Poster Presentations

PP-03

Evaluation of Nanopore DNA Sequencing Technology for Urgent HLA High-Resolution Typing of Cadaveric Donors

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Objective: The definition of human leukocyte antigen (HLA) epitopes necessitates high-resolution (HR) genotyping, often not attainable before the allocation of deceased donors. Nanopore DNA Sequencing technology (3rd generation) emerging as a promising avenue for rapid HLA HR typing.

Materials and Methods: This study evaluates nanopore sequencing for urgent HLA-HR typing of cadaveric donors. It introduces a rapid HLA typing method employing the Nanotype 24/11v2 Ruo assay (Omixon) using 11 loci (HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQA1,-DQB1,-DPA1,-DPB1) from 30 samples, paving the way for on-call deceased donor allocation. This assay employs multiplexed long-range polymerase chain reaction with library preparation within 90 minutes. Data, generated on a MinION sequencing device using a MinION flow cell type R9.4, undergoes high-accuracy base calling, followed by analysis in Nanotyper software, restricted only in exons.

Results: In comparing results with pre-typed data (AlloSeq Tx17 kit, CareDx) sequencing on MiSeq, the nanopore method yielded 100% concordance for 330 loci with the current next-generation-sequencing method, maintaining a minimum 2-field typing. The method also provided accurate data at all HLA loci in approximately 4 hours, without prolonging allocation time. The average read length was 3150 bp, with an average minimum coverage for key exons of 1630 for all HLA loci. Notably, key exon allelic imbalance for heterozygous samples at most HLA loci was over 0.6.

Conclusion: Conducting HR typing across all HLA loci for deceased organ donor allocation has notable clinical benefits: The implementation of nanopore HLA typing for deceased donors before transplantation, paired with antibody screening and identification, enhances virtual cross-match accuracy, especially for hypersensitized recipients, within a time-efficient framework.

PP-04

Eight Digit Human Leukocyte Antigen -A, -B, -C, -DRB1, -DQB1, Allele and Haplotype Frequencies with Next Generation Sequencing, Single-Center Experience

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Objectives: There is a lack of data on the 8-digit human leukocyte antigen (HLA) frequencies in the Turkish population, and there are no reports of haplotype analysis in this population. The aim of this study was to evaluate the 8-digit 5 loci (HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1) allelic data of 364 individuals in our database.

Materials and Methods: The 8-digit allele were sequenced using next generation sequencing (NGS) methods, and the resulting data were analysed using MIA FORA NGS FLEX HLA genotyping software (version 3.0). The assessment of the complete HLA data set was conducted utilising PyPop software, version 1.0.2.

Results: A total of 54 HLA-A, 96 HLA-B, 62 HLA-C, 74 HLA-DRB1, and 45 HLA-DQB1 alleles were identified. The three most frequent allele for each loci were A*02:01:01:01 (0.15247), A*24:02:01:01 (0.14973), A*01:01:01:01 (0.125); B*51:01:01:01 (0.07143), B*49:01:01:01 (0.0522), B*35:01:01:02 (0.04396); C*12:03:01:01 (0.08791), C*07:01:01:01 (0.07692), C*04:01:01:06 (0.07555); DRB1*11:04:01:01 (0.09066), DRB1*03:01:01:01 (0.08516), DRB1*07:01:01:01 (0.06593); DQB1*03:01:01:02 (0.15659), DQB1*02:01:01:01 (0.08929), DQB1*03:02:01:01 (0.08379).

Haplotype calculation showed that A*01:01:0 1:01~B*08:01:0 1:01~DRB1*03:01 :01:01 (0.01374); A*01:0 1:01:01~B*08:01: 01:01~C*07:01:01:01~DRB1*03 :01:01:01 (0.01374) and A*01:0 1:01:01~B*08:01:01:01~C*07:01 :01:01~DRB1*03:01:01 :01~DQB1*02:01: 01:01 (0.01236) were the most frequent haplotypes in our population.

Conclusion: The present study identified the frequencies of 8-digit HLA-A, -B, -C, -DRB1, and -DQB1 allele and haplotype frequencies in a Turkish population using NGS. The newly acquired data can be employed in the mapping of HLA patterns in our country, thereby providing a foundation for the design of subsequent investigations.