

High-Resolution HLA Genotyping Improves Matching, Survival Rates for Stem Cell Transplantation

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SAN FRANCISCO (GenomeWeb) – The UK's Anthony Nolan Research Institute has found that high-resolution HLA typing leads to better outcomes for stem cell transplant patients.

The institute published a [retrospective study](#) of 891 bone marrow donors and their respective recipients in the journal *Biology of Blood and Marrow Transplantation* last week and found that when patients were matched at 12 alleles across six HLA genes, five-year survival was 55 percent compared to 40 percent for patients with any degree of mismatch. In addition, the researchers found that 29 percent of patients who were previously thought to be matched via HLA typing methods that included Sanger sequencing and sequencing-specific oligonucleotide probing, actually had at least one mismatch when they were retrospectively analyzed using long-read sequencing on the Pacific Biosciences RS II platform. And, five-year survival among these patients was also lower — at 30 percent.

"We've always known that the better the match, the better the outcome," said Steven Marsh, director of bioinformatics at Anthony Nolan Research Institute and senior author of the study. "But, the HLA genes are incredibly polymorphic, and so we've been limited by the technology to identify all the HLA variants."

As such, the degree to which patients should be matched has not been fully understood, and matching has focused predominantly on the gene exons that encode for the antigen recognition domain, since those functional regions are thought to be the most relevant.

Marsh said that Anthony Nolan began evaluating sequencing technologies in 2013 and ultimately decided to focus on the PacBio technology due to its long reads, which would enable the team to phase variants, important for determining whether a given donor and recipient are a match.

The team published a [feasibility study](#) in *PLOS One* in 2015, and later that year [implemented the protocol](#) on a routine basis.

The method used is a targeted sequencing protocol of the full HLA-A, -B, and -C genes and the exonic regions of the HLA-DRB1, -DQB1, and -DPB1 genes.

In the study, the researchers applied the PacBio method retrospectively to the 891 samples that had been previously typed and for whom clinical outcomes data was available. PacBio sequencing resulted in a different matching status for 29.1 percent of donor-recipient pairs.

"That was the first thing that really stood out," said Neema Mayor, head of immunogenetics research and lead author of the study. "We thought all of these individuals had been well matched, but they were not as well-matched as we had previously thought." Then, when the team analyzed the outcomes, they found that "the individuals with mismatches that we didn't know about did significantly worse," she said.

Another important finding was the impact of intronic variation. Intronic variants are not routinely analyzed due to the belief that they are not likely to impact function and due to the already difficult nature of analyzing the HLA genes in the first place. The focus has always been on matching variants in the exons.

"It was thought that intronic variants would not have an impact, but we've never had the data to ask whether they matter or not," Marsh said. But, because the Anthony Nolan team sequenced the full genes of the HLA-A, -B, and -C genes, they could look at intronic variation, as well.

In the study, the researchers found previously unknown variants in nearly 25 percent of the pairs, many of which were in introns. Marsh said that trying to tease out the impact of these intronic mismatches from the impact of other mismatches was difficult because there were only 13 pairs that had mismatches only in intronic regions, but nonetheless, the group did find that outcomes were slightly worse in those patients compared to those with no differences, but better than those who were mismatched at one of the 12 HLA alleles.

Marsh hypothesized that the intronic variants could serve as another means for ensuring a haplotype match across the entire major histocompatibility locus and do not necessarily contribute to functional differences themselves. "The data isn't definite, but that's our hypothesis," he said, adding that previous research has shown that whole haplotyping matching of the MHC locus results in better outcomes.

The researchers also analyzed the impact of the presence of cytomegalovirus in donors and recipients. CMV is a common virus that many people are exposed to and that remains dormant but can be reactivated when a person is immunocompromised. Testing donors and recipients for CMV is important, Mayor said, because "you don't want to give a patient with dormant CMV cells from a donor who has never experienced the virus before, because then the patient may not be able to fight it," she said.

Similarly, it's important to not give a recipient who has not previously been exposed to CMV cells from a donor who has. "When picking a donor, HLA is still the primary factor" affecting compatibility, Mayor said, but "then you start bringing in other factors and the next most important factor we pick is CMV status."

"It's a very nice study and the data is very useful," Nezh Cereb, CEO and cofounder at HLA typing firm Histogenetics, said. Histogenetics offers a range of HLA typing services using both PacBio and Illumina sequencing technology. Initially, the firm [began incorporating](#) PacBio sequencing in 2014 to help phase ambiguous samples that it typed using the Illumina platform, but now the company also offers full-gene sequencing on the system.

"We do advocate for whole-gene sequencing for the HLA class I genes," he said. But, whole-gene sequencing for the class II genes is not always practical due to the size of those genes, although he noted that as the technology improves, it will become more feasible.

Cereb noted that the minimum requirement in the US for HLA typing in stem cell transplantation is to match at eight alleles across four genes — HLA-A, -B, -C, and -DRB1. But, with the advances in sequencing technology, it is becoming possible to analyze the DQB1 and DPB1 genes and to further understand their impact on outcomes.

He said that while the Anthony Nolan study did show that the greater amount of matching, the better the outcomes, trying to pinpoint the impact of genes like DQB1 and DPB1 was harder to do. "But, as the study shows, matching for more loci increases survival," he said.

"I hope that more studies like this one will follow" that can help to further understand the impact of variation within those genes, he added.

Going forward, Marsh said that the researchers aim to replicate the findings of this study in another cohort of patients. In addition, Mayor said that the researchers are looking at the impact of other genetic factors, including the KIR genes and HLA-E, on transplant outcomes.