

Genotype Variability of Human Metapneumovirus Isolated from Korean Children, 2007 to 2013

Mi-Kyung Lee, Tae-Hyoung Kim, Jun Hyung Lee
Chung-Ang University Hospital, Seoul, South Korea

Background

- **Human metapneumovirus (hMPV)**
 - Enveloped, negative-sense RNA virus of the *Paramyxoviridae* family
 - *Pneumovirinae* subfamily along with respiratory syncytial virus
 - Common viral pathogen in respiratory tract infection (RTI) of young children
 - Discovered in Netherland in 2001

Background

- **Human metapneumovirus (hMPV)**
 - Identification of acute hMPV infection
 - Serology: not adequate
 - RT-PCR : most widely used
 - Subtype
 - A1, A2 (A2a, A2b), B1, B2
 - The clinical significance of lineage classification by genotype remains unclear

Objective

- **Purpose of this study**

- evaluated the contribution of hMPV to viral RTIs and identified the genotype variability and circulating patterns of hMPV in Korean children.
- In addition, we performed sequence and phylogenetic analysis of unidentified strains.

Materials and Methods

■ Sample Collection

- From January 2007 to December 2013 (7 years)
- Nasopharyngeal aspirates from 14,853 pediatric patients
- Viral RNA extraction using Viral Gene-spin (Intron Biotechnology, Korea)
- Reverse transcription and Multiplex PCR or Realtime-PCR using RV Detection kits (Seegene, Korea)

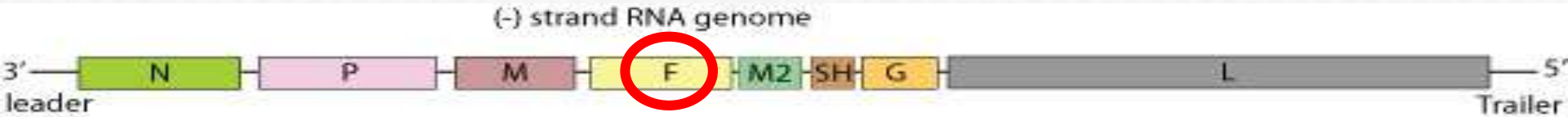
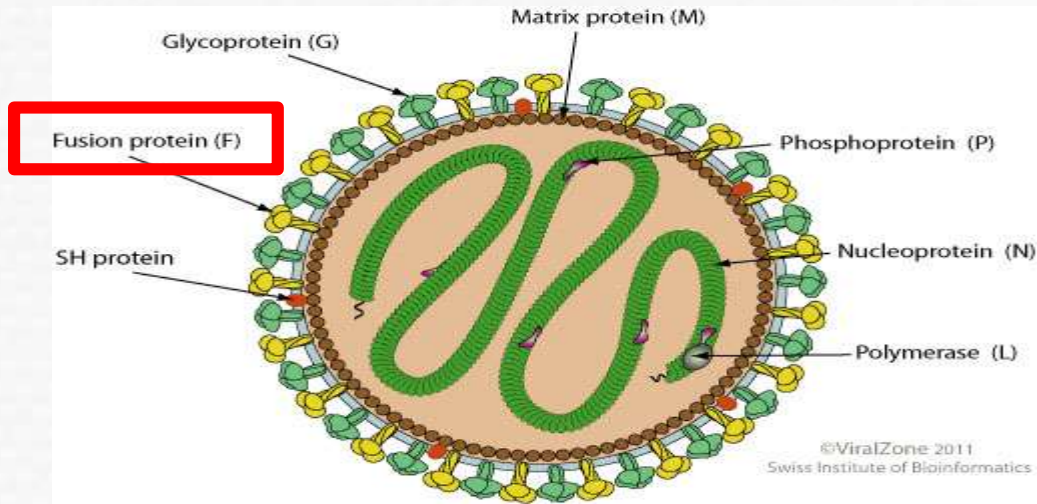
Materials and Methods

■ hMPV Genotyping

- Total 793 samples positive for hMPV
- Nested PCR-RFLP (restriction enzyme length polymorphism) analysis
 - Amplification target: F gene of hMPV
 - Restriction Enzyme: Tsp509I for 16 hrs at 65°C
 - Electrophoresis in 4% w/v agarose gel

Materials and Methods

- Enveloped virus with a nonsegmented negative-sense RNA genome

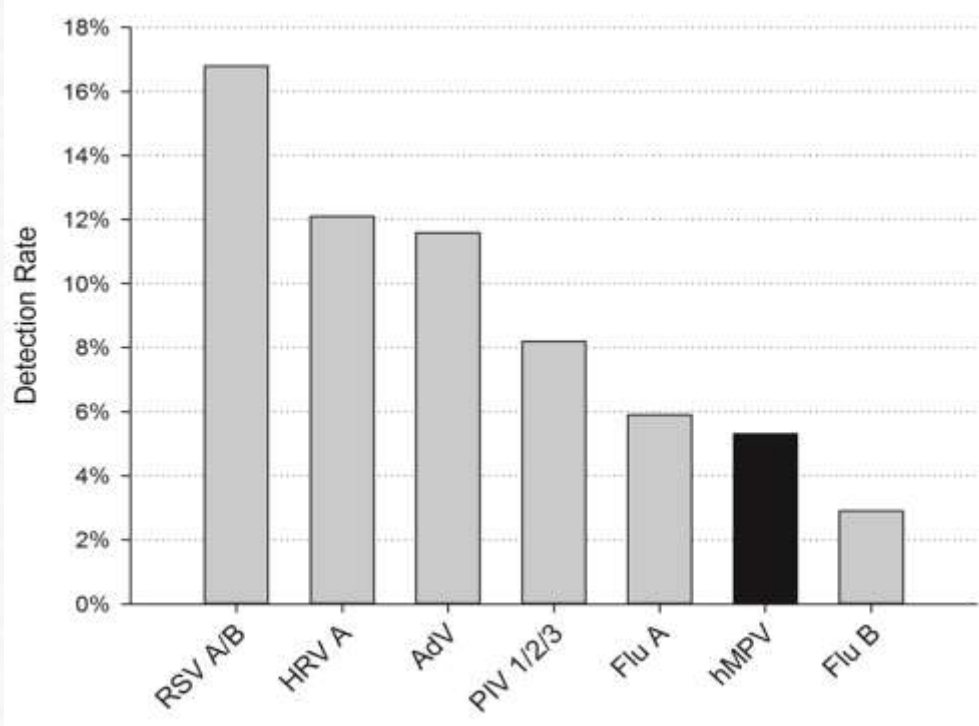


Materials and Methods

- **Sequencing & Phylogenetic Analysis (F gene)**
 - 15 samples with unknown bands in RFLP
 - 5 samples; overlapping patterns
 - 9 samples; Not Determined (ND) 1 pattern
 - 1 sample; ND2 pattern
 - Sequencing: BigDye Terminator ver. 3.1 and ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems)
 - Sequence alignment & phylogenetic Analysis: Molecular Evolutionary Genetics Analysis version 6 (MEGA6)

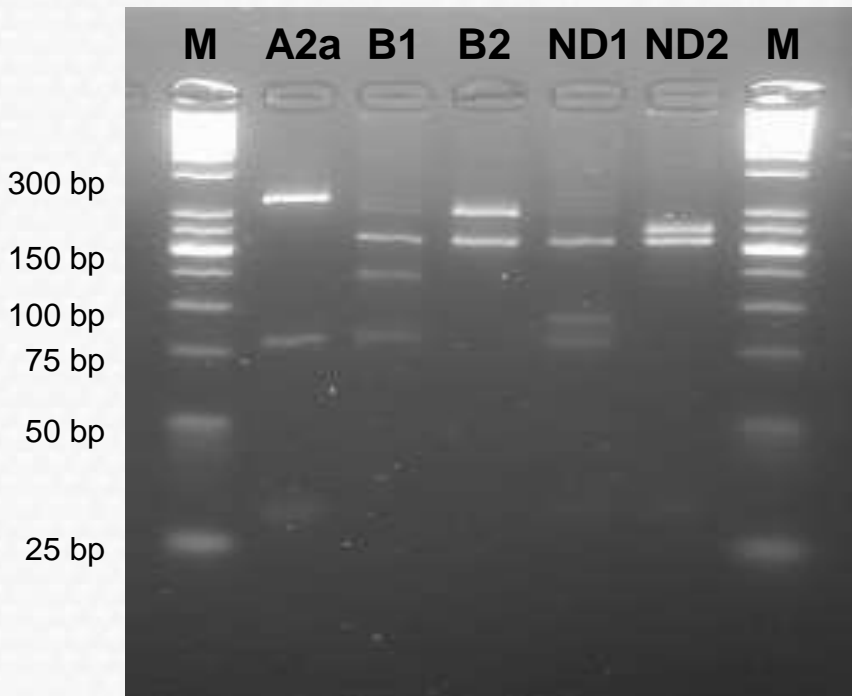
Results

- **Detection Rate of 7 respiratory viral pathogens**



During the 7 years of this study, hMPV was detected in **5.3%** (793 of 14,853) of all samples analyzed.

Results



■ Specific bands in Subtypes

- A2a: 24, 28, 76, 220 bp
- B1: 77, 116, 153 bp
- B2: 153, 193 bp
- ND1: 26, 77, 90, 156 bp
- ND2: 26, 156, 167 bp

Results

■ hMPV genotyping by nested PCR & RFLP

- Among the 793 samples which were hMPV-positive in the RV screening test, nested PCR-RFLP analysis showed distinct bands in 749 samples (94.4%).
- In 98.0% (734 of 749) of these samples, we clearly identified four hMPV genotypes (A2a, A2b, B1, and B2), but genotype A1 was not detected.

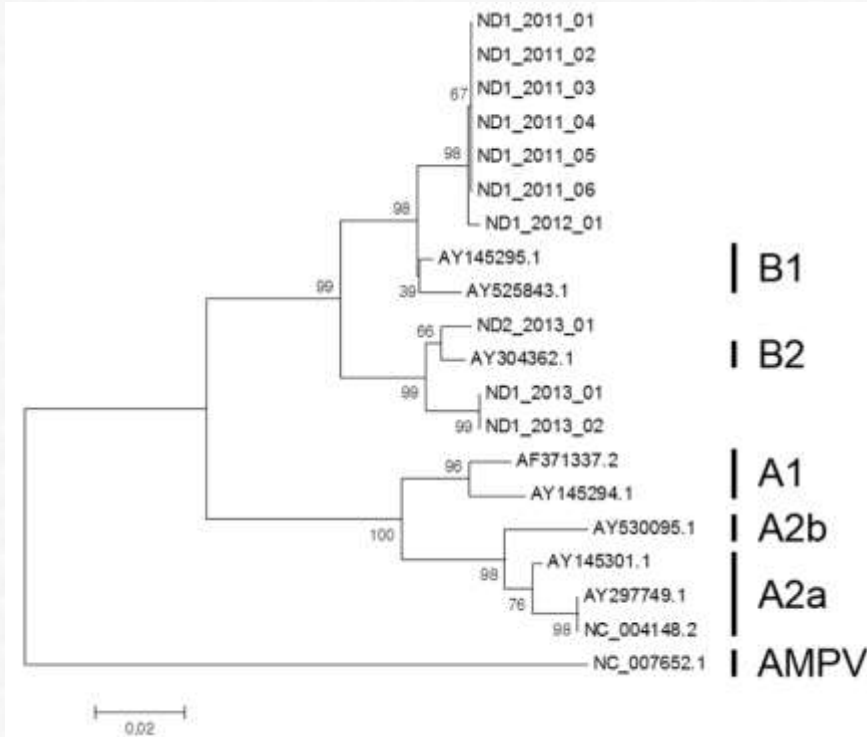
Results

■ hMPV genotypes during a 7-year period

Year	hMPV-positive samples by genotype N (%)						Total
	A1	A2a	A2b	B1	B2	ND*	
2007	0 (0.0)	41 (85.4)	0 (0.0)	0 (0.0)	7 (14.6)	0 (0.0)	48 (100.0)
2008	0 (0.0)	1 (5.3)	0 (0.0)	0 (0.0)	17 (89.5)	1 (5.3)	19 (100.0)
2009	0 (0.0)	33 (40.7)	0 (0.0)	0 (0.0)	46 (56.8)	2 (2.5)	81 (100.0)
2010	0 (0.0)	150 (79.8)	2 (1.1)	27 (14.4)	7 (3.7)	2 (1.1)	188 (100.0)
2011	0 (0.0)	60 (40.8)	0 (0.0)	76 (51.7)	5 (3.4)	6 (4.1)	147 (100.0)
2012	0 (0.0)	13 (10.0)	0 (0.0)	97 (74.6)	19 (14.6)	1 (0.8)	130 (100.0)
2013	0 (0.0)	26 (19.1)	0 (0.0)	12 (8.8)	95 (69.9)	3 (2.2)	136 (100.0)
Total	0 (0.0)	324 (43.3)	2 (0.3)	212 (28.3)	196 (26.2)	15 (2.0)	749 (100.0)

Results

■ Phylogenetic tree of 10 isolates (ND1 or ND2 patterns)



- In the ND1 pattern strains of 2011-2012, we discovered high similarity to genotype B1.
- The ND1 and ND2 pattern strains of 2013 were similar to genotype B2.

Conclusion

- The predominant genotype of hMPV changed over the 7-year period in the following order
: A2a (2007) → B2 → B2 → A2a → B1 → B1 → B2 (2013)
- This large-scale, 7-year study determined the contribution of hMPV to viral respiratory tract infections and identified the genetic diversity of hMPV in Korea.
- We expect that this study may help reveal the characteristics of hMPV propagation.