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Research paper

Complete genome sequence of mumps viruses isolated from patients with parotitis, pancreatitis and encephalitis in India

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ABSTRACT

Limited information is available regarding epidemiology of mumps in India. Mumps vaccine is not included in the Universal Immunization Program of India. The complete genome sequences of Indian mumps virus (MuV) isolates are not available, hence this study was performed. Five isolates from bilateral parotitis and pancreatitis patients from Maharashtra, a MuV isolate from unilateral parotitis patient from Tamil Nadu, and a MuV isolate from encephalitis patient from Uttar Pradesh were genotyped by the standard protocol of the World Health Organization and subsequently complete genomes were sequenced. Indian MuV genomes were compared with published MuV genomes, including reference genotypes and eight vaccine strains for the genetic differences. The SH gene analysis revealed that five MuV isolates belonged to genotype C and two belonged to genotype G strains. The percent nucleotide divergence (PND) was 1.1% amongst five MuV genotype C strains and 2.2% amongst two MuV genotype G strains. A comparison with widely used mumps Jeryl Lynn vaccine strain revealed that Indian mumps isolates had 54, 54, 53, 49, 49, 38, and 49 amino acid substitutions in Chennai-2012, Kushinagar-2013, Pune-2008, Osmanabad-2012a, Osmanabad-2012b, Pune-1986 and Pune-2012, respectively. This study reports the complete genome sequences of Indian MuV strains obtained in years 1986, 2008, 2012 and 2013 that may be useful for further studies in India and globally.

1. Introduction

Mumps is an acute viral illness characterized by fever and swelling of the parotid gland(s) that affects typically young children, adolescents, sometimes adults and may lead to complications such as deafness, orchitis, oophoritis, pancreatitis and meningo-encephalitis (Philip et al., 1995). Approximately, half of infected individuals develop classical disease and others may be asymptomatic or develop non-specific respiratory symptoms (WHO, 2005). Mumps virus (MuV) is a member of *Paramyxoviridae* family, subfamily *Paramyxovirinae* and genus *Rubulavirus*. MuV has a single-stranded, negative sense RNA genome consisting of 15,384 nucleotides that encodes seven proteins; fusion (F) and hemagglutinin–neuraminidase (HN), nucleoprotein (NP), phosphoprotein (V/P/I), matrix protein (M), large protein (L), and small hydrophobic (SH) protein (Elango et al., 1988). To strengthen the molecular epidemiology of mumps, a standard protocol to define and name distinct MuV genotypes was proposed by the World Health Organization (WHO). Accordingly, a MuV SH gene based RT-PCR was used to generate global sequence database. An updated MuV nomenclature has assigned 12 mumps genotypes i.e. A–N except E and M genotypes (WHO, 2012).

Limited reports are available regarding epidemiology of mumps, its incidence and sero-prevalence in India. Therefore, a detailed investigation on the sporadic cases or outbreak(s) of mumps from India is necessary to understand the circulation of MuV in the context of the global mumps epidemiology. Recently, a circulation of mumps genotype C strains has been reported from Karnataka, Maharashtra and Tamil Nadu states (Raut et al., 2015; Vaidya et al., 2013; Jeevan et al., 2013) and a circulation of mumps genotype G strains from the Maharashtra and Punjab states (Vaidya et al., 2013; Mishra et al., 2013). Altogether, 73 MuV complete genome sequences are available in the GenBank database (Jin et al., 2015) however, none from India. This study reports complete genome sequences of seven mumps viruses isolated from Maharashtra, Tamil Nadu and Uttar Pradesh states of India and its comparison with eight MuV vaccine genomes available in GenBank.

2. Materials and methods

2.1. Mumps patients and mumps virus isolates

The clinical presentation of mumps patients and the details of the mumps virus isolates were described in Table 1. Mumps patients were confirmed in the laboratory either by enzyme immunoassay (EIA) or

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Table 1
Clinical presentation of mumps patients and details of the mumps virus isolates.

Case	Age[a]/sex	Clinical presentations	Mumps IgM EIA	Virus isolation source	Mumps virus RT-PCR	Mumps virus isolate name
1	6/F	1–2 days fever, cough, cold and bilateral parotitis	Positive	Throat swab	Positive	Pune-2012
2	46/M	1–3 days fever, cough, bilateral parotitis, orchitis and pancreatitis	Positive	Throat swab	Positive	Pune-2008
3	7/F	1–2 days fever and bilateral parotitis	Positive	Throat swab	Positive	Osmanabad-2012a
4	11/F	1–2 days fever and bilateral parotitis	Positive	Throat swab	Positive	Osmanabad-2012b
5	8/F	1–4 days fever, cough, body ache and unilateral parotitis	Positive	Oral swab	Positive	Chennai-2012
6	NA[b]	Fever with bilateral parotitis & MuV isolated during 1986	NA[b]	NA[b]	Positive	Pune-1986
7	1/M	Encephalitis patient	Positive in CSF	CSF	Positive	Kushinagar-2013

[a] Age mentioned in years.
[b] Not available.

reverse transcriptase polymerase chain reaction (RT-PCR). The oldest MuV isolate was obtained in year 1986 followed by isolates from years 2008, 2012 and 2013. Five mumps viruses were isolated from Maharashtra state (Pune-1986, Pune-2008, Pune-2012, Osmanabad-2012a and Osmanabad-2012b) and another two mumps isolates were received from the Dr. ALM PG Institute of Basic Medical Sciences Chennai, Tamil Nadu (Chennai-2012) and NIV Gorakhpur Unit, Uttar Pradesh (Kushinagar-2013).

Virus isolations were performed in Vero cells using throat or oral swabs or cerebrospinal fluid (CSF) collected from the suspected mumps patients. Mumps virus RNA was detected in the tissue culture fluid (TCF) and mumps SH gene was sequenced to define virus genotype. Subsequently, all mumps isolates were subjected to complete genome sequencing.

2.2. RNA extraction and RT-PCR amplification

Sufficient quantity of viral RNA was extracted from 0.5 ml of TCF of each MuV isolate as per the instructions provided in the commercial kit (Qiagen, Hilden, Germany). Individual PCRs for each gene of each isolate were performed. RNA was reconstituted in nuclease free water and stored at −80 °C until amplification by RT-PCR. Previously published 46 MuV primers (Jin et al., 2000) and an additional 15 primers (Table 2) were synthesized using the GenBank sequence AF280799 (Integrated DNA Technologies, USA) and MuV genome was amplified using a set of 61 primers. A set of overlapping fragments ranging in size from 444 to 886 base pairs were amplified in each RT-PCR reaction using One-step RT-PCR with Platinum® *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA). The complementary-DNA was prepared at 55 °C for 30 min followed by hot-start at 94 °C for 2 min and 40 cycles of 94 °C for 15 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 30 s (extension) and a final extension step at 72 °C for 7 min was completed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA).

Table 2
Additional primers used for entire MuV genome amplification.

Primer ID	Sequence (5′ → 3′)	Location
MuV1-a	ACC AAG GGG AAA ATG AAG AT	1–20
MuV-F25	AAT GCA TCC CTC CAA AAT GC	5914–5933
MuV-F26r	GAA TTT CGA GGG CTC CAT CT	6612–6631
MuV F27	ATT GGA TTT CAG CAT TGT CTC	5796–5816
MuV F28r	CGG CAG GGT CAC GAG ACG TTA	6240–6260
MuV 25-a	GGA GTT TCG ATC ACT CAC TCT A	6419–6440
MuV 26r-a	CCA TCA CTG AGA TAT TGG ATT T	7305–7326
MuV33	CTC TAT CGG CCA TCC ACT CAA	7084–7104
MuV34r	GAG TGT ACT ATT GGG CAA GAC	7589–7609
MuV L-35	GCT AGT CAT TAT CTG TCC CC	9212–9231
MuV L-41	ATT GCT GAT GTG AAA CGA TT	11,336–11,355
MuV L-47	GTA TGT CAC CTG AGG ACA AA	13,350–13,369
MuV L-49	TAC AGA TTG ATT CAA GCA GG	14,024–14,043
MuV L-53	CCA ATC ATC AGA TCA TGA CA	14,770–14,789
MuV L-54r	ACC AAG GGG AGA AAG TAA AA	15,365–15,384

2.3. Sequencing and data analysis

The PCR products were purified using a QIAamp DNA minikit (Qiagen, Hilden, Germany) and sequenced using the same set of primer pairs used in RT-PCR at a concentration of 0.02 μg/μl. Twenty-five cycles of 94 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min were performed. The sequencing reactions for each gene were performed separately using the DyeDeoxy terminator sequencing kit (Applied Biosystems, Foster City, CA, USA). Furthermore, a sequencing reaction (forward and reverse) was purified using a Dye Ex 2.0 Spin Kit (Qiagen, Hilden, Germany). Genome sequences were obtained using an automated sequencer (ABI/HITACHI 3730XL, DNA Analyzer (96), Tokyo, Japan).

Both DNA strands of overlapping PCR amplicons were sequenced, the resulting sequences were compiled and consensus sequence was deduced. The complete genome sequences of mumps viruses available as on 28th April 2015 in GenBank were downloaded, compared using the MEGA version-6 to define the genetic variations in each gene along with reference genotypes (EU370206 and AY280799) and eight vaccine strains i.e. Jeryl Lynn (AF338106), RIT 4385 (FJ211584), Leningrad-3 (AY508995), L-Zagreb (AY685920), Urabe (AB000388), Hoshino (AB470486), Miyahara (AB744048) and RS-12 (JQ388690). Multiple nucleotide sequence alignment was performed by using CLUSTAL W implemented in MEGA (Kumar et al., 2004). A phylogenetic tree of each gene was constructed by using the neighbor-joining and Kimura two-parameter methods (Supplementary Fig. 1). The robustness of the grouping was assessed by using 1000 bootstrapping replicates.

3. Results

3.1. Phylogenetic analysis of mumps virus isolates

The phylogenetic analysis of complete genomes including SH gene showed five genotype C (Pune-2008, Osmanabad-2012a, Osmanabad-2012b, Chennai-2012 and Kushinagar-2013) and two genotype G (Pune-1986 and Pune-2012) viruses (Fig. 1 and Supplementary Fig. 1). The entire nucleotide sequences of Indian MuV isolates (MuVi/Pune.IND/00.86, MuVi/Pune.IND/50.08, MuVi/Pune.IND/07.12, MuVi/Osmanabad.IND/10.12/17, MuVi/Osmanabad.IND/10.12/18, MuVi/Chennai.IND/35.12 and MuVi/Kushinagar.IND/36.13) were submitted to GenBank under accession numbers; KF738114, KF843893, KF843894, KF843895, KF843896, KF738113 and KM385447.

3.2. Analysis of complete genome

The genome of Indian MuV isolates was 15,384 base pairs in length and identical to previously published MuV genomes. The genome