A newly discovered human pneumovirus isolated from young children with respiratory tract disease

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Cytopathic effects in tMK cells

Control

hMPV-infected
Electron microscopy

- Pleiomorph
- Average size 100 - 600 nm
- Nucleocapsids rarely observed
- Envelope projections of 13 - 17 nm
- Paramyxovirus
RAP-PCR procedure

1. RNA isolation
2. RAP-PCR
3. Gel electrophoresis

4. Cloning (pCR2.1)
5. Sequencing
6. BLAST
PCR and sequence analysis of vRNA

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## Genomic organisation of pneumoviruses

| Gene | APV, 13.373 bp |
|------|---------------|
| N    | P             | M             | F | M2 | SH | G | L |

| Gene | hMPV, 13.378 bp |
|------|-----------------|
| N    | P | M | F | M2 | SH | G | L |

| Gene | hRSV, 15.225 bp |
|------|-----------------|
| NS1  | NS2 | N | P | M | SH | G | F | M2 | L |
Order Mononegavirales, family Paramyxoviridae

Paramyxovirinae

Pneumovirinae

Metapneumovirus

Pneumovirus

DNA Maximum likelihood, Polymerase ORF
Phylogenetic analysis

Maximum likelihood, 100 bootstraps, 3 jumbles
Genetic diversity of hMPV isolates

F, 142 bp

APV
94-1
99-1
99-2
94-2
93-1
93-2
93-3
00-1

0.1

N, 71 bp

APV
94-1
99-1
99-2
93-2
94-2
93-4
93-1
00-1
93-3

0.1

M, 143 bp

APV
94-1
99-2
99-1
00-1
93-2
93-1
94-2
93-3
0.1
93.4

L, 102 bp

APV
94-1
99-1
99-2
93-2
93-1
93-3
00-1
99-2
94-2
93-4

0.1

Maximum likelihood, 100 bootstraps, 3 jumbles

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## Amino acid sequence homology of metapneumovirus ORFs

| Sequence identity with ORFs of hMPV isolate 00-1 | N  | P  | M  | F  | M2.1 | M2.2 | SH | G   |
|-------------------------------------------------|----|----|----|----|------|------|----|-----|
| hMPV 99-1                                       | 95 | 86 | 98 | 94 | 95   | 90   | 57 | 33  |
| APV-C                                           | 88 | 68 | 87 | 81 | 84   | 56   | N.A.| N.A.|
| APV-A                                           | 69 | 55 | 78 | 68 | 72   | 25   | 18 | 9   |
### Seroprevalence of hMPV in The Netherlands

| Age (Years) | Immunofluorescence assays | Virus neutralization assays |
|-------------|---------------------------|-----------------------------|
|             | $n$ tested | $n$ positive (%) | $n$ tested | $n$ positive (%) | Titre range |
| 0.5 - 1     | 20         | 5 (25)             | 12         | 3 (25)          | 16-32 |
| 1 - 2       | 20         | 11 (55)            | 13         | 4 (31)          | 16-32 |
| 2 - 5       | 20         | 14 (70)            | 8          | 3 (38)          | 16-512 |
| 5 - 10      | 20         | 20 (100)           | 4          | 4 (100)         | 32-256 |
| 10 - 20     | 20         | 20 (100)           | 4          | 3 (75)          | 32-128 |
| > 20        | 20         | 20 (100)           | 4          | 3 (75)          | 32-128 |
| 8 - 99¹     | 72         | 72 (100)           | 11         | 11 (100)        | 16-128 |

¹Sero-archeological analysis using sera collected in 1958
Conclusions-1

a newly discovered human metapneumovirus (hMPV)

- hMPV is associated with respiratory tract illnesses:
  - In young children (& elderly, immunocompromised, others)
  - Primarily in the winter season
  - Symptoms similar to those caused by RSV (ranging from mild respiratory symptoms to severe bronchiolitis and pneumonia)
  - From children infected with hMPV, no other human pathogens were isolated
  - hMPV vRNA is not detected in throat swabs from healthy children

- Experimental infections of animals with hMPV:
  - hMPV does not replicate in chickens and turkeys
  - hMPV replicates in macaques, and causes mild respiratory symptoms
  - hMPV replicates in guinea pigs and ferrets
Conclusions-2

A newly discovered human metapneumovirus (hMPV)

- hMPV is a putative new member of the *pneumovirinae*, genus *metapneumovirus*
- Virus characteristics (EM, CPE, HA⁻, CHCl₃-sensitive, trypsin-dependence)
- Sequence homology
- Genomic organisation

- hMPV is a virus that commonly infects humans
  - Sero-prevalence reaches 100% of humans by the age of five years
  - In the 2000 winter season, hMPV was isolated from 10% of children with respiratory tract illnesses (negative for other pathogens)
  - Human sera collected in 1958 were all positive for antibodies to hMPV
  - Reinfection with homologous/heterologous virus strains?

- hMPV diversity
  - Two genetic clusters of hMPV isolates were found in the Netherlands
  - The genetic clusters may represent different serotypes of hMPV
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