Gene zygosity determined by the Copy Number tool can help resolve typing ambiguities.

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Background

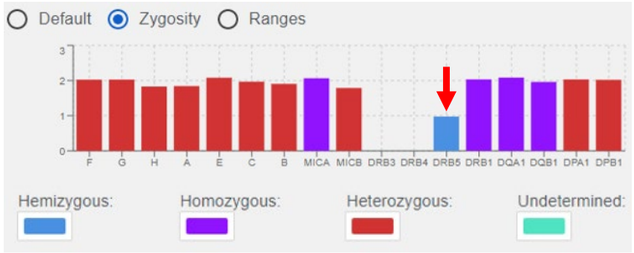
In most genomes the standard copy number (CN) for most genes is two per diploid cell with a copy inherited from each parent. Hence, individuals can be homozygous when both copies are identical or heterozygous when they are different. While most HLA genes follow these rules, certain haplotypes lack genes like DRB3, DRB4 and DRB5. Therefore, individuals can have only one copy of those genes (hemizygous). Further complexities in HLA typing emerge in situations when one of the alleles inherited have partial sequence deletions, usually resulting in null alleles. CareDx CN tool is able to estimate zygosity at each locus. Here we present three examples where this tool can help disambiguate typing without the need to use other techniques.

Methods

Fastq files were exported from AlloSeq Tx17 typing software and input on the CN tool.

Results

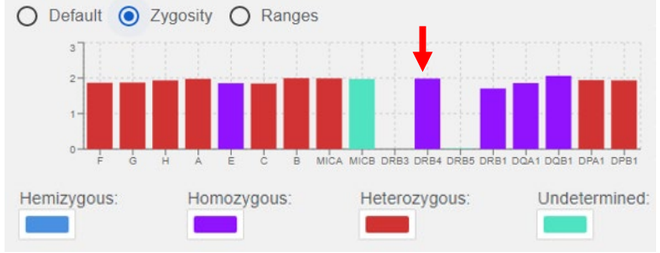
Case 1: While DRB1*15:03 is strongly associated with DRB5 (DR51), between 5-8% of individuals with DRB1*15:03 lack the DRB5 gene. For patients homozygous for DRB1*15:03, it is not possible to know if they have 1 or 2 copies of DRB5. This information is important to determine the amount of functional protein produced, which may influence the strength of the immune response.



IMGT/DRB5	01:01:01	01:01:01
IMGT/DRB1	15:03:01	15:03:01

Case 2: DRB4*03:01N carries a deletion that removes the 5' region of the gene, extending upstream up to intron 2. Hence, it will not amplify unless a long-range PCR assay includes a specific primer to bind in this region, which is not included in most of the systems currently used. In samples were 2 DRB4 copies are expected (e.g. homozygous DRB1*04, DRB1*07 or DRB1*09, or heterozygous combinations of them) typing produces ambiguous combinations: homozygous DRB4*01:01:01 or heterozygous DRB4*01:01:01/03:01N. CN tool can help to disambiguate these samples based on the zygosity of the DRB4 gene.

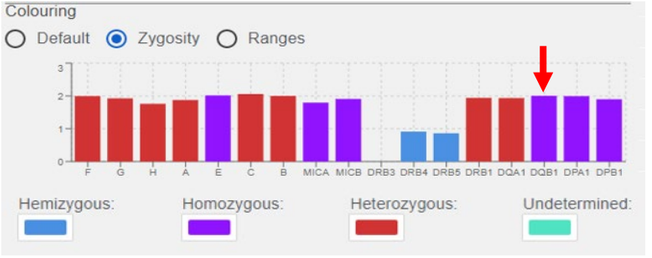
Allele 1	Allele 2	CORE	EXONS	Differences
DRB4*01:01:01	X	0	0	
DRB4*01:01:01	DRB4*03:01N	0	0	



IMGT/DRB1	09:01:02:07	09:01:02:07
IMGT/DRB3		
IMGT/DRB4	01:01:01:03	01:01:01:03

Case 3: DQB1*03:276N has two deletions. The first one spans 3.3 kb in the 5'UTR and the second one 3.7 kb starting at the end of 5'UTR until halfway through intron 1. The rest of the sequence of this allele is identical to DQB1*03:01:01:01. Unless a long-range PCR including a specific primer is performed, the null allele will not amplify, generating ambiguity between these alleles that can be resolved based on the zygosity of the DQB1 locus.

Allele 1	Allele 2	CORE	EXONS	Differences
DQB1*03:01:01	X	0	0	
DQB1*03:01:01	DQB1*03:276N	0	0	



IMGT/DQA1	03:03:01:--	05:05:01:--
IMGT/DQB1	03:--:--:--	03:--:--:--

Conclusions

CN tool estimates zygosity of HLA loci. Here we present three scenarios where this tool helps to resolve typing ambiguities based on the zygosity of the specific locus. These are common ambiguities observed but other scenarios may be possible.

References

1. IPD-IMGT/HLA Database. https://www.ebi.ac.uk/ipd/imgt/hla/
2. HLA nomenclature. https://hla.alleles.org/pages/genes/statistics/