Editorial

The power of Oxford Nanopore MinION in human leukocyte antigen immunogenetics

Marcel G. J. Tilanus

Department of Transplantation Immunology, Maastricht University Medical Center, Maastricht, the Netherlands

Correspondence to: Prof. Dr. Marcel G. J. Tilanus. Department of Transplantation Immunology, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX, Maastricht, the Netherlands. Email: m.tilanus@mumc.nl.

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Jain *et al*. described the delivery of nanopore sequencing to the genomics community (1). One of the more complicated genomic regions on chromosome 6, is the area of the human Major histocompatibility complex (MHC). The human leukocyte antigen (HLA), the human equivalent of the MHC, plays a major role in the immune defense against pathogens such as viruses and bacteria. HLA genes encode cell surface proteins which present foreign peptides to T cells and NK cells inducing an immune response to eliminate the intruder. In a transplantation setting, particularly in stem cell transplantation (SCT), the identification of HLA polymorphism of the patient and donors is crucial since matching for the four main HLA loci, HLA-A, -B, -C and -DRB1 directly influences the transplantation outcome (2). In case of the availability of mismatched donors only, typing other HLA loci is relevant (3,4). Moreover, the definition of epitopes within the HLA antigens become more crucial in SCT, whereas the presence of antibodies against epitopes of the HLA antigens of the donor is a contraindication in kidney transplantation.

Sequencing based typing for HLA alleles has become the golden standard for the identification of HLA polymorphism since its introduction in 1993 (5). Due to the high level of shared polymorphism among the alleles and the identification of numerous new alleles, a high percentage of ambiguous typing results is encountered by Sanger heterozygous sequence analysis. Short read next generation sequencing (NGS) approaches similarly result in ambiguities since these approaches use short DNA fragments (6). The single molecule sequencing approach from Oxford Nanopore defines full length HLA polymorphism of all loci simultaneously on the individual parental chromosomes (single molecules) and circumvents phasing uncertainties of polymorphic sites in its long sequence reads.

Additional SNP polymorphism adjacent to the HLA-genes or in non-coding regions is an additional risk factor in unrelated SCT. Haplotypes including SNP polymorphism increase the possibility to estimate risks prior to transplantation (7). The 4 Mb HLA region on chromosome 6 is unique in that it carries the highest polymorphism in the human genome (8) with 17,166 allelic variants defined in IMGT/HLA version 3.28.0 (9). Polymorphism of the entire 4 Mb HLA region has been addressed by sequencing 20 kb fragments obtained by region specific extraction (10). Knowledge of polymorphism of the entire HLA will refine and assign relevant SNPs and HLA gene polymorphism in transplantation and disease associations and can be addressed by the Oxford Nanopore sequencing strategy (11-13).

Knowing the full genomic polymorphism of the HLA region helps to resolve the question which particular polymorphism is functionally relevant. Considering not only the genotype but also the effect of polymorphism on phenotype is essential in risk prediction since expression levels influence the immune response. SNPs in the regulatory regions and 3'untranslated region of HLA genes can affect expression levels and influence immune responses. An example is the expression marker HLA-DP rs9277534 SNP that influences the risk on GVHD in HLA-DPB mismatched transplantations; recipients with the high expressed allele transplanted with donors with the low

expressed allele variant had a high risk on GVHD (14) and polymorphism in the HLA-C upstream region that affects the expression of HLA-C (15). Consequently, this might have effect on the T and NK cell alloreactivity and these expression differences might have clinical consequences.

Taken all together, SNPs in the HLA region, both within as well as outside the genes, become more and more important to define the best HLA matching and predict risks in transplantation outcome and disease susceptibility. A nomenclature reflecting SNPs rather than HLA alleles, or epitopes within the HLA antigens, is required to predict the immunological reactions in health and the risk stratification in transplantation and diseased individuals.

The capacity of the Nanopore MinION flow cells allows for the analysis of multiple gene polymorphism simultaneously. Genome-wide association studies (GWAS) identify multiple genes that are involved in the mechanism leading to diseases. The additive effect of multiple gene polymorphism can now be addressed (16). For instance celiac disease is associated with HLA-DQ2 and -DQ8 (17). Additional risk factors could be identified within the HLA region that adds 18% to the HLA-DQB genetic risk up to 48% (18) and thus refine the association of HLA with celiac disease. GWAS analysis of immune genes revealed several polymorphic genes with immune functions that have impact on celiac disease (19).

Risk stratification through the identification of multiple immune response related polymorphism will lead to precision medicine and individualized treatment. The characteristics of a healthy immune system depends on heritable gene variation and non-heritable influences (20). New ways of combining knowledge of individual gene system polymorphism and influence on the immune system will lead to the definition of a healthy immune system and contribute to therapy how to restore the immune system in disease.

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