Biol Blood Marrow Transplant 23 (2017) 1374–1380

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Clinical Research: Analysis

Role of Genetic Polymorphism of *ALDH2* in Hematopoietic Stem Cell Transplantation

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Article history:
Received 8 February 2017
Accepted 30 March 2017

Key Words:
Hematopoietic stem cell transplantation
Acetaldehyde
Aldehyde dehydrogenase-2
Transplantation-related mortality
Engraftment

A B S T R A C T

Aldehyde dehydrogenase 2 (*ALDH2*) is involved in critically important biological processes, such as the metabolism of aldehydes and aldehyde-induced genotoxicity in hematopoietic stem cells. Given its role in these biological processes, we hypothesized that a functional *ALDH2* polymorphism could affect transplantation outcomes after hematopoietic stem cell transplantation. Here, we analyzed the Japanese national registry data for 409 patients who underwent allogeneic bone marrow transplantation (BMT) from HLA-matched unrelated donors. To evaluate the impact of the recipient and donor *ALDH2* polymorphism on transplantation outcomes, we estimated hazard ratios (HRs) and 95% confidence intervals (CIs) adjusted for potential confounders. The recipient *ALDH2* Lys/Lys genotype was significantly associated with higher transplantation-related mortality (TRM), with an HR relative to Glu/Glu genotype of 2.45 (95% CI, 1.22 to 4.90). The recipient Lys/Lys genotype also tended to be associated with delayed platelet engraftment (HR, .66; 95% CI, .43 to 1.03). In conclusion, we observed increased TRM among recipients with the *ALDH2* Lys/Lys genotype in HLA fully matched BMT. We also observed a suggestive association with delayed platelet engraftment, which warrants further examination. These results may suggest that the recipient *ALDH2* genotype affects the metabolism of endogenous aldehydes, leading to a significant impact on transplantation outcomes.

INTRODUCTION

Aldehyde dehydrogenase-2 (*ALDH2*) is a mitochondrial enzyme that converts acetaldehyde, metabolized from ethanol, to acetate. *ALDH2* is also capable of metabolizing other aldehydes [1], however, and thereby provides an important protective enzymatic function against these toxic agents. In particular, *ALDH2* plays a key role in oxidizing endogenous aldehydes under oxidative stress, such as 4-hydroxy-2-nonenal and malondialdehyde [2]. A functional *ALDH2* polymorphism (*ALDH2*; rs671, Glu504Lys, see the website https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=671) is highly prevalent in East Asian populations [3-5] and the *ALDH2* Lys allele is associated with enzyme activity [6,7]. The Glu allele encodes a protein with normal catalytic activity, whereas the Lys allele encodes an inactive protein [8]. As a result, *ALDH2* Glu/Lys heterozygotes have far less than one-half of the *ALDH2* activity of Glu/Glu homozygotes, and Lys/Lys homozygotes have no detectable *ALDH2* activity.

Recently, Patel's laboratory reported that endogenous aldehydes might be the source of DNA damage using a Fanconi anemia (FA) mouse model lacking both *FANCD2* (1 of the FA genes) and *ALDH2* [9,10]. They showed that double knockout mice deficient in *FANCD2* and *ALDH2* display an accelerated development of leukemia and bone marrow failure (BMF), but that the single mutant mice do not. These findings also underlie the crucial role of *ALDH2* enzyme activity during embryogenesis and in protecting hematopoietic stem cells (HSCs). Hira et al. reported that *ALDH2* polymorphism (rs671) contributes to the progression of BMF in Japanese FA patients [11], supporting the idea that the phenomenon seen in the experimental model might also be applicable in humans. To date, 19 *ALDH* genes including *ALDH2* have been identified in the human genome [12]. Among them, we hypothesized that this functional *ALDH2* polymorphism (rs671) could affect transplantation outcomes after hematopoietic stem cell transplantation (HSCT) based on its role in these biological processes.

Here, we investigated the association between recipient and donor *ALDH2* genotypes (rs671) and clinical outcomes

Financial disclosure: See Acknowledgments on page 1380.
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http://dx.doi.org/10.1016/j.bbmt.2017.03.033
1083-8791/

in HLA fully matched, unrelated bone marrow transplantation (BMT) from the Japanese Marrow Donor Program (JMDP).

SUBJECTS AND METHODS

Subjects for Analysis

ALDH2 genotyping was performed on 409 unrelated donor-recipient pairs from the JMDP database that satisfied the following criteria: (1) transplant was from an HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 allele-matched donor between January 1993 and December 2005; (2) transplantation of T cell–replete marrow without in vivo use of antithymocyte globulin for graft-versus-host disease (GVHD) prophylaxis; (3) first transplantation; (4) Japanese ethnicity; and (5) survival for >7 days after transplantation. All donor-patient pairs were genotyped for all HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1alleles at the field 1 and field 2 level of the 2010 World Health Organization Nomenclature for factors of the HLA system [13]. Five recipients were not genotyped successfully, leaving 404 recipients and 409 donors for inclusion in the analysis. The final clinical survey of these patients was completed by September 2012. All patients and donors provided written informed consent to participate in this study at the time of transplantation, in accordance with the Declaration of Helsinki. This study was approved by the institutional review board of the JMDP and Aichi Cancer Center Research Institute, where this study was organized.

ALDH2 *Genotyping*

DNA of each subject was extracted from white blood cells in the buffy coat using a FlexiGene DNA kit (Qiagen, Tokyo, Japan). Genotyping of *ALDH2* (rs671) was conducted using TaqMan Assays with the Applied Biosystems 7500 Fast system (Foster City, CA). Genotyping quality was statistically assessed using the Hardy-Weinberg test and by retyping of a random sampling of 5% of subjects.

Endpoints

The primary endpoints of the study were overall survival (OS), treatment-related mortality (TRM), and relapse. Other endpoints included neutrophil engraftment, reticulocyte engraftment, platelet engraftment, acute GVHD, and chronic GVHD. *TRM* was defined as any death while the patient was in remission. Neutrophil, reticulocyte, and platelet recovery were considered to have occurred when the absolute neutrophil count was $\geq 2.5 \times 10^9$ cells/L, reticulocytes were ≥10%, and platelets were $\geq 5.0 \times 10^9$ cells/L, respectively, for 3 consecutive days. Clinical grading of acute GVHD was performed according to established criteria [14,15]. Chronic GVHD was defined as limited or extensive chronic GVHD according to the Seattle criteria [16].

Statistical Analysis

Descriptive statistics were used to summarize the variables related to patient and donor characteristics. Comparisons among groups were performed with the chi-square test. The probability of OS was estimated according to the Kaplan-Meier method [17], and groups were compared using the log-rank test. The probabilities of relapse, TRM, engraftment and GVHD were estimated based on cumulative incidence curves [18]. Competing events were death without relapse for relapse, relapse for TRM, death without engraftment for engraftment, and death without GVHD for GVHD. The groups were compared using Gray's test.

To evaluate the impact of the recipient and donor *ALDH2* genotypes (rs671) on transplantation outcomes, we estimated the hazard ratios (HRs) or subhazard ratios and 95% confidence intervals (CI) adjusted for potential confounders. A Cox proportional hazards model was used to evaluate the impact on OS, whereas the multivariable competing risk regression model [19,20] was used to evaluate the impact on the other endpoints. The following possible confounding variables were considered for OS, TRM, relapse, acute GVHD, and chronic GVHD: the recipient's age group (16 to 49 years or >50 years), the recipient's sex (male or female), sex mismatch between the recipient and donor (match, male [donor]/female [recipient], or female [donor]/male [recipient]), disease (acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, malignant lymphoma, myelodysplastic syndrome, or others), disease status before transplantation (standard or high risk), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other/missing), type of conditioning regimen (myeloablative or reduced intensity), and year of transplantation (1993 to 1999 or 2000 to 2005). ABO compatibility (match, major mismatch, minor mismatch, bidirectional or missing) and total nucleated cell dose (<2.0, 2.0 to 2.5, 2.5 to 3.0, $\geq 3.0 \times 10^8$/kg, or missing) were also considered for neutrophil, red cell, and platelet engraftment. The variables found to be significant in the univariate analyses ($P \leq .05$) were included as potential confounders in the multivariate analyses (Supplementary Table S1-S3).

Based on the report by the Center for International Blood and Marrow Transplant Research, the conditioning regimens were classified as myeloablative if they included total body irradiation (TBI) > 8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan >7.2 mg/kg, or melphalan >140 mg/m^2. Other conditioning regimens were classified as nonmyeloablative [21]. We defined acute myeloid leukemia and acute lymphocytic leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, malignant lymphoma in any complete remission, and myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases; other conditions were considered high risk.

We considered a P value < .016 (= .05/3) as statistically significant for the primary endpoints (Bonferroni adjustment for 3 primary endpoints). All analyses were performed using STATA version 13 (Stata Corp., College Station, TX).

RESULTS

Patient Characteristics

Table 1 shows patient and donor characteristics. The genotype frequencies of Glu/Glu, Glu/Lys, and Lys/Lys were 55% (223 of 404), 38% (153 of 404), and 7% (28 of 404), respectively, in the recipients, and 52% (213 of 409), 39% (159 of 409), and 9% (37 of 409), respectively, in the donors. Clinical background characteristics were not significantly different between *ALDH2* genotypes (rs671) among recipients and donors.

OS

The 5-year OS rate was 48% (95% CI, 41% to 54%) with the recipient Glu/Glu genotype, 48% (95% CI, 40% to 56%) with the recipient Glu/Lys genotype, and 39% (95% CI, 21% to 56%) with the recipient Lys/Lys genotype ($P = .631$) (Figure 1). The 5-year OS rate was 48% (95% CI, 41% to 55%) with the donor Glu/Glu genotype, 44% (95% CI, 44% to 52%) with the donor Glu/Lys genotype, and 58% (95% CI, 40% to 72%) with the donor Lys/Lys genotype ($P = .629$) (Figure 2). In multivariate analysis, recipient and donor *ALDH2* polymorphisms (rs671) were not significantly associated with the risk of overall mortality (Table 2).

TRM and Relapse

The cumulative incidence of 5-year TRM was 28% (95% CI, 22% to 34%) with the recipient Glu/Glu genotype, 26% (95% CI, 19% to 34%) with the recipient Glu/Lys genotype, and 50% (95% CI, 29% to 68%) with the recipient Lys/Lys genotype ($P = .102$) (Figure 3). Multivariate analysis revealed that the recipient *ALDH2* Lys/Lys genotype was significantly associated with a higher TRM, with an HR relative to Glu/Glu of 2.45 (95% CI, 1.22 to 4.90; $P = .012$), whereas the donor *ALDH2* genotypes did not show any significant association with a higher TRM (Table 2). When we assessed heterogeneity in TRM by recipient *ALDH2* genotype (rs671) among conditioning regimens by including a cross-product term of recipient *ALDH2* genotype (rs671) and the type of conditioning regimen, we observed significant heterogeneity between conditioning regimens with and without TBI (HR for heterogeneity = 2.00 [95% CI, 1.03 to 3.83, $P = .041$]) (Table 3).

We also analyzed the main causes of TRM by recipient *ALDH2* polymorphism (rs671) (Table 4). Among the main causes of TRM, the incidence rate of organ failure in recipients with the Lys/Lys genotype was twice that in those with the Glu/Glu genotype (5.9 versus 3.0 per 1000 person-days). The types of organ systems affected in recipients with the Lys/Lys genotype were cardiac (n = 1, 25%), pulmonary (n = 1, 25%), liver (n = 1, 25%), and multiple organs (n = 1, 25%).

In the multivariate analyses, the recipient and donor *ALDH2* genotype (rs671) did not show any significant association with the risk of relapse (Table 2).