ANALYSIS OF 37,000 CAUCASIAN SAMPLES REVEALS TIGHT LINKAGE BETWEEN SNP RS9277534 AND HIGH RESOLUTION TYPING OF HLA-DPB1

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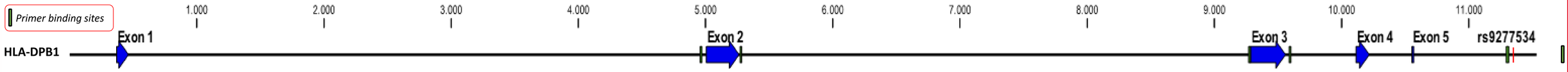
Introduction

HLA-DPB1 mismatching between patients and unrelated donors is known to increase the risk of acute graft-versus-host-disease (GvHD) after hematopoietic stem cell transplantation. If only HLA-DPB1 mismatched donors are available, the genotype defined by the Single Nucleotide Polymorphism (SNP) rs9277534 can be used to select mismatched donors that are well-tolerated. [1] However, since rs9277534 resides within the 3' untranslated region (UTR), it usually is not analyzed during DPB1 routine typing.



Methods

To verify whether rs9277534 can be inferred from standard DPB1-typing, we analyzed 37,261 samples of mostly Caucasian origin for their linkage between rs9277534 and HLA-DPB1 typing results based on sequencing exons 2 and 3. rs9277534 located in the 3' UTR was amplified by PCR and sequenced on Illumina MiSeq or Hiseq instruments. SNP calling was performed with FasType (DKMS Life Science Lab). DPB1 was typed based on sequencing exons 2 and 3 as described before [2, 3]. All samples with unclear results were reanalyzed using PacBio SMRT sequencing.

Results and Conclusion

We established the rs9277534 linkage for 40 DPB1 alleles:

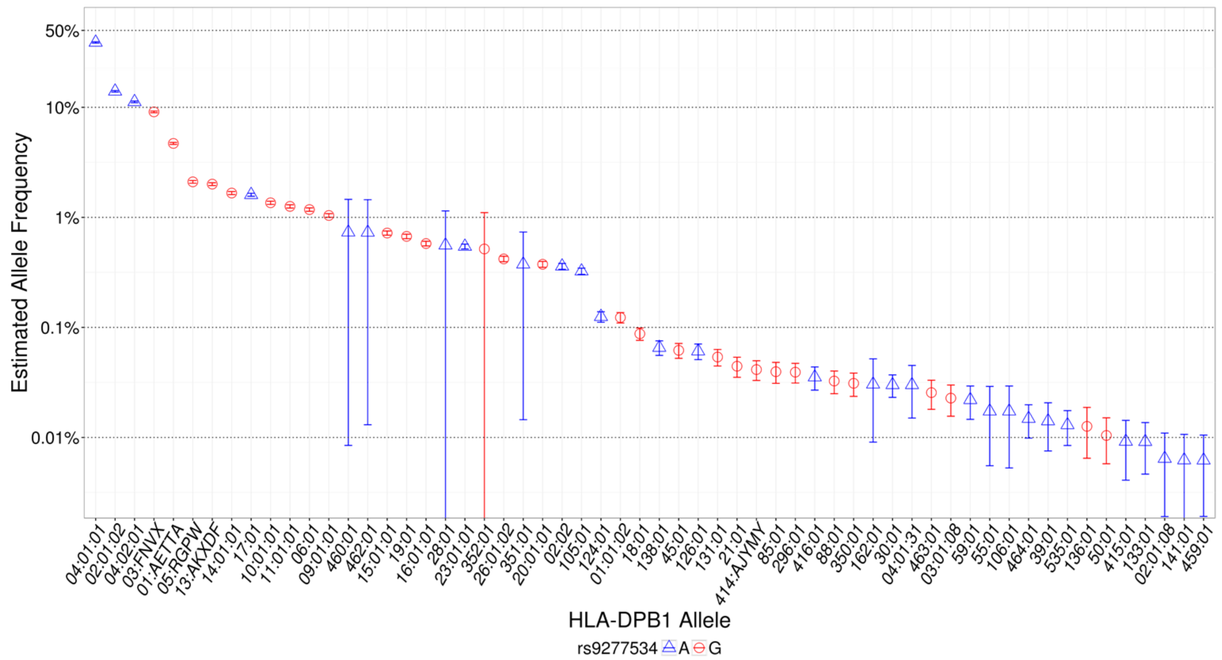
rs9277534	Known Linkages[1]	Novel Linkages
A	02:01, 02:02, 04:01, 04:02, 17:01	23:01, 24:01, 30:01, 31:01, 33:01, 40:01, 41:91, 46:01, 47:01, 51:01, 55:01, 71:01, 72:01, 81:01, 94:01, 105:01, 109:01, 124:01, 126:01, 128:01, 138:01. 464:01, 535:01
G	01:01, 03:01, 05:01, 06:01, 09:01, 10:01, 11:01, 13:01, 14:01, 15:01, 16:01, 19:01, 20:01	18:01, 21:01, 26:01, 29:01, 35:01, 36:01, 45:01, 52:01, 57:01, 78:01, 85:01, 88:01, 90:01, 104:01, 130:01, 131:01, 296:01

After primary analysis of 37,261 samples, 19 showed an unexpected rs9277534. SMRT sequencing disproved all of these as artefacts.

Expected rs9277534	Samples	Unexpected Linkage	
		Primary analysis	Secondary analysis (SMRT)
Homozygous	21,818 (59%)	17 (0,08%)	0
Heterozygous	14,971 (40%)	2 (0,013%)	0
Unknown	472 (1.3%)	-	-
	37,261	**19 (0,05%)**	**0**

In 100% of samples linkage was in full concordance with the expectation derived from the DPB1 genotyping result.

Based on the analysis of 37,261 samples, we estimate the following frequencies for the alleles with defined rs9277534 linkage:



We conclude that HLA-DPB1 typing of exons 2 and 3 is sufficient to infer the DPB1 expression marker rs9277534 with very high accuracy.

This information could be used to select HLA-DPB1-mismatched donors that do not increase the risk of GvHD, without the need for an additional pretransplantation screening for SNP rs9277534.

References

[1] Petersdorf et al. (2015) "High HLA-DP Expression and Graft-versus-Host Disease." New England Journal of Medicine 373: 599–609.
[2] Lange et al. (2014) "Cost-efficient high-throughput HLA typing by MiSeq amplicon sequencing." BMC Genomics 15:63.
[3] Lang et al. (2016) „ABO allele-level frequency estimation based on population-scale genotyping by next generation sequencing." BMC Genomics 17:374.

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