Sequencing data of cell-free DNA fragments in living-related liver transplantation for inborn errors of metabolism

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A B S T R A C T
Graft derived cell-free DNA was recently reported as a non-invasive biomarker to detect graft damage or rejection after liver transplantation. There are a number of methods for quantification of Gcf-DNA,3 including quantitative-PCR, digital droplet PCR and massively parallel sequencing (next generation sequencing). Here we present the NGS4 data and fragment size distribution of cell-free DNA in the plasma of patients with inborn errors of metabolism who underwent living-related liver transplantation. For
Living-related liver transplantation for inborn errors of metabolism: more insights please see Analysis of fragment size distribution of cell-free DNA: a potential noninvasive marker to monitor graft damage in living-related liver transplantation for inborn errors of metabolism. [1]. © 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Data

Blood specimens from 11 patients with inborn errors of metabolism (IEM) who underwent living-related liver transplantation were collected at six specific time-points (d0, d1, d7, d14, d30, and d60). All the blood samples were drawn in the morning of the given days. This article shows the sequencing data and fragment size profile of cell-free DNA in the plasma of the transplant recipients. The total sequencing reads and reads aligned to Y-chromosome of each patient are summarized in Table 1. Each plasma DNA sample has generated 5.22 ± 1.02 million (mean ± SD) sequencing reads.

Analysis of the sequencing read lengths showed that cell-free DNA fragments were routinely present in the circulating plasma with a peak size around 165 base pairs (bp) before operation. Moreover, the size became shorter at post-operative day 1 and returned to a normal size when measured at day 7 or day 14. Despite this finding, there was a noticeable profile difference among participating subjects in the overall size distribution of their cfDNA (Fig. 1). We observed that cell-free DNA fragments in the plasma after liver transplantation were composed of different groups of fragments. These groups have different size ranges and Graft-derived cell-free DNA is present mainly in the shorter fragments.
| Case | Sex | Donor | Sex-mismatched | Time-pointa | Reads aligned to chromosome Y | Reads in all chromosomes |
|------|-----|-------|----------------|-------------|-----------------------------|-------------------------|
|      |     |       |                |             | Total 105-145bp >145bp      | Total 105-145bp >145bp |
| 1    | M   | Mother| d0             | 9108        | 1604                        | 6981                     |
|      | M   | Mother| d1             | 1160        | 268                         | 799                      |
|      | M   | Mother| d7             | 1797        | 406                         | 1075                     |
|      | M   | Mother| d14            | 7105        | 653                         | 6136                     |
|      | M   | Mother| d30            | 7186        | 798                         | 5991                     |
|      | M   | Mother| d60            | 6305        | 822                         | 5050                     |
| 2    | F   | Father| d0             | NA          | NA                          | NA                       |
|      | F   | Father| d1             | 6763        | 1894                        | 4375                     |
|      | F   | Father| d7             | 3884        | 699                         | 2890                     |
|      | F   | Father| d14            | 1751        | 245                         | 1408                     |
|      | F   | Father| d30            | 346         | 49                          | 259                      |
| 3    | M   | Father| d0             | 5382        | 790                         | 4209                     |
|      | M   | Father| d1             | 5651        | 1508                        | 3705                     |
|      | M   | Father| d7             | 5443        | 858                         | 4157                     |
|      | M   | Father| d14            | 4758        | 644                         | 3925                     |
|      | M   | Father| d30            | 5918        | 754                         | 4811                     |
| 4    | F   | Mother| d0             | 93          | 23                          | 56                       |
|      | F   | Mother| d1             | 101         | 29                          | 48                       |
|      | F   | Mother| d7             | 72          | 7                           | 46                       |
|      | F   | Mother| d14            | 82          | 10                          | 63                       |
|      | F   | Mother| d30            | 107         | 14                          | 69                       |
|      | F   | Mother| d60            | 86          | 14                          | 52                       |
| 5    | F   | Father| d0             | 124         | 19                          | 89                       |
|      | F   | Father| d1             | 5166        | 1380                        | 3415                     |
|      | F   | Father| d7             | 2946        | 488                         | 2235                     |
|      | F   | Father| d14            | 1866        | 241                         | 1518                     |
|      | F   | Father| d30            | 255         | 35                          | 187                      |
| 6    | M   | Mother| d0             | 4626        | 556                         | 3752                     |
|      | M   | Mother| d1             | 1929        | 295                         | 1468                     |
|      | M   | Mother| d7             | 4413        | 718                         | 3297                     |
|      | M   | Mother| d14            | 5534        | 621                         | 4668                     |
|      | M   | Mother| d30            | 4947        | 672                         | 4008                     |
|      | M   | Mother| d60            | 5878        | 778                         | 4705                     |
| 7    | F   | Mother| d0             | 62          | 7                           | 39                       |
|      | F   | Mother| d1             | 84          | 16                          | 54                       |
|      | F   | Mother| d7             | 78          | 10                          | 46                       |
|      | F   | Mother| d14            | 124         | 11                          | 97                       |
|      | F   | Mother| d30            | 71          | 12                          | 50                       |
| 8    | M   | Mother| d0             | 5523        | 661                         | 4449                     |
|      | M   | Mother| d1             | 3796        | 854                         | 2502                     |
|      | M   | Mother| d7             | 4986        | 626                         | 3945                     |
|      | M   | Mother| d14            | 6149        | 694                         | 5095                     |
|      | M   | Mother| d30            | 7337        | 796                         | 6161                     |
|      | M   | Mother| d60            | 7469        | 1134                        | 5812                     |
| 9    | M   | Mother| d0             | 7467        | 791                         | 6156                     |
|      | M   | Mother| d1             | 2990        | 711                         | 1968                     |
|      | M   | Mother| d7             | 4894        | 801                         | 3773                     |
|      | M   | Mother| d14            | 5705        | 661                         | 4770                     |
|      | M   | Mother| d30            | 6088        | 688                         | 5073                     |
|      | M   | Mother| d60            | 7945        | 1059                        | 6336                     |
| 10   | M   | Mother| d0             | 7224        | 844                         | 5882                     |
|      | M   | Mother| d1             | 1438        | 357                         | 970                      |
|      | M   | Mother| d7             | 6117        | 697                         | 5141                     |
|      | M   | Mother| d14            | 6517        | 749                         | 5433                     |

*continued on next page*
transplantation were composed of a group with a shorter fragment size (105-145bp) and another group with a longer fragment size (160-170bp) (Fig. 1). An overlap between graft-derived cell-free DNA and recipient-derived cell-free DNA was observed in the intermediate fragment size of 145–160bp. Based on the size distribution of cell-free DNA fragments, the sequencing reads were categorized into two groups (105-145bp, >145bp) (Table 1), which were subsequently used for S/L-Frag calculation [1]. Sequencing reads from Y-chromosome in the sex-matched pairs were used for Gcf-DNA quantification [1].

Table 1 (continued)

| Case | Sex Donor | Sex-mismatched | Time-pointa | Reads aligned to chromosome Y | Reads in all chromosomes |
|------|-----------|----------------|-------------|-----------------------------|-------------------------|
|      |           |                |             | Total 105-145bp >145bp Total 105-145bp >145bp |                             |
| M    | Mother Y  | d30            |             | 5560 641 4594               | 4,743,502 587,815 3,654,160 |
| M    | Mother Y  | d60            |             | 7571 960 6017               | 7,094,356 923,938 5,205,815 |
| 11 M | Father N  | d0             |             | 5840 855 4556               | 4,714,376 692,469 3,416,575 |
| M    | Father N  | d1             |             | 6688 1423 4764              | 5,322,285 1,025,954 3,620,193 |
| M    | Father N  | d7             |             | 7048 1236 5317              | 5,990,017 925,345 4,071,872 |
| M    | Father N  | d14            |             | 7867 758 6721               | 6,097,298 605,475 4,902,337 |
| M    | Father N  | d30            |             | 7554 1051 6080              | 6,102,619 840,679 4,621,985 |
| M    | Father N  | d60            |             | 7763 1002 6253              | 6,335,392 782,798 4,843,640 |

a d0 = operation day, sampling before operation; d1 = 1 day after operation; d7 = 7 days after operation; d14 = 14 days after operation; d30 = 30 days after operation; d60 = 60 days after operation.

Fig. 1. Size distribution of cell-free DNA in the plasma of liver transplantation patients with inborn errors of metabolism (IEM) before and after operation. Each line represents the size distribution of cell-free DNA in the plasma at different dates, with d0 and d1-60 indicating pre-operation and 1–60 days post-operation, respectively.
2. Experimental design, materials and methods

2.1. Sample preparation and sequencing

Five milliliters of EDTA blood specimens were collected from 11 patients with IEMs, including Ornithine Transcarbamylase Deficiency (OTCD), Propionic Acidemia (PA), Carbamoyl Phosphate Synthetase 1 Deficiency (CPS1D), Primary Hyperoxaluria (PH), N-acetyl Glutamic-acid Synthase Deficiency (NAGSD), Ethylmalonic Encephalopathy (EE) and Methylmalonic Acidemia (MMA), at 6 specific time-points after living-related liver transplantation (i.e. day 0, day 1, day 7, day 14, day 30 and day 60). All of the procedures and informed consent were approved by the Department of Ethics Committee at the Beijing Friendship Hospital of the Capital Medical University (Beijing, China) (approval document number: 2017-P2-080-02). All the legal guardians have provided written informed consent before living donor liver transplantation. Cell-free plasma was separated from the blood samples via two centrifugations (4°C at 2500×g for 10 minutes and 4°C at 15,500×g for 10 minutes). The resultant plasma was stored at −80°C until further analysis. DNA fragment from 600 μL of cell-free plasma was extracted by using Circulating Nucleic Acid Kit (Qiagen, Germany) [1]. The libraries were constructed by Ion Plus Fragment Library Kit (Life Technologies, USA) on the Ion Proton platform and then quantified using a Qubit Fluorometer. Subsequently, the selected libraries were pooled together with different barcodes and sequenced using an Ion Proton system (Life Technologies).

2.2. Sequencing data analysis

All sequencing data were aligned to the human genome reference sequences (version: NCBI Build37/hg19) using TMAP software (version 4.6.11). Unique reads whose mapping quality scores (MAPQs) were greater than 10 and whose lengths were longer than 35 bp were used in subsequent analyses [2]. In the sex-mismatched pairs, the proportion of reads from Y-chromosome (% chrY) was calculated and then used to determine the male DNA concentration in plasma.

2.3. Fragment size analysis

The reads mapped to hg19 were converted from Binary Alignment/Map (BAM) format to Browser Extensible Data (BED) format by using BEDTools software, and the length of each read was calculated by subtracting the start of the read from its end in the BED file. The size distribution of cell-free DNA in the recipient plasma was analyzed by calculating the percentage of read counts with each fragment size (ranging from 100 to 200bp) in total read counts. The sequencing reads were then grouped by their lengths, read counts of shorter fragments (105-145bp) and longer fragments (160-170bp) were used for further study on the S/L-Frag calculation in the research paper [1].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2020.105183.
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