Next-generation sequencing for deducing donor mismatched human leukocyte antigen typing from urine of kidney transplant recipients: abridged secondary publication

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KEY MESSAGES

1. Donor human leukocyte antigen (HLA) typing is crucial for the diagnosis of antibody-mediated rejection. It facilitates prompt medical intervention to salvage the graft from failure. Recipients' urine sample is proven to be a valuable non-invasive source for the deduction of donor HLA typing.
2. Next-generation sequencing (NGS) can help resolve the donor mismatched HLA typing at high resolution and reveal additional HLA loci that were not determined during transplant workup.
3. The NGS protocol can complement the polymerase chain reaction–single specific primer method to determine the presence of donor-specific antibody and define the specificity of donor typing at allelic level.
4. Loss of donor chimerism in recipient urine sample was associated with failure in detecting donor HLA allele by NGS.
5. Further optimisation of donor DNA quantity, amplification, and NGS data analysis is warranted to enhance the detection of donor DNA material in recipient urine sample.

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Introduction

Human leukocyte antigen (HLA) plays an integral part in immune surveillance and recognition of self and non-self antigens. HLA mismatches can affect the outcome of allogeneic transplantation. Owing to complexity and diversity of HLA, determination of antigenic specificity of HLA has always been challenging. Owing to the limited availability of specific antibody sera, HLA typing by serological method has been replaced by DNA-based typing. Polymerase chain reaction–based methods have limitations of tedious workflow, low throughput, un-phased data, and ambiguity. Whereas next-generation sequencing (NGS) provides a high throughput and comprehensive HLA typing without uncertainty. High-resolution typing information and expansion of additional HLA loci enable clinicians to fine-tune immunosuppressive treatment regimen, facilitate better monitoring of graft survival, and minimise complications related to the immune system.

Kidney transplantation is the most cost-effective modality for end-stage kidney diseases. It enables better quality of life and has superior survival outcomes. However, there is a severe shortage of kidney donors in Hong Kong. Many patients opt to undergo transplantation outside of Hong Kong and then return to Hong Kong for subsequent immunosuppressive therapy and follow-up care. In the event of graft failure, renal replacement therapy and retransplant pose a heavy burden on the healthcare system.

With the use of new immunosuppressive regimen to control T-cell alloimmunity, the episode of T-cell-mediated acute rejection has dramatically reduced. Nonetheless, antibody-mediated rejection still plays a major role in acute and chronic allograft rejection. Development of de novo donor-specific antibody (DSA) also leads to acute or chronic allograft rejection. With the improvement in sensitivity and specificity of antibody identification, DSA can be detected in early emerging stage. For patients who underwent kidney transplantations in Hong Kong, DSA can be promptly identified with known donor information to facilitate early clinical intervention by adjusting the immunosuppressant dosage and minimising expensive treatments.

To determine the donor HLA typing and enable the detection of DSA to facilitate clinical management, we developed a protocol using conventional HLA

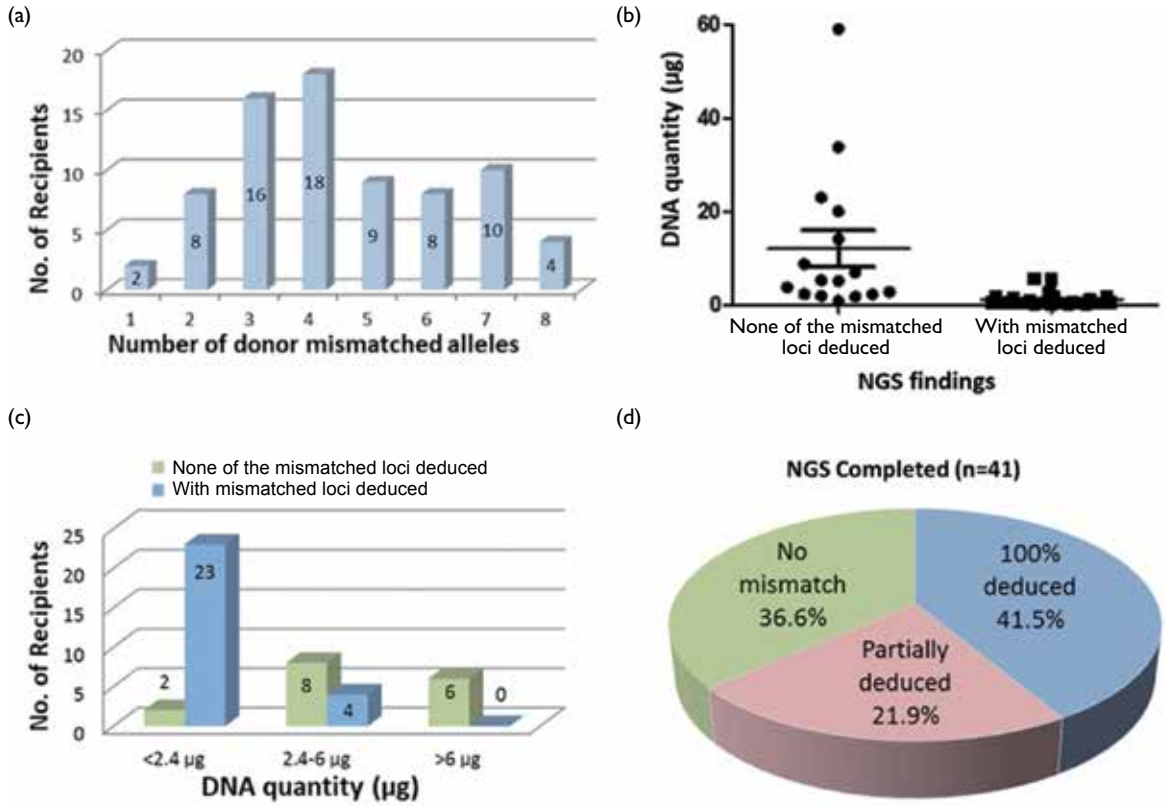

FIG. (a) Distribution of the number of mismatched human leukocyte antigen (HLA) between the recipients and the donors, (b) the association between DNA quantity and next-generation sequencing (NGS) findings of all samples, (c) distribution of the number of recipients with different amount of urine DNA quantity and the NGS results, and (d) results of deduction of donor HLA typing from recipient urine samples

typing to deduce donor mismatched HLA typing from recipient urine sample.[1] In our previous project, we have demonstrated that donor mismatched HLA typing can be successfully deduced from recipient urine samples and achieves a DSA diagnostic rate comparable with that in patients with known donor typing. However, ambiguities were found owing to the limitation of the polymerase chain reaction–single specific primer (PCR-SSP) method; some of the antigens were masked. In addition, poor DNA quality and/or inadequate amount of DNA render deduction of donor mismatched antigen unfeasible. Therefore, this study aimed to evaluate NGS for deduction of mismatched donor HLA information from recipient urine samples.

Materials and methods

A total of 75 patients who underwent kidney transplantations between 2016 and 2021 in Hong Kong with known donor HLA information and with more than one HLA mismatch at HLA-A, -B, -DRB1 or -DBQ1 loci were included. Patients with zero mismatch donor were excluded. The mean number of mismatched HLA was 4.4 alleles (Fig a).

Non-invasive urine HLA typing was deduced from the DNA material extracted from fresh early morning urine of recipients. HLA typing was performed by a NGSgo HLA typing kit (GenDx, The Netherlands) according to manufacturer instructions. The deduced mismatched donor HLA typing from the urine sample was compared with the patient's own HLA typing and known donor typing transplantation in Hong Kong. Data were analysed with the NGSMultiAnalysis tool.

Results

A total of 41 urine DNA samples with optimum DNA yield and quality were subjected to NGS analysis. The DNA quantities of the urine samples were significantly higher in those who failed to retrieve donor typing than those who could deduce donor HLA typing (P=0.0142, Fig b). The overall success rate for deducing donor mismatched HLA typing from urine samples was 63.4%. Low DNA quantity of the urine sample was associated with better result of deduction of the donor mismatched typing (Fig c). One plausible explanation could be the domination of recipient DNA in the chimeric urine

sample. Most of the amplicon and sequencing reads were dominated by the recipient DNA and hence the donor DNA failed to be detected.

Of the 26 samples with deduced HLA mismatch typing, 17 (65.4%) could detect all the mismatched alleles and nine (34.6%) could detect partial donor mismatched alleles (Fig d). The deduced HLA typing was 99.9% specific and matched with the donor typing in record. 15 (36.6%) of 41 urine samples had no mismatched donor HLA typing deduced, despite the mean number of mismatched alleles of 4.4. The number of mismatched between the three groups was not significantly different (P>0.05). This suggests that the probability of detecting deduced mismatch donor HLA typing was independent from the number of mismatched HLA alleles between the recipients and the donors.

The possible cause of failure in the deduction could be loss of donor chimerism in the urine sample. Only 0% to 4% of donor chimerism was observed from samples that failed in the deduction of donor mismatched HLA typing. However, this was independent to the time lapse after transplantation. Percentage of donor chimerism in individual mismatched allele could be determined by counting the sequence reads of the alleles. The mean number of donor alleles detected was 7.6% (range, 1.9%-25.1%). Samples with higher percentage of donor chimerism were likely to have 100% deduction of mismatched HLA alleles.

To determine whether NGS could resolve the ambiguity and quantity insufficiency issue of the PCR-SSP method, samples of recipients with unknown donor typing were retrieved. Mismatched HLA typing could be detected with a mean level of donor DNA of 5.6% (range, 2.5%-12.1%). In two samples, donor mismatched A*02:03 allele could be discriminated from the recipient A*02:07/A*02:06 allele, which could not be done so in the PCR-SSP method. Mismatched of all the eight loci have also been revealed. These results suggested that NGS could resolve the ambiguity and quantity insufficient issue of the PCR-SSP method and that additional loci could be typed simultaneously with less amount of DNA in urine sample.

Discussion

This study showed that mismatched donor HLA typing could be deduced with 99.9% specificity. However, owing to loss of donor DNA chimerism in the recipient urine samples, some donor mismatched HLA alleles were unable to be detected, probably masked by the predominant recipient DNA material. The sensitivity of detecting the donor HLA from the urine samples was 63.4%.

The superb resolution of NGS over the PCR-SSP method in terms of ambiguity resolution and typing accuracy for antibody antigen assay enables interpretation of the presence of DSA. NGS can deduce donor typing of some new HLA alleles such as HLA-C and HLA-DPB1, which plays a role in antibody-mediated rejection.[2] The additional donor HLA typing information facilitates decision making in prompt medical intervention and hence prolong graft survival.

The current NGS workflow requires high quality of template DNA, but DNA from the urine sample is usually fragmented with small molecular size, which results in a lower detection rate, compared with the PCR-SSP method. Alternative amplification or enrichment strategy may enable detection of scarce donor material in the urine sample. Further optimisation of NGS workflow and data analysis is warranted to enhance the detection of scarce donor HLA in recipient urine sample.

NGS is a viable alternative to deduce donor mismatched HLA typing from the recipient urine. It can resolve the donor typing at high resolution level and reveal the typing of additional allele simultaneously. This information is crucial for DSA interpretation and facilitation of prompt medical intervention to avoid the cost of treating chronic rejection.

Funding

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