Virus transmission during kidney transplantation assessed by virome analysis of living donor and recipient

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Solid organ transplantation and transmission of viruses

Routine screening prior to living donor kidney transplantation

**Serology**
- HIV 1/2
- HTLV I/II
- Hepatitis A/B/C/E
- HSV, CMV, EBV
- Measles, Mumps

**PCR**
- Hepatitis B/C

Little is known about other untested, apathogenic viruses a donor might carry and likely will transmit
Kidney transplant living donor/recipient pairs

**Sampling**

- University Hospital Zurich
- Enrollment of donor/recipient pairs since August 2014

- Each sampling consists of:
  - Blood
  - Urine
  - Stool

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling 1</td>
<td>Sampling 1</td>
</tr>
<tr>
<td>n = 30</td>
<td>time of transplant</td>
</tr>
<tr>
<td>Sampling 2</td>
<td></td>
</tr>
<tr>
<td>n = 30</td>
<td>4-6 weeks post-transplant</td>
</tr>
<tr>
<td>Sampling 3</td>
<td></td>
</tr>
<tr>
<td>n = 25</td>
<td>1 year post-transplant</td>
</tr>
</tbody>
</table>
Kidney transplant living donor/recipient pairs

*Evaluated symptoms*

- Immunosuppressive therapy
- Antiinfective prophylaxis
VirMet: a set of tools for viral metagenomics

VirMet is a software suite designed to help users running viral metagenomics experiments: unspecific massively parallel sequencing with the aim of discovering and characterizing the virus fraction of biological samples.

Virmet is now called with a command subcommand syntax: `virmet fetch --viral n`, for example, downloads the viral nucleotide database. Other available subcommands so far are:

- `fetch`: download genomes
- `update`: update viral/bacterial database
- `index`: index genomes
- `wolfpack`: analyze a Miseq run
- `covplot`: plot coverage for a specific organism

A short help is obtained with `virmet <subcommand> -h`.

Further detail following the menu on the left.

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**Viral metagenomic sequencing**

**Methods**

- Centrifugation
- Filtration (0.45 µm)
- Storage - 80°C
- Nuclease treatment
- Nucleic acid extraction EasyMAG system
- RNA
- cDNA synthesis
- 2nd strand synthesis
- Anchor PCR
- Library preparation Nextera XT
- Sequencing Illumina MiSeq
- Data analysis VirMet pipeline

**Random, Anchored Amplification**

- read length 150 bp
- ~ 3-5 Mio. reads/sample

**VirMet: a set of tools for viral metagenomics**

Lewandowska et al., Microbiome, 2017
Metagenomic sequencing detected BKPyV, JCPyV, HPV and TTV in blood and urine samples of donors and recipients (set 1)
TT viral loads increased in kidney transplant recipients under immunosuppression

- Lewandowska et al., 2017
- Young et al., 2015
- Görzer et al., 2015
- Görzer et al., 2014
- Jones et al., 2005
- Maggi et al., 2003
Metagenomic sequencing detected BKPyV, JCPyV, HPV and TTV in blood and urine samples of donors and recipients (set 1).

Suggested transmission of JCPyV from donors to recipients.
Sequence-specific qPCR confirmed metagenomic sequencing results (set 1)

Confirmed sequencing results but also detected additional cases
Sequence-specific qPCR identified cases of possible virus transmission (set 2)

Revealed 2 additional cases of JCPyV transmission from donor to recipient
Suggested transmission of JCPyV from donors to recipients in 7 cases
Confirmed transmission of JCPyV from donors to recipients in 6 out of 7 cases
• Metagenomic sequencing detected BKPyV, JCPyV, HPV and TTV (no other viruses were detected)

• Sequence-specific qPCR detected additional cases of BK-/JCPyV

• Phylogenetic analysis confirmed transmission of JCPyV from kidney transplant donors to recipients in 6 out of 7 cases

• TTV was detected by metagenomic sequencing and viral loads increased in kidney transplant patients under immunosuppression (qPCR)

• The role of JCPyV infection after renal transplantation is so far poorly defined

• Further studies are needed to define the impact of the donor’s virome on the recipient and predict transplant outcomes
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