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血小板输注无效与血小板抗原(HPA)多态性的相关性 *

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摘要 目的：探讨血小板输注无效(PTR)与血小板抗原(HPA)多态性的相关性。**方法：**2017 年 5 月到 2018 年 9 月选择在首都医科大学附属北京同仁医院输血科进行血小板输注的患者 187 例，检测所有患者血液的 HPA 多态性，判断 PTR 发生情况并进行相关性分析。**结果：**在 187 例患者中，发生 PTR 32 例，发生率为 17.1 %。PTR 患者的 HPA1、HPA2、HPA3、HPA4、HPA5 的 b 基因频率都显著高于非 PTR 患者(P<0.05)，也都呈基因多态性分布；非 PTR 患者都呈 aa 纯合子单性态分布。在 187 例患者中，直线相关分析显示 HPA1、HPA5 基因多态性与 PTR 有显著相关性(P<0.05)，与 HPA2、HPA3、HPA4 基因多态性无相关性(P>0.05)。Logistic 回归分析显示 HPA1 基因多态性、HPA5 基因多态性、再生障碍性贫血、骨髓增生异常综合症为导致 PTR 的影响因素(P<0.05)。**结论：**血小板输注中 PTR 比较常见，多伴随有 HPA 多态性，HPA1 和 HPA5 基因多态性、再生障碍性贫血、骨髓增生异常综合症为导致 PTR 的影响因素。

关键词：血小板输注无效；人类血小板抗原；基因多态性；相关性

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Correlation between Platelet Transfusion Refractoriness and Platelet Antigen (HPA) Polymorphism*

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ABSTRACT Objective: To investigate the association between platelet transfusion refractoriness（PTR）and platelet antigen (HPA) polymorphism. **Methods:** From May 2017 to September 2018, 187 patients who underwent platelet transfusion in Department of Blood Transfusion, Beijing Tongren Hospital, Capital Medical University were selected. The HPA polymorphism in all patients were measured, and the incidence of PTR were determined and were given correlation analysis. **Results:** In 187 patients, 32 patients of PTR occurred, the incidence rates was 17.1 %. The b gene frequencies of HPA1, HPA2, HPA3, HPA4, and HPA5 in PTR patients were significantly higher than those in non-PTR patients (P<0.05), and all of them were genetic polymorphism distribution; non-PTR patients were all aa homozygous single-sex distribution. In the 187 patients, linear correlation analysis showed that HPA1 and HPA5 gene polymorphisms were significantly associated with PTR(P<0.05), and no correlated with HPA2, HPA3, HPA4 gene polymorphisms (P>0.05). Logistic regression analysis showed that HPA1 gene polymorphism, HPA5 gene polymorphism, aplastic anemia, and myelodysplastic syndrome were the influencing factors of PTR (P<0.05). **Conclusion:** PTR are common in platelet transfusion, and are associated with HPA polymorphism. HPA1 and HPA5 polymorphism, aplastic anemia, and myelodysplastic syndrome are the influencing factors of PTR.

Key words: Platelet transfusion refractoriness; Human platelet antigen; Gene polymorphism; Correlation

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前言

血小板(plantlet，PLT)起源于巨核细胞，是人体不可或缺的血液成分，可通过粘附、聚集和释放反应，在凝血与生理性止血中发挥重要作用[1]。目前，血小板输注也比较常见，主要用于预防和治疗因 PLT 数量减少或功能障碍引起的严重出血，进而提高患者的治愈率 [2,3]。但是部分患者在多次输注 ABO 同型 PLT 后可能出现 PLT 的增加值显著小于预期，表现为血小板输注无效 (platelet transfusion refractoriness，PTR)[4,5]。PTR 发生时，输入的血小板在体内被迅速破坏，严重情况下可导致患者

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死亡。临床上引起PTR的主要原因包括非免疫因素和免疫因素，其中免疫因素包括抗-人类血小板抗原(Human Platelet Alloantigen，HPA)表达异常等[6,7]。HPA基因位于第5、6、17和22号染色体上，为共显性双等位基因遗传[8]。HPA具有高度的多态性，是由于膜糖蛋白多肽链内某一单个氨基酸被取代的结果，与血小板膜糖蛋白密切相关[9]。当前在临床上已发现10余种HPA抗原，包括HPA1、HPA3、HPA4等，其中许多是由编码基因的点突变所造成的，也与血栓性疾病有一定的相关性[10,11]。国外报道白种人易引起PTR的血小板特异性抗原有HPA1~HPA5[12]。我国上海、南京等一些地区也进行开展了血小板抗原基因分型检测，发现了HPA2、HPA3、HPA4、HPA5、HPA15的不配合率比较高[13]。但由于地域的差异，多态性分布也有一定的不同，因此本研究选择了HPA1~HPA5，探讨了血小板输注无效与HPA多态性的相关性，希望为临床进行血小板性输注提供试验依据。

1 资料与方法

1.1 研究对象

2017年5月到2018年9月选择在首都医科大学附属北京同仁医院输血科进行血小板输注的患者187例，纳入标准：遵循知情同意原则，献血者与供血者无血缘关系；均为汉族；血小板均来自健康献血者，乙肝表面抗原、丙肝病毒抗体、艾滋病病毒抗体、抗梅毒抗体均为阴性；血红蛋白、血小板计数、肝功能指标均在正常值范围内；年龄18-70岁；试验征得患者的知情同意，并与之签订临床研究知情同意书；符合首都医科大学附属北京同仁医院输血科人体试验委员会所制定的伦理学标准。排除标准：输注其他血液者；血小板输注前24 h服用两性霉素B、万古霉素者；血小板输注前1个月内输注静脉免疫球蛋白者；弥散性血管内凝血、败血症、脾肿大；妊娠与哺乳期妇女。

在187例患者中，男101例，女86例；年龄最小19岁，最大68岁，平均年龄54.10± 4.39岁；平均体重指数22.49± 2.19岁；疾病类型：白血病84例，免疫性血小板减少性紫癜26例，再生障碍性贫血34例，骨髓增生异常综合症18例，其他25例。

1.2 HPA 多态性检测

1.2.1 检测仪器与试剂 血液DNA提取试剂盒购自德国QIAGEN公司，凝胶成像仪与琼脂糖凝胶电泳仪购自美国Bio-rad公司，低温高速离心机购自美国贝克曼公司，PCR扩增仪为美国ABI9700型。

1.2.2 检测过程 采集所有患者空腹外周静脉血3-5 mL，按照提取试剂盒的说明书提取总DNA，根据HPA1、HPA2、HPA3、HPA4、HPA5的基因序列设计相应的引物并进行PCR扩增。对所有合格的PCR产物进行直接测序（均由上海生工生物工程股份有限公司完成），进行BLAST分析，分析突变情况。

1.3 PTR 判断标准

输注24 h后，若血小板恢复百分率(PPR)＜20 %或者血小板计数纠正增加指数(CCI)＜10[14]。

1.4 统计方法

选择SPSS 22.00软件，计数数据以(%)表示，对比用 x^2 检验，相关性分析采用直线相关分析，影响因素分析采用logistic回归分析，P＜0.05为差异具有统计学意义。

2 结果

2.1 PTR 发生情况

在187例患者中，发生PTR 32例，发生率为17.1 %。

2.2 HPA 基因多态性分布

PTR患者的HPA1、HPA2、HPA3、HPA4、HPA5的b基因频率都显著高于非PTR患者(P<0.05)，也都呈基因多态性分布；非PTR患者都呈aa纯合子单性态分布。具体见表1。

表1 血小板输注患者的HPA基因多态性分布(例)

Table 1 Distribution of HPA gene polymorphism in patients with platelet transfusion (n)

HPA type	PTR patient (n=32)					Non-PTR patient (n=155)				
	Genotype			Gene frequency		Genotype			Gene frequency	
	aa	ab	bb	a	b	aa	ab	bb	a	b
HPA1	16	10	6	65.6%	34.4%*	155	0	0	100.0%	0.0%
HPA2	19	8	5	71.8%	28.2%*	155	0	0	100.0%	0.0%
HPA3	21	6	5	75.0%	25.0%*	155	0	0	100.0%	0.0%
HPA4	18	10	4	71.8%	28.2%*	155	0	0	100.0%	0.0%
HPA5	16	12	4	56.3%	43.7%*	155	0	0	100.0%	0.0%

Note: Compare with the non-PTR patient, *P<0.05.

2.3 HPA 基因多态性与 PTR 的相关性

在187例患者中，直线相关分析显示HPA1、HPA5基因多态性与PTR有显著相关性(P<0.05)，与HPA2、HPA3、HPA4基因多态性无相关性(P>0.05)。具体结果见表2。

2.4 影响因素分析

在187例患者中，以PTR作为因变量，以临床资料与HPA基因多态性作为自变量，logistic回归分析显示HPA1基因多态性、HPA5基因多态性、再生障碍性贫血、骨髓增生异常综合症为导致PTR的影响因素(P<0.05)。具体情况见表3。

3 讨论

血小板输注是一种治疗血小板减少和预防出血的手段，但可能导致PTR[15]。PTR是指患者接受充足治疗剂量的血小板输注后，达到一定的血小板量，处于不应性状态[16]。由于患者出血症状的改善程度较难量化，在临床上常以CCI和PPR作为判断血小板输注效果的量化标准[17]。并且由于血小板输注24 h计

表 2 HPA 基因多态性与 PTR 的相关性(n=187)

Table 2 Correlation between HPA gene polymorphism and PTR (n=187)

HPA type	r	*P*
HPA1	0.555	0.000
HPA2	0.215	0.102
HPA3	0.194	0.166
HPA4	0.262	0.098
HPA5	0.652	0.000

表 3 影响 PTR 的因素分析(n=187)

Table 3 Factors affecting PTR (n=187)

Index	OR	*P*	95% CI
HPA1 gene polymorphism	6.188	0.027	1.299-21.167
HPA5 gene polymorphism	4.611	0.026	1.211-17.931
Aplastic anemia	5.276	0.001	2.087-13.761
Bone marrow hyperplasia syndrome	3.318	0.027	1.187-8.642

数能反映出血小板在受者体内的存活情况，为此在使用输注 24 h 后以 CCI 或 PPR 判定血小板输注效果可以减少主观因素的影响，是临床判定 PTR 最简便和最常用的指标[18]。导致 PTR 的原因包括自身免疫性疾病、ABO 血型等，尤其是多数患者需要考虑 ABO 血型，导致血小板输注效果不佳[19,20]。

血小板表面的膜糖蛋白(Glycoproteins, GP)分为质膜糖蛋白和颗粒膜糖蛋白，其能够刺激机体产生抗体(血小板抗原)[21]。基因多态性也称为单核苷酸多态性 (Single nucleotide polymorphism，SNP)，是单个氨基酸发生变化，从而导致抗原结构形式的变化，从而形成多种人类血小板同种特异抗原，包括 HPA1 到 HPA29 等[22,23]。本研究显示在 187 例患者中，发生 PTR 32 例，发生率为 17.1 %；PTR 患者的 HPA1、HPA2、HPA3、HPA4、HPA5 的 b 基因频率都显著高于非 PTR 患者，也都呈基因多态性分布；非 PTR 患者都呈 aa 纯合子单性态分布。从机制上分析，HPA 抗原性较强，输注 HPA 不合的血小板易影响血小板输注效果，特别是反复输注血小板的患者约有 60 %的几率产生 HPA 抗体[24]。还有研究显示血小板输注患者和供者血清中 HPA2、3、15 呈现出较显著的多态性分布，HPA3、15 的杂合度最高，且 HPA2、3、5、6、15 等位基因频率均呈现多态性分布[25,26]。

本研究直线相关分析显示血小板输注患者的 HPA1、HPA5 基因多态性与 PTR 有显著相关性，与 HPA2、HPA3、HPA4 基因多态性与显著相关性；logistic 回归分析显示 HPA1 基因多态性、HPA5 基因多态性、再生障碍性贫血、骨髓增生异常综合症为导致 PTR 的影响因素。在生理或病理状态下，血小板对止血均起着关键性作用[27]。特别是对白血病、骨髓衰竭和造血干细胞移植等患者输注血小板可达到止血或预防出血的目的，进而改善患者的预后，但是 PTR 也是伴随而来的难题之一[28,29]。有研究显示在免疫性 PTR 对的发生因素中，HPA 占 80 %以上，可以导致机体产生血小板同种的抗体从而引起血小板破坏，且病程更加严重[30-32]。不过本研究也有一定的不足，由于 HPA 基因多态性在人群中的分布情况与地域、人种等均有较密切的关系，而本研究只考虑一个民族与一个分部地域，导致结果可能存在偏倚，将在后续研究中深入分析。

综上所述，血小板输注中 PTR 比较常见，多伴随有 HPA 多态性，HPA1 和 HPA5 基因多态性、再生障碍性贫血、骨髓增生异常综合症为导致 PTR 的影响因素。

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