·研究原著·

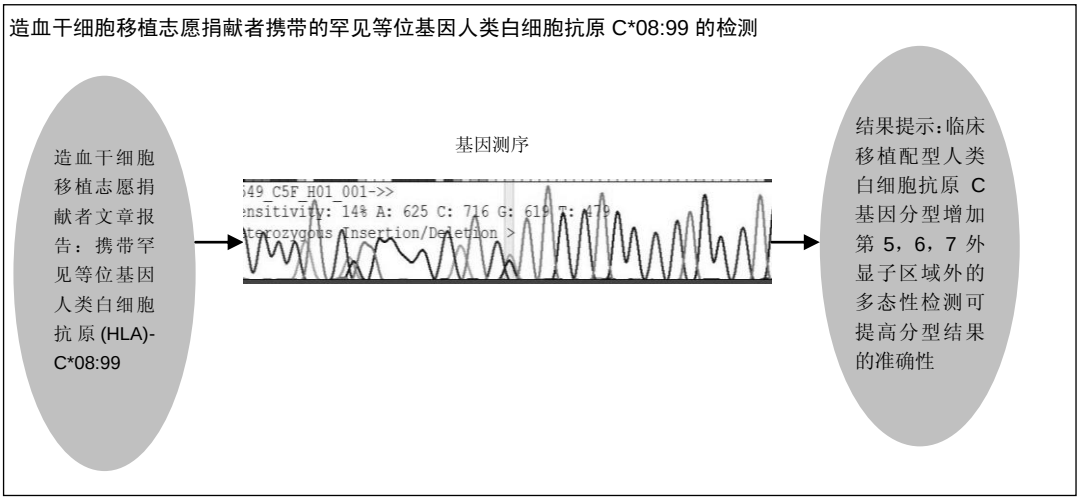
造血干细胞移植志愿捐献者罕见基因人类白细胞抗原C*08:99的测序分析

王大明，邹红岩，聂冬梅，高素青，王 飞(深圳市血液中心免疫遗传室，广东省深圳市 518035)

引用本文：*王大明，邹红岩，聂冬梅，高素青，王飞. 造血干细胞移植志愿捐献者罕见基因人类白细胞抗原 C*08:99 的测序分析[J]. 中国组织工程研究，2016，20(1):102-106.*

DOI: 10.3969/j.issn.2095-4344.2016.01.018 **ORCID:** 0000-0002-3848-3779(王大明)

文章快速阅读：



王大明，女，1978年生，黑龙江省齐齐哈尔市人，汉族，副主任技师，主要从事输血专业及免疫遗传学的研究。

通讯作者：王大明，深圳市血液中心免疫遗传室，广东省深圳市 518035

中图分类号:R394.2
文献标识码:B
文章编号:2095-4344(2016)01-00102-05
稿件接受：2015-11-29
http://WWW.crter.org

文题释义：

人类白细胞抗原：是分布在组织细胞表面的一组膜蛋白，它们的基本生物学功能是将抗原信息提呈给T细胞识别，从而启动和调节特异性免疫应答。根据分子结构、组织分布和生物学功能的差异，人类白细胞抗原抗原可以分为人类白细胞抗原Ⅰ类抗原(包括人类白细胞抗原A，B，C)和人类白细胞抗原Ⅱ类抗原(人类白细胞抗原DR，DQ，DP)2类。人类白细胞抗原由第6染色体短臂上一组紧密连锁的基因群编码。在群体中，其编码基因均表现出高度的遗传多态性，人类白细胞抗原多态性的检测称为人类白细胞抗原分型。人类白细胞抗原分型可用于器官或组织移植的组织配型、疾病遗传易感性的研究和法医学鉴定等方面。

基因测序：是一种新型基因检测技术，能够从血液或唾液中分析测定基因全序列，预测罹患多种疾病的可能性，如癌症或白血病。

摘要

背景：随着测序技术被广泛应用，器官移植配型的高分辨确认工作逐渐深入开展，人类白细胞抗原新等位基因不断涌现，但由于发现较晚，基因频率还不能准确计算，相关报道甚少，这些基因常被忽视，有时单纯根据基因频率判断分型结果，易造成分型结果的误判。

目的：检测与分析1例造血干细胞移植志愿捐献者携带的罕见等位基因人类白细胞抗原C*08:99。

方法：采用快速DNA提取试剂盒从全血样本中提取基因组DNA，经人类白细胞抗原C基因商品化测序分型试剂盒扩增，纯化后的扩增产物作为模板由试剂盒配套的第2，3和4外显子常规检测区正反向测序引物及自行研制的非常规检测区第5外显子正反向、第6外显子正向和第7外显子反向测序，经乙醇/醋酸钠/EDTA纯化的测序反应产物于ABI Prism™ 3730测序仪电泳检测，相关的Assign 3.6+分析软件予以人类白细胞抗原高分辨水平基因分型。

结果与结论：①将电泳后的数据导入Assign-SBT 3.6+分析软件，得出的分型结果为C*07:04，08:99。②结果证实，临床移植配型人类白细胞抗原C基因分型增加第5，6，7外显子区域外的多态性检测可提高分型结果的准确性，对临床组织配型工作具有重要意义。

关键词：

*干细胞；移植；人类白细胞抗原C；C*08:99；测序分型；造血干细胞移植；罕见基因；多态性；广东省自然科学基金*

主题词：

组织工程；干细胞；基因

基金资助：

广东省自然科学基金项目(c15140500000119)

Wang Da-ming, Zou Hong-yan, Nie Dong-mei, Gao Su-qing, Wang Fei (Immunogenetics Laboratory, Shenzhen Blood Center, Shenzhen 518035, Guangdong Province, China)

Wang Da-ming, Associate chief technician, Immunogenetics Laboratory, Shenzhen Blood Center, Shenzhen 518035, Guangdong Province, China

Corresponding author: Wang Da-ming, Immunogenetics Laboratory, Shenzhen Blood Center, Shenzhen 518035, Guangdong Province, China

Subject headings: Tissue Engineering; Stem Cells; Genes
Funding: the Natural Science Foundation of Guangdong Province, No. c15140500000119

Sequencing analysis of a rare human leukocyte antigen, C*08:99, from a volunteer donor of hematopoietic stem cell transplantation

Abstract

BACKGROUND: As the sequencing technology has been widely used and high-resolution confirmation of organ transplant matching has been gradually developed, new human leukocyte antigen (HLA) alleles are emerging. However, the gene frequency of some genes cannot be calculated accurately, and there are rare reports. These genes are often ignored, and it is easy to misjudge their genotypes only according to gene frequency.

OBJECTIVE: To test and analyze a rare allele, HLA-C*08:99, from a volunteer donor of hematopoietic stem cell transplantation.

METHODS: Genomic DNA was extracted automatically from the blood sample by using quick DNA purified kit and amplified by HLA-C locus commercial sequence-based typing kit. The purified PCR product was utilized as the DNA template in the sequencing reaction, and six direct sequencing reactions of PCR product covering exons 2, 3 and 4 in both directions were performed using commercial kit. Four direct sequencing reactions of PCR product covering exon 5 in both directions, exon 6 in forward direction and exon 7 in reverse direction were performed using in-house BigDye terminator cycle sequencing reaction kit. Sequencing reaction products purified by ethanol/sodium acetate/ ethylenediaminetetraacetic acid method were sequenced by ABI PrismTM3730 DNA Sequencer.

RESULTS AND CONCLUSION: The allele assignment was analyzed with Assign-SBT 3.6+ software, and the sample HLA-C typing result was C*07:04, 08:99. Increasing the sequencing analysis at exons 5, 6 and 7 of HLA-C locus will help to make clear the ambiguous SBT result and improve the accuracy of HLA-C typing when it is necessary, which shows important significance in clinical tissue matching.

Cite this article: *Wang DM, Zou HY, Nie DM, Gao SQ, Wang F. Sequencing analysis of a rare human leukocyte antigen, C*08:99, from a volunteer donor of hematopoietic stem cell transplantation. Zhongguo Zuzhi Gongcheng Yanjiu. 2016;20(1):102-106.*

0 引言 Introduction

人类白细胞抗原C基因(HLA-Cw)抗原为经典的HLA-I类分子，为移植相关的抗原，与自然杀伤细胞(NK cell)相互作用，在细胞免疫反应中起重要作用[1]。2015年7月已更新的国际免疫遗传IMGT/HLA数据库3.21.0(http://www.ebi.ac.uk/imgt/hla/stats.html)显示已发现HLA-C基因2 740个，C*08组的等位基因为162个。C*08:99于2013年11月首次被某一地区报道，美国国家骨髓库(National Marrow Donor Program，NMDP)网站(http://bioinformatics.nmdp.org/)公布的罕见等位基因资料显示,自1987年近百万份标本中检测HLA-C基因C*08:99被发现的次数为零。查阅大量国内和国外文献后，自首次报道后尚未被再次报道。

文章在为临床移植配型供/受者对HLA-A，-B，-C，-DRB1和-DQB1位点做高分辨水平结果分型时，发现供者携带HLA-C*08:99基因。此样本的HLA基因分型结果为：HLA-A*02:03，24:02；B*18:01，48:01；C*07:04，08: 99；DQB1*03:03，06:01；DRB1*09:01，15:01。现分析报告如下。

1 材料和方法 Materials and methods

1.1 设计 随机样本检测，单一样本观察实验。

1.2 时间及地点 于2005年1月至2015年12月在深圳市血液中心免疫遗传室完成。

1.3 材料

标本：来源于参加临床造血干细胞移植配型骨髓库志愿捐献者1 500例，其中女性志愿者1例携带罕见等位基因。

1.4 实验方法

1.4.1 基因组DNA制备 取5 mL抗凝外周血，采用全血基因组DNA提取试剂盒(美国QIAGEN)0.3 mL全血制备基因组DNA，剩余全血置−30 ℃冰箱冻存备用。DNA质量浓度调节在50 mg/L，纯度$A_{260\ nm/280\ nm}$值控制在1.80。琼脂糖电泳显示无降解。

1.4.2 HLA-C位点高分辨水平基因测序分型 HLA-C位点第2，3，4外显子PCR扩增：按AlleleSEQR HLA测序试剂盒说明书操作。PCR加样及扩增反应：①将相关试剂从冰箱取出，置于加样洁净台上。在一只1.5 mL的离心管内加入16 μL PCR反应缓冲液和0.3 μL Taq酶，混匀、离心。②取16 μL混合液加入反应管中，混合液中加入4 μL基因组DNA，混匀，离心。③将反应板封盖后放入9700 PCR扩增仪中，扩增反应采用20 μL反应体系，按照如下循环参数进行扩增：95 ℃ 10 min→36个循环：96 ℃ 20 s→60 ℃ 30 s→72 ℃ 3 min，→ 4 ℃。

PCR产物的直接测序：PCR产物的纯化采用ExoSAP-IT酶处理.去除多余的游离PCR引物和底物dNTPs；即在反应管中加入3 μL ExoSAP-IT.低速离心混合后，置于PCR仪37 ℃ 30 min→80 ℃ 15 min→4 ℃。

纯化处理后的产物作为测序反应模板。采用试剂盒配有的测序引物对HLA-C位点第2、第3和第4外显子的正向和反向进行序列测定。测序反应采用10 μL反应体系，包含8 μL测序引物和2 μL经ExoSAP-IT处理过的PCR产物，反应条件为：25个循环：96 ℃ 20 s→50 ℃ 30 s→60 ℃ 2 min，→ 4 ℃。HLA-C位点的第5正反向、第6外显子正向和第7外显子反向采用实验室自行研制的测序引物[3]进行测序，同为10 μL反应体系，包含8 μL测序引物和2 μL经ExoSAP-IT处理过的PCR产物，反应条件为：95 ℃ 1 min →25个循环：95 ℃ 10 s→50 ℃ 5 s→60 ℃ 4 min，→ 4 ℃。

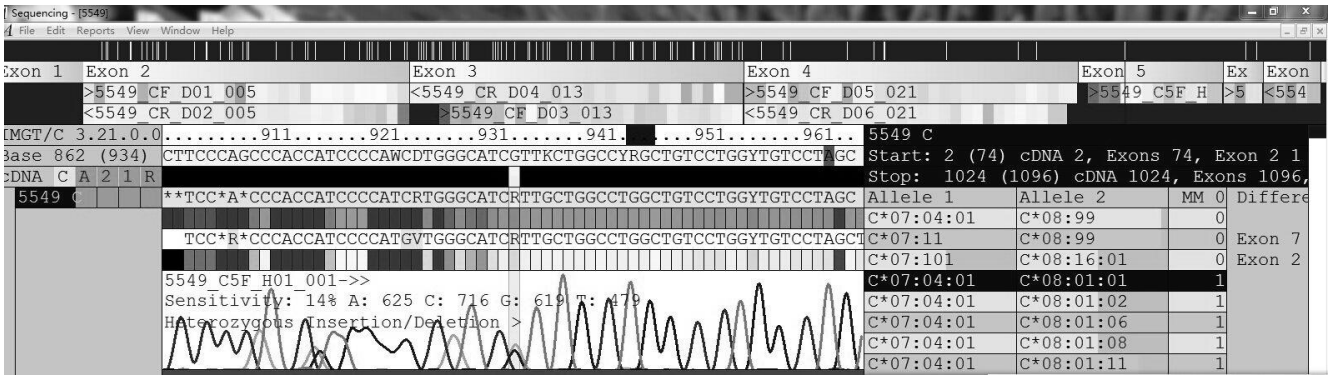


图 1　受检者 HLA-C 位点基因分型结果序列图

Figure 1　Sequence genotyping of the HLA-C locus

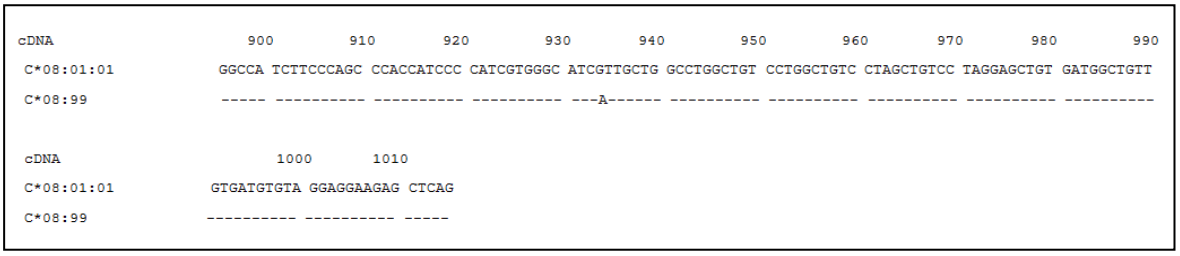


图 2　人类白细胞抗原 C 基因第 5 外显子差异碱基图

Figure 2　Differential basic groups of HLA-Cw at exon 5

图注：C*08: 99 与 C*08: 01:01 的差异碱基在第 5 外显子的 934 位，其他标记为“-”，表示 C*08: 99 与 C*08: 01:01 的其他碱基均相同。

测序反应产物采用乙醇/醋酸钠/EDTA沉淀法加入15 μL超纯甲酰胺溶液(Hi-Di Formamide)，在PCR扩增仪上95 ℃变性2.5 min。测序反应产物用ABI Prism™ 3730型基因测序仪电泳检测并收集电泳信息。

1.4.3　结果分析　采用Assign-SBT 3.6+(Conexio Genomies，Western Australia)分析软件分析受检者HLA-C位点的等位基因型。见**图1**。

1.5　主要观察指标　HLA-C基因测序分型C*08:99基因的分析结果。

2　结果　Results

HLA-C基因测序分型C*08:99基因的分析结果：将电泳后的数据导入Assign-SBT 3.6+分析软件，得出的分型结果为C*07:04，08: 99。见**图2**。

3　讨论　Discussion

HLA-C基因位于人类第6号染色体短臂。已有的临床研究资料表明，造血干细胞移植供/受者对HLA-C基因的匹配程度与受者移植结果有直接相关性[4-7]。HLA基因测序分型技术是HLA基因分型的“金标准”。目前HLA-SBT基因测序分型技术被业内公认为造血干细胞移植前HLA高分辨配型的最佳方法，但由于商品化试剂盒通常只提供第2、第3和第4外显子的正反向测序引物，只能检测此区域内的多态性，区域外的多态性无法检测。

为彻底解决HLA-Cw基因模棱两可的结果，曾有研究报道应用分子克隆和测序方法，但该方法需扩增全长序列、载体连接、转化、提取质粒、挑选阳性克隆和克隆测序等繁多环节，适合鉴定新等位基因，但不适合临床需及时报告基因分型结果的常规检测。因C*08:99发现较晚，现有的序列特异性引物(SSP)试剂盒不能鉴定C*08:99，实验利用HLA-C基因商品化测序分型盒中剩余的PCR产物，针对HLA-C基因第5，6，7外显子进行直接测序，可解决HLA-C第2，3，4外显子区域外出现的模棱两可结果和确认少见或罕见等位基因，为临床移植配型提供了一个有效、便捷和低耗的实验方法。

等位基因C*08:99于2013年11月在某一地区发现，因此为准确地判定此结果的正确性，文章加做了第5，6，7外显子的测序，与国际免疫遗传IMGT/HLA数据库提供的全长序列比对，完全相符，判定此结果正确。美国国家骨髓库(National Marrow Donor Program，NMDP)网站(http：//bioinformatics. nmdp.org/)公布的罕见等位基因资料显示，自1987年近百万份标本检测HLA-C基因C*08:99被发现的次数为零。查阅大量国内和国外文献后，自首次报道后尚未见再次被报道。虽然C*08:99发现比较晚，但在2年内的时间在中国被检测到第2次。此基因是否有可能在中国人群中为较常见基因，由于近6年骨髓库入库数据刚刚从低分辨水平转为高分辨水平，一些在中国继首次被发现后再次出现而在美国国家骨髓库公布的数据中却显示发现次数极少或被划为罕见的等位基因也陆续浮出水面，例如B*51:39，C*07:63，C*08:22，C*03:100，DRB1*12:10等[8]，这几年骨髓库随着

各分库上传数据经过统计学分析等位基因频率发现C*08:22和DRB1*12:10为中国常见等位基因。至于C*08:99等位基因频率是否会大于1/50 000，需要HLA-C基因高分辨入库数据逐渐提高到一定数量[9]，才可定论。

大量国外文献表明，造血干细胞移植时同一位点某些等位基因可以错配，而某些等位基因错配会导致严重的不良反应[10]。近些年，罕见等位基因及新等位基因层出不穷[11-12]，其基因频率正逐渐被重视，尤其是对于患者检索无关(无血缘关系)造血干细胞移植的供者及研究患者移植后愈后生存情况有着十分重要的意义。虽然C*08:99发现比较晚(2013年11月首次宣布发现此基因)，自首次发现到再次被检测到约两年左右，其基因频率待HLA-C高分数据积累到一定程度方可知晓。国内外大量研究表明，人类白细胞抗原的在异基因造血干细胞移植的高分辨组织配型方面有着非常重要的作用[13-50]，尤其是某些基因位点的高分辨分型结果可允许错配，可为患者争取最佳移植时间及提高生活质量起到极其重要作用。

致谢：衷心感谢深圳市血液中心免疫遗传研究室的实验者们协助整理数据及撰写论文。

作者贡献：第二作者进行实验设计。第一作者进行实验操作，资料收集，数据整理和成文。第三作者进行实验评估。第四作者进行审校。

利益冲突：所有作者共同认可文章无相关利益冲突。

伦理问题：实验获单位伦理委员会批准，符合医学伦理要求。

文章查重：文章出版前已经过 CNKI 反剽窃文献检测系统进行 3 次查重。

文章外审：本刊实行双盲外审制度，文章经国内小同行外审专家审核，符合本刊发稿宗旨。

作者声明：文章第一作者对研究和撰写的论文中出现的不端行为承担责任。论文中涉及的原始图片、数据(包括计算机数据库)记录及样本已按照有关规定保存、分享和销毁，可接受核查。

文章版权：文章出版前杂志已与全体作者授权人签署了版权相关协议。

4 参考文献 References

[1] Colonna M, Brooks EG, Ferrara GB, et al. Generation of allospecific natural killer cells by stimuluLation across a polymorphism of HLA-C. Science. 1993;260(5111):1121-1124.

[2] Li J, Qu X, Zhang C, et al. Characterization of 236 novel alleles at the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci from China Marrow Donor Program. Tissue Antigens. 2011; 78:267-270.

[3] 高素青,徐筠娉,王大明,等. HLA-Cw基因常规检测区外第1、5、6、7外显子核苷酸序列测定的研究及临床应用[J].中华检验医学杂志,2011,33(6):513-516.

[4] Petemdorf EW, Longton GM, Anasetti C, et al. Association of HLA-C disparity with graft failure after marrow transplantation from unrelated donors. Blood. 1997;89:1818-1823.

[5] Ferrara GB, Bacigalupo A, Lamparelli T, et al. Bone marrow transplantation from unrelated donors:the impact of mismatches with substitutions at position 116 of the human|eukocyte antigen class I heavy chain. Blood. 2001; 98:3150-3155.

[6] Chalandon Y, Tiercy JM, Schanz U, et al. Impact of high resolution matching in allogeneie unrelated donor stem cell transplantation in Switzerland. Bone Marrow Transplant. 2006;37:909-916.

[7] Loiseau P, Busson M, Balere ML, et al. HLA Association with hematopoielie stem cell transplantation outcome:the number of mismatches at HLA-A, -B, -C, -DRBI, or -DQBI is strongly associated with overall survival. Biol Blood Marrow Transplant. 2007;13:965-974.

[8] 王大明,邹红岩,何柳媚,等.一例供者携带的C* 03:100等位基因的测序分析[J].国际输血及血液学杂志,2012,35(5):390-392.

[9] Cano P, Klitz W, Mack SJ, et al. Common and well-documented HLA alleles: report of the Ad-Hoc committee of the american society for histocompatiblity and immunogenetics. Hum Immunol. 2007;68:392-417.

[10] Kobayashi S, Kikuta A, Ito M, et al. Loss of mismatched HLA in myeloid/NK cell precursor acute leukemia relapse after T cell-replete haploidentical hematopoietic stem cell transplantation. Pediatric Blood Cancer. 2014;61(10): 1880-1882.

[11] 王大明,何柳媚,邹红岩,等. 4例人类白细胞抗原罕见等位基因的结果分析[J].中国组织工程研究,2013,17(44):7809-7814.

[12] Wang DM, Xu YP, Deng ZH. Identification of a novel HLA-C*01 variant allele, C*01:46. Tissue Antigens. 2011;78: 70-71.

[13] Saber W, Opie S, Rizzo JD, et al. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. Blood. 2012;119(17):3908-3916.

[14] Spellman S, Klein J, Haagenson M, et al. Scoring HLA class I mismatches by HistoCheck does not predict clinical outcome in unrelated hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18(5):739-746.

[15] Askar M, Sobecks R, Morishima Y, et al. Predictions in the face of clinical reality:HistoCheck versus high-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease. Biol Blood Marrow Transplant. 2011;17(9):1409-1415.

[16] Petersdorf EW, Malkki M, Horowitz MM, et al. Mapping MHC haplotype effects in unrelated donor hematopoietic cell transplantation. Blood. 2013;121(10):1896-1905.

[17] ratwohl A, Brand R, Niederwieser D, et al. Introduction of a quality management system and outcome after hematopoietic stem-cell transplantation. J Clin Oncol. 2011;29(15):1980-1986.

[18] Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLAmatching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110(13):4576-83.

[19] Arora M, Weisdorf D, Stephen R. HLA-identical sibling compared with 8/8 matched and mismatched unrelated donor bone marrow transplant for chronic phase chronic myeloid leukemia. J Clin Oncol. 2009;27:1644-1652.

[20] Shikari H, Amparo F, Saboo U, et al. Onset of ocular graft-versus-host disease symptoms after allogeneic hematopoietic stem cell transplantation. Cornea. 2015; 34(3):243-247.

[21] Zheng CC, Zhu XY, Tang BL, et al. Comparative analysis of unrelated cord blood transplantation and HLA-matched sibling hematopoietic stem cell transplantation in children with high-risk or advanced acute leukemia. Ann Hematol. 2015; 94(3):473-480.
[22] Remberger M, Beelen DW, Fauser A, et al. Increased risk of extensive chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplantation using unrelated donors. Blood. 2005;105:548-551.
[23] 23. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002;11(99):4200-4206.
[24] Greinix HT, Fae I, Schneider B, et al. Impact of HLA class I high-resolution mismatches on chronic graft-versus-host disease and survival of patients given hematopoietic stem cell grafts from unrelated donors. Bone Marrow Transplant. 2005;35:57-62.
[25] Weisdorf DJ, Anasetti C, Antin JH, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: Comparative analysis of unrelated versus matched sibling donor transplantation. Blood. 2002;99:1971-1977.
[26] Mohamed M. Immunology of hematopoietic stem cell transplantation. Immunol Invest. 2014;43(8):1-30
[27] Hladun R, Badell I, González M, et al. Survival analysis of hematopoietic stem cell transplantation in children with primary immunodeficiency in Spain. An Pediatr (Barc). 2014;82(2):62-67.
[28] Alois G, Helen B, Jakob P. Hematopoietic stem cell transplantation activity in Europe. Curr Opin Hematol. 2013;20(6):485-493.
[29] Marcelo A. Fernandez V, Wang T, et al. Identication of a permissible HLA mismatch in hematopoietic stem cell transplantation. Blood. 2014;123(8):1270-1278.
[30] Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004;104:1923-1930.
[31] Brunstein CG. Wanted: prospective studies of alternative donors. Biol Blood Marrow Transplant. 2015;21(6):957-958.
[32] Perusco A, Gamba C, Galarza P, et al. HLA antigenic and haplotype frequencies estimated in hematopoietic progenitor cell donors from Argentina. Transplant Proc. 2014;46(9):3064-3067.
[33] Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014;371(4):339-348.
[34] Grubic Z, Burek Kamenaric M, et al. HLA-A, HLA-B and HLA-DRB1 allele and haplotype diversity among volunteer bone marrow donors from Croatia. Int J Immunogenet. 2014;41(3):211-221.
[35] Testi M, Andreani M, Locatelli F, et al. Influence of the HLA characteristics of Italian patients on donor search outcome in unrelated hematopoietic stem cell transplantation. Tissue Antigens. 2014;84(2):198-205.
[36] Gourraud PA, Balère ML, Faucher C, et al. HLA phenotypes of candidates for HSCT: comparing transplanted versus non-transplanted candidates, resulting in the predictive estimation of the probability to find a 10/10 HLA matched donor. Tissue Antigens. 2014;83(1):17-26.
[37] Bemer MJ, Sorror M, Sandmaier BM, et al. A pilot pharmacologic biomarker study in HLA-haploidentical hematopoietic cell transplant recipients. Cancer Chemother Pharmacol. 2013;72(3):607-618.
[38] Bäuerlein CA, Riedel SS, Baker J, et al. A diagnostic window for the treatment of acute graft-versus-host disease prior to visible clinical symptoms in a murine model. BMC Med. 2013; 11:134.
[39] Hombrink P, Hassan C, Kester MG, et al. Discovery of T cell epitopes implementing HLA-peptidomics into a reverse immunology approach. J Immunol. 2013;190(8):3869-3877.
[40] Grubic Z, Stingl K, Zunec R. Heterogeneity of HLA-DRB1*04 alleles and haplotypes in the Croatian population. Tissue Antigens. 2012;80(3):219-223.
[41] Thiel U, Wolf P, Wawer A, et al. Human leukocyte antigen distribution in German Caucasians with advanced Ewing's sarcoma. Klin Padiatr. 2012;224(6):353-358.
[42] Kuwatsuka Y, Kohno A, Terakura S, et al. Phase II study of dose-modified busulfan by real-time targeting in allogeneic hematopoietic stem cell transplantation for myeloid malignancy. Cancer Sci. 2012;103(9):1688-1694.
[43] Mori T, Kato J, Shimizu T, et al. Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation from unrelated donors. Biol Blood Marrow Transplant. 2012; 18(2):229-234.
[44] Sobecks RM, Askar M, Thomas D, et al. Cytomegalovirus reactivation after matched sibling donor reduced-intensity conditioning allogeneic hematopoietic stem cell transplant correlates with donor killer immunoglobulin-like receptor genotype. Exp Clin Transplant. 2011;9(1):7-13.
[45] Viswanathan C, Roy A, Damodaran D, et al. Optimization of the inventory size of the public cord blood program-the Indian context. J Assoc Physicians India. 2010;58:608-611.
[46] Rosenmayr A, Pointner-Prager M, Mitterschiffthaler A, et al. What are a patient's current chances of finding a matched unrelated donor? Twenty years' central search experience in a small country. Bone Marrow Transplant. 2012;47(2): 172-180.
[47] Kanda J, Lopez RD, Rizzieri DA. Alemtuzumab for the prevention and treatment of graft-versus-host disease. Int J Hematol. 2011;93(5):586-593.
[48] Testi M, Lai S, Orrù S, et al. Distribution of HLA alleles and haplotypes in the Maldivian population. Tissue Antigens. 2011;77(3):235-238.
[49] Balas A, García-Sánchez F, Vicario JL. Allelic and haplotypic HLA frequency distribution in Spanish hematopoietic patients. Implications for unrelated donor searching. Tissue Antigens. 2011;77(1):45-53.
[50] Willemze R, Ruggeri A, Purtill D, et al. Is there an impact of killer cell immunoglobulin-like receptors and KIR-ligand incompatibilities on outcomes after unrelated cord blood stem cell transplantation? Best Pract Res Clin Haematol. 2010; 23(2):283-290.