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Identification of the rs9277534 HLA-DP expression marker by next generation sequencing for the selection of unrelated donors for hematopoietic cell transplantation

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ABSTRACT

Mismatching of an unrelated donor against a high-expression HLA-DPB1 recipient allele is associated with a high risk of graft-versus-host disease and mortality. The Seattle Cancer Care Alliance (SCCA) and Fred Hutchinson Cancer Research Center transplant program employs an algorithm to match for HLA-A, B, C, DRB1, DQB1 and DPB1 alleles (12/12) and to avoid, whenever possible, donor mismatching against a recipient high-expression HLA-DPB1 allele. HLA-DPB1 expression is associated with the rs9277534 A/G polymorphism located in the 3′UTR of the HLA-DPB1 gene. Next generation sequencing of HLA-DPB1 using the Illumina TruSight HLA V2 Sequencing Panel and Conexio Assign software analyses provides information on rs9277534 variants without the need for any additional SNP testing. Here we present the molecular location of rs9277534 in NGS data and discuss the challenges to resolve HLA-DPB1 ambiguities.

1. Introduction

HLA matching at high resolution for HLA-A, B, C, and DRB1 is associated with increased overall survival and decreased transplant-related mortality and graft-versus-host disease (GVHD) [1,2]. Current guidelines for matching hematopoietic cell transplant (HCT) donors include high resolution HLA matching defined as identity for nucleotide sequences within the antigen recognition domains (ARD) of exon 2 and 3 for HLA Class I and exon 2 for HLA Class II. The ARD provides specificity for the peptides being presented to the T-cell receptor [3]. The clinical impact of variations outside the ARD is thought to be minimal [4–7]. However, recent studies demonstrate that variations in regulatory regions are clinically relevant. Mismatching between a high-expression recipient HLA-DPB1 and a low-expression donor HLA-DPB1 allele is associated with a high risk of GVHD [8].

HLA-DPB1 expression level is associated with rs9277534 located in the 3′ untranslated region (3′UTR) of the HLA-DPB1 gene. This SNP was previously found to correlate with progression of chronic hepatitis B virus (HBV) infection [9] in European and African-American populations. HLA-DPB1 surface protein levels and mRNA levels in individuals with the rs9277534GG genotype were significantly higher compared to individuals with AG or AA genotypes. The correlation of rs9277534 genotype and HLA-DPB1 mRNA expression levels were confirmed in transplant patients and their unrelated donors [8]. Even in HLA-12/12-matched transplants, the presence of the rs9277534G allele is associated with a higher risk of acute GvHD compared to the absence of rs9277534G (hazard ratio for 146 HLA-DPB1-matched rs9277534AG transplants vs 420 rs9277534AA transplants was 1.86; for 22 rs9277534GG transplants vs 420 rs9277534AA transplants was 1.53 as demonstrated by Petersdorf et al.). The authors suggested that the minor histocompatibility antigens presented by highly-expressed HLA molecules on the recipient's cells provoke robust graft-versus-host alloreactivity from the donor cells compared with HLA molecules expressed at low levels on the cell surface [8].

With the exception of whole gene sequencing approaches, most routine HLA genotyping methods do not cover the HLA-DPB1 3′UTR and an additional screening would increase the cost of HLA genotyping in the clinical setting for donor selection. Schöne et al. recently published a study demonstrating that the rs9277534 genotype can be accurately predicted from the nucleotide sequence of HLA-DPB1 exon 3 determined in the course of high-resolution HLA-DPB1 genotyping by next-generation sequencing (NGS) [10].

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We report the use of next-generation sequencing (NGS) for high resolution HLA typing as informative for rs9277534 variants without additional SNP testing. Although NGS provides phase-resolved HLA typing for 11 loci, the HLA-DPB1 locus still has the highest ambiguity rate, which presents challenges for donor selection based on the HLA-DPB1 expression level.

2. Materials and methods

2.1. Specimens

Ninety three cases including 35 combinations of ambiguities were selected from total of 2193 HLA typing results performed by NGS between 2017 and 2018 in our lab.

2.2. 2.2. HLA typing method

NGS is performed using Illumina TruSight HLA V2 Sequencing Panel, which covers whole gene for HLA-A, B, C, DQA1, DQB1 and DPA1 loci; exon 2 through exon 5 for HLA-DRB1/3/4/5 loci; and the end of intron 1 (0.9 kb) through 3′UTR for HLA-DPB1 locus. Selected cases were retrospectively analyzed using Conexio Assign software (IMGT library 3.26–3.33) for identifying rs9277534 genotype. Web based NCBI dbSNP GeneView section is used as an independent tool to verify the genomic coordinate for rs9277534. Each HLA-DPB1 allele was mapped and assigned as low- or high-expression, as described in previous studies [8–10].

3. Results

3.1. Mapping the position of rs9277534

The genomic coordinate of rs9277534 as documented in the NCBI dbSNP GeneView database [11] is used as a reference for assigning the rs9277534 genotype. The rs9277534 SNP is located at 33,087,030 bp on 6p21 and encodes an “A” or “G” allele. The Trusight HLA V2 NGS panel yields a 9.7 kb amplicon for HLA-DPB1 gene, which spans the end of intron 1 through the 3’UTR as shown in Fig. 1. The rs9277534 SNP is found at 11,361 bp within the HLA-DPB1 locus, which corresponds to 792 bp within the 3′UTR. As highlighted in the red box (Fig. 2), the consensus rs9277534 is “R” which designates that either “A” or “G” variants could exist at the position and is consistent with the NCBI dbSNP database. As shown in our analyses output, the SNP genotypes for DPB1*04:01 and DPB1*03:01 alleles are rs9277534A and rs9277534G, respectively, which confirms the previously reported SNP genotypes for these alleles.

3.2. Verification of HLA-DPB1 expression markers predicted by exon 3 sequence features

It has been reported that 2 most common sequence features in exon 3 defined by 7 SNPs predict HLA-DPB1 expression markers [10]. Reference SNP identification numbers of the 7 SNPs are rs1126537, rs1126541, rs1042187, rs1042212, rs1042331, rs104335, and rs1071597, and their cDNA locations within the HLA-DPB1 gene are 96.2; 98.3; 107.1; 118.3; 167.3; 170.2 and 179.3, respectively. We have used exon 3 sequence features linked to the rs9277534 as a quality control to identify rs9277534 expressions in over 40 samples. We have verified that the exon 3 sequence feature “GTTGTCT” was linked to rs9277534A and “ACCACTC” was linked to rs9277534G (Table 1). Of note, base call for rs104335 linked to rs9277534G is “T” in our data as well as in IMGT consensus, however it was previously reported as “A” by Schöne et al. [10], which probably was a “typo” mistake.

3.3. Identification of the rs9277534 expression markers by 3′UTR sequencing data

We have identified expression markers for rs9277534 in ten samples representing ten alleles not previously reported by Schöne et al. (Table 2) using 3′UTR empirical data generated by NGS. The rs9277534 expression markers of the newly identified ten alleles were confirmed by exon 3 sequence features linked to the SNP as well as IMGT reference sequences at the 3′UTR. All ten alleles rs9277534 expression markers were 100% concordant with the predictions by exon 3 sequence features. Five of the ten alleles rs9277534 expression markers were also confirmed by 3′UTR sequences in IMGT database; however, the remaining five alleles full sequencing data were not available in the IMGT database.

3.4. Current ambiguities in HLA-DPB1 NGS typing

Thirty-five combinations of ambiguities have been detected in our NGS typing (from March 2017 to April 2018) performed in 2193 samples by three field analyses. Of these, seven combinations (20%) have been resolved by library updates and seven (20%) have been resolved by extended 4-field analyses; however, new ambiguities continue to appear as new alleles are named to the IMGT database (Table 3). Many (49%) of these ambiguities are amplicon ambiguities, caused by lack of coverage from 5′UTR to the end of intron 1 in the TruSight HLA V2 sequencing panel. For example, DPB1*13:01/107:01, in which the differences are in exon 1, (cDNA −22.1 and −14.2), cannot be resolved by our NGS. The second most common type of ambiguity (17%) is conditional due to the homology between the alleles, which leads to large gaps in heterozygous base positions and causes phase ambiguity. For example, DPB1*04:01:01 paired with *04:02:01 combinations have over 4 kb homology in intron 2, which present cis-trans ambiguities with certain pairs (ex: DPB1*126:01; and DPB1*105:01). The amplicon ambiguities cannot be resolved without complete sequence information across the entire gene. However, some of the conditional ambiguities can be resolved by extended 4-field analyses, depending on the variants paired in the combination. For example, using Conexio Assign software, 3-field analysis can yield ambiguous results (e.g. DPB1*04:01/126:01 with DPB1*04:02/105:01). However, 4-field analyses can resolve the ambiguities when certain allele pairs are present. In Fig. 3, HLA-DPB1*04:01:01:10 is paired with one of the DPB1*04:02:01:01/03/06 variants, excluding the DPB1*126:01 and *105:01 pair due to 3 mismatches in introns and 2 phase mismatches. Although extended 4-field analyses may help to resolve some ambiguities, it may give more “variant” ambiguities in other combinations.

3.5. HLA-DPB1 allele vs HLA-DPB1 expression level ambiguities

There are two different kinds of ambiguities when the level of expression of the HLA-DPB1 alleles is considered. All DPB1 alleles in the string may have the same expression (all low or high: DPB1*04:01/126:01 [A/A], *04:02/105:01 [A/A] or DPB1*03:01/651:01 [G/G], *104:01/10:01 [G/G], respectively) (Table 3). Alternatively, the string of ambiguities may contain a mixture of high- and low-expression alleles (DPB1*104:01/124:01 [G/A], *02:01/547:01 [A/G]). When all alleles within an ambiguity string have the same level of expression, complete resolution of the ambiguity might not alter the level of expression of the patient/donor HLA-DPB1 mismatch; however, when the ambiguity string contains a mixture of high- and low-expression alleles, resolution of the allele is needed to define whether the mismatch is high or low-risk. Most ambiguities may be resolved with complete sequence data for each allele and remains an important area of investigation for future studies.

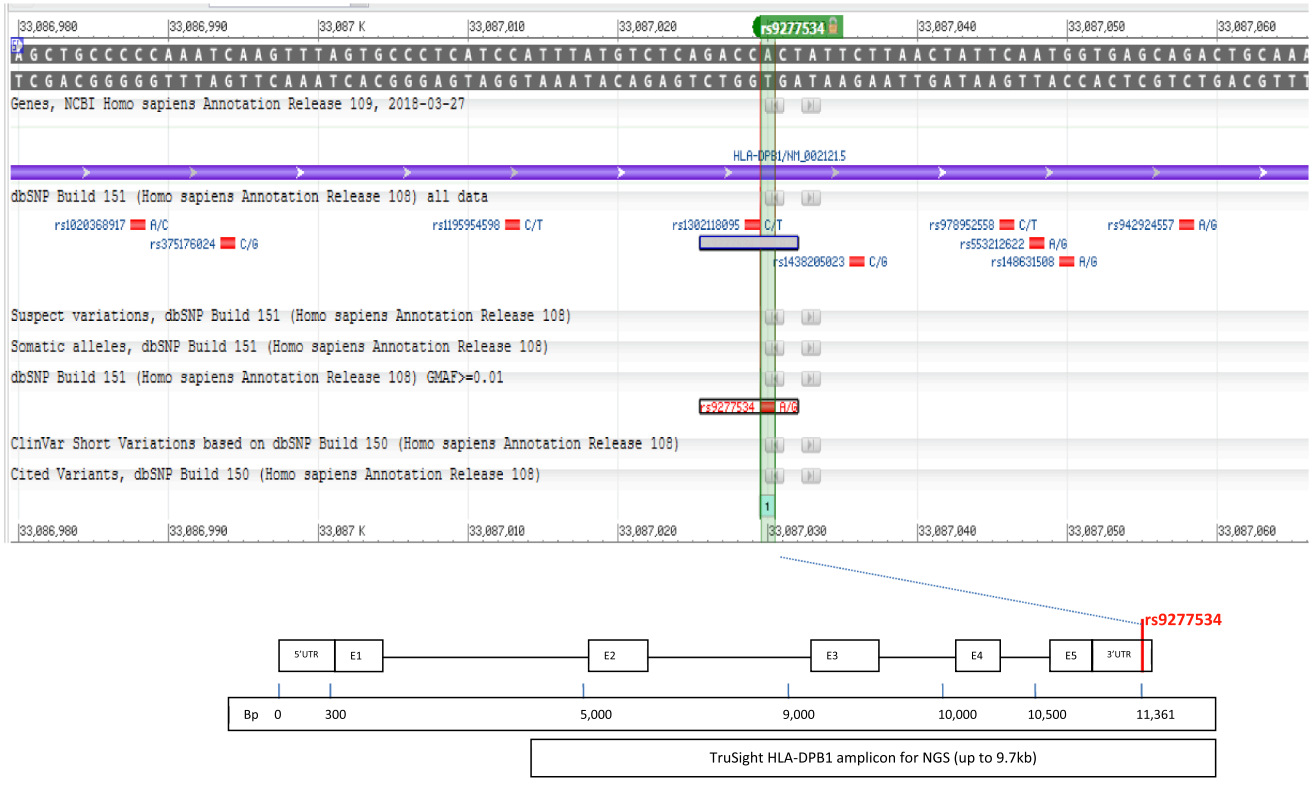


Fig. 1. Molecular location of HLA-DPB1 rs9277534 on NCBI dbSNP GeneView database and within HLA-DPB1 gene at 3′UTR. rs9277534 consensus variants are A/G as highlighted at the position of 33,087,030 bp on GeneView database and 11,361 bp within HLA-DPB1 gene. Surrounding sequences highlighted in gray under the genomic coordinate were used to verify the exact location of rs9277534 within 3′UTR in our NGS analyses output. The current coverage of HLA-DPB1 gene by TruSight HLA V2 Sequencing Panel is displayed in the bottom of the figure. E1–E5 refer exons 1–5.

4. Discussion

The degree of donor HLA matching is predictive of clinical outcome after HCT. Currently, HLA matching of unrelated donors is focused on HLA allele level matching within the ARD, i.e. matching at exons 2 and 3 for HLA class I and exon 2 for HLA class II.

TruSight HLA V2 Sequencing Panel covers all required regions for most of the HLA loci except HLA-DRB and DPB1 loci, since the primers are not yet designed to cover 5′UTR, exon 1 and intron 1. The large size of intron 1 in HLA-DRB1/3/4/5 (7673 bp–9564 bp) and DPB1 (4636 bp) might prevent the design of primers for these regions located outside of ARD, which leads to amplicon ambiguities in these loci. Conditional ambiguities also occur in allele combinations with large gaps (1 kb or more) between heterozygous base positions used to phase

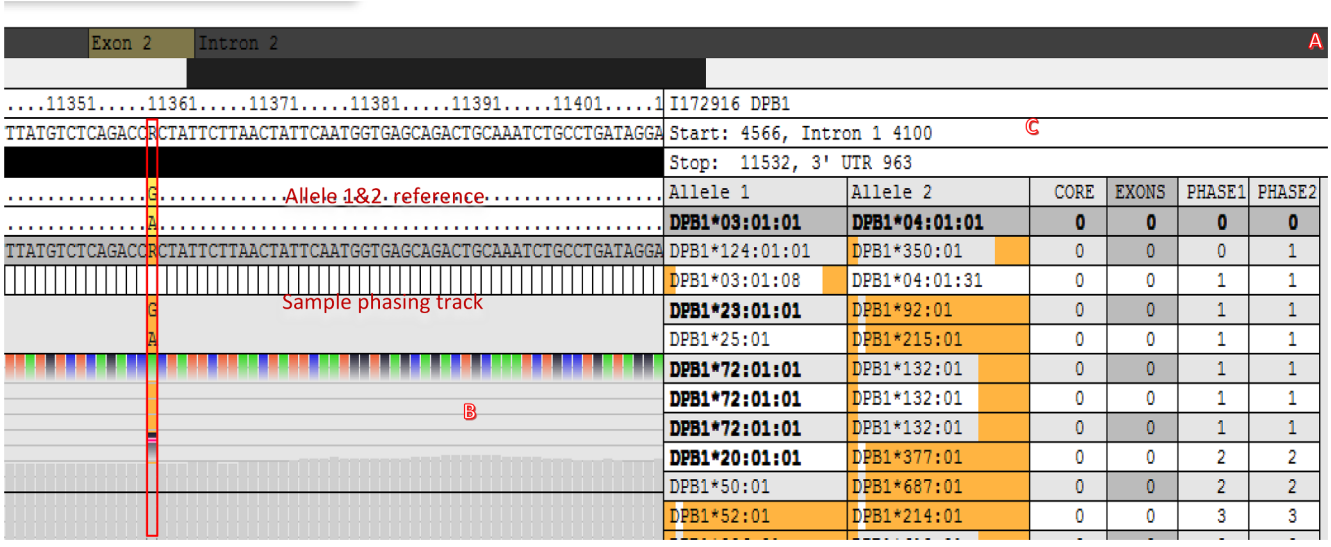


Fig. 2. Conexio Assign analyses output. Confidence Plot and Locus Structure in the top (A), Sequences Panel in the left (B) and Results Panel in the right (C). HLA-DPB1*03:01:01, *04:01:01 pair is un-ambiguously assigned without any mismatches in exons and phasing. Locus consensus and sample base calls at position 11,361 at 3′UTR are highlighted with red box. rs9277534 genotype for HLA-DPB1*03:01:01 is G and HLA-DPB1*04:01:01 is A, presented in the sample phasing track correlates with the Allele 1&2 reference base calls.

Table 1
Molecular locations and genotypes of rs9277534 and 7 SNPs associated with rs9277534.

HLA-DPB1 Expression Marker	Exon 3 SNPs associated with rs9277534							rs9277534
	rs1126537 c.96.2	rs1126541 c.98.3	rs1042187 c.107.1	rs1042212 c.118.3	rs1042331 c.167.3	rs104335 c.170.2	rs1071597 c.179.3	11361 bp within DPB1 gene or 792 bp in 3′UTR
High	A	C	C	A	C	T	C	G
Low	G	T	T	G	T	C	T	A

Molecular locations of 7 SNPs associated with rs9277534 in cDNA positions at exon 3 are shown. Molecular location of rs9277534 is at 11361 bp within the entire HLA-DPB1 gene, which correlates to the nucleotide position of 792 bp within the 3′UTR of HLA-DPB1 gene. The rs9277534G is associated with high expression HLA-DPB1 alleles, whereas rs9277534A is associated with low expression HLA-DPB1 alleles. The rs9277534G-linked sequence feature of exon 3 is ACCACTC and rs9277534A-linked sequence feature of exon 3 is GTTGTCT.

Table 2
Identification of HLA-DPB1 expression markers by 3′UTR sequencing data.

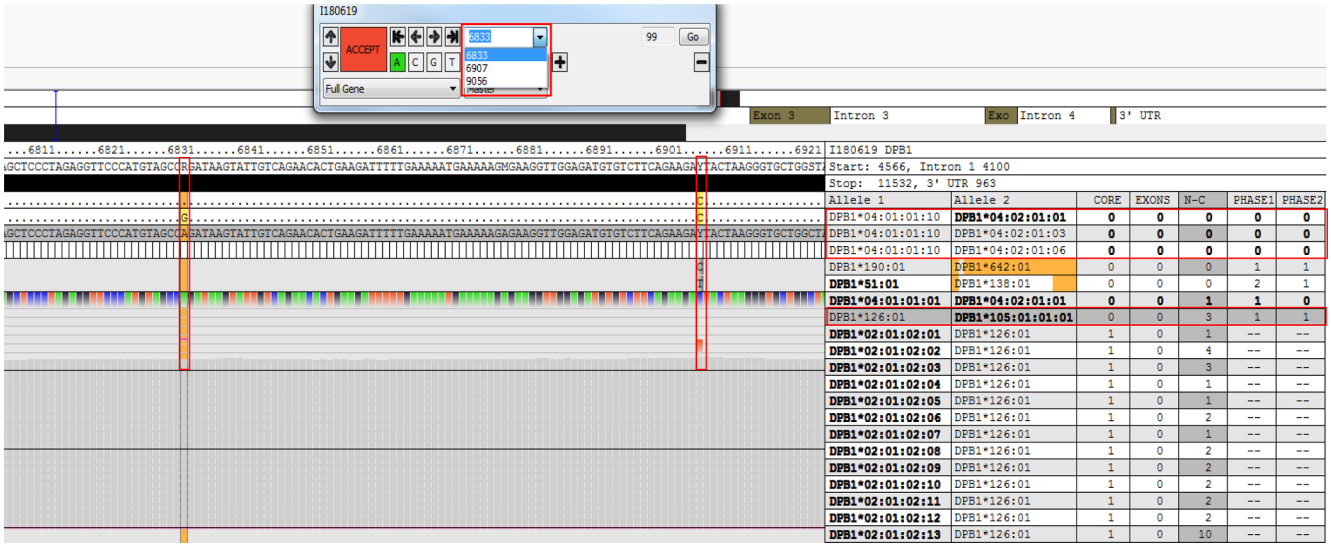
HLA-DPB1 Alleles	*640:01	*48:01	*145:01	*572:01	*515:01	*583:01	*108:01	*584:01	*65:01	*106:01
rs9277534	A	A	A	G	G	A	A	G	G	A
Exon 3 SF	A-linked	A-linked	A-linked	G-linked	G-linked	A-linked	A-linked	G-linked	G-linked	A-linked
IMGT Reference data for rs9277534	A	A	–	G	–	–	–	G	–	A

rs9277534 genotyping for 10 HLA-DBP1 alleles newly identified by NGS 3′UTR empirical data are shown. rs9277534 genotypes for these alleles are concordant with the predicted genotypes using exon 3 sequence features (SF) as well as IMGT reference data. "–" represents the alleles, where 3′UTR data for rs9277534 are not available in IMGT.

Table 3
HLA-DPB1 ambiguity list and rs9277534 genotype identified by NGS at 3′ untranslated region.

HLA-DPB1 ambiguity	Rs9277534 genotype	Note	HLA-DPB1 ambiguity	Rs9277534 genotype	Note
01:01/11:01 ***417:01/654:01***	GG	Conditional ambiguity	**13:01/*107:01*** **01:01:01**	GG	Amplicon ambiguity
01:01/*162:01*† 02:01/***461:01***†	GA	Resolved by library update and N-C analysis	**13:01/*107:01*** 02:02:01	GA	Amplicon ambiguity
02:01/416:01† 124:01†/*351:01*	AA	By N-C analysis (27 mm at N-C, 2 phase mm)	**13:01/*107:01*** 02:01:02	GA	Amplicon ambiguity
03:01/*351:01* 04:02/**463:01**	GA	Amplicon ambiguity. No coverage on 5′UTR, I1	**13:01/*107:01*** **03:01:01**	GG	Amplicon ambiguity
03:01/*351:01* 126:01/**350:01**	GA	Conditional Cis-trans amb. in exons, no full IMGT data	**13:01/*107:01*** 04:01:01	GA	Amplicon ambiguity
03:01/**04:01** ***650:01***/**10:01**	GG	Resolved by IMGT 3.29, new var. amb. by 3.33	**13:01/*107:01***/133:01 04:01/**350:01**	GA	Amplicon ambiguity
03:01/124:01† 04:01/**350:01**†	GA	By N-C analysis	**13:01/*107:01*** 04:02:01	GA	Amplicon ambiguity
04:01/**350:01**† **14:01**/*651:01*†	AG	By N-C analysis	**13:01/*107:01*/** ***519:01*** **05:01/*135:01***	GG	Amplicon ambiguity
04:02/105:01† 02:01/416:01†	AA	By N-C analysis	**13:01/*107:01*** **10:01:01**	GG	Amplicon ambiguity
04:02/105:01 04:01/126:01	AA	Conditional ambiguity	**13:01/*107:01*** **11:01:01**	GG	Amplicon ambiguity
04:02/105:01 17:01/*460:01*	AA	Conditional ambiguity	**13:01/*107:01*** **14:01:01**	GG	Amplicon ambiguity
04:02/105:01 23:01/138:01	AA	Conditional ambiguity	**13:01/*107:01*** **21:01**	GG	Amplicon ambiguity
04:02/105:01 124:01/*351:01*	AA	Resolved by IMGT3.29, new var. amb. by 3.33	**13:01/*107:01*** **26:01:02**	GG	Amplicon ambiguity
19:01†/*106:01* **34:01**	AG	Resolved by library update	**13:01/*107:01*** 105:01	GA	Amplicon ambiguity
35:01:01 105:01/**463:01**†	GA	Resolved by library update	**104:01**/124:01† 02:01/**414:01**†	GA	By N-C analysis N-C 31 mm
13:01/*107:01* **104:01:01**	GG	Amplicon ambiguity	**104:01**/124:01 02:02/***547:01***	GA	Conditional ambiguity
13:01/*107:01* 04:02:01	GA	Amplicon ambiguity	**104:01**/124:01† 04:01/**350:01**†	GA	Resolved by library update
			104:01/124:01† 71:01:01	GA	Resolved by library update

Bolded: Confirmed high expression alleles, published. ***Bolded italics***: Predicted high expression alleles by exon 3 SF.
Not bolded: Confirmed low expression alleles, published. *Not bolded italics*: Predicted low expression alleles by exon 3 SF.
Var.amb: Variant ambiguity. †Ambiguities resolved. Underlined: Ambiguities resolved, but new library creates more variant ambiguities at 4th field in some combinations. N-C: Non-coding regions in NGS typing, mm: mismatch, I1: Intron 1.



Allele 1	Allele 2	CORE	EXONS	N-C	PHASE1	PHASE2
DPB1*04:01:01:10	**DPB1*04:02:01:01**	**0**	**0**	**0**	**0**	**0**
DPB1*04:01:01:10	DPB1*04:02:01:03	**0**	**0**	**0**	**0**	**0**
DPB1*04:01:01:10	DPB1*04:02:01:06	**0**	**0**	**0**	**0**	**0**
DPB1*190:01	DPB1*642:01	0	0	0	1	1
DPB1*51:01	DPB1*138:01	0	0	0	2	1
DPB1*04:01:01:01	**DPB1*04:02:01:01**	**0**	**0**	**1**	**1**	**0**
DPB1*126:01	**DPB1*105:01:01:01**	0	0	3	1	1
DPB1*02:01:02:01	DPB1*126:01	1	0	1	--	--
DPB1*02:01:02:02	DPB1*126:01	1	0	4	--	--
DPB1*02:01:02:03	DPB1*126:01	1	0	3	--	--
DPB1*02:01:02:04	DPB1*126:01	1	0	1	--	--
DPB1*02:01:02:05	DPB1*126:01	1	0	1	--	--
DPB1*02:01:02:06	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:07	DPB1*126:01	1	0	1	--	--
DPB1*02:01:02:08	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:09	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:10	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:11	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:12	DPB1*126:01	1	0	2	--	--
DPB1*02:01:02:13	DPB1*126:01	1	0	10	--	--

Fig. 3. Four field analysis output of HLA-DPB1 NGS typing. Un-ambiguous 3-field result for the HLA-DPB1*04:01:01, *04:02:01 allele pair was assigned by 4-field analysis of Conexio Assign software using IMGT library 3.29.0 that was ambiguous by 3-field analysis (DPB1*04:01:01/126:01, *04:02:01/105:01). The HLA-DPB1*126:01, *105:01:01:01 pair was excluded by 3 positions (at 6833, 6907 and 9056 bp, shown in the mismatch list field of the Navigator) in Intron 2 in addition to two phase-mismatches as highlighted in red boxes. Briefly, if HLA-DPB1*126:01, *105:01:01:01 is present, then AG at 6833 bp, CC at 6907 bp and GG at 9056 bp should be observed; however, NGS data shows AA, CT and AG at those positions, respectively. Although 4-field analysis resolved the 3-field ambiguity, some variant ambiguities at the fourth field still exist as illustrated in Results Panel (DPB1*04:01:01:10, *04:02:01:01/03/06).

alleles [12]. This occurs especially in HLA-DPB1, in which certain combinations (e.g., DPB1*04:01:01:01, *04:02:01:02/05/10 or DPB1*126:01:01:01, *105:01:01:02/04/07) are lacking heterozygous positions between exon 2 and exon 3 for over 4 kb and therefore in some variants DPB1*04:01:01/126:01:01, *04:02:01/105:01:01 ambiguities could not be excluded. To augment the commercially available NGS HLA typing kits, Shiina and Ehrenberg developed cost-effective HLA typing primers for long-range PCR based NGS to span the entire HLA gene [13,14]. In current clinical practice, HLA ambiguities can be reported in G groups, which define alleles that share identical nucleotide sequences within the ARD or P groups, which define alleles that differ in nucleotide sequences in core exons but that encode identical ARD [3]. Therefore, HLA ambiguities within the same G or P group do not require resolution except for non-expressed null alleles by current NMDP guidelines [15].

Regardless of the allele level ambiguity, direct ascertainment of rs9277534 through NGS typing paves the way for unambiguous assignment of the HLA-DP expression level without additional SNP testing. However, HLA-DPB1 alleles within the same G group may not always share identical expression levels, presenting challenges for donor selection, especially when the ambiguous alleles are common. Future improvements in NGS coverage should reduce these ambiguities.

Mayor et al. recently demonstrated that overall survival was significantly improved in patients with HLA-12/12 ultra-high resolution HLA matching at the full gene level for HLA class I (including regions outside of the ARD, introns and UTRs) and within coding DNA sequences (exons only) for HLA class II genes compared to any mismatching at this level of resolution [16]. In addition, recent disease association studies have shown that variants in non-coding regions of HLA-DPB1 correlate with the risk of infection and autoimmune diseases [8,9] implying that these variants play role in HLA-DP expression. It has also been demonstrated that high expression of HLA-C allele is associated with protection against HIV; in contrast, high expression of HLA-C allele has a deleterious effect in Crohn's disease [17]. In unrelated donor HCT, an increased level of expression of patient's mismatched HLA-C allele is associated with high risk of grade III–IV acute GVHD, non-relapse mortality and mortality [18]. These findings collectively show that variants outside the ARD could have functional impacts through different pathways.

Current HLA matching for HCT is based on 10/10 match at HLA-A, B, C, DR and DQ loci. Due to the presence of recombination hotspots between HLA-DQB1 and DPB1 loci, HLA-DPB1 disparity is frequently observed. In the recent comprehensive review of HLA-DPB1 matching in HCT, Fleischhauer et al. emphasized that limited alloreactivity is sufficient for graft-versus-leukemia effect, whereas aggressive alloreactivity can lead clinically uncontrollable GVHD. The permissive mismatches, on the other hand, may minimize the risk of GVHD while maintaining GVL effect [19]. Recent studies also strongly recommend avoiding non-permissive HLA-DPB1 mismatches to decrease transplant-related mortality and acute GVHD [20,21]. Two different models have already been developed to predict permissible HLA-DPB1 alleles using (1) T-cell epitopes (TCE) and (2) Predicted Indirectly ReCognizable HLA-DPB1 Epitopes (PIRCHE) [22–24]. The TCE model is based on differences within the ARD and hypervariable regions of T-cell receptor, and thus directly recognizes peptides. In contrast, the PIRCHE model indirectly recognizes polymorphic peptides derived from the mismatched HLA alleles found in all exons. HLA-DPB1 non-permissive mismatches predicted by the TCE model are associated with significantly greater TRM and overall mortality compared to HLA-DPB1 permissive mismatches; however, both permissive and non-permissive HLA-DPB1 mismatches were equally associated with increased risk of GVHD [25,26]. Increased numbers of PIRCHEs correlated with an increased risk of acute GVHD after HCT and de novo development of donor specific antibodies after kidney transplantation [27]. In addition to these models based on the structural polymorphisms, a new approach developed by Petersdorf et al. demonstrates the functional significance of polymorphisms in noncoding MHC regions [8]. Their study showed that A or G variants of HLA-DPB1 rs9277534 located within the 3′UTR is associated with HLA-DPB1 expression levels; HCT from donors with the low-expressing alleles mismatched with recipients with the high-expressing alleles had a high risk of acute GVHD [8]. Morishima et al. also demonstrated that rs9277534 represented a highly conserved HLA-DPB1 exon 3 to 3′UTR region and may provoke acute GVHD differently than the TCE mismatching algorithm, which is informative for exon 2 polymorphisms [28]. Future studies considering both structural and functional consequences of mismatched allele variants may enhance the selection of mismatched donors.

5. Summary

Identification of the rs9277534 genotype in routine HLA typing by NGS provides information for avoiding mismatching against high-expression HLA-DPB1 alleles in the recipient, without additional SNP testing.

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