Long-Read Oxford Nanopore Sequencing Resolves Novel Full-Length HLA-DPB1 Alleles

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Aim

- Unambiguous high-resolution HLA typing is critical for matching in hematopoietic stem cell and solid organ transplantation. While short-read next-generation sequencing (NGS) reliably achieves two-field typing, it often introduces phase ambiguity and incomplete gene coverage, particularly for genes with large intronic regions such as HLA-DPB1.
- Oxford Nanopore Technologies (ONT) enables long-read sequencing without fragmentation of long-range PCR amplicons, allowing full-length gene coverage. We report three cases with mutations in *HLA-DPB1* detected by short-read NGS and further characterized using whole-gene DPB1 primers and ONT sequencing to obtain full-length gene sequences.

Methods

- Genomic DNA were extracted from three cases were transplant candidates from blood and/or buccal swabs using 11 HLA loci were amplified using long-range PCR (GenDx, MX11-3) followed by sequencing on the Illumina MiSeq platform.
- Genomic DNA from each sample was further amplified using NGS-Pronto and whole-gene DPB1 primers (GenDx) and sequenced on the GridION platform.
- Data analysis was performed using NGSengine v4.1.2 (GenDx, Utrecht, The Netherlands) with the IPD-IMGT/HLA database version 3.62.

Conclusions

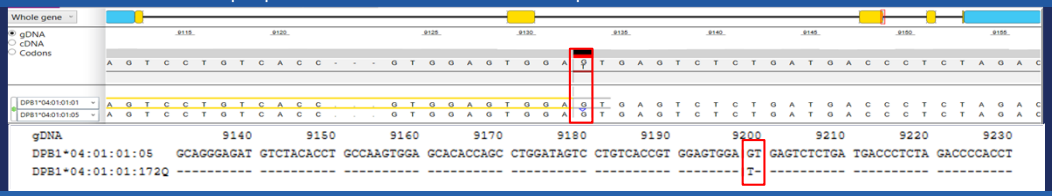
- Full-length PCR amplification combined with ONT long-read sequencing enables direct characterization of *HLA-DPB1* variants across entire gene amplicons.
- These cases demonstrate achieving full-length genomic sequences and resolving phase ambiguity using ONT long-read sequencing.
- This approach facilitates accurate allele definition , resolves phasing ambiguity and supports submission of novel sequences to reference databases.
- Three novel alleles were assigned as DPB1*04:01:01:172Q, DPB1*1932:01N and DPB1*1946:01

Results

- ONT long-read sequencing confirms the mutations detected by short-read sequencing by Illumina MiSeq platform

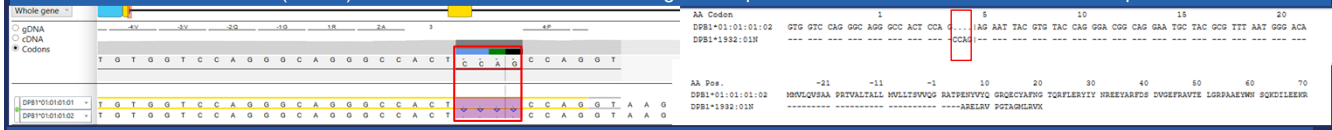
Case 1: *DPB1*04:01:01:172Q*

- A mutation GT>TT at the splice site of intron 3 in DPB1*04:01:01:05. It may disrupt normal RNA splicing, potentially leading to exon skipping, intron retention, or activation of cryptic splice sites, thereby altering the expressed DPB1 transcript.
- This mutation was confirmed in both peripheral blood and buccal swab samples.



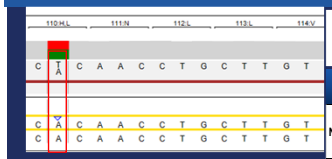
Case 2: DPB1*1932:01N

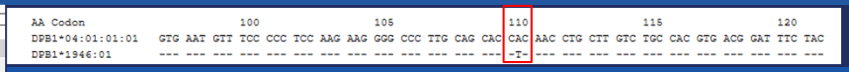
- A four-nucleotide insertion (CCAG) in exon 1 of *DPB1**01:01:01:02 at genomic position 96 causes frameshift and form stop codon TAA



Case 3: DPB1*1946:01

- A nucleotide substitution (A>T) at codon 110 CAC > CTC in exon 3 of *DPB1**04:01:01:01, resulting in an amino acid substitution from Histidine to leucine





Assignment of Novel Alleles

Novel allele	Most similar allele	GeneBank accession number	IPD-IMGT/HLA Database submission number	Race	HLA genotype
DPB1*04:01:01:172Q	DPB1*04:01:01:05	PZ213881	HSW10103055	European descent	A*03:01, 11:01; B*07:02, 35:01, C*04:01, 07:02 DRB1*01:01, 15:01; DRB5*01:01; DQA1*01:01, 01:02; DQB1*05:01, 06:02; DPA1*01:03, 01:03; DPB1*04:01, **04:01:01:172Q**
DPB1*1932:01N	DPB1*01:01:01:02	PZ213879	HSW10103057	African American	A*30:02, 74:01; B*14:02, 45:01; C*06:02,08:02; DRB1*09:01, 15:03; DRB4*01:01; DRB5*01:01; DQA1*01:02, 03:03; DQB1*02:02, 06:02; DPA1*02:02, 02:02; DPB1*01:01, **1932:01N**
DPB1*1946:01	DPB1*04:01:01:01	PZ213880	HSW10103054	Hispanic	A*02:05, 68:01; B*35:17, 58:01; C*04:01, 07:18; DRB1*03:01, 08:02; DRB3*02:02; DQA1*04:01,05:01;DQB1*02:01,04:02; DPA1*01:03,02:01; DPB1*14:01,**1946:01**

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