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Brief Articles

Identifying Inherited and Acquired Genetic Factors Involved in Poor Stem Cell Mobilization and Donor-Derived Malignancy

Katarzyna Rojek [1], Eric Nickels [1], Barbara Neistadt [1], Rafael Marquez [1], Amittha Wickrema [1], Andrew Artz [1], Koen van Besien [1,†], Richard A. Larson [1], Ming K. Lee [2], Jeremy P. Segal [3], Mary-Claire King [2], Tom Walsh [2], Akiko Shimamura [4,‡], Sioban B. Keel [5], Jane E. Churpek [1,6], Lucy A. Godley [1,6,*]

[1] *Section of Hematology/Oncology, Department of Medicine, and The University of Chicago Comprehensive Cancer Center, The University of Chicago, Chicago, Illinois*
[2] *Department of Medicine, Division of Medical Genetics, and Department of Genome Sciences, University of Washington, Seattle, Washington*
[3] *Division of Genomic and Molecular Pathology, Department of Pathology, University of Chicago, Chicago, Illinois*
[4] *Clinical Research Division, Fred Hutchinson Cancer Research Center, Pediatric Hematology/Oncology, Seattle Children's Hospital, and Department of Pediatrics, University of Washington, Seattle, Washington*
[5] *Department of Medicine, Division of Hematology, University of Washington, Seattle, Washington*
[6] *Center for Clinical Cancer Genetics, The University of Chicago, Chicago, Illinois*

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A B S T R A C T

Analysis of the clinical characteristics of hematopoietic stem cell transplant (HSCT) donors has proven beneficial for identifying cases of heritable hematopoietic disorders. This study examines poor peripheral blood hematopoietic stem cell mobilization after granulocyte colony–stimulating factor administration among 328 donors as a potential marker for suspected familial predisposition to myeloid malignancies. Here, we present data comparing the clinical characteristics of poor-mobilizing versus nonpoor-mobilizing donors and the results of panel-based sequencing of hematopoietic genes in poor-mobilizing donors. From this analysis, we identified a novel case of a donor-derived myelodysplastic syndrome in an HSCT recipient that is consistent with clonal evolution of *TET2*-mutated clonal hematopoiesis of indeterminate potential (CHIP) within the donor. This study demonstrates the potential risk of using hematopoietic stem cells from a donor with CHIP and raises the question of whether there should be increased screening measures to identify such donors.

INTRODUCTION

Mobilized peripheral blood stem cells (PBSC) are the most frequent source of hematopoietic stem cells (HSC) used for allogeneic HSC transplantation (HSCT). However, genetic factors contributing to donors who mobilize PBSC poorly, and how this affects transplantation outcomes, are not well understood. Previously, our laboratory identified a case of germline predisposition to myeloid malignancies by studying related allogeneic stem cell donors who had baseline unexplained thrombocytopenia as a marker for identifying familial myelodysplastic syndrome (MDS)/acute leukemia predisposition syndromes [1]. Because poor mobilization has been observed in individuals with other heritable hematopoietic disorders [2], we hypothesized that we could identify additional individuals and families at high risk for having a germline predisposition allele by examining related allogeneic HSC donors who mobilized low numbers of PBSCs. Here, we present data of the results of panel-based sequencing of hematopoietic genes in poor-mobilizing donors.

MATERIALS AND METHODS

Subjects and Samples

We evaluated 328 HLA-matched related HSC donors who underwent PBSC mobilization at The University of Chicago from 2001 to 2011 for transplantation into a first-degree relative with a hematopoietic malignancy. $CD34^+$ cells in the peripheral blood (PB) were measured by flow cytometry on day 5 of mobilization after 4 to 5 days of granulocyte colony–stimulating factor (G-CSF) at 10 mcg/kg/day. We defined *poor mobilizers* as those whose $CD34^+$ cell numbers fell within the lowest quartile among all donors, which

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* Correspondence and reprint requests: Lucy A. Godley, MD, PhD, Section of Hematology/Oncology, Department of Medicine, The University of Chicago, 5841 S. Maryland Avenue MC2115, Chicago, IL 60637-1470.
E-mail address: lgodley@medicine.bsd.uchicago.edu (L.A. Godley).
† Current Address: Koen van Besien, Department of Hematology and Medical Oncology, Weill Cornell Medical College/ New York Presbyterian Hospital, New York, NY.
‡ Current Address: Akiko Shimamura, Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA.
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included those with day 1 PB CD34+ counts ≤55.0 cells/μL). Supplemental Table S1 lists all donors who had abnormalities demonstrable in the screening complete blood cell count, regardless of mobilization parameters. Retrospective analysis of matched related transplantation data was approved by The University of Chicago institutional review board, and informed consent was obtained from 28 subjects identified as poor mobilizers who had samples available for sequencing.

Targeted Gene Panel Sequencing

Genomic DNA isolated from each donor's mobilized PBSC product collected before transplantation was screened for mutations utilizing MarrowSeq (The University of Washington Medical Center Genetics and Solid Tumor Diagnostic Laboratory, Seattle, WA), a targeted next-generation sequencing panel (300X to 500X coverage), which includes 142 genes responsible for inherited and acquired bone marrow failure syndromes and MDS [3]. Targeted gene capture, sequencing, and analysis were performed using established protocols, and variants that were potentially damaging were confirmed by Sanger sequencing [3]. OncoHeme (The University of Chicago Department of Pathology, Chicago, IL), a second deep-sequencing next-generation sequencing panel (1000X coverage) targeting the exons of 54 genes recurrently mutated in MDS/acute leukemia, was used to identify additional acquired mutations in 1 donor/recipient pair.

RESULTS

We analyzed the clinical parameters documented on day 1 of PB collection for all matched related donors presenting for transplantation. Baseline characteristics of donors falling into the poor-mobilizer and nonpoor-mobilizer categories are given in Table 1 and Supplemental Table S2. *Poor mobilizers* were defined as those whose PB CD34+ cells/μL on the first day after 5 days of G-CSF administration ("day 1") fell within the lowest quartile of the 328 donors. Among the 82 poor mobilizers, genomic DNA samples and consent were available from 28 donors (Figure 1). Poor mobilizers were significantly older ($P < .001$), had higher mean corpuscular volume (MCVs) ($P < .001$), lower total white blood cell counts ($P = .03$), and lower platelet counts ($P = .02$) than nonpoor mobilizers (Table 1).

Among the 28 poor mobilizers sequenced using MarrowSeq (The University of Washington Medical Center Genetics and Solid Tumor Diagnostic Laboratory), clearly damaging mutations were identified in 2 individuals (7%). The first

Table 1
Poor Mobilizer and Nonpoor Mobilizer Donor Clinical Characteristics

Characteristic	All Donors (n = 328)	Poor Mobilizers (n = 82)	Nonpoor Mobilizers (n = 246)	*P* Value (Poor mobilizers versus nonpoor mobilizers)
Day 1 PB CD34+ counts, median, cells/μL	86.0 range (2.0-421.9)	40.7 IQR (29.9-48.3)	107.5 IQR (75.4-141.6)	
Female	45.43%	45.12%	45.53%	.95
Age, median, yr	47.5 range (13-74)	55 IQR (43-62)	46 IQR (37-54)	< .001
WBC, median, K/μL	6.6* range (3.1-13.7)	6.45* IQR (5.2-7.9)	6.7* IQR (5.6-8.1)	.03
Platelet count, median, K/μL	250* range (109-459)	240* IQR (201-275)	255 IQR (218-293)	.02
MCV, median, fL	89.3* range (64.7-107.6)	90.8* IQR (87.9-94.3)	88.8* IQR (85.6-91.5)	< .001

Day 1 PB CD34+ counts were obtained after mobilization of PB HSC by G-CSF. WBC counts, platelet counts, and MCV values were taken before mobilization. IQR indicates interquartile range.

* Pre-donation CBC values missing for one donor.

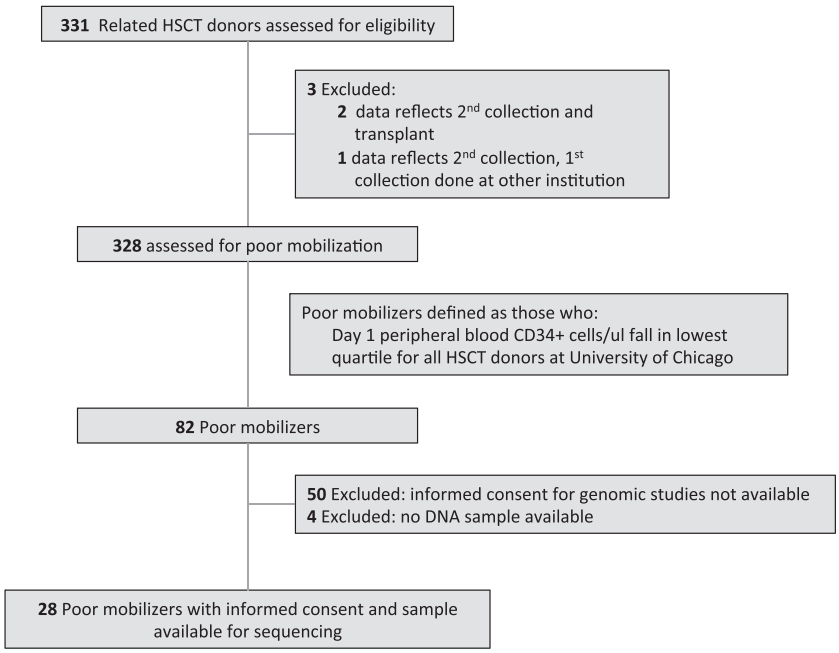

Figure 1. Flow diagram of sample analysis. Flow diagram shows how related donors were deemed eligible for this study.