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Extended HLA Haplotypes and Their Impact on DPB1 Matching of Unrelated Hematologic Stem Cell Transplant Donors

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Although HLA-DPB1 has long been considered of lesser importance in the selection of an unrelated donor (URD) hematologic stem cell transplantation, currently in many instances the DPB1 type of the donor is relevant or even critical. At present, however, only a minority of registry donors are DPB1 typed. It is also unclear to what extent the DPB1 alleles are linked to the 5-locus HLA-A-, B-, C-, DRB1, -DQB1 haplotypes. We sought to study whether there is such a linkage by using donors in the Finnish Stem Cell Registry as the study population. The 6-locus HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 haplotype frequencies were estimated from a group of 43,365 Finnish registry donors using the German National Bone Marrow Registry algorithm. Five-locus haplotype (HLA-A, -B, -C, -DRB1, -DQB1) and HLA-DPB1 allele frequencies were calculated as marginal frequencies of the estimated 6-locus haplotype frequencies. The Finnish average frequency of individual DPB1 alleles was compared with their respective frequencies in association with individual 5-locus HLA haplotypes (haplotype-specific frequencies). Finally, the probability of DPB1 matching in 10/10 matched URD transplants was assessed. Haplotype-specific DPB1 frequencies differed significantly from the average DPB1 frequencies in 81 of 100 most frequent Finnish 5-locus HLA haplotypes, including some infrequent DPB1 alleles that were associated almost exclusively with certain individual 5-locus haplotypes. Five-locus haplotypes that are enriched in Finland but rare among other Europeans carried stronger DPB1 associations than haplotypes that are frequent European-wide. Finally, 10/10 matched transplants from domestic registry donors were significantly more likely to also be DPB1 matched than those from foreign donors. The results indicate an extension of linkage disequilibrium in the MHC complex in the Finnish population. With continuing upfront DPB1 typing of registry donors, it will be possible to perform similar extended 6-locus haplotype frequency estimations also in other registries. The associations are likely to be population specific but may be weaker in more heterogeneous populations. In the future the results might be used to predict the probability of DPB1 match or permissive/nonpermissive DPB1 mismatch for non-DPB1 typed donors in registry donor searches.

INTRODUCTION

Allogenic hematologic stem cell transplant (HSCT) is a well-established treatment for many hematologic malignancies and for several life-threatening nonmalignant diseases such as severe aplastic anemia, thalassemia major, and severe congenital immunodeficiencies [1]. HLA matching between the donor and recipient is crucial for optimal transplant outcomes, and HLA identical siblings are usually considered optimal donors, whenever one is available [2]. HLA identical siblings carry identical chromosomes 6 with the patient and thus share the same alleles with the patient in all 6 classic HLA loci: HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. However, any 1 sibling has only a 25% probability of being HLA identical with the patient, and therefore most European patients do not have an available HLA identical sibling. For these patients, matched unrelated donors (URDs) are usually the primary choice [2].

Because the outcome of HSCT is strongly influenced by the degree of HLA matching [3–9], a registry donor matched for HLA-A, -B, -C, -DRB1, and -DQB1 at 2-field resolution, designated as 10/10 matched, is usually considered optimal for URD transplant. Although the HLA-DPB1 gene belongs to the δ-block of the MHC, it is separated from the other HLA class II genes by a recombination hot spot [10–13], and consequently

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finding DPB1 matched (12/12 matched) donors for a significant proportion of patients has been assumed to be unrealistic. This has not been considered an obstacle for successful HSCT, because mismatching at the DPB1 locus has been deemed less relevant for survival than mismatching at other classic HLA loci: Although DPB1 mismatching heightens the risk of graft-versus-host disease, it also lowers the risk of leukemia relapse [14]. It has thus not seemed to have an impact on the overall survival probability of otherwise well-matched leukemia patients [4,15,16]. Therefore, for years matching at DPB1 has not been widely used for donor selection, and typing donors for the DPB1 locus has not been worth the extra cost.

Individual studies, however, have reported an impact of DPB1 mismatching on overall survival in some patient subgroups [17–19], some DPB1 mismatches [20], or less than 8/8 matched URD transplants [16], and since the first decade of the 2000s a number of studies have shown that some DPB1 mismatches are well tolerated and others are not [9,21–24]. Transplants with well-tolerated (permissive) DPB1 mismatches seem to carry outcomes similar to DPB1 matched transplants, whereas nonpermissive mismatches result in higher transplant-related and overall mortality. The permissiveness of a DPB1 mismatch can be determined by the expression levels of the mismatched DPB1 alleles, relative immunogenicity of T cell epitopes of the patient and donor DPB1 alleles, or a functional distance score between them [21,22,25–27]. However, irrespective of the grouping method, the patient and the donor must first be DPB1 typed.

It has also become apparent that the presence of preformed donor-specific HLA antibodies in the HSCT patient multiplies the risk of graft rejection [28]. For patients carrying DPB1 antibodies, DPB1 typing of their registry donors is thus recommended. In addition, although most HSCTs are performed because of hematologic malignancies, many nonmalignant diseases are also currently treated with HSCT [1], and their proportion is increasing [29,30]. A graft-versus-leukemia effect is not needed in these transplant indications, and graft-versus-host reactivity is therefore purely detrimental. These patients represent a group where not merely a permissive DPB1 mismatch but an actual DPB1 match is the optimal goal.

Concurrently with these developments, HLA typing of newly recruited registry donors has become more affordable because of high-throughput HLA sequencing methods. All this has made the upfront DPB1 typing of registry donors both clinically and economically justifiable.

As a consequence, newly recruited registry donors are currently often DPB1 typed upfront, but most (73.4% in 2017 [31]) of the more than 30 million registry donors worldwide were recruited at a time when this was considered an unnecessary cost. It might therefore be helpful to be able to predict the probability of such donors' DPB1 mismatch permissiveness or DPB1 match at the time of a registry search. Under the assumption of a weak linkage with other classic HLA loci, the probability of DPB1 matching or permissive mismatching mostly depends on the average frequency of the patient's DPB1 alleles in the donor population. However, as donor HLA data in worldwide registries also increasingly include DPB1 typing data, it may be possible to reassess the traditional concept of HLA haplotypes consisting of loci from HLA-A to -DQB1 only and extend them to include DPB1.

Earlier studies have shown that the small and linguistically isolated Finnish population in Northeastern Europe carries some unique HLA characteristics [32,33]. These characteristics can be summarized as homogeneity, restricted allele repertoire and a number of HLA-A-B-C-DRB1-DQB1 haplotypes that are frequent in Finland but rare or very rare in other ethnic Europeans (specifically in the large registries like Germany and the United States). In a previous report we designated these haplotypes as Finnish Enriched Rare (FER) haplotypes [34]. We hypothesized that because of these characteristics, significant associations between HLA-A-B-C-DRB1-DQB1 haplotypes and DPB1 alleles might exist in Finns, even if the associations are less easily discerned in more heterogeneous populations. We also hypothesized that the associations might be stronger in FER haplotypes compared with haplotypes that are common also among other Europeans. Finally, we also surmised that because of the specific HLA constitution, Finnish donors might have a higher probability of DPB1 matching with Finnish patients than foreign donors.

METHODS

Study Population

Haplotype frequencies were estimated from anonymized HLA data of 43,365 Finnish stem cell donors; 15,288 of these donors had been DPB1 typed, most (13,247 donors) at G-group level resolution, 154 donors at 3-field resolution, and 1703 at 2-field high resolution. The DPB1 typing results of 184 donors carried National Marrow Donor Program codes. All DPB1 typed donors were high resolution typed for HLA-A, -B, -C, -DRB1, and -DQB1 loci, and more than 12,000 (80%) had been recruited after the previous haplotype analysis; this group of recently recruited 6-locus high resolution typed donors increased the number of high resolution typed individuals from 2917 to 15288, compared with a previously published study [34]. For 17,060 donors, their serologic typing was mapped to low resolution molecular HLA-A and -B types (XX-codes) according to the World Marrow Donor Association guidelines [35], because the haplotype frequency estimation algorithm requires HLA input in molecular notation. Restriction to fully high resolution typed individuals only and omission of not fully typed or low resolution typed donors would have introduced the risk of bias into the analysis [36].

Haplotype and Allele Frequency Estimations

The 6-locus HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 high resolution haplotype frequencies were estimated using the German National Bone Marrow Registry haplotype frequency estimation algorithm, which is based on the Expectation-Maximization (EM) method, and has successfully completed the matching validation of the World Marrow Donor Association [37].

The overall DPB1 allele frequencies in the Finnish Stem Cell Registry were calculated by adding up the frequencies of all HLA-A-B-C-DRB1-DQB1-DPB1 haplotypes carrying the same DPB1 allele (marginal frequencies) and confirmed by comparing the results with the overall DPB1 allele frequencies in the original registry data.

Because the variation of HLA alleles is immense, 6-locus HLA haplotypes with lower frequencies are present in small numbers even in relatively large materials. Thus, it was not feasible to use infrequent haplotypes for further statistical analyses. Accordingly, we decided to examine the DPB1 associations of the 100 most frequent HLA-A-B-C-DRB1-DQB1 (5-locus) haplotypes in more detail. These haplotypes have a combined frequency of 62% in Finland, and as a result 14% of the Finnish population carry such an HLA haplotype combination that neither haplotype is among the 100 most frequent. The association frequency of each DPB1 allele with the 100 most common 5-locus haplotypes was first assessed individually. This was followed by the assessment of the haplotype-specific frequencies of DPB1 alleles, whereby each 5-locus haplotype is given the conditional probability for each DPB1 allele. For example, the most frequent Finnish 5-locus haplotype (A*03:01; B*35:01; C*04:01; DRB1*01:01; DQB1*05:01) is associated with DPB1*04:02 in 67% of cases, the haplotype-specific frequency of DPB1*04:02 thus being .67, although the average population frequency of DPB1*04:02 is only .2.

The FER haplotypes were assessed from the updated haplotype frequencies using previously published criteria [34]: The haplotype is ranked among the 100 most frequent A-B-C-DRB1-DQB1 haplotypes in Finland, the frequency of the haplotype is lower than .0003 in both the German and the National Marrow Donor Program white populations, and the respective frequency is at least 8 times higher in the Finnish population.

The association of DPB1 alleles with each haplotype was analyzed with Fisher's exact test by comparing the haplotype-specific DPB1 allele frequency with the frequency of the same allele in the study population (n = 30,576). The obtained P values were adjusted for the total number of conducted tests using the Bonferroni method. Adjusted $P < .05$ was considered significant. The data were analyzed and plotted using R v3.4.4 [38] with libraries *ggplot2* v3.1.0 [39], *data.table* v1.11.8 [40], and *gplots* v3.0.1 [41]. The haplotype-against-DP hierarchical clustering (Figure 1) was generated using the *heatmap.2* function with default settings.

Frequency Validation by Phased Haplotypes

The EM algorithm uses unphased input data to estimate the haplotype frequency. In each iteration the algorithm evaluates if the found frequencies

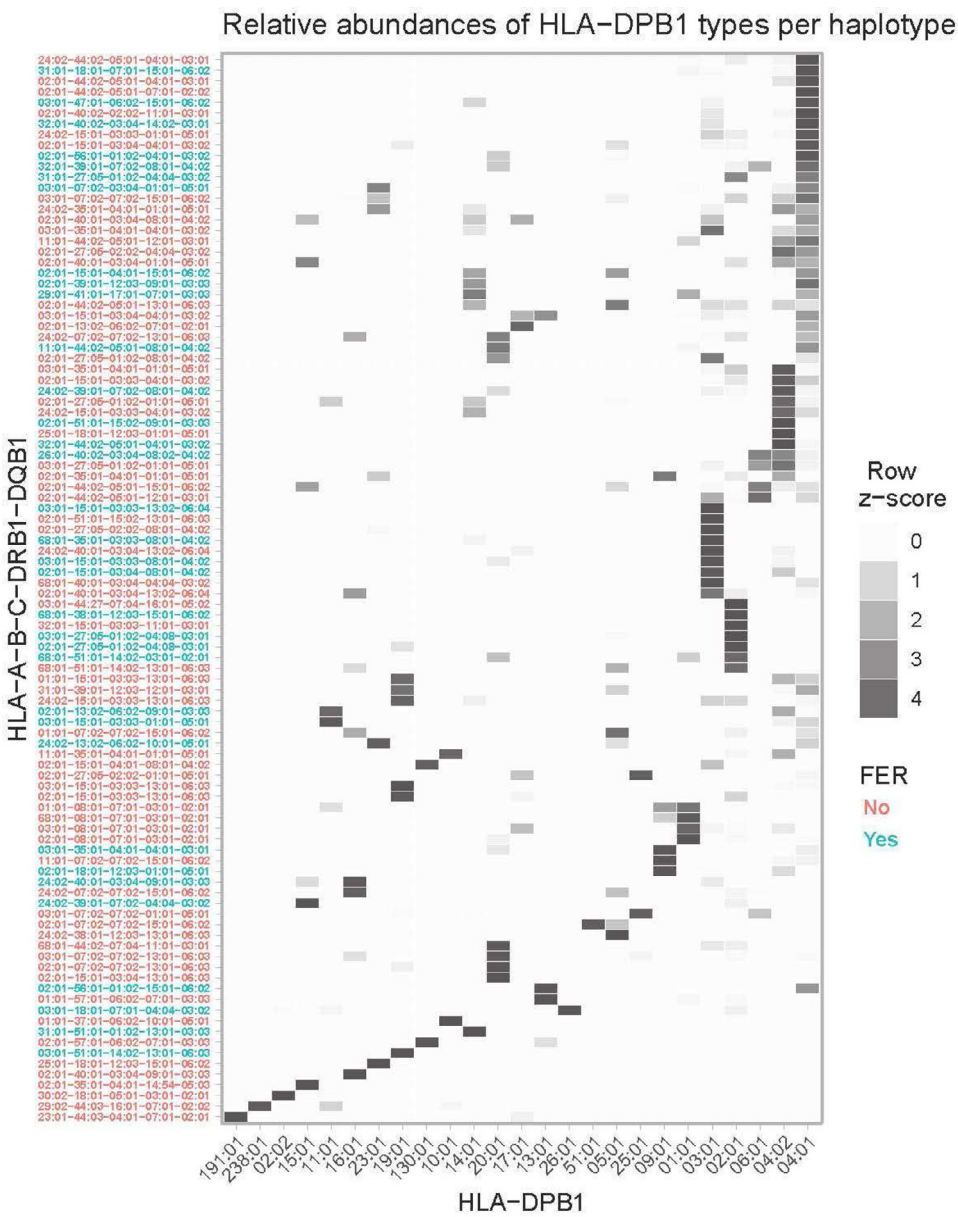

Figure 1. Relative abundance of DPB1 alleles in each haplotype. DPB1 allele frequencies are scaled within each haplotype and shown as standard deviations from the mean (ie, row z-score). The rows and columns are organized by hierarchical clustering to show DPB1 alleles with similar haplotype distribution and haplotypes with similar DPB1 allele distribution near each other. FER and non-FER haplotypes are differentiated by the *y*-axis label color.

of the haplotypes explain the input data. Because the input data do not include phase information, the algorithm cannot know which 2 haplotypes truly explain the donor's genotype, and all haplotypes possibly forming a genotype have to be taken into account. Therefore, it is to be expected that the EM algorithm might use a combination of other haplotypes to explain a phased genotype than the actual 2 haplotypes.

To validate the EM algorithm, an independent data set was constructed out of 990 URD transplanted patients. The set included the 769 patients with 10/10 matched transplants, but to achieve a reasonable number of family data and to avoid the bias of only using patients with frequent HLA haplotypes, patients who had been transplanted with less well-matched donors within the same time period were also included in the analysis. Of the 990 patients, 261 did not have any HLA typed relatives, and for an additional 170 patients the relative's HLA typing results were not conclusive (siblings with no shared HLA haplotypes or identical HLA haplotypes or shared alleles in the mismatched HLA haplotype). Thus, 559 patients of the 990 could be included in the validation set. For these 559 patients the family segregation provided 2 phased 5 locus haplotypes and the patients' DPB1 values. Because family data were not available for DPB1, the patients' DPB1 values could be phased only for patients who were homozygous for DPB1 (n = 159). The observed 5- and 6-locus haplotype frequencies were obtained by counting the haplotypes in the independent data set. The estimated frequency was calculated with the EM algorithm using the Finnish data set of the study. A 95% confidence interval (CI) was constructed around the observed frequency.

Comparison with Other Populations

With little published data available on 6-locus haplotype frequencies of other European registries, only a perfunctory comparison with other European populations was possible. This was performed by comparing the international registry donor search results [42] for 3 common phenotypes, each a homozygous carrier of a different ancestral HLA haplotype, common both in Finland and several other European populations. Each of the 2 searches yielded more than 1000 DPB1 typed registry donors worldwide, and hence the frequencies of DPB1 alleles associated with the haplotypes (number of chromosomes > 2000) could thus reliably be assessed.

Registry Donor Searches for Patients Carrying Frequent Non-FER Haplotypes

To further analyze the potential similarities between 1 (ie, Finnish) population and the average world donor population, 84 registry donor searches were performed in the World Marrow Donor Association (WMDA) search and match database. Patients for the searches were chosen with the following criteria:

they belonged to the patient validation data set, thus carrying a pair of known 5-locus HLA haplotypes; both haplotypes of the patient were among the 100 most frequent in Finland but did not belong to the group of FER haplotypes; and the probability of any 10/10 matched white donor of carrying the exact same haplotypes as the patient was >80% [43], thus minimizing the probability of the potential DPB1 mismatches being due to different phasing of the patient's and donors' alleles. Whenever an individual patient had less than 100 DPB1 typed international donors, all such donors were included in the DPB1 comparison. If the number of international donors exceeded that, 100 consecutive DPB1 typed donors were included in the analysis. Finnish DPB1 typed donors were assessed separately. Only donors with a 98% to 100% probability of being 10/10 matched with the patient were included. The proportion of 12/12 (DPB1-) matched donors was calculated separately for each search.

DPB1 Matching in URD Transplants

To evaluate the probabilities of finding a DPB1 matched domestic and DPB1 matched foreign unrelated donor, all unrelated donor transplants performed in Finland between 2003 and 2016 were analyzed. Only 10/10 (HLA-A-, -B, -C, -DRB1, and -DQB1) matched transplants were included in the analysis. The 10/10 matched transplants were then divided into 6 categories according to whether the donor had been domestic or foreign and whether the transplant had been DPB1 matched or 1 or 2 DPB1 allele mismatched. The statistical significance of the differences was calculated by the 2-tailed Fisher's exact test [44].

RESULTS

Haplotype Frequencies

Calculated from a larger population sample than previously, the frequencies of HLA*A-B-C-DRB1-DQB1 haplotypes remained quite similar to the frequencies we reported previously [34]. As before, 34 HLA 5-locus haplotypes where classified as FER haplotypes. Three of the previously reported FER haplotypes from the more infrequent end of the list were now ranked outside the 100 most common in Finland (101st, 102nd, and 106th) but were replaced by 3 other haplotypes fulfilling the criteria.

Frequency Validation by Phased Haplotypes

For the validation, the observed frequencies of 1118 phased 5-locus haplotypes were compared against the respective results from the haplotype frequency estimation; for the 6-locus haplotypes only 318 haplotypes were available for the validation. Seventy-seven distinct haplotypes occurred 3 times or more in the 5-locus validation data set. Of these, only 3 estimated frequencies were not within the boundary of the 95% CI, with 2 of these frequencies situated at the lower boundary of the CI. Overall, the observed frequencies were mostly a little higher in the phased data set than the estimated frequencies, which was to be expected, because the smaller sample size in the observed set enhances the frequencies of the present haplotypes, while disregarding all haplotypes not occurring in the set.

Only 21 6-locus haplotypes occurred 3 or more times in the 6-locus validation data set, and an additional 17 haplotypes occurred twice. Despite the very limited number of haplotypes, all estimated frequencies for these haplotypes were within the 95% CI.

DPB1 Allele Frequencies in the Finnish Population

In the Finnish population there were only 7 DPB1 alleles with an average allele frequency higher than .01: DPB1*04:01 (f = .400), DPB1*04:02 (f = .201), DPB1*02:01 (f = .137), DPB1*03:01 (f = .124), DPB1*01:01 (f = .054), DPB1*05:01 (f = .024), and DPB1*14:01 (f = .012). According to Pypop.org [45], individual DPB1 allele frequencies are quite similar all across Southwestern, Central, and Northern Europe, with DPB1*04:01 the most common DPB1 allele not only in Finland but across all Europe. With such wide variation between the overall frequencies of different DPB1 alleles and with the assumed very weak linkage disequilibrium (LD), one would expect DPB1*04:01 to be the most common DPB1 allele for most if not all HLA-A-B-C-DRB1-DQB1 haplotypes.

Haplotype-Specific DPB1 Frequencies

Nonetheless, when the DPB1 associations of individual 5-locus haplotypes were assessed (Figure 1, Supplementary Table S1), DPB1*04:01 was found to be the most frequent DPB1 association for only 50 of the 100 most frequent 5-locus haplotypes. Of the other DPB1 alleles, DPB1*01:01 was the most frequent for 4 haplotypes (all these haplotypes consisting of B*08:01, C*07:01, DRB1*03:01, and DQB1*02:01, with only HLA-A allele varying); DPB1*02:01 for 11 haplotypes, DPB1*03:01 for 13 haplotypes, DPB1*04:02 15 haplotypes, and both DPB1*05:01 and DPB1*14:01 for 2 haplotypes. Even DPB1 alleles 15:01, 16:01, and 19:01 with overall population frequencies as low as .0051 to .0053 were the most frequent DPB1 alleles for one 5-locus haplotype each.

On the other hand, the only DPB1 allele that was represented in association with all 100 5-locus haplotypes was DPB1*04:01. However, even DPB1*04:01, which has an average population frequency of .4, had a haplotype-specific frequency that varied from as low as .019 to as high as 1.0 per individual 5-locus haplotype. In contrast to DPB1*04:01, the rest of the DPB1 alleles did not seem to be associated with individual 5-locus haplotypes; the number of such haplotypes was 17 for DPB1*04:02 and 21, 31, 60, 67, and 77 for DPB1*02:01, DPB1*03:01, DPB1*01:01, DPB1*05:01, and DPB1*14:01, respectively. The clustering of 5-locus haplotypes according to the associated DPB1 alleles in Figure 1 emphasizes the presence of extended class II haplotypes (ie, DPB1*03:01 in association with DRB1*13 and DRB1*08).

Thus, the relative frequency of any 1 specific DPB1 allele varied substantially per haplotype, and a high proportion of 5-locus haplotypes were mainly associated with 1 specific DPB1 allele: the median of the highest 5-locus haplotype-specific DPB1 allele frequencies was .71 (range, .29 to 1) (Figure 2). The average population frequency of the specific DPB1 allele did not have a strong influence on this: In the 50 5-locus haplotypes in which DPB1*04:01 was the most frequent DPB1 allele, the median of haplotype-specific frequency of DPB1*04:01 was .74 (range, .35 to 1). For the other 50 5-locus haplotypes, the median of the highest DPB1 allele frequencies was .70 (range, .29 to .95). When the 5-locus haplotypes were divided into FER haplotypes and non-FER haplotypes, there was, however, a pronounced difference (Figure 2). For FER haplotypes the median was .81 (range, .40 to .96), whereas for non-FER haplotypes the median was .62 (range, .29 to 1), suggesting a stronger interconnection of DPB1 alleles with the 5-locus haplotypes in the FER setting.

Statistical Significance of DPB1 Associations

We then assessed the statistical significance of the haplotype-specific DPB1 frequencies (Figure 3 and Supplementary Figure S1). Among the 100 most frequent 5-locus HLA-haplotypes, 81 haplotypes carried at least 1 statistically significant DPB1 association. Of the 34 FER haplotypes all carried at least 1, whereas of the 66 non-FER haplotypes 19 did not ($P = .0002$), confirming the initial hypothesis of stronger DPB1 associations for FER haplotypes.

DPB1 Associations in Other Populations

In the Finnish population the ancestral haplotypes AH 8.1, AH 35.2, and AH 7.1 were significantly associated with DPB1*01:01, DPB1*04:02, and DPB1*05:01, respectively. These haplotypes are extremely common in Finland but also

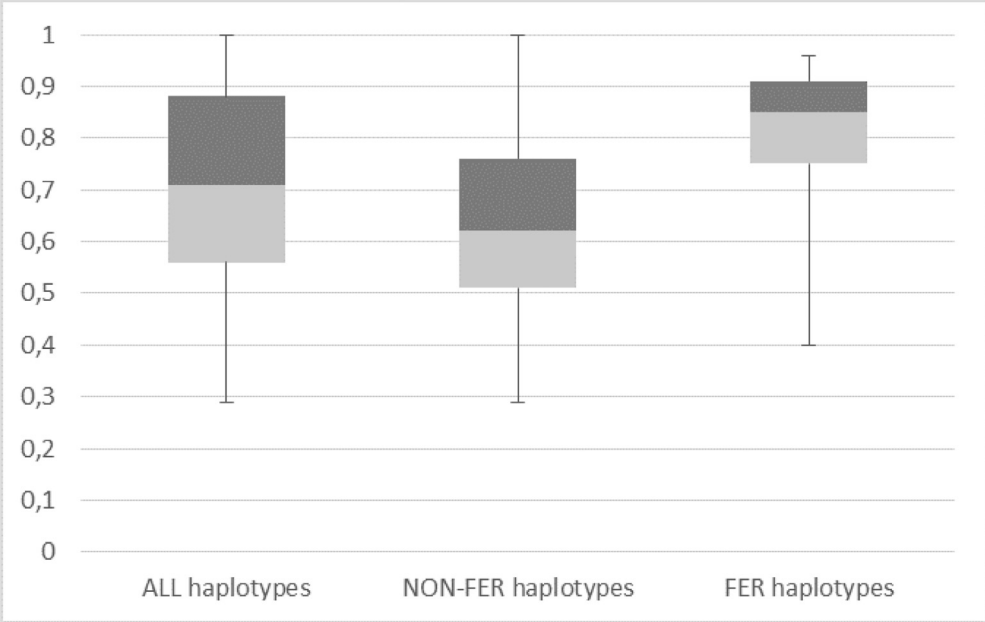

Figure 2. Ranges, medians, and upper and lower quartiles of the highest 5-locus haplotype-specific DPB1 allele frequency, depicted for all 100 HLA haplotypes (left) and divided into non-FER haplotype group (middle) and FER haplotype group (right).

elsewhere in Europe. To find out whether indications of similar DPB1 associations in other European populations exist, 3 worldwide donor searches [42] for common HLA phenotypes were run and analyzed. Each phenotype was homozygous for 1 of these 3 ancestral haplotypes. The DPB1 alleles of 344 British donors (688 haplotypes) and 848 German donors (1696 haplotypes) carrying AH 8.1 were assessed from the registry donor reports. In connection with AH 8.1, the frequency of DPB1*01:01 was .26 in the United Kingdom and .34 in Germany, whereas the average DPB1*01:01 frequencies in these populations were .08 and .04 to .05 respectively [46]. Similarly, for AH 35.2 the frequency of DPB1*04:02 was greater than .4 in Germany, Britain, Poland, and Sweden, the frequency thus being 4- to 10-fold compared with the average frequency of DPB1*04:02 in these countries [46], and DPB1*05:01 was 5- to 6-fold over-represented in connection with AH 7.1 in the United Kingdom, Germany, and Norway [47]. The results indicate that all DPB1 associations described in this study are not unique to Finland.

Registry Donor Searches for Patients Carrying Frequent Non-FER Haplotypes

Of the 84 searches performed in the WMDA search and match database, only 1 resulted in no DPB1 typed donors worldwide, and for 67 patients (80%) there were ≥100 DPB1 typed 10/10 matched international donors. In contrast, because of the relatively small size of the Finnish registry, 11 patients (13%) had no DPB1 typed Finnish donors and only 2 had ≥100; the median of DPB1 typed Finnish donors per patient was 9. Of the 84 searches, 79 (94%) produced at least 1 DPB1 matched donor for the patient. Even in these current searches, the DPB1 matching probability of Finnish versus international donors was markedly different: Of the total 1521 Finnish donors, 452 (29.7%) were DPB1 matched with the patient, whereas for the 7407 international donors the number was 1308 (14.1%, $P < .0001$). Thirty-four patients (40.5%) carried DPB1 alleles that were deemed as the most likely pair by the EM analysis. The probability of finding a 12/12 matched donor was higher for these patients; for them, the proportion of DPB1 matched Finnish donors was 372 of 872 (42.7%) and international donors 633 of 2994 (21.1%). The individual most frequent DPB1 allele combination in this sample of 84 patients was DPB1*04:01 homozygous, carried by 16 patients (19%). The DPB1*04:01 homozygosity frequency among the international 10/10 matched donors for these patients varied between 1 of 28 (3.7%) and 55 of 100 (55.0%, $P = .0001$). Similarly, for the 10 patients carrying the allele pair DPB1*02:01, 04:01, the proportion of DPB1 matched donors varied between 2 of 100 (2.0%) and 44 of 100 (44.0%, $P < .0001$) and for the 12 patients with allele pair DPB1*04:01, 04:02 between 4 of 100 (4.0%) and 36 of 100 (36.0%, $P < .0001$). The results show that even in a mixed international donor pool, the frequency of DPB1 alleles varies significantly according to the haplotypes in question.

DPB1 Matching in URD Transplants

To uncover the impact of DPB1 associations on the matching level in clinical practice, we finally retrospectively assessed the proportions of DPB1 matched (12/12 matched) and 1 or 2 allele DPB1 mismatched (11/12 or 10/12 matched, respectively) URD HSCTs performed in Finland between 2003 and 2016. In total, 769 10/10 matched transplants were performed during this period; 235 (30.6%) were from domestic and 534 (69.4%) from foreign donors. Of transplants from a domestic donor, 97 (41.3%) were DPB1 matched, whereas a match at DPB1 occurred in 133 transplants (24.9%, $P < .0001$) with a foreign donor. For the rest of the 10/10 matched transplants, 122 Finnish donors (51.9%) were 11/12 matched and only 16 (6.8%) 10/12 matched, whereas of foreign donors 309 (57.9%) had been 11/12 matched and as many as 92 (17.2%) 10/12 matched. There was a marked difference between the 3 Finnish transplant centers, 1 of which has been prioritizing DPB1 matched registry donors for several years, whereas the other 2 have prioritized other donor characteristics over DPB1 match. Of the transplants performed in the transplant center with DPB1 priority, 66 of 140 domestic transplants (47.1%) and 90 of 311 foreign transplants (28.9%) had been DPB1 matched ($P = .0003$); for the other 2 transplant centers together the

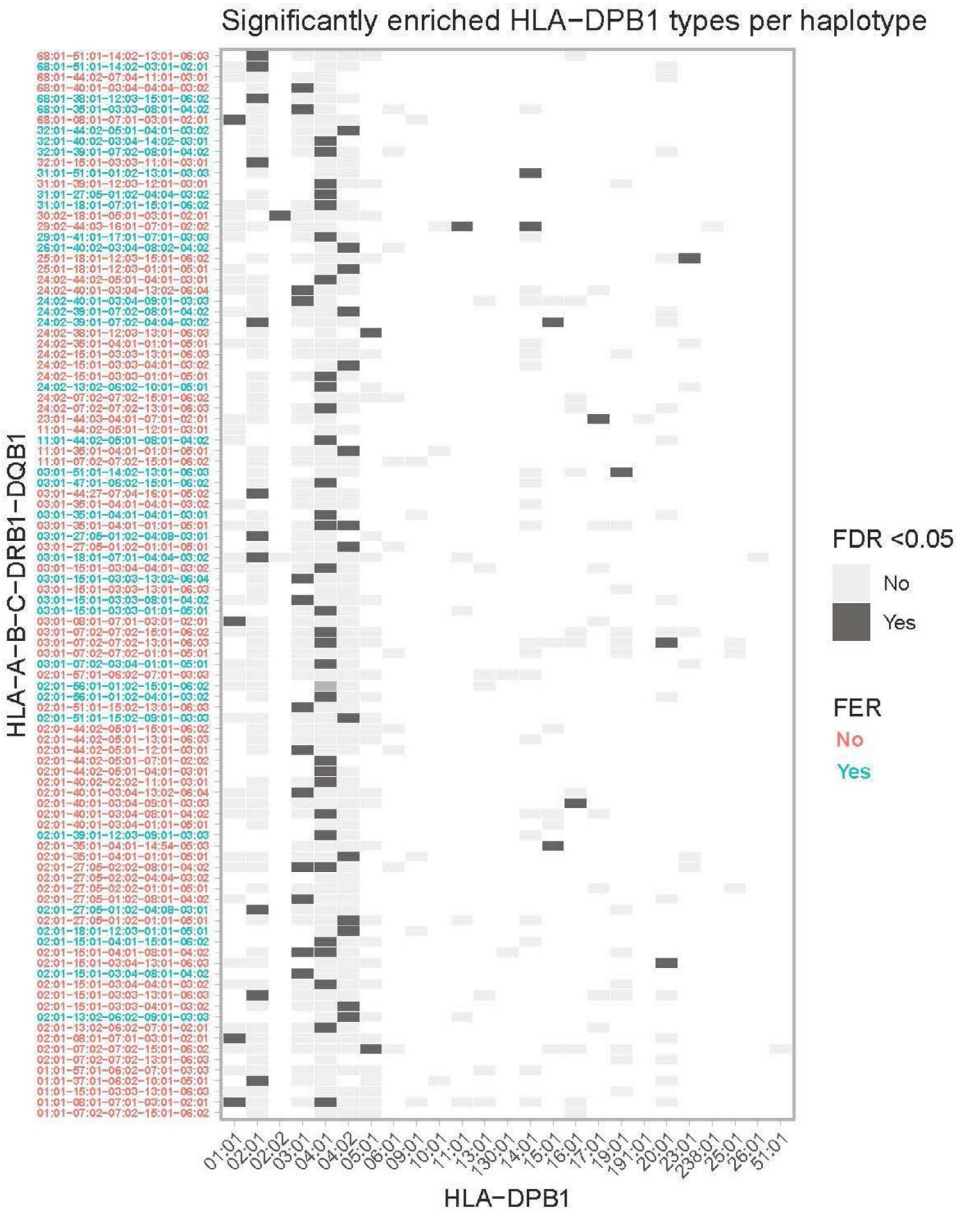

Figure 3. Statistically significant DPB1 allele enrichments in each haplotype. The *P* value significance cut-off is defined as the false discovery rate (FDR) below .05, calculated by using the Bonferroni correction for multiple testing. FER and non-FER haplotypes are differentiated by the *y*-axis label color.

respective numbers were 31 of 95 (32.6%) and 43 of 223 (19.3%, $P = .013$) (Figure 4). The results indicate that even when DPB1 match is not specifically solicited, domestic donors are perchance significantly more often DPB1 matched than foreign donors. Transplant center 1 had been able to minimize also the frequency of 2 DPB1 mismatches: 3 (2.1%) of their 10/10 matched domestic transplants had been mismatched for both DPB1 alleles, whereas the number was 35 (11.3%) for foreign donors ($P = .0008$) (Figure 4).

DISCUSSION

The idea for this study arose from the everyday work of an HLA laboratory and stem cell registry. We had observed an abundance of donors carrying an identical infrequent DPB1 allele in some donor searches and a paucity of permissively DPB1-mismatched donors for patients carrying very frequent DPB1 alleles in others. Instances of a conspicuous proportion of DPB1 matched registry donors seemed to occur more often between Finnish patients and Finnish donors, but they were not infrequent even internationally. Despite such observations, we are aware of only few studies reporting HLA haplotype frequencies extending to the DPB1 locus. Wennerström et al. [48] reported extended DRB1 (HLA class II) haplotypes from a Finnish population sample of 150 individuals in 2013. More recently, Norwegian and Russian Karelian 6-locus haplotype frequencies were reported [47,49]. To our knowledge, no studies on the strength of the linkage disequilibrium between 5-locus HLA haplotypes and DPB1 have been published. There are probably several reasons for this.

Until 2010 DPB1 matching was not widely used as a donor selection criterion. Thus, upfront DPB1 typing of new registry donors has not seemed worthwhile, and the number of DPB1 typed individuals in any specific population has long remained very limited. Because of the immense variability of the HLA alleles, assessing and studying 6-locus haplotypes requires a large high resolution typed population. Furthermore, the DPB1

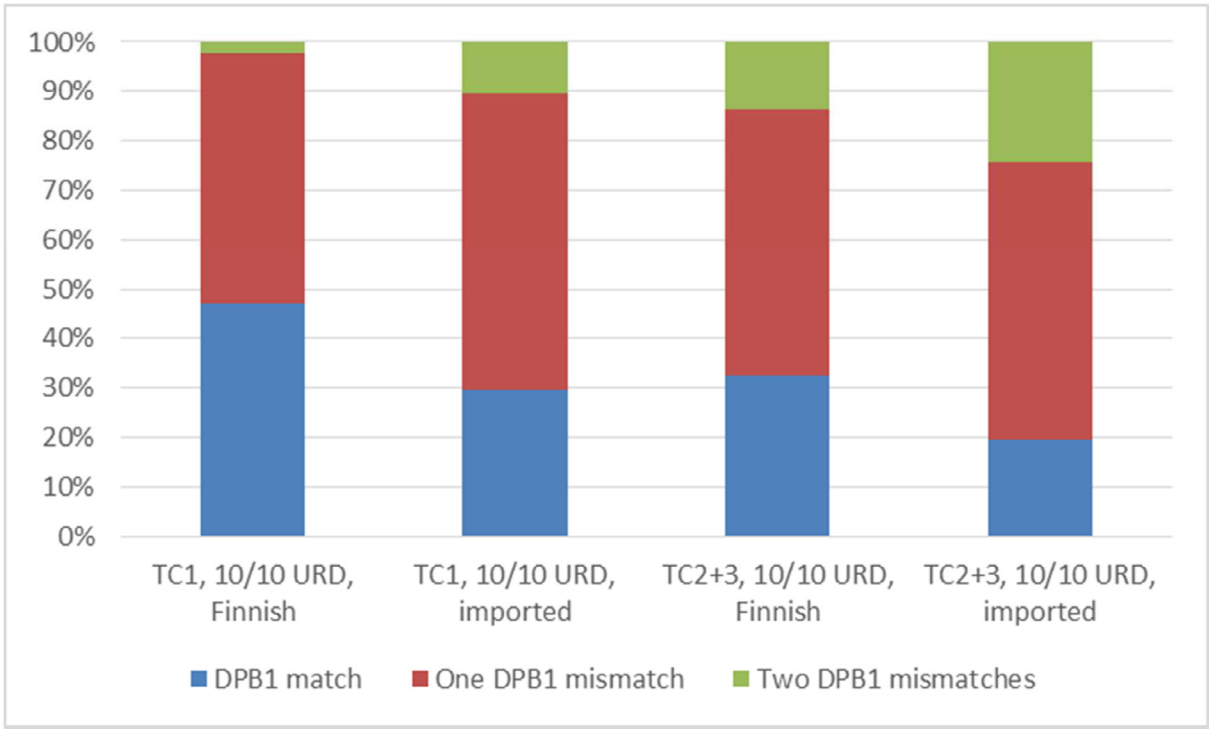

Figure 4. Proportions of DPB1 matched and 1 or 2 DPB1 allele mismatched transplants among the 10/10 matched URD HSCTs in Finland 2004 to 2016 (n = 769). Transplant center 1 (TC1) has had a practice of prioritizing 12/12 matched donors and avoiding 2 DPB1 mismatches, whereas transplant centers 2 and 3 (TC 2+3) have not.

locus is separated from the nearest classic HLA locus, DQB1, by approximately 400 kilobases [50], and there is a relatively active recombination hot spot situated between them [12,13] . When donor–recipient pairs have retrospectively been typed for the DPB1 locus, less than 20% of 10/10 matched transplant pairs have been also DPB1 matched [4,9,15], which has not supported a notion of a strong linkage between DPB1 and other classic HLA loci. Recent changes in registry donor typing practices have, however, made DPB1 association studies possible.

The results of this study show that in Finland significant associations exist between the classic 5-locus HLA haplotypes and the DPB1 locus. In 83 of 100 of the most frequent 5-locus haplotypes, 1 individual DPB1 allele dominated with a frequency > .50, the dominating DPB1 allele being 1 of 9 different DPB1 alleles with widely varying frequencies in the whole population. In 81 of 100 haplotypes the association was statistically significant. In the light of the previously reported recombination hot spots alone [10–12], this is somewhat surprising, but it seems that substantial linkage disequilibrium can often be seen also between adjacent haplotype blocks, making longer range LD possible [51], and this is more frequently found in populations that have undergone strong bottleneck events [52]. The relatively isolated Finnish population, also known to have suffered several famines during past centuries due to Finland's subarctic climate conditions, certainly fulfills the criteria of sharp bottlenecks. This may partly explain the results of this study. The fact that we found significantly stronger DPB1 associations for HLA haplotypes that are specifically enriched in Finland and rare among other Europeans also supports the impact of past population bottlenecks on our results.

Registry donors are a very large group of independently HLA typed individuals and, considering the variability of HLA alleles, thus optimal for HLA haplotype frequency estimations. However, for registry donors only the unphased HLA alleles are actually known. Thus, the problem of phasing the alleles of each individual into 2 HLA haplotypes has to be resolved. In this study this was done using the German National Bone Marrow Registry EM algorithm. We sought to validate the results of this algorithm by assessing the HLA haplotype frequencies in an independent data set of 559 HLA typed HSCT patients, whose HLA haplotypes we were able to reliably phase with knowledge of the HLA typing results of their relatives. Because HLA mismatched relatives have not been DPB1 typed in Finland, only 5-locus haplotype frequencies could be unequivocally calculated for all patients; for 6-locus haplotype frequencies only a subset of 159 DPB1 homozygous patients was available. With such a limited size of the population, the fundamental problem with rare haplotypes must be faced: In a sample of size n, a rare haplotype with a true frequency smaller than $1/n$ can never be truly estimated. If the haplotype is not present in the sample, the true value will be underestimated, but if it happens to be present, the true value will be overestimated. Because of this problem of rare appearances in the sample, only haplotypes occurring at least 3 times in the sample [53] were considered in the 5-locus validation data set. These frequencies were well in line with the estimated frequencies: Only 3 of 77 5-locus estimated frequencies were not within the boundary of the 95% CI, and in spite of the very limited number of 6-locus haplotype frequencies available for comparison, all estimated 6-locus frequencies were within the 95% CI. We can conclude that the EM method gives reasonable results when compared with the independent data set with regard to the 5-locus and even 6-locus haplotypes, although because of the lack of family DPB1 data the number of unequivocally determined 6-locus haplotypes was very low.

The ethnicity of individual donors is not asked or recorded in the Finnish Stem Cell registry, and it is probable that some individual donors represent other ethnic groups among the study population. It is, however, unlikely that they would have had a significant impact on the results, as the proportion of other than

Finnish born individuals in the Finnish population is currently less than 7%, and the biggest immigrant groups are from the neighboring countries [54] and likely under-represented in the registry.

The 5-locus haplotype DPB1 associations have a very practical impact on the Finnish stem cell transplant patients. From 2004 to 2016, 41.3% of 10/10 matched stem cell transplants from a Finnish donor had actually been also DPB1 (12/12) matched, whereas the proportion had been 24.9% for foreign donors. In addition to the classic 5 HLA loci, since the 1990s the Finnish Red Cross Blood Service HLA laboratory has performed additional DPB1 typing of all registry donor verification typing samples, and therefore for 2 decades the Finnish transplant centers have been able to consider the DPB1 matching along with the other HLA loci. The 1 transplant center, which has for years prioritized 12/12 matched transplants, has actually achieved this level of matching in almost 50% of transplants from domestic donors, whereas for foreign donors the proportion was less than 30% ($P = .0003$). It is noteworthy that these numbers have been achieved mainly at a time when registry donors were not DPB1 typed upfront; the DPB1 type of the donors was only revealed on verification typing. It is thus likely that with the recent availability of an increasing number of DPB1 typed donors worldwide, DPB1 matched donors will be available upfront for an even higher proportion of patients, as was indeed shown by the 84 registry donor searches that were performed for patients carrying 2 non-FER haplotypes. The difference of DPB1 matching between domestic and foreign donors could be explained by either stronger LD between HLA haplotype blocks in the Finnish population, DPB1 associations that are specific for Finns, or probably both. In previous international studies discussing DPB1 mismatching [9,15] the likelihood of DPB1 matching in 10/10 matched transplants has been slightly below 20%, but the likelihood has been statistically significantly higher for 10/10 matched than less well-matched pairs. Our 84 registry donor searches also show that the same DPB1 allele pairs are seen with highly variable frequencies with different 5-locus haplotype combinations. These findings give further support for the notion that LD between haplotype blocks exists also among other populations, but further studies are needed to confirm specific DPB1 associations and their strength.

Since 2015 all newly recruited donors of the Finnish Stem Cell Registry have been DPB1 typed. This has rapidly increased the proportion of DPB1 typed individuals in the registry, making this study possible. Even in the present situation, however, the number of HLA DPB1 typed donors (15,288) was somewhat limiting to this study because of the immense variation in the HLA genes. Consequently, we decided to limit the detailed 6-locus haplotype analysis to the 100 most frequent Finnish 5-locus haplotypes and exclude the less frequent haplotypes from the study. To reach statistically significant results in more heterogeneous populations, even higher numbers of high resolution typed donors will probably be required. With the current paucity of 6-locus haplotype data from other populations, comprehensive comparisons between different populations were not feasible. However, as most European and North American newly recruited donors are presently DPB1 typed upfront [42], there is an increasing amount of data for such analyses. This study suggests that considering the several ways in which the DPB1 type of the donor can be used today, predicting the probability of DPB1 permissiveness—or even matching—of less well-typed donors might be possible in the near future.

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SUPPLEMENTARY MATERIALS

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