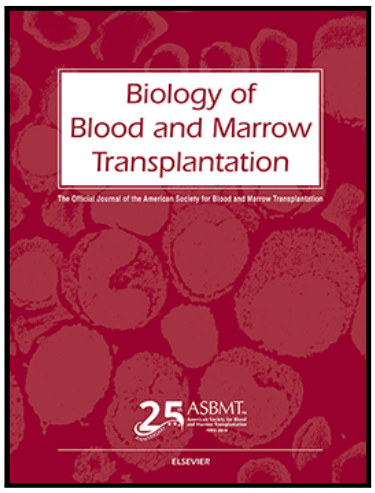

Increased MHC matching by C4 gene compatibility in URD HSCT

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Highlights

- HLA matched URD donors often result to haplotype match in an isolate population
- C4 gene can be used as a HLA haplotype determinant in HSCT
- There is a need for population specific stem cell registries

Increased MHC matching by C4 gene compatibility in URD HSCT

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Abstract

Human leukocyte antigen (HLA) matching is a prerequisite for successful allogeneic hematopoietic stem cell transplantation (HSCT) as it lowers the occurrence and severity of graft versus host disease (GvHD). However, matching a few alleles of the classical HLA genes only may not ensure matching of the entire major histocompatibility complex (MHC) region. HLA haplotype matching has been reported to be beneficial in HSCT due to the variation relevant to GvHD risk in the non-HLA region. As polymorphism in the MHC is highly population-specific, we hypothesized that donors from the Finnish registry are more likely matched at a higher level for the Finnish patients than donors from other registries. In the present study we determined 25 single nucleotide polymorphisms (SNPs) of the complement component 4 (C4) gene in the gamma block segment of MHC from 115 Finnish HSCT patients and their Finnish (n=201) and non-Finnish (n=280) donor candidates. Full matching of HLA alleles and C4 SNPs, independently or additively, occurred more likely in the Finnish - Finnish group as compared with the Finnish - non-Finnish group ($P< 0.003$). This was most striking in cases with HLA haplotypes typical of the Finnish population. Patients with ancestral HLA haplotypes (AH) were more likely to find a full HLA and C4 matched donor, regardless of donors' origin as compared with patients without AH ($P<0.0001$). Despite the clear differences at the population level, we could not find a statistical association between C4 matching and clinical outcome. The results suggest that screening C4 SNPs can be advantageous when an extended MHC matching or HLA haplotype matching in HSCT is required. This study also supports the need for small population-specific stem cell registries.

Introduction

Matching the classical human leukocyte antigens (HLA) at HLA-A, HLA-C, HLA-B, HLA-DRB1, and HLA-DQB1 is a prerequisite for a successful unrelated donor hematopoietic stem cell transplantation (URD HSCT) in order to evade graft versus host disease (GvHD), a life threatening condition. Unrelated donors are found from volunteer stem cell registries. In case adequate numbers of donors cannot be found from a national stem cell registry, donors are also searched from international registries. Depending on a patients' HLA type usually 4-8 donors are requested for confirmatory HLA typing. As HLA haplotype information is usually not available, donor selection is mainly based on allele matching at the five classical HLA genes together with donor age, sex and CMV status.

The major histocompatibility complex, MHC, encompasses 4 Mbp of DNA sequence at 6p21.3 and is divided in three classes based on roles of the genes in the immune system. MHC classes I and II contain the genes of the HLA molecules that represent peptides to T-cells. MHC class III, located between MHC classes I and II, includes e.g. the complement component C4 genes. The strikingly strong linkage disequilibrium (LD) in MHC[1–5] is thought to control the diversity of haplotypes in order to keep functionally coordinated sets of alleles together[6]. Despite the strong LD, a few recombination hot spots are located within the MHC region[4,7–11]. Their locations have been found to be the same across populations, although some appear to be haplotype or population specific[4,8,12]. These hot spots create segmented blocks in the MHC; α-, β-, γ–and δ-blocks containing HLA-A, HLA-B and –C, complement genes and HLA-DRB1 and -DQB1 genes, respectively. Even though these blocks can shuffle and combine to form novel assemblies, some very fixed block combinations exist due to the strong LD[9,13–15].

The block structure and positive LD that occurs in the MHC region enable long stretches of DNA to be inherited as ancestral haplotypes (AH)[13,16]. Many of the common MHC haplotypes in Caucasians are either ancestral haplotypes, some ranging from HLA-A up to HLA DQB1, or recombinants of AHs. Thus, the complement C4 alleles are often inherited together with the flanking HLA-B and HLA-DR/DQ alleles in the European Caucasian population[10,17–19] due to the positive LD. These conserved haplotypes of different size together with other HLA haplotypes are present at varying frequency in populations from different ethnic and/or geographical origins[20–22]. Moreover, there is variation in HLA haplotype frequencies

inside distinct populations as well[23–26]. The assortment of HLA haplotypes is enormous as the frequency of the most common HLA haplotypes in a population is usually only a few percentages and the majority of the haplotypes is found in very low frequencies[20,27].

Ethnicity of a patient affects not only the probability of finding an HLA-A, -B, -C, -DRB1, -DQB1 allele-matched unrelated donor but also the probability of finding an HLA haplotype matched donor[27–31]. There is growing evidence that also matching of non-classical HLA genes, or non-HLA genes in the HLA region, is associated with better HSCT outcome[32,33], as also does matching of entire HLA haplotypes[31,34–38]. On the other hand, mismatching may be beneficial as HLA-C, -DPB1 and MICA discrepancy has been reported to protect from relapsing[32,39].

The Finnish population is of mainly European genetic origin[40,41], but there are, however, many genetic features that differentiates the Finns from other Europeans[40,42]. This is due to a relatively small founder population, historical population bottle necks and genetic isolation[43–47]. These events are suggested to explain the reduced HLA allele pool[26,48] and specific HLA haplotypes[49] in the Finnish population.

Based on the specific HLA haplotype spectrum in the Finnish population we wanted to evaluate whether HSCT donor candidates to Finnish patients have different HLA haplotypes depending on the candidates' origin, regardless of the apparent classical HLA allele matching. We focused on the complement component 4 (C4) in the gamma-block segment of the class III region, a rarely researched area of MHC in HSCT. Match status of 25 single nucleotide polymorphisms (SNPs) at C4 gene was evaluated in 115 Finnish HSCT patients and in all their 481 donor candidates. C4 and HLA matching grades, independently and additively, were compared between Finnish and non-Finnish donor groups as well as the effect of mismatching on the outcome of HSCT.

Subjects and methods

115 Finnish patients that had received hematopoietic stem cell transplant from a registry donor between the years 2003-2016 were chosen for the study. Only patients with donor candidates from both the Finnish registry (hereafter FI donors), and other registries (hereafter non-FI donors) were selected for the

study. Every donor candidate that was invited for confirmatory HLA typing (N=481), despite of known prior HLA mismatch, was included in the study. The donors represented 12 different registries; 201 were from the Finnish registry and 280 from other registries. The study material was divided into two sets when appropriate; patients with putative Finnish (FI) or non-Finnish (non-FI) donor, according to donor's registry. Clinical data was available from 105 HSCT pairs. Demographic details of the study subjects and clinical outcomes of patients, including GvHD grading and relapse, are described in Table 1.

This study was carried out in accordance with the recommendations of the Ethical Committees of Helsinki and Turku University Hospitals with written, informed, consent from living patients and Finnish donors. Finnish Supervisory Authority for Welfare and Health (Valvira) granted a permit to study those of whom consent was not possible to ask (deceased or historical subjects).

Clinical HLA typing

Genomic DNA from the white blood cell fraction of the whole blood or from the whole blood was extracted either with QiaAmp Blood Mini kit or with QIAsymphony DSP DNA Midi Kit (Qiagen GmbH, Germany).

HLA typing was performed at the HLA laboratory of the Finnish Red Cross Blood Service, using procedures accredited by the European Federation for Immunogenetics (EFI). All the patients and donor candidates of the study were typed for one-field and two-field resolution level by SSO (Lipa, Innogenetics, UK) or rSSO-Luminex technology (Labtype, One Lambda, Inc., CA, USA) and PCR-SSP (Micro SSP™ Generic HLA Class I/II DNA Typing Trays, One Lambda, Inc.; Olerup SSP® genotyping, Olerup SSP AB, Stockholm, Sweden). Sequence-based typing for determining the HLA alleles at two-field resolution was performed with AlleleSEQR PCR/Sequencing kits (Atria Genetics, Hayward, CA, USA), using the ABI 3130xl genetic analyser (Applied Biosystems, Thermo Fisher Scientific, MA, USA) and h the Assign 3.5+ software (Conexio Genomics Pty Ltd, Fremantle, Australia).

C4 SNP matching

MHC class III matching was performed with commercial Gammatype™ typing kit (KD-PD8.0-1(96), Conexio-Genomics, CareDx). The kit consists of a panel of 25 different primer pairs that are targeted at complement component C4 gene in the MHC class III region; 23 targeted primer pairs for SNPs at C4 gene and two primer pairs for C4A and C4B genes. The results are interpreted by presence or absence of a particular SNP at C4 gene without indication of zygosity. PCR conditions for each reaction were performed according to the manufacturer's instructions. The parameters for the electrophoresis were as following: 2% agarose gel (SeaKem® LE Agarose 50005, Lot No: 0000576343, Lonza), 150V and 30-40 min.

The reagent details are confidential and, therefore, the particular SNPs the kit detects remain unidentified. Specific characteristics of the SNPs is not possible to provide in this study. Information on ancestral haplotype (AH) defining SNPs (AH7.1, 8.1, 13.1, 18.1, 38.1, 42.1, 44.2, 44.4, 46.2, 47.1, 52.1, 54.1, 55.1, 57.1, 62.1.) was kindly provided by Dr. Bruno Vanherberghen with CareDx's approval. Due to these technical restrictions and confidentiality issues the actual haplotyping is not performed. This study provides additional information exclusively for the match grade of the C4 and HLA genes between a transplantation pair, not phasing of the genes, and therefore the results are interpreted as putative haplotype matching."

Matching models

Five separate matching models were defined based on the number of classical HLA genes and MHC classes included (Table 2). In the 5-HLA gene matching model the match status in a putative HSCT pair was assessed according to the clinical HLA-A,-B,-C,-DRB1 and -DQB1 allele assignment (10/10 matching). The HLA-DPB1 gene was included in the 6-HLA gene matching model (12/12 matching). Differentially to the two HLA matching models above, SNP matching at the complement component C4 gene was applied in the C4 matching model. Finally, the 5- and 6-HLA gene matching models were combined with the C4 matching model and are hereafter referred to as 5- and 6-HLA gene haplotype models.

AH and FER haplotype matching

The set of possible HLA haplotypes of the 115 patients were first constructed by reflecting a patient's HLA type and allele combination to the known HLA haplotype frequencies in the Finnish population (our unpublished data). The combination of HLA haplotypes with the highest probability based on their frequency was selected as patients' putative haplotype assembly. Patients were grouped into the ancestral haplotype (AH) positive set on the condition that they were positive for an AH tagging SNP and had the corresponding HLA-B, HLA-DRB1 and HLA-DQB1 types. If a patient was negative for an AH SNP and/or did not have the corresponding HLA type, patients were classified as AH negative, regardless of the patient-donor match status. Other classical HLA loci (HLA-A and HLA-DPB1) were ruled out of this analysis due to the known recombination sites between different genomic blocks close to these genes. Patients were also divided into Finnish enriched rare (FER) positive or FER negative groups comparing the putative haplotypes according to the published FER haplotypes list[49]. As there is a known active recombination hot spot near to the HLA-DPB1 gene, the FER and AH compatibility was restricted to the 10/10 HLA matching.

Statistical analysis

The alpha level was set at .05 for statistical tests in the population study. Chi square tests were performed to compare HLA, C4 and haplotype matching between the Finnish and non-Finnish donor groups using GraphPad Prism software v.7.02.

The effect of different mismatch/match conditions (HLA-DPB1 and C4, independently and additively) on clinical outcomes of 105 patients was investigated. Patients with no acute or chronic GvHD (a/cGvHD) were compared to patients with aGvHD grades 1-4 and to patients with limited or extensive forms of cGvHD. Relapsed patients were compared to non-relapsed patients (presence/absence). For each mismatch/match condition, we computed the odds ratio (Wald's unconditional maximum likelihood estimation) of observing a negative clinical outcome for patients with a mismatch compared to patients

with a full match. We computed confidence intervals using the Baptista-Pike mid-p method. We used Fisher's exact test to determine statistical significance. In addition, we carried out non-inferiority testing[50] on these odds-ratios. We defined inferiority margins on the change in proportion of negative outcome with values of δ = .1 for relapse and δ = 0.25 for aGvHD and cGvHD. From these proportion inferiority margin we computed an odds-ratio threshold for each mismatch/match condition and clinical outcome pair. The mismatch condition was considered to be non-inferior to the match condition if the upper bound of the 90% confidence interval on the odds-ratio was smaller than the odds-ratio threshold. Confidence intervals were computed using the R library *ORCI*[51].

The survival analysis was carried out by first analysing the data using a random forest (RF) survival model to evaluate the contributions of different variables. The RF analysis was performed using the R library *ranger* v0.10.0[52] with default settings. The variable importances and their sampling variances were estimated through jackknife resampling (Figure S2). Four of the top variables (i.e. donor age, patient age, GT match and cGvHD) were selected for subsequent analysis with cox and Kaplan-Meier models implemented in the R library *survival* v.2.42-3[53] (Figure S3). The data was managed and plotted using the R libraries *tidyverse* v.1.2.1[54] , *data.table* v.1.10.4-3[55] and *ggpubr* v0.1.6[56].

The R code implementing the analyses are available in GitHub (https://github.com/FRCBS/Gammatype).

Results

HLA matching

Of the 481 patient-donor candidate pairs altogether, 399 (83.0%) pairs were fully 10/10 matched for HLA-A, -B, -C, -DRB1 and DQB1 genes (the 5-HLA gene matching model, Table 2). Mismatching occurred most often at HLA-C or HLA-DQB1 (10% and 8.1%, respectively, data not shown). The proportion of the 10/10 HLA-matched donors was higher in the FI donor group than in the non-FI donor group (89.1% vs 78.6%; P=0.003, OR 0.45, 95% CI 0.27-0.77). For seven patients (6.1%) fully 10/10 HLA-matched donors were found solely in the FI donor group. Two patients (1.7%) remained without any 10/10 HLA-matched donor candidate.

In the 6-HLA gene model, with the HLA-DPB1 gene included, only 108 (23.0%) fully 12/12 HLA-matched pairs were found (Table 2). The proportion of HLA-matched pairs was equal in both donor groups, with 11.0% for FI donors and 11.9% for non-FI donors (P=0.4) .Thus, mismatching occurred mostly at the HLA-DPB1 gene (73.4%) in this model.

C4 matching

Altogether, 481 patient-donor candidate pairs were screened for the match status in the C4 gene. Of them, 263 patient–donor candidate pairs (54.7%) were fully matched for the 25 SNPs in the C4 gene, the others were matched for 17 to 24 of the SNPs (Table 2). The proportion of full C4 match in the FI patient–FI donor group was higher (77.1%) than in FI patient-non-FI donor group (38.6%) (P<0.0001, OR 0.19 95%, CI 0.12-0.28). Distribution of the number of C4 SNPs mismatches, however, did not differ between the two groups (Table 2).

Added value of C4 matching in HLA matched patient donor candidate pairs

We analysed whether SNPs in the C4 gene can reveal genetic differences of the MHC in the HLA-A, -B, -C, -DRB1 and –DQB1 (10/10) matched patient–donor candidate pairs (N= 399). The relative number of the fully matched pairs reduced remarkably when both HLA and C4 matches were included; in the 5-HLA genes model, 83% of the pairs were matched while the share was 52% in the 5-HLA gene haplotype model (Table 2). A full C4 match occurred with a higher frequency in fully HLA matched FI patient – FI donor pairs (83.8%) than FI patient - non-FI donor pairs (45.5%)(Figure 1a); the difference is statistically significant (P<0.0001, OR 0.16, CI 0.10-0.26). Therefore, C4 mismatching was the main differentiator between the FI and non-FI donor groups in the putative haplotype model with 5 HLA genes.

To further expand the MHC matching, HLA-DPB1 was included in the putative 6-HLA gene haplotype model, i.e. 10/10 matched patient-donor candidate pairs with HLA-DPB1 result (N=399). Altogether 108 pairs (27.1%) were 12/12 HLA matched, of which 71 (65.7%) were also C4 matched (Table 2). The share

of pairs with both HLA and C4 match was higher in the FI patient - FI donor group (N= 44/52, 84.5%) than in the non-FI donor group (N=27/56, 48.2%), with P<0.0001 (OR 0.17, 95% CI 0.07-0.4) (Figure 1b). It is of note also that in the DPB1 *mismatch* group (N= 291), the majority of the C4 matched pairs were from the FI – FI group (106/179, 59.2%). The difference in distribution between the two donor groups was again significant (106/127, 83.5% vs 73/164, 44.5%%; P<0.0001, OR 0.16, 95% CI 0.09-0.28).

Effect of Finnish Enriched Rare (FER) haplotypes

Altogether 38 (33.0%) of the 115 patients had one (N=36) or two (N=2) FER haplotypes. Patients with a FER haplotype were less likely to find a 10/10 HLA-matched donor (P=0.0003; OR 2.5, 95% CI 1.5-4.1) or a 10/10 HLA and C4 matched donor *(*P=0.0048; OR 1.76, 95% CI 1.2-2.6) than patients without FER. However, patients with a FER haplotype were more likely to have 10/10 HLA and C4 matched FI donor candidates than non-FI donor candidates (P<0.0001, Chi square) (Figure 2a). Thus, finding a fully 10/10 HLA and 25 C4 SNP matched donor for a patient with a FER haplotype was highly dependent on the donor registry.

Ancestral haplotype matching

The most frequent Finnish HLA haplotype AH35.2 (frequency=0.08) was found homozygous in four patients in the study set. All the 15 donor candidates (FI N=11, non-FI N=4) for these four patients were 10/10 HLA matched and fully C4 matched. HLA haplotype AH8.1 appeared homozygous in one patient; all 4 donor candidates (FI N=3, non-FI N=1) were a full 10/10 HLA and 25 C4 SNP match for this patient.

The ancestral haplotype AH 57.1 occurred heterozygous in three patients and their donor candidates (N=16). Regardless of the origin of the 16 donor candidates (FI N=2, non-FI N=14), all of them were fully 10/10 HLA and 25 C4 SNP matched.

The impact of the ancestral haplotype in the donor search was significant (Figure 2b). Patients positive and matched for AH-associated C4 SNPs were more likely to find 10/10 HLA matched donors than

patients negative for the AH tagging SNPs (P=0.0029; OR 2.2, 95% CI 1.3-3.8). Patients with at least one AH were more likely to have a full 10/10 HLA and 25 C4 SNP matched donor as compared to patients without any AH specific SNPs (P<0.0001; Chi-square test).

The impact of the HLA and C4 match on clinical outcome

We tested the effect HLA and C4 matching status on clinical outcomes (cGvHD, aGvHD and Relapse) using available clinical data for 105 10/10 HLA matched HSCT. We computed the odds ratios (and their 95% confidence intervals) of observing an adverse clinical outcome for patients with a mismatch compared to patients with a full match for four mismatch/match conditions (HLA-DPB1 and C4, independently and additively). We used Fisher's exact test to test for the presence of an effect of mismatch/match status on clinical outcomes. No statistically significant differences in clinical outcomes were found. Since the absence of significant result does not imply the absence or presence of inferiority between mismatch/match conditions, we also ran non-inferiority analyses (Figure S1). Mismatched conditions were considered non-inferior to matched conditions for relapse in the C4 mismatch vs match condition, for cGvHD in the HLA-DPB1 mismatch vs match condition and in the HLA-DPB1 mismatch vs match with C4 matched condition, as well as for aGvHD in the C4 mismatch vs match condition.

All available variables were initially screened for potential importance for survival using a random forest model. The most important variables were patient and donor age, cGvHD, diagnosis and total matching over the 25 C4 SNPs (GT match). These variables excluding diagnosis were selected for survival analysis using Kaplan-Meier curves and Cox regression analysis. None of the variables reached statistical significance after multiple testing adjustment, but there was a trend towards higher survival rates of about three years post transplantation for patients that were under 53 years, exhibited cGvHD or had a C4 mismatch (Figures S2 and S3).

Discussion

It is well established that matching alleles of the classical HLA- A, B, C, DR and -DQ genes is beneficial in HSCT[57]. Lately, several studies have suggested that increased GvHD risk after transplantation is related to HLA-DPB1 mismatches[58,59] . Matching merely a set of classical HLA class I and II genes may not reveal possible haplotype difference in unrelated HSCT as HLA genes encompass only a small fraction of the whole MHC segment. Since haplotype data is not usually available from registry donors, the standard HLA-matched URD pairs[60,61] or even sibling pairs[38], may carry hidden mismatches in the MHC region. Matching the entire HLA haplotypes has been reported to significantly decrease the risk of GvHD and increase the overall survival in allogenic HSCT. Conversely the incompatibility of extended MHC haplotypes significantly impairs GvHD and overall survival, emphasizing the importance of matching the entire MHC region[35,38,60–62].

In this study, based on population history and haplotype frequencies, we hypothesized that Finnish registry donors are more likely to be not only HLA matched but also HLA haplotype matched compared to non-Finnish registry donors. The small founder population, several genetic bottlenecks and isolations by density and language have created a special genetic structure in Finns[63] and may have contributed to the MHC constitution as well. Finnish HLA haplotype frequencies are known to differ from those of the neighbouring populations and, in fact, several common Finnish haplotypes do not exist elsewhere in Europe[26,48,49]. To identify possible haplotype matches, we used a 25 C4 SNPs panel as the high variation both in structure and sequence of the C4 gene[64,65] and its location at γ -block between the β- and δ-blocks in the MHC region support its use as a haplotype determinant. In addition, the positive LD of the C4 with its surrounding loci, HLA-B[17] and HLA-DRB1[10] , further highlights its usability in haplotype matching.

According to our results, a Finnish URD is more likely to be matched with a Finnish patient than a non-Finnish donor regardless of MHC class. When each gene was individually studied, there was higher incidence of HLA-A, -B, -C, -DRB1, –DQB1 and C4 matching in the FI donor group than in the non-FI group. The observed mismatches at HLA-C and HLA-DQB1 is in accordance with reported haplotype differences between populations as population-specific HLA combinations are usually focused at these genes[66,67]. The idea of different HLA haplotype compositions between populations is further supported by

our finding of C4 matching being significantly lower in the non-FI donor group compared with the FI group. HLA-DPB1 was an exception since there was no difference in matching between the two groups. This is explained by the active recombination hotspot between HLA-DQ and HLA–DP genes[4,8,68].

The probability of finding a putative haplotype matched donor (i.e. both HLA- and C4-matched) was significantly different between the two donor groups for the benefit of Finnish donors. We, however, underline that no real haplotyping was performed in the study due to the limitations of the C4 SNP typing method and therefore referred haplotype models in this study are speculative. It is of note that matching was better with a Finnish donor in any match/mismatch setting as they had a higher incidence of being C4 matched despite being HLA mismatched. Our findings are congruent with the idea of the block structure of MHC where novel haplotypes are formed by shuffling the genomic blocks[14,15]. Thus, based on these results, selecting a HLA matched Finnish donor for a Finnish patient may result either in the individual MHC block match or even in the entire MHC segment match.

The putative haplotype match with an FI donor concerns especially the small group of patients that have a Finnish Enriched Rare (FER) haplotype. In this group, matching reached all the way from HLA-A to HLA-DQB1 and, usually, also up to HLA-DPB1 gene (data not shown) despite of the known active recombination site just before HLA-DPB1[4,7,8]. Fairly limited sample size together with low effective population size[69] in the study may explain the results of relatively high number of 12/12 matches especially in the FER group. Most isolates show substantially higher levels of LD than outbred populations[70]. The fixation of haplotypes is high in small populations due to recombination between ancestral haplotypes themselves[71,72]. The full matches in the FER group may also be explained simply by relatively recent introduction of these haplotypes into the Finnish population.

Even though many HLA haplotypes are population specific, some haplotypes are found to be invariable and preserved across several populations, even in distant ones. For example the ancestral haplotype AH 57.1 (HLA-A1-C6-B57-DR7-DQ3) is found in populations of European, Asian and African origins[19,27,28,73,74]. Therefore a fixed haplotype may give specific frames for the remaining haplotype ensuring a haplotype match together with HLA match. This idea is supported by our findings that a proportion of HLA and C4 matched donors is higher in the group of patients with putative ancestral

haplotype than in the group with no AH. Also, ancestral haplotypes 35.2 and 8.1 were highly conserved as no variation of 25 SNPs at C4 gene or HLA-A,-B,-C, DRB1 and -DQB1 at two field resolution were observed in 19 homozygous donors from both donor groups. The haplotype structure was disrupted at HLA-DPB1 gene, as expected.

The specific MHC constitution of the Finnish population would favour to prefer a Finnish donor for a Finnish patient in order to minimize the risk of GvHD. Also, as non-classical HLA and haplotype matches are reported to reduce the risk of HSCT complications[35,36,39,60–62], genetic and clinical data was combined to evaluate the effects of C4 and haplotype mismatching on GvHD, relapse and survival. However, we didn't find any statistically significant association between C4 compatibility and HSCT outcome, which is consistent with recent reports[75,76], though controversial results have also been reported[77]. It is of note that the impact of this MHC segment cannot completely be excluded based on our study as the relatively low number (N=105) of actual transplantation pairs available did not afford us sufficient power to detect smaller but nevertheless clinically significant differences. A larger dataset of transplantation pairs would be required to confirm the questionable role of C4 as such in HSCT. In any case, this study suggests that C4 region can be used as a HLA haplotype marker, which can be beneficial for HSCT patients as HLA haplotype matching has been reported to reduce complications after HSCT [38,60,62].

As the frequencies of HLA haplotypes can vary greatly between populations, demand for registries representing various ethnic origins of unrelated donors exists. When matching an unrelated registry donor to a Finnish patient prior to the HSCT, the unique HLA constitution of Finns may display challenges. Of the Finnish patients, up to 4% find a HLA-matched donor from the Finnish Stem Cell Registry only[49]. Therefore, the need for a national stem cell registry within such a distinct population is necessary[26]. In a reverse situation, non-Finnish patients with low frequency HLA haplotypes that are enriched in Finland can benefit from the FSCR. The results of this study endorse the Finnish populations' well-known role as a genetic outlier amongst other Caucasian populations.

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Authorship contributions

J.C., J.P. and S.K. designed the study. J.C. and S.K. managed DNA samples and performed C4 SNP and HLA typing. J.C., S.K., J.R. and M.L. performed the statistical and data analyses. U.S., M.P., R.N. and M.I.-R. collected and interpreted the clinical data. J.C., J.R., M.L., J.P and S.K. interpreted the results and wrote the manuscript.

Data availability statement

Data reported in this study is not available due to the limitations set by the Ethical Committee.

Ethical approval and informed consent

All experiments were carried out in accordance with relevant guidelines and regulations defined in the Finnish legislation. The Ethical Committee of the Helsinki University Hospital and Finnish Supervisory Authority for Welfare and Health (Valvira) have approved the study.

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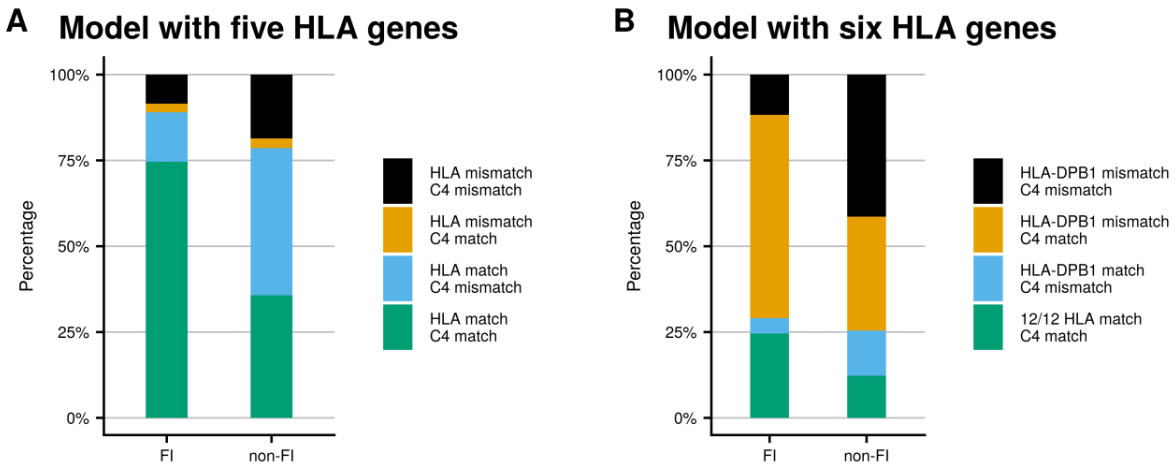

Figure 1. The C4 match in the 5- and 6-HLA gene models. a) Distribution of the C4 match in the Finnish and non-Finnish donor groups in the 5-HLA gene matching model. b) Distribution of the C4 match in the Finnish and non-Finnish donor groups in the 6-HLA gene matching model. In both models the Finnish donors were more likely to result in a C4 match than the non-Finnish donors for a Finnish patient. FI=Finnish donor, non-FI=donor from worldwide registries, HLA=human leucocyte antigen, C4=complement component gene 4.

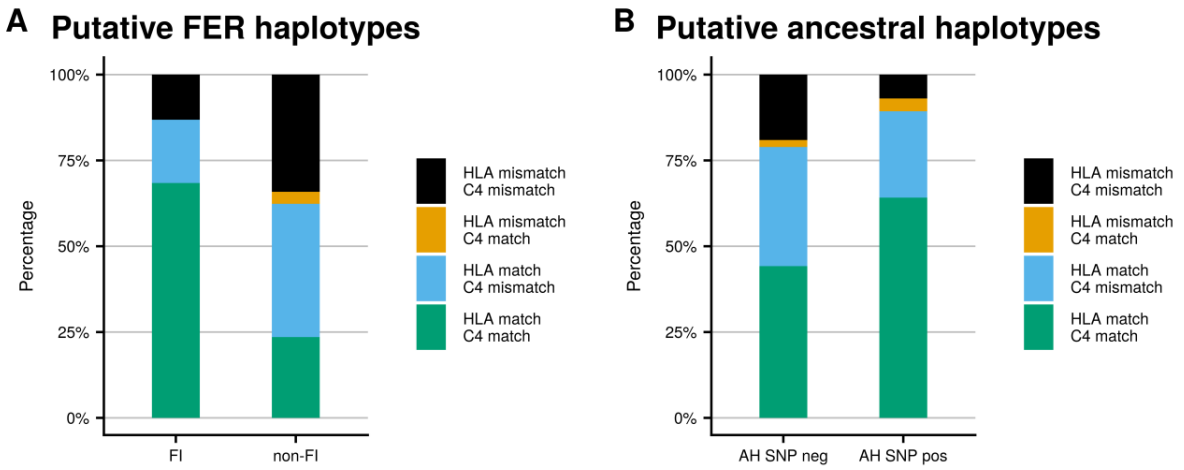

Figure 2. The C4 match in the extended haplotypes. a) Distribution of Finnish enriched rare haplotype in Finnish and non-Finnish donor groups. Finnish patient with FER is more likely to find a 10/10 HLA matched and C4 matched donor from the Finnish registry than other registries. b) Distribution of HLA match and C4 match grades in ancestral haplotype positive and negative donor groups. Patients with AH tagging SNP are more likely to find a 10/10 HLA matched and C4 matched donor than recipients without AH tagging SNP. FER haplotype=Finnish enriched rare haplotype, FI=Finnish donor, non-FI=donor from worldwide registries, HLA=human leucocyte, antigen, C4=complement component gene 4, AH=ancestral haplotype.

Table 1. Donor and patient characteristics

Donors		n
registry	Finland	201
	USA	20
	Germany	242
	Poland	2
	Great Britain	3
	France	2
	Norway	2
	Sweden	4
	Denmark	1
	Australia	1
	Canada	1
	Switzerland	1
	non-Finnish, registry NA	1
	all	**481**

Study subjects		donor n	patient n
age	<20	2	2
	20-40	75	20
	41-60	28	57
	>60	0	26
CMV	pos	60	75
	neg	45	30
gender	M	82	59
	F	23	46
ABO	A	52	46
	B	9	12
	AB	7	10
	O	37	37
Rh	pos	89	89
	neg	16	16

Patients		n
diagnosis	ALL	18
	AML	34
	AUL	1
	CLL	4
	CML	2
	HL	3
	leukemia	1
	mantle cell lymphoma	1
	MB Hodking	1
	MDS	9
	MDS/AML	2
	MM	11
	myelofibrosis	6
	myeloma	4
	NHL	5
	SAA	1
	T-ALL	1
	T-PLL	1
	all	**105**

Stem cell source	n
peripheral blood	91
bone marrow	14

Clinical outcome	n
no aGvHD	53
aGvHD 1-4	50
aGvHD na	2
no cGvHD	52
cGvHD 1-2	43
cGvHD na	10
no relapse	65
relapse	37
relapse na	3

population study; recipients n=115, donors n=481
clinical study; n=105 recipient/donor pairs

Table 2. HLA and C4 match in the study population

Matching model	match grade	all donors n (%)	FI donors n (%)	non-FI donors n (%)
5 HLA genes				
HLA-A,-B,-C,-DRB1,-DQB1	10/10 match	399 (83.0)	179 (89.1)	220 (78.6)
n=481 subjects	any mismatch grade	82 (17.0)	22 (10.9)	60 (21.4)
	9/10 match	48 (10.0)	14 (7.0)	34 (12.1)
	8/10 match	24 (5.0)	8 (4.0)	16 (5.7)
	7/10 match	9 (1.9)	0 (0.0)	9 (3.2)
	6/10 match	1 (0.2)	0 (0.0)	1 (0.4)
6 HLA genes				
HLA-A,-B,-C,-DRB1,-DQB1,-DPB1	12/12 match	108 (23.0)	52 (11.0)	56 (11.9)
n=470 subjects	any mismatch grade	362 (77.0)	144 (30.6)	218 (46.4)
	11/12 match	241 (51.3)	113 (24.0)	128 (27.2)
	10/12 match	85 (18.1)	25 (5.3)	60 (12.8)
	9/12 match	20 (4.3)	5 (1.1)	15 (3.2)
	8/12 match	10 (1.9)	1 (0.2)	9 (1.9)
	7/12 match	4 (0.9)	0 (0.0)	4 (0.9)
	6/12 match	2 (0.4)	0 (0.0)	2 (0.4)
	no HLA-DPB1 result	11 (2.3)	5 (1.1)	6 (1.3)
C4 gene				
25 SNPs	25/25 match	263 (54.7)	155 (77.1)	108 (38.6)
n=481 subjects	any mismatch grade	218 (45.3)	46 (22.9)	172 (61.4)
	24/25 match	39 (17.9)	9(19.6)	30(17.4)
	23/25 match	36 (16.5)	4(8.7)	32(18.6)
	22/25 match	45 (20.6)	12(26.1)	33(19.2)
	21/25 match	26 (11.9)	6(13.0)	20(11.6)
	20/25 match	28 (12.8)	9(19.6)	19(11.0)
	19/25 match	22 (10.1)	3(6.5)	19(11.0)
	18/25 match	19 (8.7)	3(6.5)	16(9.3)
	17/25 match	3 (1.4)	0.0	3(1.7)
putative haplotype model, 5 HLA genes				
HLA-A,-B,-C,-DRB1,-DQB1, C4	C4 match, 10/10 HLA match	250 (52.0)	150 (74.6)	100 (35.7)
n=481 subjects	any mismatch grade	231 (48.0)	51 (25.4)	180 (64.3)
	C4 match, HLA mismatch	13 (2.7)	5 (2.5)	8 (2.9)
	C4 mismatch, HLA match	149 (31.0)	29 (14.4)	120 (42.9)
	C4 mismatch, HLA mismatch	69 (14.3)	17 (8.5)	52 (18.6)
putative haplotype model, 6 HLA genes				
HLA-A,-B,-C,-DRB1,-DQB1,-DPB1, C4	C4 match, 12/12 HLA match	71 (17.8)	44 (24.6)	27 (12.3)
n=399 subjects	any mismatch grade	328 (82.2)	135 (75.4)	193 (87.7)
	C4 match, HLA mismatch	179 (44.9)	106 (59.2)	73 (33.2)
	C4 mismatch, HLA match	37 (9.3)	8 (4.5)	29 (13.2)
	C4 mismatch, HLA mismatch	112 (28.1)	21 (11.7)	91 (41.4)

HLA-A,-B,-C,-DRB1,-DQB1 HLA-typed samples; recipients n=115, donors n=481 (FI n=201, non-FI n=280)
HLA-A,-B,-C,-DRB1,-DQB1-DPB1 HLA-typed samples; recipients n=115, donors n=470 (FI n=196, non-FI n=274)