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Brief Articles

Assessment by Extended-Coverage Next-Generation Sequencing Typing of DPA1 and DPB1 Mismatches in Siblings Matching at HLA-A, -B, -C, -DRB1, and -DQ Loci

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Allogeneic hematopoietic stem cell transplant from an HLA matched sibling donor is usually the preferable choice. The use of next-generation sequencing (NGS) for HLA typing in clinical practice provides broader coverage and higher resolution of HLA genes. We evaluated the frequency of DPB1 crossing-over events among patients and potential related donors typed with NGS. From July 2016 to January 2018, 593 patients and 2385 siblings were typed. We evaluated sibling matching status in 546 patients, and 44.8% of these patients had siblings that matched at HLA-A, -B, -C, -DRB1, and -DQB1 loci. In 306 patient–HLA matched sibling pairs, we found 6 pairs (1.96%) with 1 DPB1 mismatch, and 5 of these pairs included an additional mismatch in DPA1. No additional mismatches were observed at the low expression loci. Using the T cell epitope algorithm, 4 of these DP mismatches were classified as permissive, 1 as nonpermissive in the host-versus-graft direction, and 1 as nonpermissive in the graft-versus-host direction. The frequency of DPB1 and DPA1 mismatches is low, and their impact in related donor transplants is not well established. Although DP typing in related transplants goes beyond guidelines, it is especially relevant for sensitized patients. NGS-based HLA typing provides full gene coverage, and its use in clinical practice can enable better donor selection.

INTRODUCTION

Allogeneic hematopoietic stem cell transplant (HSCT) is a curative therapy for several malignant and nonmalignant hematologic disorders. Despite the improvements in transplant outcomes with alternative donors, an HLA matched sibling donor is still the preferable choice, although this is available for less than 30% of patients.

The selection of a fully HLA matched sibling donor has been recommended to be based on HLA typing for loci A and B at intermediate or high resolution and HLA-DRB1 at high resolution by molecular methods when family haplotypes can be established. If it is not possible to obtain complete information about haplotype segregation from all family members tested, then extended C locus typing is desirable [1].

Crossing-over events in the HLA region are relatively common. In a recent study of 232 quartet families (928 haplotypes) typed by next-generation sequencing (NGS), we detected 16 recombinant haplotypes that resulted from crossing-over events between HLA-A-C (n = 5), B-DRB (n = 5), and DQB1-DPA1 (n = 5) as well as between C-B (n = 1) loci, resulting in a recombination fraction > 1.7% [2].

The impact of HLA DP mismatches has been thoroughly analyzed in the context of unrelated donor HSCT, and several studies have shown its impact of increasing the risk of graft-versus-host disease (GVHD) [3]. In a study performed more than a decade ago, HLA-DPB1 typing by reference strand-mediated conformation analysis detected 5.3% of disparity in HLA-DPB1 among sibling pairs matched at HLA-A, -B, and -DRB1 loci [4]. DP matching in putative HLA identical siblings was not evaluated routinely until recently when the application of NGS to HLA typing in clinical laboratories affords high throughput and extended sequence coverage.

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In our practice we evaluate the full HLA match grade including DP and anti-HLA antibody compatibility in allogeneic transplants with related and unrelated donors. This testing goes beyond the testing guidelines [1] and has proven to be useful in detecting unexpected mismatches. A 51-year-old Hispanic woman with myelodysplastic syndrome was evaluated for HSCT at the Stanford Histocompatibility Laboratory. She had a 73-year-old sister who matched at all HLA loci with the exception of differences at both DPA1 and DPB1 loci. At these loci the patient carried DPA1*01:03, 02:01 and DPB1*02:01, 11:01, whereas the sibling carried DPA1*02:01, 02:01 and DPB1*11:01, 17:01. The patient was highly sensitized with a combined calculated panel reactive antibody of 100%. The solid-phase assay using single antigen beads showed reactivity against multiple DPB1 alleles of a well-defined cross-reactive group defined by the presence of amino acid "E" at residues 56 of DPB1 [5]; this epitope is present in DPB1*17:01 (Mean fluorescence intensity (MFI) = 3202). Because of the possible increased risk for primary graft failure due to these mild/moderate donor-specific antibodies and the older age of this related donor, it was considered that a young 10/10 HLA-matched unrelated donor who was antibody compatible or with a lower donor-specific antibody would be a better donor option.

This case illustrates the value of the application of NGS to obtain extended HLA typing including DP loci; these tests are of importance when the patient shows humoral sensitization. Our observation indicates that no assumptions should be made about compatibility and matching between partially typed putative "HLA identical." This is particularly applicable when the patient has anti-HLA antibody reactivity against antigens encoded in untested loci.

METHODS

From July 2016 to January 2018, 593 patients and 2385 siblings were typed in the Stanford Histocompatibility Laboratory. All patients were typed at HLA loci A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, and DPB1 using NGS methods (Mia Fora; Immucor, Inc, Norcross, GA) and performed following the manufacturer's instructions [6]. Long-range PCR amplified the entire genes of all class I loci (5'Untranslated region (5'UTR) to 3'UTR) and key regions of class II loci: DPA1, 5'UTR to intron 4; DPB1, intron 1 to intron 4; DQA1, 5'UTR to intron 4; DQB1, 5'UTR to intron 5.

The initial testing for assessing HLA potential match grade of siblings included intermediate resolution level typing of alleles at HLA-A, -B, and -DRB1 loci by PCR amplification combined with oligonucleotide hybridization specific sequence of oligonucleotides probes (SSOP). All matched sibling donors by SSOP were typed using NGS.

RESULTS

We evaluated sibling matching in 546 patients; 44.8% of these patients had siblings that matched at HLA-A, -B, -C, -DRB1, and -DQ loci: 199 patients had 1, 35 patients 2, 7 patients 3, and 4 patients had 4 matched siblings. In 306 patient–sibling pairs, we found 6 pairs (1.96%) in which we observed 1 DPB1 mismatch (Table 1). Five of these pairs included an additional mismatch in DPA1. The only pair matching in DPA1 had an allele mismatch in DPB1. No additional mismatches in alleles were observed at the low expression DRB3/4/5, DQA1, and DQB1 loci.

Table 1
Analysis of Potential Recipient–Matching Donor Pairs

	No. of Cases (%)
Recipient–donor pair	306 (100)
Matching A/B/C/DRB1/DQA1/DQB1/ DRB3/4/5/DPA1/DPB1	288 (94)
One mismatch in A/B/C/DRB1/DQB1	9 (2.9)
DPB1 mismatch	6 (1.9)

Using the T cell epitope algorithm [7], 4 of these DP mismatches were classified as permissive, 1 as nonpermissive in the host-versus-graft direction, and 1 as nonpermissive in the graft-versus-host direction. None of the patients with a donor with 1 mismatch in DP allele had another 10/10 matched sibling (Table 2). Two patients underwent allogeneic HSCT from matched sibling donors with DP mismatch.

Because of limitations in the family size we could not fully define haplotypes in all cases. Despite these limitations we identified 9 possible crossing-over events in 546 patients and their families including 6 cases with DP mismatches plus 2 cases with putative crossing-over events between HLA-A and -C loci and 1 case with a putative recombination between the B and DRB1 loci.

DISCUSSION

A mismatched related donor (a related donor with a mismatch in 1 of the HLA-A, -B, -C, -DRB1, and -DQB1 loci) is considered an acceptable option when a matched related donor is not available. Transplant outcomes comparing 1 antigen or allele mismatched related donor and matched unrelated donor were assessed in a few studies using different methodologies and with conflicting results in acute leukemia [8,9]. A study using high resolution at 10 loci found a higher incidence of graft failure and nonrelapse mortality among mismatched related donors, with a tendency of higher GVHD, resulting in better overall survival for matched unrelated donors [8].

In our study we found a lower frequency of DPB1 mismatches using NGS HLA typing than that found in a previous study [4] in which DPB1 disparities were detected by reference strand-mediated conformation analysis. A recent study suggested that the latter methodology when compared with NGS testing resulted in a high discrepancy rate of 47 discrepancies in 891 transplant pairs for assessing of matching between patients and donors [10]. The observation of this frequency of mismatches between siblings at DP loci most likely resulted from crossing-over events in the intervening region between DQB1 and DPA1; the interval between these loci has a relatively large physical distance in which recombination hot spots have been identified [11]. There is strong linkage disequilibrium between DPA1 and DPB1 loci; the finding of only 1 single match at DPA1 among DPB1 mismatched pairs results from the well-known associations of several DPA1 alleles with more than 1 DPB1 alleles as assessed in our recent study.

The impact of DPB1 mismatches in sibling donor transplants was assessed in a prior study in which patients were typed only at HLA-A, -B, -DRB1, and -DPB1 [9]. This report found an increased incidence of acute GVHD in transplants mismatched at DPB1 compared with transplants fully matched at this locus. In this study HLA-DQB1 typing was performed in the pairs with a mismatch at DPB1. No additional mismatches were found, and the increased risk for acute GVHD could therefore be ascribed only to the effect of DPB1 mismatches.

High-resolution HLA typing is typically performed after low-resolution HLA typing for initial match grade assessment. The high-throughput nature of NGS technology applied in this report enables accurate high-resolution HLA typing in a relatively short period of time (turnaround time of 3 or 4 days). The NGS technology to perform HLA typing has the advantage of resolving virtually all phase ambiguities at all HLA loci with the exception of DPB1, eliminating the unwieldy and time-consuming processes of performing tiered tests required for resolution of ambiguities. DPB1 ambiguities including exon 2/exon 3

Table 2
DPB1 Mismatched Cases

Patient	DPA1 Mismatch	Mismatch in Other LEL	TCE Algorithm	Direction	Another MSD Typed	Transplant	Donor
1	Yes	No	Permissive		No	Yes	mmDP sibling
2	Yes	No	Nonpermissive	HVG	No	Yes	mmDP sibling
3	Yes	No	Permissive		No	No	–
4	Yes	No	Permissive		No	No	–
5	No	No	Permissive		No	No	–
6	Yes	No	Nonpermissive	GVH	No	Yes	Haplo sibling

LEL indicates low expression loci (DRB3/4/5, DQA1/DQB1, DPA1/DPB1); TCE, T-cell-epitope; MSD, matched sibling donor; mmDP, DP mismatch; HVG, host-versus-graft; GVH, graft-versus-host.

shuffling result from lack of phasing of these exons because of the absence of informative single nucleotide polymorphisms (SNPs) in the intervening intron. With the use of long-range PCR, it is possible to analyze the nucleotide sequences of all exons, intervening introns, and flanking regions of the HLA genes. This process allows for unambiguous genotype assignment [11]. The impact of mismatches of allele variants differing at coding regions of antigen-recognition sites has been well established; in contrast, the impact of variants differing at coding regions outside of antigen-recognition sites and/or introns is largely unknown. Recent work indicates that variations in downstream exon 2 of DPB1 show a close correlation with the levels of expression of DPB1 molecules [12], indicating that genetic regions outside exon 2 that determine the structure of the peptide binding regions have regulatory roles in determining DPB1 expression levels. Extended coverage afforded by NGS typing allows the typing for polymorphisms that correlate exactly with SNPs at the 3'UTR region; testing for downstream variations beyond exon 2 allows the classification of expression levels and their putative immunogenicity [13] of mismatched DPB1 alleles.

The frequency of DPB1 and DPA1 mismatches is low, and the impact of these mismatches in related donor transplants is not well established. Although these loci are not routinely typed at many centers, anti-HLA antibodies should be tested routinely, in particular if some of the HLA loci are not tested. It is particularly important to assess humoral sensitization in patient who have been exposed to HLA allo-antigens. The adoption of full gene coverage NGS-based HLA typing is likely to become the gold standard test for determining compatibility in both HSCT and solid organ transplant because it can afford the highest resolution and gene coverage that allows for full evaluation of the nature of HLA allele and epitope mismatches. The high-throughput nature of NGS technologies extends sequence coverage; in addition, NGS-based typing allows testing for alleles at HLA loci that have not been tested routinely (DRB3/4/5, DQA1, DPA1). The additional testing of these loci can be performed without significant increases in both labor and cost. In addition, the extended gene coverage afforded by NGS HLA typing allows the characterization of expression levels of mismatched alleles and the detection of possible known and novel deleterious mutations that may result in no expression of alleles, unraveling additional HLA mismatches that many currently used tests with limited sequence coverage do not detect. These NGS tests paired with detailed antibody testing assessing reactivity for HLA epitopes allows a full assessment of compatibility between patients and donors, allowing for optimal matching categorization, prioritization, and selection of donors.

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