doi: 10.13241/j.cnki.pmb.2020.08.025

白血病融合基因 EVI1 的多态性与白血病发生风险的相关性 *

石 媛 毕小朵 杨光路 李晓华 付俊鲜 李天霞 张称心△

(内蒙古医科大学附属医院儿科 内蒙古 呼和浩特 010110)

摘要 目的:探讨白血病融合基因亲嗜性病毒整合位点 1(ecotropic viral integration site-1,EVI1)的多态性与白血病发生风险的相关性。**方法**:选取本院 2017 年 2 月 ~2019 年 2 月收治的骨刺患儿 90 例作为研究组,同期选择健康人群 83 例作为对照组。清晨空腹抽取两组入选者的外周静脉血 2 mL,采用 PCR 方法检测两组入选者 EVI1 的多态性情况,调查一般资料并进行相关性分析。**结果**:EVI1 rs17561 基因共有 CC、CA、AA 三种基因型, 两组入选者的 EVI1 rs17561 基因分布均符合 Hardy-Weinberg 平衡定律,研究对象具有群体代表性。两组入选者 EVI1 rs17561 基因型分布差异具有统计学意义(P<0.05),研究组的 EVI1 rs17561 基因 CC 基因型显著高于对照组(90.0 % vs. 75.9 %, P<0.05),研究组的等位基因 C 频率(显著高于对照组(96.7 % vs. 80.7%, P<0.05)。在 90 例骨刺患儿中,6 例患儿确诊为白血病,检出率为 6.7 %,均为 CC 基因型。研究组患儿 EVI1 rs17561 基因的 CC 基因型与血小板计数、危险度分层、诊断分型显著相关 (P<0.05)。多元 Logistic 回归分析显示血小板计数、危险度分层、诊断分型为影响 EVI1 rs17561CC 基因型的主要因素(P<0.05)。**结论**:白血病患儿融合基因 EVI1 多态性比较常见,多表现为 rs17561CC 等位基因,此等位基因可能与白血病患者的血小板计数、危险度分层、诊断分型显著相关,其中血小板计数、危险度分层、诊断分型为影响 EVI1 rs17561CC 基因型的主要因素。

关键词:白血病;融合基因;亲嗜性病毒整合位点 1;基因多态性;相关性

中图分类号:R733.7 **文献标识码**:A **文章编号**:1673-6273(2020)05-1515-04

Correlation between Leukemia Fusion Gene EVI1 Polymorphism and Risk of Leukemia*

SHI Yuan, BI Xiao-duo, YANG Guang-lu, LI Xiao-hua, FU Jun-xian, LI Tian-xia, ZHANG Chen-xin△

(Department of Pediatrics, Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, 010110, China)

ABSTRACT Objective: To investigate the association between leukemia fusion gene eukaryotic fusion site 1 (EVI1) polymorphism and risk of leukemia. **Methods:** Ninety children children with spurs admitted to our hospital from February 2017 to February 2019 were selected as study groups, and eighty- three healthy people were selected as control group. The 2 mL of peripheral venous blood of the two group in the morning were collected, the polymorphism of EVI1 in the two groups were detected by PCR. The general data were investigated and were given correlation analysis. **Results:** The EVI1 rs17561 gene has three genotypes of CC, CA and AA, and the EVI1 rs17561 gene distribution of the two groups were consistent with the Hardy-Weinberg equilibrium law that so the subjects were representative. The difference of EVI1 rs17561 genotype distribution compared between the two groups were statistically significant (P<0.05), and the EVI1 rs17561 gene CC genotype in the study group was significantly higher than that in the control group (90.0% vs. 75.9 %, P<0.05), and the frequency of allele C in the study group was significantly higher than that in the control group (96.7% vs. 80.7 %, P<0.05). Among the ninety children with bone spurs, 6 patients were diagnosed with leukemia, and the detection rate was 6.7 %, all of which were CC genotypes. In the leukemia group. The CC genotype of EVI1 rs17561 gene in the study group was significantly correlated with platelet count, risk stratification, and diagnostic typing (P<0.05). Multivariate logistic regression analysis showed that platelet count, risk stratification and diagnostic typing were the main factors affected the EVI1 rs17561CC genotype (P<0.05). **Conclusion:** The fusion gene EVI1 polymorphism in children with leukemia is more common, and it is mostly represented by the rs17561CC allele. This allele may be significantly related to platelet count, risk stratification, and diagnosis typing in patients with leukemia. Stratification and diagnosis are the main factors affecting the genotype of EVI1 rs17561CC.

Key words: Leukemia; Fusion gene; Ecotropic integration site 1; Gene polymorphism; Correlation

Chinese Library Classification(CLC): R733.7 Document code: A

Article ID: 1673-6273(2020)05-1515-04

* 基金项目:内蒙古自治区高等学校青年科技英才计划 A 类项目(NJYT-17-A19)

作者简介:石媛(1988-),女,硕士研究生,住院医师,研究方向:小儿血液肿瘤,电话:15034772366,E-mail:shiyuan_0471@163.com

△ 通讯作者:张称心(1973-),男,硕士研究生,副主任医师,研究方向:小儿血液肿瘤,

电话:1363471 6661,E-mail:zhangcx1973@126.com

(收稿日期:2019-11-04 接受日期:2019-11-28)

前言

白血病是一种源于造血干细胞癌变的疾病，具有治疗困难、容易复发的特点。白血病的发生是一个复杂而多步骤的过程，可能与遗传学和环境因素的作用有关[1-3]。已有研究显示克隆性染色体数量、结构异常可形成具有肿瘤特性的融合基因，也可使一些在细胞生长 / 凋亡调控过程中起重要作用的基因表达失控，从而干扰细胞增生、生存或分化调节，最终导致白血病发生 [4,5]。早期判断患者的病情与分型有利于进行个性化治疗，促进改善患者的预后[6]。

白血病细胞及分子遗传学改变作为白血病诊断、预后预测的标记已经得到广泛认同，目前可采用的方法包括荧光原位杂交技术 (Fluorescence in situ hybridization, FISH) 检测、PCR 检查、染色体核型分析、基因组测序等[7-9]。亲嗜性病毒整合位点 1 (ecotropic viral integration site-1，EVI1) 基因定位于人类染色体 3q26，是一个位点特异的 DNA 结合蛋白，编码一个相对分子质量 14.5 万道尔顿的锌指蛋白转录因子，参与 RNA 的转录调节，对造血干细胞的增殖和存活起重要作用[10,11]。有研究显示 EVI1 存在一定的基因多态性，与机体的脂质代谢、骨代谢有一定的相关性，同时可能涉及人类疾病的易感性[12-14]。本研究主要探讨了白血病融合基因 EVI1 的多态性与白血病发生风险的相关性，现总结报道如下。

1 资料与方法

1.1 研究对象

本研究经医院伦理委员会审核批准与所有入选者的知情同意。选取本院 2017 年 2 月到 2019 年 2 月收治的骨刺患儿 90 例作为研究组，纳入标准：年龄 4-16 岁；检查前未接受过激素等任何抗白血病治疗。。排除标准：合并恶性肿瘤患者；伴有遗传代谢性疾病患者；伴有其他基因疾病患者。同期选择健康人群 83 例作为对照组。

研究组中，男 47 例，女 43 例；年龄最小 4 岁，最大 16 岁，平 7.15± 1.28 岁；平均体重指数 18.72± 2.18 kg/m^2。血红蛋白 75.20± 2.19 g/L，白细胞计数(60.24± 2.66)× 10^9 个 /L，血小板计数(56.39± 4.11)× 10^9 个 /L。对照组中，男 42 例，女 41 例；平均年龄 7.66± 1.32 岁；平均体重指数 17.88± 2.22 kg/m^2。

1.2 EVI1 多态性检测

抽取两组入选者的清晨空腹外周静脉血 2 mL，提取 DNA (厦门施科生物科技有限公司提供)，放置于 -20℃低温箱保存备用。Taqman 探针由北京优博兰基因技术有限公司生成提供)，上游引物序列：5'ACATTGCTCAGGAAGC-TAAAAG-GTG3'，下游引物序列：5'TGACCTAG-GCTTGAT-GATTCTAAA3' (上海基康生物技术有限公司合成)。采用 Bio-Rad CFX manager 软件对 EVI1 rs17561 基因的基因型 PCR 扩增结果进行分析。

1.3 调查资料

调查所有入选者的性别、年龄、体重指数等资料，记录白血病患者的血红蛋白、白细胞计数、血小板计数、危险度分层与诊断分型等。

1.4 统计学方法

采用 SPSS 21.00 统计学软件进行数据分析，两组入选者的 EVI1 rs17561 基因分布采用 Hardy-Weinberg(H-W)进行平衡检验，计数数据对比采用 χ^2 检验分，计量数据对比采用 t 检验，相关性分析采用 Pearson 相关系数，同时采用多元 Logistic 回归分析探讨相关性，检验水准为 α=0.05。

2 结果

2.1 平衡检验结果

EVI1 rs17561 基因共有 CC、CA、AA 三种基因型，两组入选者的 EVI1 rs17561 基因分布均符合 Hardy-Weinberg 平衡定律，研究对象具有群体代表性。

2.2 两组 EVI1 rs17561 基因型和等位基因分布比较

两组入选者 EVI1 rs17561 基因型分布差异具有统计学意义(P<0.05)，研究组的 EVI1 rs17561 基因 CC 基因型(90.0 %)显著高于对照组(75.9 %，P<0.05)，等位基因 C 频率(96.7 %)显著高于对照组(80.7 %，P<0.05)。见表 1。

表 1 两组 EVI1 rs17561 基因型和等位基因分布比较[例(%)]

Table 1 Comparison of the EVI1 rs17561 genotype and allele distribution between the two groups [n(%)]

Groups	Genotype			Allel	
	CC	CA	AA	C	A
Study group(n=90)	81(90.0)*	9(10.0)	0(0.00)	87(96.7)*	3(3.33)
Control group (n=83)	63(75.9)	12(14.5)	8(9.6)	67(80.7)	16(19.3)

Note: Compared with the control group, *P<0.05.

2.3 白血病的检出率

90 例骨刺患儿中，6 例患儿确诊为白血病，检出率为 6.7%，均为 CC 基因型。其中，急性淋巴细胞白血病的 4 例，慢粒性粒细胞白血病 1 例，急性粒细胞白血病 M2 型 1 例。

2.4 研究组患者 EVI1 rs17561 基因多态性与临床指标的相关性

研究组中，Pearson 相关分析显示 EVI1 rs17561 基因的 CC 基因型与血小板计数、危险度分层、诊断分型显著相关(P<0.05)，见表 2。

以 EVI1 rs17561CC 基因型的发生作为应变量，以血小板计数、危险度分层、诊断分型作为自变量，多元 Logistic 回归分析显示血小板计数、危险度分层、诊断分型为影响 EVI1 rs17561CC 基因型的主要因素(P<0.05)。见表 3。

3 讨论

白血病是一类有着不同行为和不同治疗反应的血液系统

表 2 研究组患者 EVI1 rs17561 基因多态性与临床指标的相关性(n=90)

Table 2 Correlation between EVI1 rs17561 gene polymorphism and clinical indicators inthe study group (n=90)

Index	Platelet count	Risk stratification	Diagnostic classification
r	0.562	0.633	0.598
P	0.003	0.000	0.001

表 3 影响白血病 EVI1 rs17561CC 基因型的多元 Logistic 回归分析(n=90)

Table 3 Multivariate logistic regression analysis of genotypes affecting leukemia EVI1 rs17561CC (n=90)

Index	B	*P*	OR	95.0%CI
Platelet count	2.893	0.024	16.836	2.071~104.28
Risk stratification	3.126	0.017	19.374	1.848~302.16
Diagnostic classification	1.190	0.461	3.742	0.183~32.683

恶性肿瘤,发病率约为 3-5/10 万,主要由正常髓系细胞分化发育过程中造血祖细胞恶性变转化而来[15]。该病多数患者伴随有克隆性染色体数量、结构异常。已有研究显示机体染色体突变可形成具有肿瘤特性的融合基因，导致相关基因表达失控,从而干扰细胞增殖、分化、成熟、凋亡,诱发白血病的发生[16,17]。

EVI1 基因是一个具有 DNA 结合活性的锌指结构转录因子,位于染色体 3q26,可与不同的蛋白形成复合体,调控一些信号通路中关键基因的转录[18]。异常的 EVI1 表达引起骨髓细胞增生过度和全血细胞减少[19]。EVI1 基因也可控制胚胎的发育,对造血干细胞的增殖和存活起重要作用,参与白血病干细胞的产生[20,21]。本研究显示 EVI1 rs17561 基因共有 CC、CA、AA 三种基因型，两组入选者的 EVI1 rs17561 基因分布均符合 Hardy-Weinberg 平衡定律;研究组的 EVI1 rs17561 基因 CC 基因型、等位基因 C 频率(96.7 %)均显著高于对照组(80.7 %)。此外,90 例骨刺患儿中,6 例患儿确诊为白血病，均为 CC 基因型。从机制上分析,rs17561 作为 EVI1 基因上的非同义单核苷酸多态性(single nucleotide polymorphism,SNP),当主要等位基因 C 改变为次要等位基因 A 时,则可产生变异体 A114S,从而将 rs17561 基因 114 位置上的编码氨基酸由丙氨酸(Alanine,Ala)转变为丝氨酸(Serine,Ser)[22]。该突变还可增强炎症及免疫反应,从而提高炎性相关疾病的易感性,比如增加子宫内膜异位症、恶性肿瘤的易感性[23]。

白血病的治疗需先采取诱导化疗使病情达到完全缓解后再行进一步化疗或骨髓移植[24]。但危险度分层、诊断分型术常出现无核分裂象的标本而使异常核型检出率显著降低，检测费用也比较贵,故难以普遍开展[25,26]。EVI1 基因一个位点特异的 DNA 结合蛋白,主要控制胚胎的发育,也可参与 RNA 转录调节,对造血干细胞的增殖和存活起重要作用[27,28]。EVI1 rs17561 在多种炎症和免疫性疾病中有着重要促进作用,其原因可能与 rs17561 的主要等位基因上 C 突变为次要等位基因 A,形成的 A114S 具有促进炎症因子释放作用,进而促进炎症反应,同时可能涉及人类疾病的易感性[29-31]。本研究结果显示白血病患者 EVI1 rs17561 基因的 CC 基因型与血小板计数、危险度分层、诊断分型显著相关;多元 Logistic 回归分析显示血小板计数、危险度分层、诊断分型为影响 EVI1 rs17561CC 基因型的主要因素,表明 EVI1 rs17561 基因多态性与患者的病情显著相关。其影响机制可能与主要等位基因在机体发挥的某种调控作用有关,但本研究未发现其具体调控机制,需要后续进一步研究证实。

综上所述,白血病患者中融合基因 EVI1 多态性比较常见,多表现为 rs17561CC 等位基因,此等位基因可能与白血病患者的血小板计数、危险度分层、诊断分型显著相关,其中血小板计数、危险度分层、诊断分型为影响 EVI1 rs17561CC 基因型的主要因素。

参 考 文 献(References)

[1] Akhter A, Farooq F, Elyamany G, et al. Acute Myeloid Leukemia (AML): Upregulation of BAALC/MN1/MLLT11/EVI1 Gene Cluster Relate With Poor Overall Survival and a Possible Linkage With Coexpression of MYC/BCL2 Proteins [J]. Appl Immunohistochem Mol Morphol, 2018, 26(7): 483-488

[2] Benajiba L, Alexe G, Su A, et al. Creatine kinase pathway inhibition alters GSK3 and WNT signaling in EVI1-positive AML[J]. Leukemia, 2019, 33(3): 800-804

[3] Bozzetti C, Turkmen S, Richter U, et al. A Rare Case of Acute Myeloid Leukemia with a t (2;3) Chromosomal Translocation Characterized by Thrombophilia and Chemoresistance [J]. J Clin Exp Hematop, 2016, 56(1): 64-68

[4] Chen Y, Ernst P. Hematopoietic transformation in the absence of MLL1/KMT2A: distinctions in target gene reactivation[J]. Cell Cycle, 2019, 18(14): 1525-1531

[5] Du W, He J, Zhou W, et al. High IL2RA mRNA expression is an independent adverse prognostic biomarker in core binding factor and intermediate-risk acute myeloid leukemia [J]. J Transl Med, 2019, 17 (1): e191

[6] Fenouille N, Bassil C F, Ben-Sahra I, et al. The creatine kinase pathway is a metabolic vulnerability in EVI1-positive acute myeloid leukemia[J]. Nat Med, 2017, 23(3): 301-313

[7] Hofman I JF, Van Duin M, De Bruyne E, et al. RPL5 on 1p22.1 is recurrently deleted in multiple myeloma and its expression is linked to bortezomib response[J]. Leukemia, 2017, 31(8): 1706-1714

[8] Hu T, Shen J, Liu W, et al. Multiple myeloma secondary to acute lymphoblastic leukemia: A case report [J]. Medicine (Baltimore), 2019, 98(1): e14018

[9] Hu Z, Medeiros LJ, Wang W, et al. 3q26.2/EVI1 rearrangement is

associated with poor prognosis in classical Philadelphia chromosome-negative myeloproliferative neoplasms[J]. Mod Pathol, 2017, 30(7): 940-951

[10] Jiang M, Zou X, Huang W. Ecotropic viral integration site 1 regulates the progression of acute myeloid leukemia via MS4A3-mediated TGFbeta/EMT signaling pathway[J]. Oncol Lett, 2018, 16(2): 2701-2708

[11] Kim YJ, Yang JJ, Han Y, et al. A Rare Case of ETV6/MECOM Rearrangement in Therapy-Related Acute Myeloid Leukemia with t (3;12) and Monosomy 7[J]. Clin Lab, 2017, 63(2): 415-418

[12] Kuila N, Nayak KB, Halder A, et al. Ecotropic viral integration site I regulates alpha1, 6-fucosyl transferase expression and blocks erythropoiesis in chronic myeloid leukemia [J]. Leuk Lymphoma, 2017, 58(8): 1941-1947

[13] Lawce H, Szabo E, Torimaru Y, et al. MECOM (EVI1) Rearrangements: A Review and Case Report of Two MDS Patients with Complex 3q Inversion/Deletions[J]. J Assoc Genet Technol, 2017, 43(1): 9-14

[14] Liu K, Tirado CA. A t (3;8) (q26.2;q24) involving the EVI1 (MECOM) Gene[J]. J Assoc Genet Technol, 2018, 44(3): 92-99

[15] Maicas M, Vazquez I, Alis R, et al. The MDS and EVI1 complex locus (MECOM) isoforms regulate their own transcription and have different roles in the transformation of hematopoietic stem and progenitor cells[J]. Biochim Biophys Acta Gene Regul Mech, 2017, 1860(6): 721-729

[16] Manachai N, Saito Y, Nakahata S, et al. Activation of EVI1 transcription by the LEF1/beta-catenin complex with p53-alteration in myeloid blast crisis of chronic myeloid leukemia[J]. Biochem Biophys Res Commun, 2017, 482(4): 994-1000

[17] Mittal N, Li L, Sheng Y, et al. A critical role of epigenetic inactivation of miR-9 in EVI1(high) pediatric AML[J]. Mol Cancer, 2019, 18(1): e30

[18] Nakamura Y, Ichikawa M, Oda H, et al. RUNX1-EVI1 induces dysplastic hematopoiesis and acute leukemia of the megakaryocytic lineage in mice[J]. Leuk Res, 2018, 74: 14-20

[19] Nakata Y, Ueda T, Nagamachi A, et al. Acquired expression of Cbl (Q367P) in mice induces dysplastic myelopoiesis mimicking chronic myelomonocytic leukemia[J]. Blood, 2017, 129(15): 2148-2160

[20] Paredes R, Schneider M, Stevens A, et al. Erratum: EVI1 carboxy-terminal phosphorylation is ATM-mediated and sustains transcriptional modulation and self-renewal via enhanced CtBP1 association [J]. Nucleic Acids Res, 2018, 46(16): e8663

[21] Paredes R, Schneider M, Stevens A, et al. EVI1 carboxy-terminal phosphorylation is ATM-mediated and sustains transcriptional modulation and self-renewal via enhanced CtBP1 association [J]. Nucleic Acids Res, 2018, 46(15): 7662-7674

[22] Sadeghian MH, Rezaei Dezaki Z. Prognostic Value of EVI1 Expression in Pediatric Acute Myeloid Leukemia: A Systematic Review[J]. Iran J Pathol, 2018, 13(3): 294-300

[23] Saha HR, Kaneda-Nakashima K, Shimosaki S, et al. Suppression of GPR56 expression by pyrrole-imidazole polyamide represents a novel therapeutic drug for AML with high EVI1 expression[J]. Sci Rep, 2018, 8(1): e13741

[24] Sato K, Sakai H, Uchida A, et al. Acute myeloid leukemia with t (3;8) (q26.2;q24), a simple variant of 3q26.2/EVI1 translocation[J]. Rinsho Ketsueki, 2017, 58(4): 315-322

[25] Shiba N. Current diagnosis and treatment for pediatric acute myeloid leukemia[J]. Rinsho Ketsueki, 2017, 58(4): 389-399

[26] Stavropoulou V, Kaspar S, Brault L, et al. MLL-AF9 Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome [J]. Cancer Cell, 2016, 30(1): 43-58

[27] Suekane A, Saito Y, Nakahata S, et al. CGRP-CRLR/RAMP1 signal is important for stress-induced hematopoiesis[J]. Sci Rep, 2019, 9(1): e429

[28] Suguna E, Farhana R, Kanimozhi E, et al. Acute Myeloid Leukemia: Diagnosis and Management Based on Current Molecular Genetics Approach[J]. Cardiovasc Hematol Disord Drug Targets, 2018, 18(3): 199-207

[29] Wafa A, S AL, Aljapawe A, et al. A new adult AML case with an extremely complex karyotype, remission and relapse combined with high hyperdiploidy of a normal chromosome set in secondary AML [J]. BMC Hematol, 2018, 18: 21

[30] Wang H, Schaefer T, Konantz M, et al. Prominent Oncogenic Roles of EVI1 in Breast Carcinoma[J]. Cancer Res, 2017, 77(8): 2148-2160

[31] Yamamoto K, Yakushijin K, Ichikawa H, et al. Coexpression of ETV6/MDS1/EVI1 and ETV6/EVI1 fusion transcripts in acute myeloid leukemia with t (3;12) (q26.2;p13) and thrombocytosis[J]. Leuk Lymphoma, 2019, 60(5): 1294-1298