

Umbilical Cord Blood Units Cryopreserved in the Public Cord Blood Bank: A Breakthrough in iPSC Haplobanking?

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Eun Youn Roh^{1,2,3,*} , Sohee Oh^{4,*}, Jong Hyun Yoon^{1,2,3},
Byoung Jae Kim^{2,5}, Eun Young Song³, and Sue Shin^{1,2,3}

Abstract

The use of induced pluripotent stem cells (iPSCs) is an emerging therapeutic option for precision medicine. Cord blood (CB) cells with lower immunogenicity, fewer genomic changes, and persistent epigenetic memory might be ideal candidates for iPSC production. Based on the human leukocyte antigen (HLA) distribution of cord blood units (CBUs) in the public CB bank, we estimated the coverage of the Korean population with HLA-homozygous iPSCs to repurpose cryopreserved CBUs. We analyzed a total of 27,904 Korean CBUs donated to the public CB bank. Low-to-intermediate resolution typing was performed for HLA-A, -B, and -DRB1 alleles, and individuals possessing homozygous HLA haplotypes were identified by direct counting. Moreover, the matching probabilities for zero-mismatch transplantation were calculated for 27,904 CBUs and 50,000,000 potential Korean patients. Among the preserved CBUs, 15 HLA-A, 40 HLA-B, and 13 HLA-DRB1 alleles as well as 48 homozygous HLA-A-B-DRB1 haplotypes were identified at serological equivalents (2 digits). The 48 identified homozygous haplotypes cumulatively matched 78.18% of the 27,904 Korean CB donors as zero HLA-mismatch iPSC sources. Among the combinations of 1,699 haplotypes with frequencies greater than 0.001%, assuming a population of 50 million, those 48 haplotypes can provide a match for 78.37% of potential Korean recipients. A practicable number of HLA-A, -B, and -DRB1 homozygous iPSC lines derived from CBUs may be an efficient option in allogeneic iPSC therapy because this type of haplobanking may provide cell lines with optimal HLA matching for up to three-quarters of the Korean population.

Keywords

cord blood, human leukocyte antigen (HLA), homozygous, iPSC (induced pluripotent stem cell), haplobanking, Korean

Introduction

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs), are characterized by self-renewal and the ability to give rise to all types of cells in the body (somatic cells). Although ESCs have potential for cellular therapies, their use poses a moral dilemma. Human induced pluripotent stem cell (iPSC) technology, which earned Dr. Yamanaka a Nobel Prize in Medicine, overcomes the ethical limitations on the use of ESCs¹. Indeed, as the cell sources of iPSCs are isolated from adult tissues, iPSCs are more ethically acceptable than ESCs and are advantageous due to the ability to select discrete genetic characteristics from adult donors². To date, numerous studies have been conducted on how iPSCs may contribute to precision medicine. Although various types of somatic cells have been successfully reprogrammed, including cells from the peripheral blood, bone marrow, and umbilical cord blood (UCB)^{3–6}, it is still unclear whether there is an optimal source for iPSCs, given the epigenetic memory,

¹ Department of Laboratory Medicine, Seoul National University Boramae Medical Center, Republic of Korea

² Seoul Metropolitan Public Cord Blood Bank-ALLCORD, Republic of Korea

³ Department of Laboratory Medicine, Seoul National University College of Medicine, Republic of Korea

⁴ Department of Biostatistics, Seoul National University Boramae Medical Center, Republic of Korea

⁵ Department of Obstetrics and Gynecology, Seoul National University Boramae Medical Center, Republic of Korea

* Both the authors contributed equally to this study as co-first authors.

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Corresponding Author:

Sue Shin, Department of Laboratory Medicine, Seoul National University Boramae Medical Center, 20 Boramaero 5-gil, Dongjak-gu, Seoul 07061, Republic of Korea.

Email: jeannie@snu.ac.kr.



ease of collection, environmental insults, and unique characteristics of these cells.

Two types of iPSC therapeutic models, namely, autologous and allogeneic, have been proposed, and each one has its pros and cons. In the autologous model, the patient's own cells are isolated, corrected, and differentiated for therapy; this approach is performed "on demand" and is uneconomical for large-scale precision medicine. In the allogeneic model, which is less time consuming than the autologous model, the patient must obtain histocompatible cells from unrelated donors. The degree of immunological response from allogeneic transplants correlates with the extent of human leukocyte antigen (HLA) matching⁷; therefore, a full HLA match or zero mismatch (known as beneficial matching) between the patient and iPSC is mandatory to avoid rejection. Thus, the concept of a hybrid model, an iPSC repository with HLA-homozygous types to minimize the immune response, has been introduced^{8,9}. A small number of selected iPSC lines will be sufficient to provide zero HLA mismatch. Although a large budget is required to select homozygous donors and establish a repository, these cell lines will be compatible with a large proportion of the population and well tolerated by a large number of individuals¹⁰.

For the past quarter century, cord blood (CB) has been an increasingly important cell source for hematopoietic stem cell transplantation (HSCT). CB contains large quantities of stem cells that can be easily reprogrammed. Since CB contains adult stem cells obtained from the youngest donors, the cells can proliferate more rapidly and for a longer time than older donors' cells. Furthermore, because these naïve cells have not been exposed to environmental insults, they have fewer genetic mutations than those of adults^{11,12}. CB stem cell populations also have higher levels of telomerase and deoxyribonucleic acid (DNA) repair machinery than adult stem cells¹³. Finally, less mature cells such as CB cells seem to be less immunogenic, resulting in less graft-versus-host disease. These superior attributes of CB cells render them an ideal candidate for a source of iPSC production. Public cord blood banks (CBBs) have been established to manage donated cord blood units (CBUs) for unrelated HSCT. As mentioned above, the vulnerabilities of an HLA-homozygote iPSC repository are the excessive HLA typing assays for donor selection and the high cost of the registry. Therefore, CBUs in public CBBs can be a good choice for this model. Ready-to-use cryopreserved CBUs enable the quick application of cells in reprogramming. CBBs record information on each donor's HLA profile, medical history, and laboratory test results for infectious diseases, thus enabling convenient identification of appropriate individual CBUs without the effort and expense of recruiting and screening donors. In Korea, the first large-scale public CBB (Seoul Metropolitan Governmental Public Cord Blood Bank—ALLCORD) was launched in 2006 with the support of the regional government¹⁴, in which 27,904 qualified CBUs were preserved as of December 2018.

Aim of This Study

Based on the HLA distribution of CBUs, we estimated the coverage of the Korean population by HLA-homozygous iPSCs to repurpose cryopreserved CBUs in public CBBs as sources of iPSCs.

Materials and Methods

Sample Population

To estimate the possible donor HLA types, we analyzed a total of 27,904 CBUs. All subjects voluntarily donated CBUs to the Seoul Metropolitan Public Cord Blood Bank from May 2006 to December 2018 with written informed consent. Collection and processing of the CBUs was regulated by the "Act on CB Management and Research" and was performed according to standard operating procedures as previously reported¹⁵. This study was informed of exemption determinations from the institutional review board of the Seoul National University Boramae Hospital (07-2018-33).

DNA-Based HLA Typing

Genomic DNA was extracted from anticoagulated CB using a Puregene DNA purification kit (Gentra Systems, Minneapolis, MN, USA). Each sample was typed for the HLA-A, -B, and -DRB1 alleles at low-to-intermediate resolution by the polymerase chain reaction with sequence-specific oligonucleotide probe typing method using Dynal Reli SSO typing kits (Invitrogen, Bromborough, UK) according to the manufacturer's protocols¹⁶. Based on the consensus of the Korean marrow donor program, 2-digit level DNA typing results were converted to their serologic equivalents, especially for HLA-B alleles. For example, a "B*15:01/15:28/15:38, B*40:02/40:03" typing result was assigned as B*15 (62), B*40 (61). The assignment of serologic equivalents was performed on the basis of the World Health Organization HLA nomenclature¹⁷ and the international ImMunoGeneTics information system (IMGT)/HLA database (<http://www.ebi.ac.uk/imgt/hla/>).

Statistical Analyses

The statistical analyses for the matching probabilities for zero-mismatch transplantation were conducted using the functions in *gap* and *genetics* packages (R version 3.5.3, R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>), as previously reported¹⁸. The three-stage procedure is as follows. Stage 1: Allele frequencies (AFs), Hardy–Weinberg equilibrium (HWE), and three-locus haplotype frequencies (HFs) were obtained using ARLEQUIN 3.5 software (Computational and Molecular Population Genetics Lab, Swiss Institute of Bioinformatics, Berne, Switzerland)^{19–21}, and individuals possessing homozygous HLA haplotypes were identified. Stage 2: An

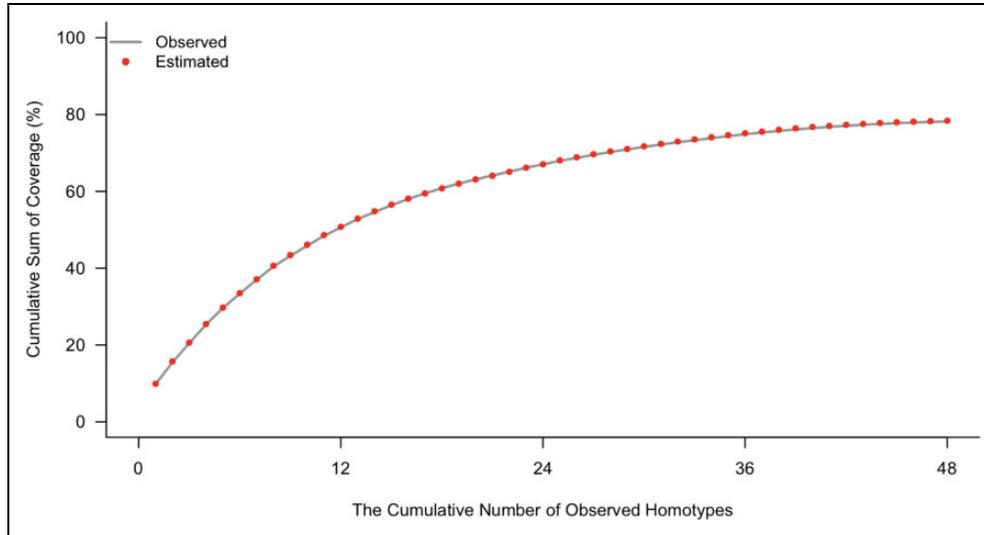


Figure 1. The 48 homozygous HLA-A, -B, and -DRB1 types identified in the public cord blood bank (ALLCORD) inventory provide zero HLA mismatch for 78.2% of 27,904 Korean recipients (black line) and 78.4% of 50,000,000 estimated potential Korean recipients (dotted line). HLA: human leukocyte antigen.

artificial patient pool was generated from HFs assuming a population size of 50 million. With the combinations of frequent haplotypes (HF with frequencies greater than 0.001%), HLA DNA genotype patterns were obtained. Stage 3: In order to investigate iPSC utilization, the observed homozygous phenotypes were used to match potential patients in both the actual population represented by the CBU data and the simulated Korean patient pool from Stage 2. Any potential patient with all HLA-A, -B, and -DRB1 alleles of the designated homozygous haplotype was considered a match. The matching probabilities for zero-mismatch transplantation (homozygous donor to haplo-identical heterozygous recipient or HLA-identical recipient) were computed using the bootstrapping method with 100 replicates¹⁸.

Results

HLA Distribution Among 27,904 CBUs and Estimated Haplotype Frequency Representing the Korean Population

AFs and HFs are shown in supplementary Tables S1 and S2. From 27,904 CBUs, a total of 15 HLA-A, 40 HLA-B, and 13 HLA-DRB1 alleles were identified at the serological equivalents (2-digit resolution). According to the results of exact tests of HWE, the study population was consistent with HWE, with *P* values of 0.863, 0.707, and 0.097 for the A, B, and DRB1 alleles, respectively. A few alleles were predominant for each locus, such as *A*02*, *A*11*, *A*24*, *A*33*, *B*15:01* (B62), *B*44*, *DRB1*04*, *DRB1*09*, *DRB1*13*, and *DRB1*15*, all of which were in the highest frequency range of more than 10.0%. The HLA allele distribution was

skewed, as only 5 HLA-A alleles with an AF of more than 5.0% accounted for 85.0% of the cumulative AF values. Similarly, 8 HLA-B and 9 HLA-DRB1 alleles accounted for 62.9% and 90.5% of the cumulative AF, respectively (supplementary Table S1). A total of 3,560 distinct A-B-DRB1 HLA haplotypes were estimated. For the 27,904 Korean CBUs, the top 1,699 HLA-A-B-DRB1 haplotypes had a frequency of at least 0.001% and a cumulative frequency of 99.9%. The most common A-B-DRB1 haplotypes with an HF of more than 1.0% were as follows: *A*33-B*44-DRB1*13* (4.61%), *A*33-B*58-DRB1*13* (3.22%), *A*24-B*07-DRB1*01* (2.50%), *A*33-B*44-DRB1*07* (2.44%), *A*30-B*13-DRB1*07* (2.17%), *A*24-B*52-DRB1*15* (2.13%), *A*11-B*15(B62)-DRB1*04* (2.08%), *A*02-B*46-DRB1*08* (1.83%), *A*33-B*58-DRB1*03* (1.35%), *A*02-B*27-DRB1*01* (1.32%), *A*24-B*54-DRB1*04* (1.20%), *A*24-B*62-DRB1*04* (1.20%), and *A*02-B*15(B62)-DRB1*04* (1.05%) (supplementary Table S2).

HLA-Homozygous Haplotypes Observed Among 27,904 Korean CBUs

The observed homozygous haplotypes were searched, and the probability of zero mismatch was calculated among the 27,904 Korean CBUs. When it is assumed that the preserved CBU donors are potential patients, a total of 48 homozygous HLA-A-B-DRB1 haplotypes that were observed among the stored CBUs would cumulatively match 78.18% of the 27,904 potential patients as zero HLA mismatch iPSC donors (source). Phenotypes that matched a haplotype were removed from subsequent searches to avoid double counting. This matching was continued for all phenotypes in the CBU population, and a curve showing the

Table 1. Homozygous HLA-A, -B, and -DRB1 Types Observed in the Public Cord Blood Bank Inventory (27,904 Units) and the Ability of These Types to Provide Zero HLA Mismatch for Potential Korean Patients.

Rank	Homozygous haplotypes	Ability for zero HLA mismatch among Koreans (%)	
		27,904 Koreans	50,000,000 potential patients
1	A*33-B*44-DRB1*13	9.77	9.92
2	A*33-B*58-DRB1*13	15.50	15.75
3	A*24-B*07-DRB1*01	20.47	20.63
4	A*02-B*15 (62)-DRB1*04	25.35	25.47
5	A*33-B*44-DRB1*07	29.64	29.74
6	A*24-B*52-DRB1*15	33.37	33.50
7	A*02-B*46-DRB1*08	36.97	37.10
8	A*30-B*13-DRB1*07	40.44	40.64
9	A*11-B*15 (62)-DRB1*04	43.19	43.43
10	A*02-B*40 (61)-DRB1*09	45.86	46.10
11	A*24-B*54-DRB1*04	48.42	48.62
12	A*02-B*51-DRB1*04	50.63	50.78
13	A*02-B*27-DRB1*01	52.79	52.89
14	A*33-B*58-DRB1*03	54.61	54.81
15	A*24-B*51-DRB1*09	56.38	56.54
16	A*02-B*13-DRB1*12	58.02	58.10
17	A*01-B*37-DRB1*10	59.48	59.47
18	A*24-B*15 (62)-DRB1*04	60.81	60.77
19	A*02-B*51-DRB1*14	62.03	62.01
20	A*24-B*35-DRB1*04	63.11	63.11
21	A*02-B*46-DRB1*09	64.12	64.07
22	A*02-B*40 (61)-DRB1*14	65.13	65.09
23	A*24-B*40 (61)-DRB1*09	66.12	66.15
24	A*02-B*51-DRB1*15	67.05	67.05
25	A*02-B*54-DRB1*04	67.92	68.07
26	A*26-B*15 (62)-DRB1*15	68.72	68.87
27	A*24-B*59-DRB1*04	69.50	69.67
28	A*02-B*40 (61)-DRB1*08	70.24	70.39
29	A*24-B*51-DRB1*12	70.93	71.03
30	A*03-B*44-DRB1*13	71.57	71.74
31	A*02-B*35-DRB1*11	72.19	72.35
32	A*02-B*48-DRB1*14	72.78	72.97
33	A*26-B*40 (61)-DRB1*09	73.32	73.52
34	A*26-B*15 (62)-DRB1*14	73.85	74.08
35	A*24-B*40 (61)-DRB1*04	74.38	74.63
36	A*02-B*54-DRB1*08	74.88	75.14
37	A*24-B*40 (61)-DRB1*12	75.33	75.55
38	A*24-B*51-DRB1*14	75.73	76.03
39	A*24-B*40 (60)-DRB1*09	76.12	76.42
40	A*24-B*51-DRB1*15	76.46	76.75
41	A*32-B*44-DRB1*04	76.79	77.03
42	A*26-B*40 (61)-DRB1*04	77.07	77.31
43	A*24-B*40 (61)-DRB1*14	77.33	77.56
44	A*24-B*13-DRB1*12	77.57	77.81
45	A*11-B*44-DRB1*13	77.75	77.99
46	A*02-B*55-DRB1*11	77.92	78.14
47	A*24-B*13-DRB1*07	78.06	78.26
48	A*32-B*44-DRB1*12	78.18	78.37

CBU: cord blood unit; HLA: human leukocyte antigen.

Description in parentheses indicates HLA antigen specificities in the HLA-B system. Forty-eight unique homozygous haplotypes obtained by direct counting are listed in order of decreasing cumulative coverage (probability of zero mismatch) for 27,904 Koreans represented by CBU donors and for 50,000,000 potential Korean recipients.

match coverage was generated (Fig. 1, solid line). The entire list of 48 homozygous haplotypes, ranked by their ability to provide zero HLA mismatch, is shown in Table 1.

The two highest-ranking homozygous HLA haplotypes were HLA-A*33-B*44-DRB1*13 and HLA-A*33-B*58-DRB1*13, which provided zero HLA mismatch for

15.50% of potential recipients in the sampled CBU population. The top 5 and top 10 homozygous HLA haplotypes covered 29.64% and 45.86% of Koreans, respectively (Table 1).

Utility Estimation of HLA-Homozygous Haplotypes for 50,000,000 Potential Korean Recipients

For practical purposes and to give the greatest chance of matching, we required each of the HLA combinations to be represented at least 0.001% among the CBUs, resulting in 1,699 haplotypes that could generate theoretical combinations in a utility estimation for 50 million Korean individuals. From combinations of the 1,699 haplotypes, 25,144,999 individual HLA DNA genotypes were generated after assuming a population of 50 million. Since the majority of the population is heterozygous, for a fully heterozygous HLA-A, -B, and -DRB1 recipient expressing two common HLA haplotypes, zero HLA mismatch can theoretically be achieved from eight different HLA-homozygous donors. We estimated the coverage of a given number of homozygous haplotypes from the pool of 50 million Korean individuals with the 25,144,999 hypothetical HLA genotypes using the bootstrap method. If iPSC lines are generated from the 48 previously identified HLA A-B-DRB1 homozygote CBUs, they can provide zero HLA mismatch for 78.37% of the 50,000,000 potential Korean recipients. The top 10 highest-ranked homozygous types cumulatively cover 46.10% of potential recipients, and a cell bank populated with as few as 20 iPSC lines of the highest-ranked types would provide zero HLA mismatch for 63.11% of potential Korean recipients (Table 1). This matching was continued for all phenotypes in the estimated populations, and a curve showing the match coverage was generated (Fig. 1, dotted line).

Discussion

To properly cover the HLA diversity of a particular population, public CBBs need to establish and maintain a large CBU inventory. To date, the use of CBUs has been mainly limited to HSCT. However, preliminary results of recent studies have suggested the possibility of CB-derived cellular therapies as novel therapeutic models for incurable and refractory disorders; for example, patients with cerebral palsy were recently safely treated with CB cell transfusion^{22,23}. Stem cells in CB promote endogenous cellular repair in hypoxic-ischemic encephalopathy of preterm babies and in stroke-damaged adult brain²⁴ and can be utilized to treat various disorders such as inherited leukodystrophies²⁵. In addition to these approaches, CBUs are expected to be excellent iPSC sources in the era of precision medicine. The use of iPSCs has emerged as a novel therapeutic option. The function of iPSCs is to generate differentiated cells to replace the damaged tissue in degenerative disorders such as liver cirrhosis, Parkinson disease, cardiac disease, or

diabetes²⁶, and recent advances in the development of genome-editing technologies have allowed the range of potential therapies to be expanded²⁷. Among the various iPSC sources, cryopreserved CB is a good option for many reasons, including the following: CBUs with assigned HLA types are available for use to generate histocompatible iPSC lines²⁸, and these young and naïve cells are highly efficient and have low immunogenicity, resulting in minimal genomic insults^{11,12}. There are several additional advantages to using CB for iPSC generation. CBUs, especially in public CBBs, are collected and processed in an approved manner for clinical use, and the quality control criteria for CBU processing, including freezing, storage, and release, are already in place and can be used in the initial steps of iPSC haplobanking development. Previously, we found that approximately 95% probability is achieved with 51,000 Korean CBUs under 5/6 matching conditions with 3-locus low-resolution typing (HLA-A, -B, and -DRB1 antigen levels)¹⁸. Moreover, with high-resolution matching at 3 loci (HLA-A, -B, and -DRB1 allele levels), the estimated number of CBUs needed is increased to 100,000²⁹. In contrast to HSCT, haplobanking of HLA-homozygous iPSCs has been proposed to significantly reduce the number of required CBUs⁸, which may simplify HLA matching, provide matches for a considerable percentage of target populations, and expand therapeutic approaches using iPSCs.

When comparing the AFs and HFIs in our study with those of previous studies, we found that there were no differences in the HLA distribution among different Korean populations^{16,18}. We determined that homozygous HLA-A-B-DRB1 combinations actually exist by directly counting the HLA-typed 27,904 CBUs in ALLCORD; these could, in principle, be sourced for iPSC lines designed to achieve zero HLA-A, -B, and -DRB1 mismatch in the Korean population. Although this total of 27,904 CBUs represents a large sample, we attempted to show the similarity between observations based on CBU inventories and estimates in a larger sized population (closer to the size of the actual Korean population). Forty-eight of the homozygous HLA haplotypes identified could provide zero HLA mismatch for more than 78% of Korean CB donors, and these results remained similar with the assumption of 50 million people. Therefore, those homozygous HLA haplotypes would be good sources of iPSC lines for the Korean population.

Several studies revealed different homozygous types and different matching probabilities, according to the HLA distributions of given populations (i.e., ethnic groups). Fifty of the highest-ranked homozygous HLA types identified in the 17 million individuals from the Bone Marrow Donors Worldwide registry could provide zero mismatch for 79% of 10,000 potential UK recipients³⁰, and the 100 haplotypes were able to meet the demand of 49.0% to 55.6% of a population in California³¹. Studies in both multiethnic and mixed-ancestry populations showed quite similar haplotypes, including the top ranked types (A1-B8-DR3, A2-B44-DR4, A3-B7-DR15, A2-B7-DR15, A1-B57-DR7, etc.);

however, the homozygous haplotypes identified in the present study are quite different (*A*33-B*44-DRB1*13*, *A*33-B*58-DRB1*13*, *A*24-B*07-DRB1*01*, *A*02-B*15(62)-DRB1*04*, *A*33-B*44-DRB1*07*, etc.) due to different HLA distributions among ethnic groups. The Korean population shows considerably restricted HLA distribution due to homogenous ethnicity. Previous analysis showed that 1.02% of CBUs were donated from non-Koreans or individuals with mixed ancestry (Korean + Asian, 0.74%; Korean + Caucasian, 0.08%; Asian + Asian, 0.18%; Asian + Caucasian, 0.01%), with the majority of non-Koreans being Asians; thus, rare types in Koreans were hardly observed¹⁶. Recent statistics from the Korean Statistical Information Service indicated that as of 2015, foreign residents comprise only 3.4% of the total population in Korea (<http://kosis.kr>)³². Therefore, compared to multiracial and mixed-ancestry populations^{30,31}, the Korean population would have an increased probability of matching when using homozygous iPSC lines. A recently reported Korean study using CBUs of 4,205 individuals showed similar results to the present study with regard to HFs and the cumulative coverage of the top 10 ranked HLA-homozygous haplotypes (41.1% vs 46% in the present study) among the HLA haplotypes initially identified³³. Theoretically, only 12 types of HLA-homozygous haplotypes in a CBB could sufficiently provide zero mismatch for half of the population.

By contrast, Japanese results showed that 30 and 50 iPSC lines would be able to match 82.2% and 90.7% of the population, respectively³⁴, which revealed higher coverage rates than those reported in this study with given types of HLA-homozygous haplotypes (30 lines match 82.2% of the Japanese population vs 72% of the Korean population), which is probably due to Japanese individuals having a more restricted HLA distribution than Koreans. These findings indicate that the optimal homozygous HLA types required to populate an iPSC bank would vary for different ethnic groups.

The Seoul Metropolitan Governmental Public Cord Blood Bank (ALLCORD) is a nonprofit organization and has the largest CB inventory size among public CBBs in Korea. Although the CBU supply of ALLCORD accounted for 71% to 97% of nationwide cord blood transplantations (CBTs) in Korea, only 408 of the 27,904 cryopreserved units have been transplanted into 287 patients as of September 2018. Since only a small percentage of CBUs are used for HSCT compared to the entire CBU inventory (which is a fundamental weakness of public CBBs), expansion of the use of cryopreserved CBUs should be deliberated. In particular, CBUs with a low total nucleated cell (TNC) count need to be considered in terms of promoting and expanding their use. The “Cord Blood Management and Research Act (CB Act)” was enacted in 2011 to establish a quality assurance system and government support for Korean public CBBs, and the current criteria for efficacy and safety are under the “CB Act”³⁵ (available from URL: <http://law.go.kr/lisInfoP.do?lsiSeq=103414#0000>). The current criterion for efficacy

is TNC count $\geq 7 \times 10^8$, and a recent study suggested upward modification up to \geq TNC count 8×10^8 . Clinicians select CBUs with higher TNCs if the histocompatibility level is identical; thus, CBUs with relatively lower TNC counts are unlikely to be picked. Since the existing law does not permit iPSC generation using suitable and preserved CB products, the relevant laws need to be revised before CBUs can be practically used as a source of iPSCs.

There are some limitations to the present study. Since CBUs in ALLCORD are classified by HLA-A, -B, and -DRB1 with 2-digit resolution (based on the consensus in Korea), we estimated the coverage of cell lines considering 3-locus matching, but this does not guarantee allelic level matching or matching for other loci such as HLA-C and HLA-DQ, which may cause immunological rejection. Thus, iPSC transplantation that matches only at the HLA specificity level described in this study may also require an immunosuppression regimen and in-depth follow-up. There is a lack of consensus regarding the level of histocompatibility that should be required for cell transplantation. However, because iPSC-derived tissues lack highly immunogenic dendritic cells that stimulate rejection and because major histocompatibility complex class II is absent from iPSCs³⁶, the low resolution of HLA-A, -B, and -DRB1 matching is known to give the maximum benefit for iPSC-based transplantation; this is in contrast to allogeneic HSCT, which requires an 8/8 allele match for the HLA-A, -B, -C, and -DRB1 loci according to U.S. standards; the HLA-A, -B, -C, -DRB1, and -DQB1 loci to have a 10/10 allele match according to European standards; or a $\geq 4/6$ allele match (HLA-A, -B, and -DRB1) for CBT^{37,38}.

We do not assess the killer-cell immunoglobulin-like receptor (KIR) types of CBUs for banking; therefore, KIR ligand status is another issue that could not be addressed in this study. The importance of the KIR family for transplantation and the role of these ligands in the rejection of HLA-matched transplants are becoming increasingly evident; for example, Ichise et al. demonstrated that natural killer (NK) cells have the potential to reject iPSC-derived tissue in a homo-to-hetero transplantation setting when KIR ligands are mismatched³⁹. But the immune-suppressive drugs used to suppress the immune reaction caused by minor histocompatibility mismatch are likely to have additional effects on NK cell-mediated immune reactions. In addition, homo-to-hetero CBTs have been reported to achieve excellent outcomes in a previous study, although KIR ligand mismatch cases were not included⁴⁰, and the data regarding the impact of KIR ligand status on clinical outcomes in Korean adult patients who received unrelated HSCT are inconclusive⁴¹. However, to reduce the use of immune-suppressive agents and improve the outcomes of homo-to-hetero transplantations performed using HLA-homo iPSCs, the assessment of KIR ligand status should be considered. Additionally, KIR ligand-mismatched transplantation cases will require intensified follow-up examinations.

ABO blood group incompatibility remains an important barrier for transplantation because of an increased risk of hyperacute rejection, but this issue was not considered in this study. Although ABO blood incompatibility has become a hurdle that can be overcome in transplantation due to improvements in various protocols based on transient depletion of preformed anti-A and/or anti-B antibodies and the modulation of B-cell immunity⁴², ABO-compatible or blood group O iPSCs should be prioritized to reduce the immunogenic burden in clinical applications⁴³. Considering that HLA-homozygous CBUs obtained from blood group O donors are selected as universal iPSC donors, the real coverage could be lower than our estimation.

Since Korea is one of the most homogeneous societies in the world, with 97% of its residents being of Korean ethnicity, a relatively smaller number of cell lines are required to match a larger proportion of the population in Korea than would be required in multiethnic nations, such as the United States or Brazil. However, the rapid growth of foreign residents (from 1.48% in 2007 to 3.39% in 2015) suggests that more haplotypes will be needed in the future³².

In future studies, we will analyze the combined HSCT donor registry data, Korean Bone Marrow Donor Registry, and public CBB data. Bone marrow registries with HLA typing results and informed consent can be additional candidates for iPSC haplobanking (although the cells are not preserved and the refusal rate is considerable), and a more homozygous haplotype may be identified with a large-scale combined analysis. In the expanded analysis, we expect a more sophisticated assessment and elucidation of the list of HLA-homozygous haplotypes covering almost the entire Korean population. To date, nearly 800,000 fully characterized CBUs are stored in public CBBs worldwide and are ready for use in creating histocompatible iPSC stocks^{28,44}. Owing to improvements in induction and differentiation techniques, HLA-homozygous CBUs would be a good source for iPSCs, and the accumulation of research data using iPSCs obtained from cryopreserved CBUs will support the establishment of a bank of CB-derived homozygous iPSC lines that will provide an opportunity to apply cellular products in practical precision medicine.

Conclusion

From the above analysis, we conclude that banking an appropriate number of HLA-A, -B, and -DRB1 homozygous iPSC lines generated from CBUs may be an economical and effective option in the allogeneic model of iPSC therapy because such haplobanking may provide iPSC stocks with beneficial HLA matching for up to three-quarters of the Korean population. Although the potential of CB in HSCT has been largely realized, the use of CB in the field of cellular therapy might emerge as one of the major, promising advances toward new therapies in the coming decades. These results can be used to support political decisions on the repurposing of cryopreserved CBUs in public CBBs as a favorable source

of iPSCs for patients requiring iPSC therapy in Koreans or other related ethnicities worldwide.

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Ethical Approval

This study received an exemption determination from the institutional review board of the Seoul National University Boramae Hospital (07-2018-33).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with a protocol that received an exemption determination from the institutional review board of the Seoul National University Boramae Hospital (07-2018-33).

Statement of Informed Consent

Informed consent for the patient information to be published in this article was not applicable because the HLA data was anonymized.

Declaration of Conflicting Interests

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ORCID iD

Eun Youn Roh  <https://orcid.org/0000-0002-1787-7509>

Supplemental Material

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