GENDX

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A new NGS-based typing strategy for whole-gene HLA-DRA

Introduction

The human *HLA-DRA* gene encodes for the HLA class II histocompatibility antigen DRα chain, which functions as a heterodimer with one of four beta chains DRβ1, DRβ3, DRβ4 or DRβ5. Unlike the highly polymorphic DRβ genes, the DRα locus shows very little sequence variation with only two protein variants encoded by 7 alleles (IMGT/HLA database 3.35.0). To allow for genotyping of DRA by means of Next-Generation Sequencing (NGS), we developed a whole-gene amplification strategy using NGSgo-AmpX DRA primers.

Materials & Methods

NGSgo-AmpX DRA was used to generate amplicons that are processed in the NGSgo® library preparation procedure (GenDx) that were sequenced on a MiSeq (Illumina) platform using paired-end sequencing (2x151 bp). Allele assignment was performed with NGSengine® software using an *HLA-DRA* database from IMGT/HLA database version 3.35.0.

Results

A total of 90 samples from two panels were amplified with NGSgo-AmpX DRA (Figure 1). The first panel was the IHWG Sequence Polymorphism (SP) reference panel (n=51) of which a subset of 13 cell lines has DRA pretypes available. The second panel was a collection of gDNA samples extracted from blood of African origin (n=39) of which the DRA alleles were unknown. All samples showed strong PCR products of the expected size of ~5.6 kb demonstrating robust amplification of whole-gene DRA. All amplicons were sequenced and results were analyzed in NGSengine® software, showing typing results of high-to-allelic resolution. Concordance for the 13 cell lines with known DRA alleles was found to be 100% at two-field resolution (Table 1). Strikingly, when considering the third and fourth field, 7 out of 13 cell lines showed the presence of new alleles. Most new alleles represented different intron variants, but also one exon variant was observed (IHWG9021). In general, the sequence variation in DRA was higher in the introns than in the exons (Figure 2), suggesting that the exon sequences are indeed highly conserved.

When analyzing the allele frequencies in all 90 samples, the DRA*01:01:01 allele group (and all intron variants) showed the highest frequency of 63.9% (Table 2). The second most frequent allele group was DRA*01:02:02 (27.2%). Interestingly, two new alleles with the same exonic SNP were frequently observed (5.0% and 0.6%). Seven samples showed the exact same variant allele of DRA*01:02:02 as seen in IHWG9021 with a new SNP in exon 1 [46G>C]. Another sample (IHWG9415) also showed this exact same SNP and an additional intron variation, representing a second new allele. This exon SNP is a nonsynonymous change resulting in amino acid change from valine to leucine (Table 3). This new allele represents a third DRA protein, besides the DRA*01:01 and DRA*01:02 proteins. The functional relevance of this new SNP in exon 1, encoding for the HLA-DRA leader peptide, remains unknown.

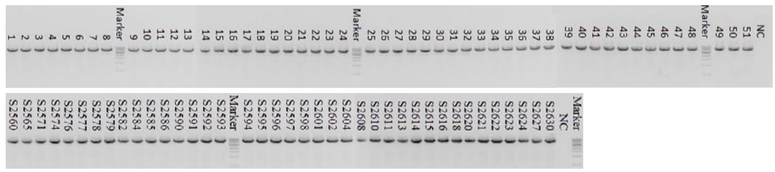

Figure 1. Full length (~5.6 kb) amplification of *HLA-DRA* using NGSgo-AmpX DRA. Results are shown for the IHWG SP panel (top panel) and samples of African origin (bottom panel).

Conclusions

The NGSgo-AmpX DRA assay provides reliable, high-resolution and high-throughput DRA genotyping. Sequencing of 90 samples has uncovered multiple new coding and noncoding SNPs that have not been reported before. These new alleles will be candidates for submission to the IMGT/HLA database. Further application of the NGSgo-AmpX DRA assay should benefit immunogenetic studies in the context of infectious or autoimmune diseases.

Table 1. Typing concordance of cell lines with known *HLA-DRA* genotypes. New alleles are shown in purple.

Sample ID	*HLA-DRA* pretype	*HLA-DRA* typing result	Concordant	Comment
IHWG9009	01:01	01:01:01:XX, 01:01:01:XX	Yes	Intron variant [1317A>G]
IHWG9014	01:02	01:02:03, 01:02:03	Yes	
IHWG9016	01:01	01:01:01:01, 01:01:01:01	Yes	
IHWG9021	01:02	01:XX:XX:01, 01:XX:XX:01	Yes	Variant of HLA-DRA*01:02:02 with SNP in exon 1 [46G>C] and multiple intronic SNPs (not listed)
IHWG9032	01:01	01:01:01:03, 01:01:01:03	Yes	
IHWG9040	01:01	01:01:01:XX, 01:01:01:XX	Yes	Intron variant [868G>A]
IHWG9045	01:01, 01:02	01:01:01:XX, 01:02:XX	Yes	Two novel intron variants with 2 and 64 intronic SNPs respectively (not listed)
IHWG9047	01:01	01:01:01:XX, 01:01:01:XX	Yes	Intron variant [-295G>A], [-288T>C], [-260T>C], [285G>A], [317C>A]
IHWG9048	01:01	01:01:01:03, 01:01:01:03	Yes	
IHWG9052	01:01	01:01:02, 01:01:02	Yes	
IHWG9056	01:01, 01:02	01:01:01:03, 01:02:02:XX	Yes	Intron variant [768G>A]
IHWG9068	01:02	01:02:02:XX, 01:02:02:XX	Yes	Intron variant with 66 novel intronic SNPs (not listed).
IHWG9092	01:01	01:01:01:03, 01:01:01:03	Yes	

Table 2. Frequencies of *HLA-DRA* alleles. New coding variants are shown in purple. New intron variants are not listed.

HLA-DRA allele	Count	%
01:01:01:01	41	22.8 %
01:01:01:02	40	22.2 %
01:01:01:03	34	18.9 %
01:01:02	2	1.1 %
01:02:02	49	27.2 %
01:02:03	4	2.2 %
01:XX:XX:01	9	5.0 %
01:XX:XX:02	1	0.6 %

Table 3. Summary of two novel *HLA-DRA* alleles (coding variants).

Sample ID	New allele	Comment
IHWG9021, IHWG9371, S2565, S2574, S2598, S2618, S2627, S2630	01:XX:XX:01	Variant of HLA-DRA*01:02:02 with multiple SNPs in introns and exon 1. Nonsynonymous change in exon 1 at [46 G>C] results in amino acid change from valine to leucine.
IHWG9415	01:XX:XX:02	Variant of DRA*01:02:02. Similar to the new allele listed above, but with one additional SNP in intron 1.

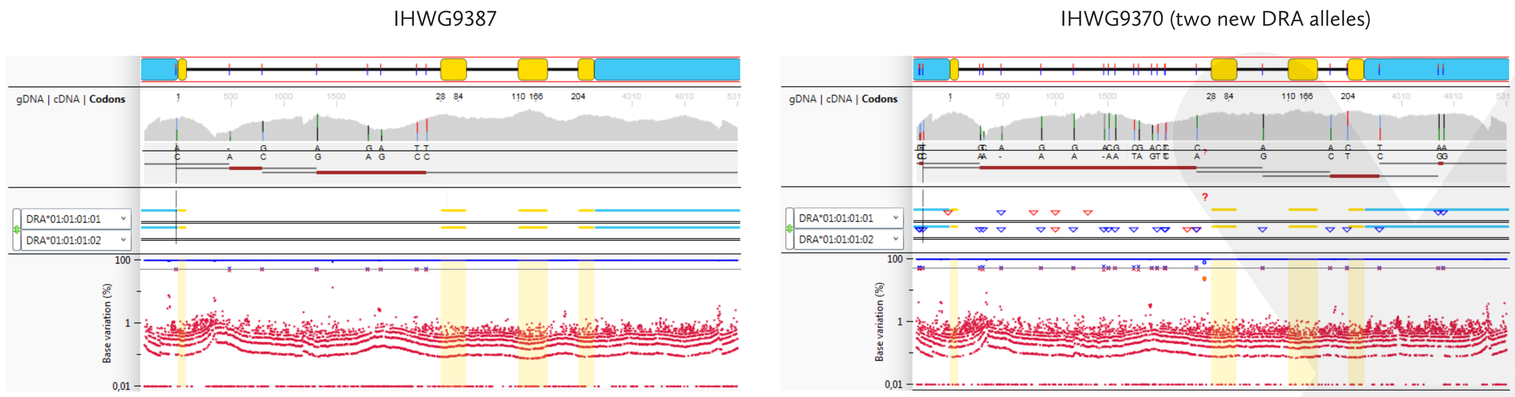

Figure 2. Examples of *HLA-DRA* typing results for the IHWG SP panel in NGSengine software. IHWG9387 has a perfect match with DRA*01:01:01:01, 01:01:01:02, whereas IHWG9370 also matches with DRA*01:01:01:01, 01:01:01:02, but has multiple new intronic SNPs (indicated with triangles) that have not been reported before.

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