

LETTER TO THE EDITOR

Glycoprotein B gene-based phylogenetic analysis of porcine cytomegalovirus isolates

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Porcine cytomegalovirus (PCMV) is a ubiquitous and undesired pathogen for pig health and cause of donor organs contamination used for xenotransplantation. However, the molecular knowledge of the virus is still very lacking. In the present study, we sequenced and analyzed the complete nucleotide sequences of glycoprotein B (gB) gene of PCMV strains from clinic samples in China. For the first time, based on the phylogenetic analysis of complete nucleotide sequences of gB, PCMV strains were revealed to have evolved into two distinct genogroups: B6-related clade I and OF1-related clade II. Also, special amino acid alterations of “TA-P-S-D” to “ST-S-P-N” were found in gB protein of B6-related clade I and OF1-related clade II strains, respectively, indicating the existence of subgroups of PCMV with obvious genetic differentiation. Moreover, the PCMV strains in the two clades are discovered to be co-circulating in China.

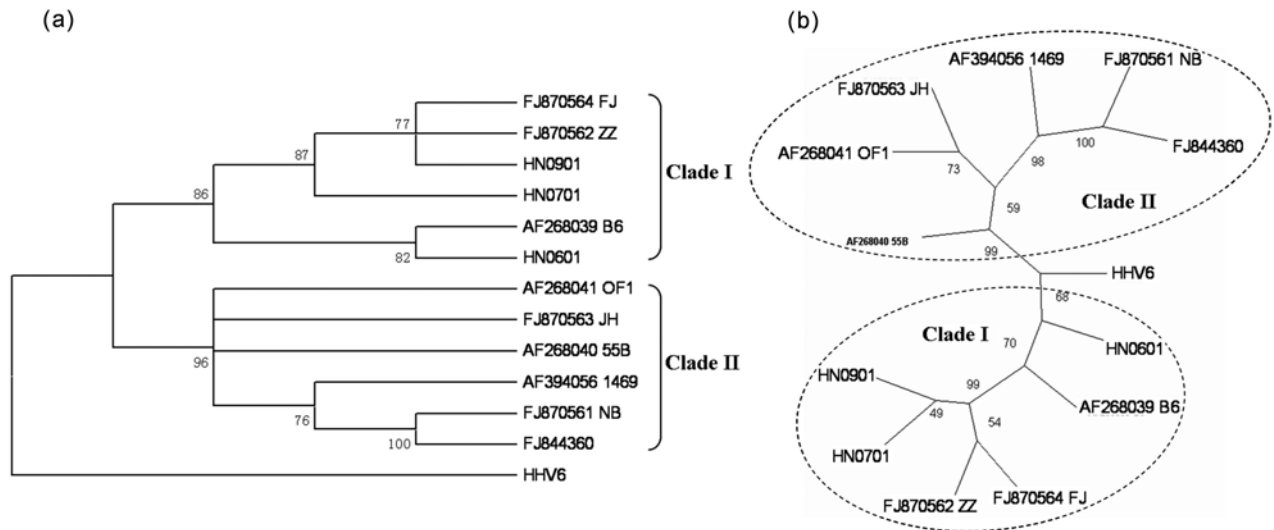
PCMV was first described as “inclusion body rhinitis” in 1955 (1). It can produce both fatal and asymptomatic generalized infections (2). The affected neonatal piglets became lethargic and pale with anasarca, with widespread haemorrhages, particularly in the lungs and kidneys. Other usual symptoms were runtting, rhinitis, pneumonia and poor weight gain. Asymptomatic infection was observed in older

pigs, with basophilic intranuclear inclusions and associated cytomegaly occurred in epithelial cells, particularly in the mucous glands of the nasal mucosa and kidney tubule cells (2–4). It can also cause *in utero* infections in sows leading to abortion, loss of fetuses and birth of weak piglets (5, 6). PCMV has been included as a member of the subfamily *Betaherpesvirinae*, and regarded as one member of the genus *Roseolovirus*, together with human herpes virus (HHV)-6 and -7, based on its biological properties such as restricted host range, protracted replication cycle which results in slow enlargement of infected cells (7), and genetic data (8–10). PCMV was reported to be distributed worldwide and to be highly prevalent in individual herds based on serological and PCR assay (2).

Lungs of pigs with respiratory disease from pig farms in two locations (Xiangtan city and Changsha city) of Hunan province, China were collected. The total DNA was extracted from the homogenized tissue samples according to the routine phenol/chloroform procedures. One primer pair for PCMV detection (PCMVDF: 5'-GAATACACTGAAGCAAGCGA TGATG-3', PCMVDR: 5'-AATCTCGGCAGCGGAAAA ACAT-3'), and three primer pairs for gB gene amplification (PCMV1F: 5'-ACCTGTTAATCGG ACCCGTGAT-3'; PCMV1R: 5'-TTATCCCTGGTTTCTAATCTCG-3'; PCMV2F: 5'-GAATGGTCAATGTTTTTCCGCTG-3'; PCMV2R: 5'-TAACCGTTTTTCGTGTAATGTAAT-3'; PCMV3F: 5'-TTGCGGGTAAGGAGGCGTATT-3'; PCMV3R: 5'-GTTTCGCTGGGCACTTCACAG-3') were designed based on the known sequences from GenBank by Primer 5.0. The PCR products were purified and cloned into

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Abbreviations: gB = glycoprotein B; PCMV = porcine cytomegalovirus



Phylogenetic analysis with nucleotide sequences of the complete gB gene sequences of PCMV strains

The trees were constructed by the UPGMA (a) and neighbor-joining (b) methods with 1000 bootstrapped replicates by MEGA 4 and PAUP 4.0 beta, respectively. Bootstrap percentages are indicated at the internodes. Besides three strains obtained in the present study, the GenBank Acc. numbers of other PCMV strains with complete gB sequences available are as follows: B6 (UK, AF268039), OF-1 (Japan, AF268041), 55B (Spain, AF268040), 1469 (Sweden, AF394056), JH (China, FJ870563), ZZ (China, FJ870562), NB (China, FJ870561), FJ (FJ, FJ870564), FJ844360 (China), FJ595497 (China). The nucleotide sequences of gB gene of HHV-6 (Acc. No. X83413) was used as an outgroup to determine the root of the tree.

pMD-18 T vector (TaKaRa) and transformed into *Escherichia coli* top10 competent cells. Positive clones of transformed cells were identified and sequenced from both ends. Assembly of sequenced contigs and sequence alignment were performed with DNAMAN version 7 (Lynnon Corporation). Three of six lung samples were PCR-positive with the PCMV detection primers and named HN0601 (from Bainihu pig farm), HN0901 (from Zhenghong pig farm) and HN0701 (from Zhenghong pig farm). The complete gB sequences of three strains were amplified, sequenced, and deposited in GenBank under the accession numbers: HQ686080, HQ686081 and FJ595497. Phylogenetic trees were conducted using the neighbor-joining (NJ) and UPGMA approaches implemented in PAUP* v4.0 beta and MEGA version 4, respectively (11, 12). The consensus tree was obtained after bootstrap analysis with 1000 replications, and values above 50% indicated above the internodes. Pairwise distances between sequences were calculated by MEGA version 4 (12).

The sizes of gB gene of the three present strains are the same (2580 nucleotides), and the identities of nucleotide and deduced amino acid (aa) sequences between them were 98.6–99.4% and 98.3–99.1%, respectively. Thirteen amino acid alterations and two aa indels (glutamine (Q) or asparagine (N) resulting from sequence repeat or deletion) were found between the three polypeptides (data not shown). Compared with other known gB amino acid sequences available in GenBank, the present three sequences showed identities of 97.1%–99.5% with them, and the amino acid

identities between all of the gB sequences were 96.3%–100%. Interestingly, at the amino-terminus of the protein, special amino acid substitutions of “TA-P-S-D” were first revealed in strains of 55B (Spain), OF-1 (Japan), 1469 (Sweden), JH (China), NB (China) and FJ844360 (China), while in strains of HN0601, HN0901, B6 (UK), ZZ (China), and HN0701 (China), they were “ST-S-P-N”. We assume, that those aa changes could represent genetic marker indicating different evolutionary story of the strains.

Phylogenetic analysis was conducted based on twelve complete nucleotide sequences of gB gene obtained in the present study and available in GenBank. These sequences were from five countries, including 8 sequences from China, and four from other countries (United Kingdom, Spain, Sweden and Japan, one from each state). Strikingly, phylogenetic trees based on the 12 complete gB sequences revealed two distinct clades with high bootstrap support values (Figure), *i.e.* clade I includes six strains: B6 (UK), ZZ (China), FJ (China), HN0601, HN0901 and HN0701 (China), and clade II consists of remaining six strains: OF-1 (Japan), 55B (Spain), 1469 (Sweden), JH (China), NB (China) and FJ844360 (China).

Generally, from special aa substitutions and phylogenetic analysis of gB, the present data provided the strong evidence, for the first time, that PCMV have evolve into two genogroups: the B6-related clade I and the OF1-related clade II. Moreover, these two groups are revealed to be co-circulating in China. Based on the limited data, B6-related clade I strains seem to be more prevalent in China. Five Chinese strains in

clade I were from three different parts of China: south-central China (HN0901, HN0601 and HN0701), central China (ZZ) and southeastern China (FJ), while the three Chinese strains in clade II are all from the same province (Zhejiang province) of eastern China. However, much more data from different parts of China and abroad are very needful to further demonstrate the evolutionary history of PCMV.

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