性对外伤性脑梗死(PTCI)患者血小板聚合率及疗效的影响 *

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摘要 目的: 外伤性脑梗死(posttraumatic cerebral infarction, PTCI)是颅脑损伤的常见并发症之一, P2Y₁₂ 受体介导血小板聚集是血栓形成的重要通路,与血小板聚集形成密切相关。本研究探讨外伤性脑梗死发生发展与血小板膜 P2Y₁₂ 受体基因 T744C 基因多态性的关系。**方法:** 用聚合酶链反应(PCR)和限制性酶切片段长度多态性(RFLP)技术对 186 例外伤性脑梗死患者 P2Y₁₂ 受体基因 T744C 多态性进行分析。分别在治疗前和治疗后对所有颅脑外伤患者的病情 GCS 评分,并按基因型分组对照分析结果。**结果:** 血小板膜 T744C 血小板膜 T744C 基因型基因频率分别为 TT 基因型 59.14%、TC 型 32.26%、CC 型 8.60%,T 等位基因 75.27%、C 等位基因 24.73%;其中 TT 基因型对奥扎格雷反应较敏感,GCS 评分预后好;而 CC 型对奥扎格雷反应性低,预后差。**结论:** T744C 基因多态性中 CC 基因型可能导致外伤性脑梗死临床及预后存在明显的个体差异, 与其对抗血小板药物抵抗有关。T744C 的 C 等位基因可能是脑梗死的遗传危险因素,开展相关遗传学风险研究,对于进一步缓解脑梗症状、改善预后具有重要意义。

关键词: 血小板; 外伤; 脑梗死; 基因; 多态性

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Role of the T744C Polymorphism of the P2Y₁₂ Gene on Platelet Response in Patients with Traumatic Cerebral Infarction and Efficacy*

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ABSTRACT Objective: Traumatic cerebral infarction (PTCI) is a common complication of traumatic brain injury, which is closely related to platelet aggregation. The aim of this study was to evaluate the effect of polymorphisms affecting the P2Y₁₂ receptor (P2Y₁₂ T744C) on modulating platelet function in acute PTCI patients on regular treatment. P2Y₁₂ receptor-mediated platelet aggregation is an important pathway for thrombosis. This article explores the traumatic cerebral infarction film development and platelet P2Y₁₂ receptor gene T744C gene polymorphism relationship. **Methods:** GCS score evaluating the cure effect of PTCI patients. PCR-RFLP analysis of 186 cases of P2Y₁₂ receptor gene T744C polymorphism. **Results:** The platelet membrane T744C genotype gene frequencies 59.14% of the TT genotype, TC 32.26% CC 8.60%, the T alleles 75.27% and C alleles 24.73%. Among people with TT genotype, the GCS score and prognosis are better than CC group. What's more, the CC genotype reduces the ability of ozagrel to inhibit platelet aggregation. **Conclusion:** T744C gene polymorphism CC genotype is a high risk of the disease. T744C C allele may be a genetic risk factor of cerebral infarction. More studies are needed to determine the possible interaction between the genetics factors, platelet response to ozagrel and long term prognosis.

Key words: Platelets; Trauma; Cerebral infarction; Gene; Polymorphism

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前言

外伤性脑梗死 (Post traumatic cerebral infarction, PTCI) 是颅脑损伤严重的并发症之一, 其发生率较高, 而及时、早期、有效的治疗与护理对外伤性脑梗死患者的康复至关重要^[1-2]。脑梗塞是由于脑动脉粥样硬化, 血管内膜损伤使脑动脉管腔狭窄, 进而因多种因素使局部血栓形成, 使动脉狭窄加重或完全闭塞, 导致脑组织缺血^[3-5]。脑血管受损后局部血管收缩以及脑外

伤引起脑水肿致颅内压增高均可使血流量减少、血流缓慢^[6,7], 同时血管损伤释放组织凝血活酶等^[8-10], 促使血小板粘附、聚集而形成血栓, 导致脑梗死^[11]。本文对 P2Y₁₂ 基因 T744C 单核苷酸多态位点与 PTCI 病人中开展研究, 探讨 P2Y₁₂ 基因 T744C 多态性与 PTCI 病人血小板功能及预后的关系。

1 资料与方法

1.1 一般资料

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本研究将 2008 年 1 月 1 日至 2012 年 12 月 12 日间五年内本院收治的 186 例颅脑损伤后并发脑梗死患者作为研究对象,民族均为汉族,且患者之间无血缘关系。其中男 101 例,女 85 例,年龄 20~65 岁,平均 38.3 岁。经头颅 CT 及 MRI 确诊其颅内存在脑梗死部位,以基底节内囊区及枕叶、小脑及脑干等多见。所有纳入研究病例均为单纯性脑梗死,本研究中排除标准为重型颅脑损伤并发的大面积脑梗死患者。

1.2 基因鉴定

本研究采用成熟的多聚酶链限制性片段长度多态性(PCR-RFLP)方法检测内含子 T744C 基因多态性^[2]。PCR 引物为上游:5'-TTTGGGGAATTTAAGTGCTAC-3' 下游为 5'-CCA-CAATAGGCAGCTATAA TG-3'。若凝胶电泳仅显示 252 bp 一条带,则该个体为 P2Y12(-744TT)野生型(W/W)。如凝胶电泳图谱显示 163 和 89 bp 两条带,则该个体为 P2Y12(744CC)突变型纯合子(M/M)。如显示 252, 163 和 89 bp 三条带,则该个体为 P2Y12(744CC)突变型杂合子(W/M)。(本研究所有分子生物学实验均在复旦大学医学院公共实验室及利用相关科研设备完成。)

1.3 实验评价

本研究采用比浊法(TPA)检测血小板聚集率。血小板聚集能力观察指标为体外检测 5 μ M ADP 诱导的体外血小板聚集率的变化。患者于入院后在清晨从前臂静脉采血 6 ml 到 3% 枸橼酸钠抗凝的塑料管内,并于采血后 2 h 内完成 ADP 诱导的血小板聚集率的测定,得到聚集率 A%。另取其中 2 ml 血液,预先加入 0.2 μ M 奥扎格雷,后加入 5 μ M ADP 诱导血小板聚集,得到聚集率 B%。此时,在奥扎格雷条件下 ADP 血小板聚集率降低的程度为(A%-B%),即为所得。本次所有实验诱导血小板聚集 ADP 采用 5 μ M 浓度。GCS 指数(Glasgow coma scale)是临床上评估病人昏迷程度的指标,本研究参照国际标准 GCS 法对收治的颅脑损伤患者入院时 GCS 评分与预后作比较分析。

1.4 统计学分析

本研究中采用 SPSS 13.0 统计软件进行统计学分析,计量资料用均值 $\pm$ 标准差($\bar{x} \pm s$)表示,组间比较采用单因素方差分析。计数资料用百分构成比表示,采用 χ^2 检验。同时,卡方检验用于分析基因多态性分布是否符合 Hardy-Weinberg 平衡。若 $P < 0.05$ 差异被认为有统计学意义。

2 结果

表 1 中国人群 186 例中 P2Y12 受体 T744C 位点基因型和基因频率

Table 1 The P2Y12 receptor T744C genotype and gene frequencies of 186 cases in chinese population

SNP 位点 Single Nucleotide Polymorphism	数量 N	基因型 Genotype			基因型频率(%) Genotype frequency(%)	
		野生 / 野生型	野生 / 突变型	突变 / 突变型	野生型	突变型
		W/W	W/M	M/M	W	M
rs2046934(T744C)	186	110	60	16	75.27	24.73
所占比例(%) Proportion(%)	100	59.14	32.26	8.60		

表 1 中显示 T744C 血小板膜 T744C 基因型基因频率分别为 TT 基因型 59.14%、TC 型 32.26%、CC 型 8.60%,T 等位基因 75.27%、C 等位基因 24.73%。该突变经过 Hardy-Weinberg 遗传平衡定律检验,三个位点基因多态性在中国健康人群分布符合遗传平衡,具有群体代表性。

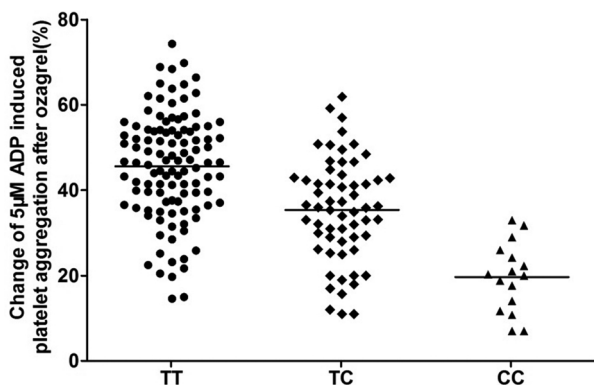


图 1 应用 0.2 μ M 奥扎格雷后抑制 5 μ M ADP 诱导的血小板聚集率的变化程度

Fig. 1 The change of 5 μ M ADP-induced platelet aggregation rate after the application 0.2 μ M ozagrel

如图 1 中所示在 T744C 基因型中 TT 型野生纯合子患者,奥扎格雷能抑制 5 μ M ADP 诱导的血小板聚集率的变化,其平均值为 45.12%。在 TC 杂合子中,奥扎格雷能抑制 5 μ M ADP 诱导的血小板聚集率的变化率的平均值为 36.15%。在 CC 突变纯合子中,其 ADP 诱导的血小板聚集率的变化程度平均值为 19.67%,三者之间相比均有显著性差异($P < 0.001$)。P2Y12 的 T744C 突变型纯合子在体外应用奥扎格雷后血小板聚集率降低的程度小于野生型个体($P < 0.05$)。

如表 2 显示,在治疗前,各组野生纯合型(W/W, n=110),突变杂合型(W/M, n=60)和突变纯合型(M/M, n=16)病人 GCS 评分之间无显著性差异($P > 0.05$)。其中, T744C 基因型中 TT 型野生纯合子患者和 TC 型野生杂合子患者 GCS 评分疗效前后对比均有显著性差异($P < 0.05$)。由上表可知,在常规治疗后 24 h 时,野生纯合型组合突变杂合型组治疗后与治疗前相比,其 GCS 评价均有显著性差异,且野生纯合型组比野生杂合型组更为显著($P < 0.01$ vs. $P < 0.05$)而突变纯合型患者其 GCS 评分无显著改变($P > 0.05$)。

3 讨论

PTCI 是一种高风险疾病,其成因是主要由血管内膜损伤

表 2 治疗前和经过治疗 24 h 后各组个体的 GCS 评分改变情况

Table 2 The treatment effects of GCS score of each group after and before the situation

GCS 评分 Glasgow Coma Scale scores	T744C 基因型 T744C gene polymorphism		
	野生 / 野生型 W/W	野生 / 突变型 W/M	突变 / 突变型 M/M
治疗前 Before treatment	5.89± 1.23	5.91± 1.32	5.99± 1.50
治疗后 After treatment	8.98± 1.16	7.15± 2.77	6.51± 1.79
P 值 P value	P<0.01	P<0.05	P>0.05

使脑动脉管腔狭窄等多种因素使局部血栓形成,使动脉狭窄加重或完全闭塞,导致脑组织缺血缺氧坏死,并引起神经功能障碍^[13-15]。本研究发现血小板 P2Y₁₂ 受体 T744C 基因多态性中 CC 基因型可能导致 PTCI 临床及早期预后出现明显的个体差异,其内源性机制可能与其对抗血小板药物抵抗有关。在白种人群中 P2Y₁₂ 基因 4 个位点的基因突变(C139T, T744c, ins801A, G52T)呈完全连锁状态,其被分别命名为 H1(野生型)和 H2(突变型),其中 H2 突变型包括 T744c 突变型。Motovska 等^[16]研究显示多种 P2Y₁₂ 受体多态性对 ADP 诱导冠心病患者的血小板反应性的基线值无显著影响。相反,Fontana 等^[17]在健康人中开展的研究表明 P2Y₁₂ 受体多态性在血小板功能中的作用关键,H2 单倍型人群的动脉粥样硬化 / 或血小板聚集的危险性增加。以上提示冠心病病理条件下,其 P2Y₁₂ 功能 - 疗效可能不同。与任何类型的脑损伤一样^[18],PTCI 可以激活凝血系统,并可能在某些情况下加剧梗死所造成的伤害。因此,我们的研究显示对 T744C 多态性对奥扎格雷后抑制 ADP 诱导的血小板聚集率的变化程度有显著影响,提示在 PTCI 的急性病理条件下,P2Y₁₂ 受体功能在体内角色可能更为关键,而其 T744C 突变可能导致临床奥扎格雷疗效降低。

另外由图 1 观察到 C 等位基因的抗血小板作用呈现基因剂量效应(TT>TC>CC),该基因剂量效应特征更进一步支持了 C 等位基因突变导致 PTCI 病人抗血小板功能的假设。而且,临床疗效评价中对基因型分组后的疗效 GCS 评分后观察到,T744C 的 C 等位基因可能是 PTCI 的遗传危险因素,具有 C 等位基因型组的病人 24 h 内的预后较差。与我们研究相一致的是,Fontana 等开展的病例对照研究^[19]显示 P2Y₁₂ 受体 H2 单倍型与外周动脉疾病(PAD)患者存在正相关。PTCI 早期治疗关系到后期神经功能损害后的自然恢复和预防进一步的严重脑损害,其早期疗效评价较重要^[20-23]。如能对开展相关遗传学风险研究,开展积极的应对措施,对于进一步缓解脑梗症状、改善预后具有重要意义。本研究提示临床中 CC 纯合子病人可以作为一个临床基因标记物,应重点关注该组病人并积极防止 PTCI 的血栓形成等。由于条件限制实验样本数有限,其结论有待扩大样本量及多中心的临床试验来进一步确定和验证。

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