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Rapid donor-specific single nucleotide variation detection by nanopore sequencing of ex vivo lung perfusate

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BACKGROUND: Donor-derived cell-free DNA (ddcfDNA) has been shown to be useful in monitoring lung graft health, and single nucleotide variations (SNVs) between donor and recipient are used to identify ddcfDNA in post-transplant recipient blood. One limitation is the need to map donor or recipient SNVs prior to calculating %ddcfDNA. In this study, we use Nanopore sequencing of ex vivo lung perfusion perfusate cfDNA to map donor SNVs and validate it using standard short-read whole genome sequencing (WGS). Methods: cfDNA was extracted from 11 clinical ex vivo lung perfusion perfusate samples and sequenced using a Nanopore sequencer. SNVs were identified by comparison to a reference genome and then filtered for homozygous calls overlapping the 1000 Genomes SNP database. Matching short-read WGS was performed on 6 matching samples to act as a gold standard. Following mapping, %ddcfDNA was calculated in cell-free DNA (cfDNA) collected from matching post-lung transplant recipient plasma using SNVs called by Nanopore vs SNVs called by short-read WGS and compared for accuracy.

RESULTS: Nanopore sequencing yielded genomic data with a median coverage of 4.88x (range 2.27-8.79) using a single flow cell with a median run length of 72 hours. The median general error rate of the sequence was 5.55% (range 4.98%-6.49%), and the median number of SNV with a depth > 3 and overlap with the 1000 Genomes SNP database was 246,993 (range 99,357-313,721). The positive predictive value of SNVs identified using 2X to 6X coverage cutoffs ranged from 66.6% to 96.9%, with a median value of 90.3% at 6X coverage. Correlation analysis showed a strong correlation between results by Nanopore sequencing and results by WGS for detecting %ddcfDNA in post-transplant plasma (R^2 = 0.996, $p < 0.001$).

CONCLUSIONS: Despite the lower sequencing accuracy and depth obtained from Nanopore sequencing, a high positive predictive value can be achieved in a set of donor-specific SNVs when appropriately filtered by read depth and overlap with SNP databases. This demonstrates the potential for the

Abbreviations: cfDNA, cell-free DNA; ddcfDNA, donor-derived cell-free DNA; EVLP, ex vivo lung perfusion; PPV, positive predictive value; SNP, single nucleotide polymorphism; SNV, single nucleotide variation; TLTP, Toronto Lung Transplant Program; TPR, true positive rate; WGS, whole genome sequencing

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use of Nanopore sequencing to generate personalized donor cfDNA maps for use in post-operative donor-derived plasma cfDNA identification.

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Background

Lung transplantation is the final treatment for many end-stage lung diseases; however, despite immunosuppression, there remain many threats to the graft following transplant, including rejection and infection, among others. Ideally, a non-invasive strategy for regular lung monitoring would allow for early therapeutic intervention leading to improved post-transplant outcomes. One promising non-invasive test harnesses cell-free DNA (cfDNA) in the post-transplant recipients' blood. cfDNA are small fragments of DNA that are released into the circulation following cellular injury by either rupture of cell membranes, cell apoptosis, necrosis, or active secretion from cells.[1,2] Consequently, the finding of donor-derived cfDNA (ddcfDNA) in recipient plasma may indicate donor cell injury, and recent multicenter trials have confirmed the usefulness of percent ddcfDNA (% ddcfDNA) in monitoring the health of the lung graft.[3–6]

To distinguish donor from recipient cfDNA in the blood, single nucleotide variations (SNVs) between the genomes of the donor and recipient are generally used.[7] As each gene has two alleles, the ideal SNV separating donor from recipient would be homozygous. To map the alleles, both donor and recipient would need to be sequenced by either targeted, exome, or whole genome sequencing (WGS), which is time-consuming and costly.[7–10] As a compromise, current methodology for ddcfDNA testing utilizes a panel of common single nucleotide polymorphisms (SNP) with the expectation that random donor-recipient pairings will differ at at least a subset of these SNP loci, allowing for % ddcfDNA to be calculated. These "genotype-free" analyses exist by statistically modeling SNPs using a mixture model, assumptions of normality, and an expectation-maximization algorithm with a preference for homozygous SNPs between donor and recipient due to mutual exclusion. However, these assumptions break down at higher levels of ddcfDNA, and improved calculation of %ddcfDNA requires an accurate genotype of either a donor or a recipient.

A recently developed sequencing technology known as Nanopore sequencing may facilitate genotyping.[11–13] Nanopore sequencing relies on inferring the nucleotide base from changes in the ionic current across a membrane as a single DNA molecule passes through a protein nanopore.[11] Samples require minimal preparation, and sequences are read out in real-time and are therefore timely and not labor intensive. However, limitations of Nanopore sequencing include the requirement of high input DNA amount and lower read accuracy than conventional short-read sequencing methodologies.

Ex vivo lung perfusion (EVLP) is performed for pre-transplant assessment, preservation, and reconditioning of donor lungs.[14–16] During EVLP, ddcfDNA is released from the lungs into the perfusate,[17] making it possible to easily collect a large sample of cfDNA non-invasively and banked frozen long-term. We hypothesized that we could rapidly generate a donor-specific SNV map using Nanopore sequencing of the perfusate in a transplant-relevant timeframe.

In this study, we investigated the feasibility of using the Nanopore sequencer to call donor SNVs from EVLP perfusate and validated it by short-read WGS of lung donor tissue and post-transplant plasma to verify its accuracy.

Materials and methods

Patient samples

EVLP perfusate samples taken at the final hour of perfusion from 11 clinical EVLP cases performed between April 2018 and March 2022 at Toronto General Hospital were used in this study. Perfusate supernatants following centrifugation at 4 °C for 5 min at 400 G were collected, snap frozen, and stored at −80 °C prior to cfDNA testing.

Clinical data was obtained from the Toronto Lung Transplant Program database. This study was approved by the University Health Network research ethics board (REB No. 12-5488, 11-0509) and also the ethics board of Trillium Gift of Life Network, the local organ procurement organization. All patients provided written informed consent.

The study concept and schematic diagram of this study are shown in Figure 1.

Perfusate sample processing (DNA extraction and evaluation)

cfDNA was extracted from approximately 1.5-4 ml of perfusate by using QIAamp MinElute ccfDNA kit (Cat. # 55284, Qiagen, Hilden, Germany). 1 µl each of the extracted DNA samples was used for Qubit quantification and quality control (QC) by Agilent 2100 Bioanalyzer (Agilent, CA, USA) using High Sensitivity DNA Chips based on the manufacturer's recommended protocol.

DNA sequencing by Nanopore MinION device and bioinformatics analysis

Library preparation and adapter ligation according to the amplicons were performed using the Oxford Nanopore Ligation Sequencing Kit (SQK-LSK110, Oxford Nanopore Technologies (ONT), Oxford, UK) and followed the Nanopore

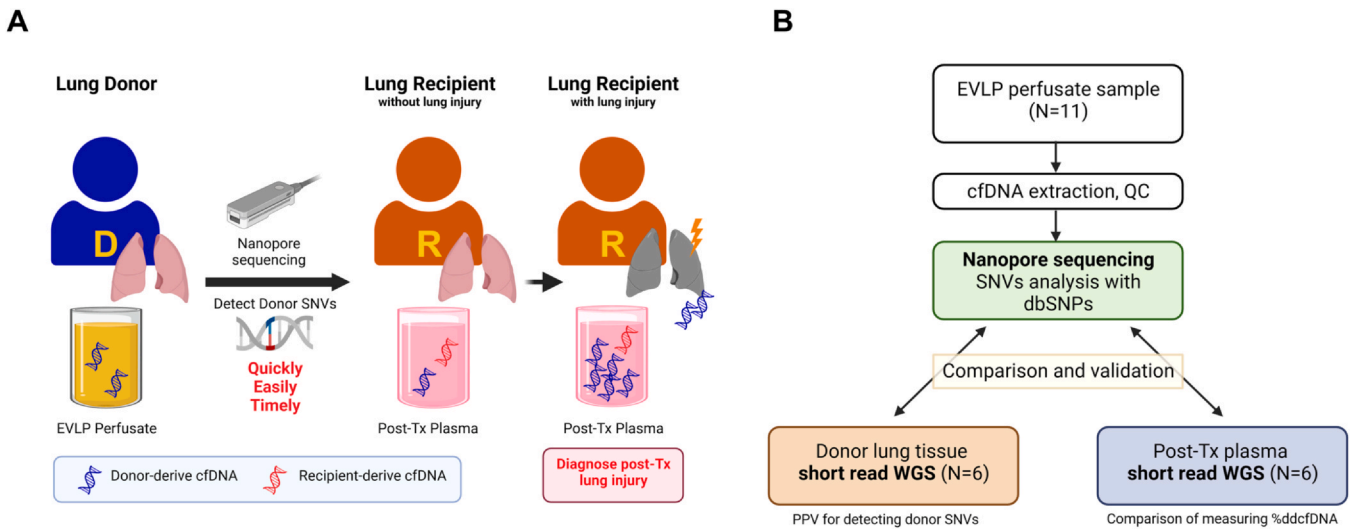

Figure 1 *Study concept and schematic diagram of this study.* **(A)** Study concept. Donor-derived single nucleotide variations (SNVs) are identified from donor-derived cell-free DNA (cfDNA) in Ex-vivo lung perfusion (EVLP) perfusate by Nanopore sequencing. The detected donor SNVs map is used to diagnose post-transplant lung injury in a timely manner. **(B)** Schematic diagram of this study. A total of 11 clinical EVLP cases were used. cfDNA was extracted from EVLP perfusate and Nanopore sequencing was performed. The results were compared for 6 of these cases to assess the accuracy of Nanopore sequencing against whole genome sequencing (WGS) in detecting donor SNVs. Furthermore, accuracy in detecting donor-derived cfDNA (ddcfDNA) in post-transplant plasma was evaluated between Nanopore and WGS method.

protocol (version: GDE_9108_v110_revL_10Nov2022). For the library preparation, 650-2,000 ng of DNA samples were used depending on sample availability. After adapter ligation, the concentration was checked, and the final DNA library was prepared by adding 37.5 µl Sequencing Buffer and 25.5 µl Loading Beads (LBII) to the DNA library. The DNA library was then run on a FLO-MIN106D flow cell (Spot-On Flow Cell, R9 Version, ONT, Oxford, UK) using the Nanopore MinION sequencing device (Oxford Nanopore Technologies, Oxford, UK). To achieve optimal sequencing of perfusate DNA, sequencing duration was adjusted between 17 hours and 6 days for each case.

Once the sequencing was complete on the MinION flow cell, using MinKNOW, the raw electrical signal from the Nanopore sequencing was stored as fast5 files, then base called into nucleotide sequence using Oxford Nanopore's Guppy basecaller version 10.1.0. The resulting FASTQ files were then aligned to human genome reference GRCh38 using Minimap2 version 2.24-r1122. Samtools version 1.14 was used to sort and index the bam file output from Minimap2. SNPs were identified using Clair3 version 1.0.5 and overlapped with the 1,000 Genomes (1000G version 102 filtered for bialleleic SNPs only and frequency greater than 1%) as a reference to increase confidence in the SNP call due to the relatively low read depth of ~5X. QC and average depth were performed with Qualimap version 2.2.1.

SNVs comparison with short-read WGS and comparison of accuracy in detecting ddcfDNA in post transplant plasma

Short-read WGS was performed on 6 matching donor tissue samples and post-transplant plasma. Frozen donor lung tissue samples and post-transplant plasma samples from matched cases were obtained from the Toronto Lung Transplant Program biobank and DNA was extracted using DNeasy Blood & Tissue Kit (Cat.# 69504, QIAGEN) and QIAamp MinElute ccfDNA kit (Cat. # 55284, Qiagen, Hilden, Germany), respectively. The lung tissue samples were collected at the end of EVLP, snap-frozen, and stored at −80 °C prior to analysis. The post-transplant blood samples were collected 24 hours after transplantation, centrifuged at 1,500 G for 10 min, and the supernatant collected as plasma and stored at −80 °C prior to analysis.

After QC by Bioanalyzer, 100 ng of genomic DNA per sample was fragmented using the Covaris M220, and the libraries were subsequently generated using the KAPA HyperPrep Kit (Cat.# 07962363001, Roche) according to the manufacturer's instructions. Final libraries were pooled in equimolar quantities and sequenced on an Illumina NovaSeq 6000 platform following the recommended protocol to generate paired-end reads of 150 bases in length.

The plasma and donor lung-tissue WGS results were mapped to GRCh38 using BWA-mem version BWA-0.7.17 (r1188), sorted with samtools v1.16.1, and duplicates marked with Picard 2.27.4. Read pairs marked as duplicates were discarded for the remaining analyses. Nanopore-identified SNVs' positive predicted value (PPV) was ascertained by selecting homozygous SNVs (allelic fraction > 0.9), with a minimum mapping quality of 5 and overlapping with the 1000 Genomes SNP database (Figure S1). The filtering for known homozygous SNVs maximized the overlap with homozygous SNVs identified in the short-read sequencing of the matched six cases. For all SNV calling steps from short-read sequencing, we required a minimum base quality of 20 and properly mapped read pairs with a mapping quality of at least 35. SNVs overlapping 1,000

Genomes were identified from the matched WGS samples, and sites with 20X coverage and 0 reference base counts were retained (homozygous SNVs). The 1,000 Genomes SNVs identified by WGS or nanopore were then used to quantify reference and alternative allele counts in the matched plasma samples. These SNVs were filtered to require at least 20X coverage.

The SNV map generated by Nanopore sequencing was compared to the map generated by short-read WGS in calculating %ddcfDNA in post-transplant plasma samples. To quantify %ddcfDNA, we fit 3 gaussians to the donor homozygous allele fraction profiles and then selected the SNVs that fell into the gaussian distribution representing recipient reference SNVs. Finally, we took the mean AF for the donor homozygous and recipient reference SNVs as the %ddcfDNA.

Samtools view was used to downsample the mapped reads for a donor genome and recipient genome from short-read sequencing and combined at different percentages to establish a "ground truth." Donor SNVs identified by Nanopore were then used to calculate a %ddcfDNA and the mean absolute error was calculated.

Statistical analysis

The correlations were tested with the Pearson correlation coefficient analysis. Differences were considered significant at $p < 0.05$ (2-sided). All statistical analyses were performed using GraphPad Prism 7.04 (San Diego, CA, USA).

Results

Nanopore sequencing

Donor patient characteristics are shown in Table 1. Library preparation took approximately 3.5 hours using the Oxford Nanopore Ligation Sequencing Kit from the perfusate sample. The read generation, estimated bases (median 24.7 giga-bases (Gb); range 9.79-32.99 Gb), run length (median 72 hours; range 17-144 hours), guanine-cytosine percentage, mean coverage, and general error rate obtained from Nanopore sequencing by using a single flow cell are shown in Table 2.

The obtained reads were mapped to the human genome with an approximate alignment rate of 99%, demonstrating that perfusate DNA was widely derived from the entire chromosome, rather than from a specific region of the human genome.

28,822,668 SNVs were identified over 11 samples, and the median number of SNP with a depth > 3 and overlap with the 1000 Genomes SNP database was 246,993 per sample (range 99,357-313,721, Table 2).

Comparison of SNVs obtained with Nanopore vs SNVs from short-read WGS

SNVs by Nanopore sequencing compared to the matching short-read WGS are shown in Figure 2. The number of SNVs obtained by Nanopore sequencing showed an increase in false negatives and a decrease in false positives and true positives as coverage cutoff increased from 2X coverage to 6X coverage. The PPV of SNVs identified in 2X to 6X coverage regions ranged from 66.6% to 96.9%, depending on the number of results obtained from sequencing, with a median value of 90.3% at 6X coverage. True positive rate decreased with each increase in coverage from 2X to 6X, with a median of 6.5% (range, 0%-20.9%) at 6X.

Table 1 Patient Characteristics of the Study Population

Patient characteristics	$N = 11$
Age, years, median (range)	54 (28-71)
Sex	
Female	1
Male	10
Body mass index, median (range)	28.09 (21.37-44.82)
Donor type: DCD/DBD	7/4
Cause of death, (%)	
Anoxia/cardiac arrest	6 (54.5)
Cerebrovascular/stroke	2 (18.2)
Head trauma	1 (9.1)
Other/unknown	2 (18.2)
Timing of EVLP sample used (1/2/3/4 hours)	0/1/4/6
Lung outcome, declined/transplant	5/6

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; EVLP, ex vivo lung perfusion; FiO2, fractional inspired O2 concentration; PaO2, partial pressure of oxygen.

Accuracy in calculating % ddcfDNA in post-transplant plasma

Percent ddcfDNA calculations using SNVs from Nanopores were similar to the gold standard short-read WGS SNVs for % ddcfDNA calculation in post-transplant plasma (Figure 3A). Correlation analysis showed a strong correlation between results by Nanopore sequencing and results by short-read WGS for detecting % ddcfDNA in post-transplant plasma (Figure 3B, R^2 = 0.996, $p < 0.001$). To confirm accuracy at low % ddcfDNA, mapped reads from short-read sequencing data of donor and recipient genomes were combined at (1%, 2%, 5%, 10%, 15%, 25%, 30%, and 40%) as simulated levels of ddcfDNA. The mean absolute error was < 3% across 0%-40% ddcfDNA and < 1% at levels under 10% ddcfDNA, suggesting high accuracy even at low percentages of ddcfDNA (Figure S2).

Discussion

In this study, using Nanopore sequencing with a single flow cell, we achieved up to 32 Gb reads in EVLP perfusate samples, with 99% of the reads mapping to the human genome. This resulted in 10X coverage in certain locations and a median of 4.89X coverage overall with a median sequencing time of 72 hours from perfusate draw and

Table 2 Summary of Sequencing Characteristics of Each Case

Case	Reads generated (M)	Estimated bases (GB)	Run length	Lung outcome	SNP overlap with 1,000 genomes	GC percentage	Coverage mean (X)	General error rate
1	13.87	9.79	3 days/23 hours/26 min	Transplanted	99,357	41.30%	2.27	5.60%
2	10.08	9.92	0 days/17 hours/1 min	Transplanted	181,241	40.30%	2.95	4.98%
3	14.31	11.81	1 days/2 hours/1 min	Declined	191,669	40.30%	3.12	5.36%
4	32.04	15.75	2 days/22 hours/36 min	Declined	248,246	40.40%	4.38	5.40%
5	17.62	12.87	3 days/0 hour/1 min	Declined	217,164	40.60%	3.31	5.18%
6	28.6	26.41	3 days/0 hour/1 min	Transplanted	233,285	40.40%	4.89	6.49%
7	36.79	32.25	3 days/0 hour/1 min	Transplanted	313,721	40.60%	8.19	5.34%
8	23.05	32.99	3 days/0 hour/1 min	Declined	290,405	39.80%	8.79	6.01%
9	23.98	29.78	3 days/0 hour/1 min	Declined	268,310	40.70%	6.61	6.35%
10	33.82	31.61	6 days/0 hour/4 min	Transplanted	246,993	41.00%	6.55	6.42%
11	34.16	24.7	5 days/0 hour/1 min	Transplanted	257,621	40.90%	4.89	5.55%

Abbreviations: GB, Gigabyte; GC, guanine-cytosine; M, million; SNP, Single-nucleotide polymorphism.

demonstrates our ability to rapidly and comprehensively sequence EVLP perfusate cfDNA using Nanopore. In addition, Nanopore sequencing allowed us to search for donor-specific SNVs from cfDNA in EVLP perfusate, and comparison with gold-standard short-read WGS demonstrated significant overlap, supporting this approach. Furthermore, we showed that% ddcfDNA in post-transplant plasma can be calculated as accurately as the conventional WGS method using donor-derived SNVs identified by using Nanopore sequencing with EVLP perfusate. While the current cost of a Nanopore flowcell is similar to that of short-read sequencers, we were able to perform all the sequencing within our academic lab without the need for a core sequencing facility. To the best of our knowledge, this is the first study to sequence cfDNA in EVLP perfusate using Nanopore sequencing and to show the possibility of rapidly detecting donor SNVs in the relevant timeframe of lung transplantation.

First, we used Nanopore sequencing to explore whether cfDNA sequencing of EVLP perfusate could be used to map donor-derived SNVs. EVLP perfusate volume is high, and thus we could obtain a large amount of cfDNA at once noninvasively. It is therefore very compatible with analyses that require high-volume DNA input, such as Nanopore sequencing. While cfDNA in post-transplant plasma is a mixture of donor- and recipient-derived cfDNA,[7] the cfDNA in EVLP perfusate is 100% ddcfDNA, and donor blood and tissue may not always be available, the ability to map donor-derived SNVs from perfusate is another advantage. In this study, we validated the SNVs obtained by Nanopore sequencing against perfusate by comparing them with short-read WGS of donor lung tissue and showed the accuracy of the SNVs obtained by Nanopore sequencing. One of the disadvantages of Nanopore sequencing is the high sequencing error rate;[18] however, we have shown that our analysis method using only homozygous SNVs filtered for overlap with the 1000 Genome SNP database can overcome the sequencing error rate of Nanopore sequencing and identify donor-derived SNVs with high accuracy. Furthermore, we have shown that the donor-derived SNVs identified here can be used to identify ddcfDNA in plasma after transplantation with a high degree of accuracy comparable to that of conventional WGS methods.

A major problem with conventional sequencing is that it takes more than a week[8] to identify SNVs in donors and recipients.[7,9,10] One option to alleviate this problem is the SNVs panel,[19] which is currently available commercially but is not donor-specific and has accuracy challenges, and this panel can also take days to weeks to run.[19] The current study showed that sequencing libraries could be prepared in 3.5 hours from perfusate, and sequencing within 3 days by using a single flow cell yielded enough SNVs to be used to discriminate donors from recipients. Although there are still issues regarding the time required for Nanopore sequencing, sequencing speed can also be further adjusted simply by adding flow cells, and entire human genomes were recently sequenced in 8 hours from sample collection to result in a pediatric ICU setting using a large number of flow cells[13] which will allow analysis within the timeframe of

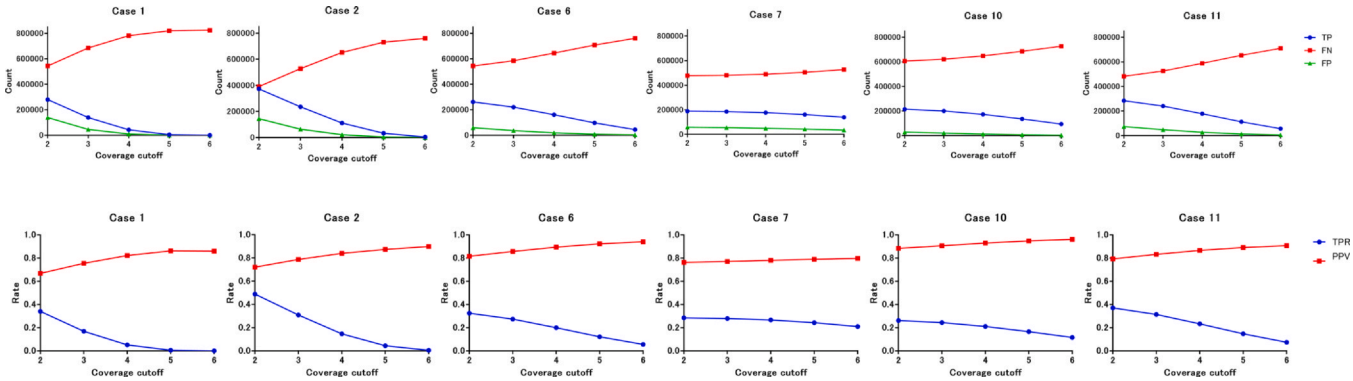

Figure 2 *Results of Nanopore sequencing to the matching short-read WGS.* The number of single nucleotide variations (SNVs) obtained by Nanopore sequencing showed an increase in true positives (TP) and a decrease in false negatives (FN) and false positives (FP) as coverages increased from X2 coverage to X6 coverage. The positive predictive value (PPV) of SNVs identified in 2X to 6X coverage regions ranged from 66.6% to 96.9%, with a median value of 90.3% at 6X coverage. TPR, true positive rate.

A

Percent of ddcfDNA detected in plasma after Tx

Case	WGS	Nanopore
1	31.0%	27.5%
2	41.0%	36.5%
6	8.0%	7.5%
7	29.5%	28.5%
10	56.0%	51.5%
11	21.5%	19.5%

B

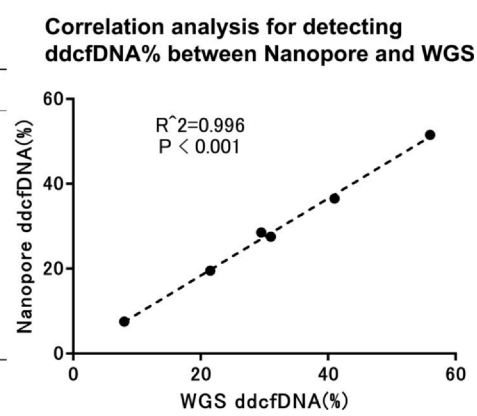

Figure 3 *Results of accuracy in detecting ddcfDNA in post-transplant plasma.* **(A)** The nanopore results showed similar results to the whole genome sequencing (WGS) library for donor-derived cfDNA (ddcfDNA) detection in post-transplant plasma. **(B)** Correlation analysis showed a strong correlation between results by Nanopore sequencing and results by WGS for detecting ddcfDNA% (R^2 = 0.996, $p < 0.001$).

the transplant. Further optimization of the DNA extraction process and library preparation may shorten the time from perfusate to the start of sequencing.

Several limitations associated with the present study warrant mention. This strategy remains only applicable to donor lungs undergoing EVLP, and we recognize that not all donor lungs undergo EVLP, but nanopore sequencing of DNA from donor PBMC may also be possible. Moreover, we acknowledge that this study remains a proof-of-concept for donor SNV calling by Nanopore and may not be fully clinically useful today. As sequencing technologies mature, it may yet be more cost-effective to simply sequence the donor (or recipient) by short-read sequencing at the time of procurement. Nonetheless, we show here the use of long-read Nanopore and the bioinformatic processes to obtain donor SNVs at high accuracy. As technologies mature, minimal-processing long-read sequencing may yet evolve into a clinically viable option. We omitted technical replicates of the next-generation sequencing technologies utilized given the low error rates, but replicates may yet have identified sequencing errors in short-read sequencing, though this should be mostly overcome by the coverage and the high variant allele frequency expected between normal genomes.[20] We also acknowledge that this is a proof-of-concept description of long-read sequencing for SNV calling. The clinical implications of recipient %ddcfDNA is best left to larger multicenter trials; however, this manuscript adds an alternative strategy for its calculation.

In conclusion, nanopore sequencing allowed us to easily and timely search for donor SNVs from cfDNA in EVLP perfusate. Despite the lower sequencing accuracy and depth obtained from Nanopore sequencing, a high PPV can be achieved in a set of donor-specific SNVs when appropriately filtered by read depth and overlapped with SNP databases. This demonstrates the potential for the use of nanopore sequencing to generate personalized donor cfDNA maps for use in postoperative donor-derived plasma cfDNA identification.

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Author contributions

Conceived and designed the analysis: HY, PZ, JS, GWW, JCY, Collected the data: HY, JA, AS, Performed the analysis: HY, JA, PZ, SK, JS, GWW, JCY, Wrote the paper: HY, JA, GWW, JCY.

Conflicts of interest statement

SK is a shareholder of Traferox Technologies Inc., and consultant for Lung Bioengineering. JCY is a consultant for Traferox Technologies Inc.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2025.100433.

References

1. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426-37.
2. Corcoran RB, Chabner BA. Cell-free DNA analysis in cancer. New England J Med 2019;380:501-2.
3. De Vlaminck I, Martin L, Kertesz M, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Nat Acad Sci U. S. A. 2015;112:13336-41.
4. Gielis EM, Ledeganck KJ, De Winter BY, et al. Cell-free DNA: an upcoming biomarker in transplantation. Am J Transpl 2015;15:2541-51.
5. Tsuji N, Agbor-Enoh S. Cell-free DNA beyond a biomarker for rejection: biological trigger of tissue injury and potential therapeutics. J Heart Lung Trans 2021;40:405-13.
6. Rosenheck JP, Keller BC, Fehringer G, Demko ZP, Bohrade SM, Ross DJ. Why cell-free DNA can be a "Game Changer" for lung allograft monitoring for rejection and infection. Curr Pulmonol Rep 2022;11:75-85.
7. De Vlaminck I, Valantine HA, Snyder TM, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med 2014;6:241ra77.
8. Shukla N, Levine MF, Gundem G, et al. Feasibility of whole genome and transcriptome profiling in pediatric and young adult cancers. Nat Commun 2022;13:2485.
9. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: an overview. Human Immunol 2021;82:801-11.
10. Khush KK. Clinical utility of donor-derived cell-free DNA testing in cardiac transplantation. J Heart Lung Transpl 2021;40:397-404.
11. Oxford Nanopore Technologies. Available at: https://nanoporetech.com/.
12. Simpson JT, Workman RE, Zuzarte PC, David M, Dursi LJ, Timp W. Detecting DNA cytosine methylation using nanopore sequencing. Nat Methods 2017;14:407-10.
13. Gorzynski JE, Goenka SD, Shafin K, et al. Ultrarapid nanopore genome sequencing in a critical care setting. New Engl J Med 2022;386:700-2.
14. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. New Engl J Med 2011;364:1431-40.
15. Peel JK, Pullenayegum EM, Naimark D, et al. Evaluating the impact of ex vivo lung perfusion on organ transplantation: a retrospective cohort study. Ann Surg 2023;278:288-96.
16. Valenza F, Rosso L, Coppola S, et al. Ex vivo lung perfusion to improve donor lung function and increase the number of organs available for transplantation. Transpl Int 2014;27:553-61.
17. Kanou T, Nakahira K, Choi AM, et al. Cell-free DNA in human ex vivo lung perfusate as a potential biomarker to predict the risk of primary graft dysfunction in lung transplantation. J Thorac Cardiovasc Surg 2021;162:490-499.e2.
18. Wang Y, Zhao Y, Bollas A, Wang Y, Au KF. Nanopore sequencing technology, bioinformatics and applications. Nat Biotechnol 2021;39:1348-65.
19. Grskovic M, Hiller DJ, Eubank LA, et al. Validation of a clinical-grade assay to measure donor-derived cell-free DNA in solid organ transplant recipients. J Mole Diagn JMD 2016;18:890-902.
20. Robasky K, Lewis NE, Church GM. The role of replicates for error mitigation in next-generation sequencing. Nat Rev Genet 2013;15:56-62.