

## Genetic and antigenic analysis of a recombinant foot-and-mouth disease virus

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**Genetic and antigenic analysis of a recombinant foot-and-mouth disease virus has been carried out in relation to the two representative isolates from the parental genotypes involved in recombination in the coat protein genes, and the vaccine strains currently being used in the country. The recombinant (IND 170/88) is divergent antigenically ( $r$  value  $< 0.2$ ) and genetically (6.9–13.3% at nucleotide level and 5.2–7.4% at amino acid level) from the two vaccine strains (IND 490/97 and IND 17/77). However, it maintained antigenic closeness ( $r$  value 1.0) with one parental genotype (IND 40/00 of genotype VII) and was divergent ( $r$  value 0.35) from the other (IND 233/99 of genotype VI). The antigenic analysis in relation to the critical amino acid residues involved in virus neutralization has been discussed, but no correlation could be made between genetic divergence and antigenic relationships. This could be due to modulation of overall antigenicity of the virus by amino acid substitutions occurring at positions neighbouring the known antigenically critical residues.**

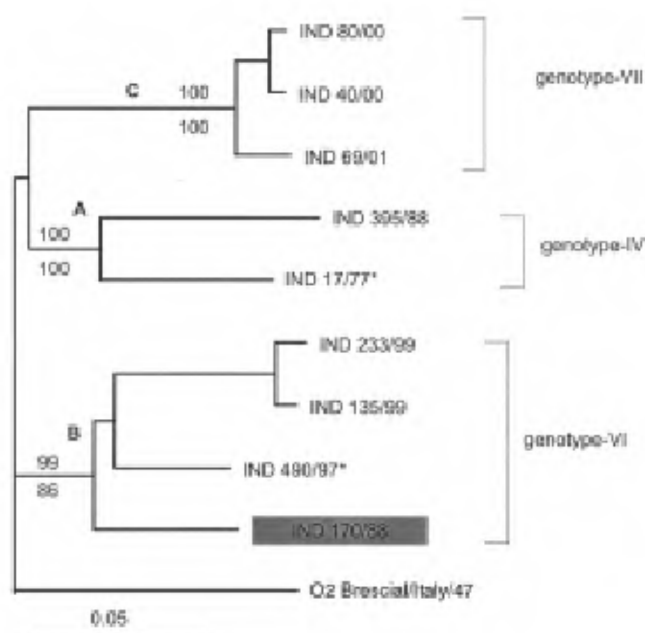
FOOT-and-mouth disease (FMD) remains one of the most economically important diseases of domestic animals including cattle, sheep, goats, buffalo and pig. The disease is caused by the foot-and-mouth disease virus (FMDV), a member of the genus *Aphthovirus* under the family Picornaviridae<sup>1</sup>. The characteristic feature of FMDV, like other RNA viruses, is its diversity, which has been reflected by the presence of seven distinct serotypes (O, A, C, Asia 1 and SAT1–3) and multiple subtypes worldwide<sup>2</sup>. The virus population replicates as a pool of related but non-identical genomes, termed viral quasispecies<sup>3,4</sup>. The genetic heterogeneity in a quasi-species population is the result of error-prone replication due to lack of proofreading activity of the RNA-polymerase enzyme. From the quasi-species population, divergent strains are selected either in the presence or absence of immune pressure<sup>5,6</sup>. Recent reports provide evidence for positive (Darwinian) selection on the capsid protein genes, which leads to antigenic diversity in FMDV<sup>7,8</sup>. Besides the above mechanisms, homologous recombination also plays an important role in the diversity of the virus<sup>9</sup>. Till recently, it was the 3' end of the genome coding for non-structural proteins where recom-

bination was observed in different Picornaviruses<sup>9–11</sup>. More recently, we have reported recombination in the structural protein genes of a type-A FMDV isolated in India, by comparative sequence analysis of the complete capsid-coding (2.2 kb) region<sup>12</sup>. In India, the disease situation due to serotype-A is more complex as a result of existence of multiple genotypes<sup>13</sup>. Often, two or more genotypes have been shown to cocirculate within a given period. This genetic heterogeneity exhibited by type-A viruses may result in antigenic diversity. Preliminary serological analysis on some of the representative isolates of genotypes VI and VII has revealed antigenic differences from one of the vaccine strains (IND 17/77)<sup>14</sup>, thus stressing the need for regular monitoring of the field strains and periodic updating of the vaccine strain(s). The process of updating may get complicated if recombinants arise as a result of co-infection of susceptible host with multiple genotypes. Keeping this in mind, we have analysed the genetic and antigenic relationship of a recombinant virus in relation to the vaccine strains and the representative isolates, one each from the two parental genotypes (genotypes VI and VII) involved in the recombination. The result provides evidence for antigenic and genetic diversity of the recombinant from the two vaccine strains. One parental isolate maintained good antigenic relationship with the recombinant, whereas the second one remained divergent.

The viruses used in this study were adapted and passaged in the BHK-21 cell monolayers. The clarified, infected, cell-culture supernatant containing the virus was stored in aliquotes at  $-70^{\circ}\text{C}$  for subsequent use. The vaccine strains (IND 17/77 and IND 490/97) and the field isolates [IND 233/99 (genotype VI) and IND 40/00 (genotype VII)] were purified and quantified according to the methods described previously<sup>15,16</sup>. IND 17/77 and IND 490/97 (WBN 17/82) are the serotype-A vaccine strains used respectively, by Indian Veterinary Research Institute, Bangalore Campus and Indian Immunologicals Ltd, Hyderabad. For neutralization test, antisera against the purified and acetylethyleneimine-inactivated virus (146S) particles were raised in rabbits by the method described earlier<sup>17</sup>. Two-dimensional microneutralization test (2D-MNT) was performed according to Rweyemamu *et al.*<sup>18</sup>, with BHK 21 cells as the indicator system. The end-point titre of the serum was expressed as a reciprocal of the final dilution of serum in the virus/serum mixture, which neutralizes 100 TCID<sub>50</sub> in 50% of the wells. Viral RNA of vaccine strain IND 490/97 was extracted from the infected supernatant using Total RNA isolation kit (Qiagen). Reverse transcription-PCR and cycle sequencing of the capsid-coding (P1) region were carried out as described earlier<sup>12,19</sup>. The previously determined P1 sequences of IND 17/77, IND 395/88, IND 135/99, IND 233/99, IND 40/00, IND 80/00, IND 69/01 and IND 170/88 (recombinant) were used to reconstruct the phylogenetic relationship<sup>12,19</sup>. The nt and deduced amino acid

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(aa) sequences were aligned using the Megalign program from DNASTAR package (DNASTAR Inc., USA). Phylogenetic analysis was carried out using the program Phylip 3.5c (ref. 20). The neighbour-joining method was applied with Kimura 2-parameter option and a transition/transversion ratio of 3.23, estimated from the nt data set using the program Tree-Puzzle 5.0 (ref. 21). The final output of the tree was generated using TreeView 1.5.2 (ref. 22). It is a general belief that more the sequence length better the phylogenetic information. In the tree, the isolates were distributed in three major lineages (designated as A, B and C) with high (86–100%) confidence values (Figure 1), and were clustered as in the 1D (VP1-encoding) gene-based analysis to designate the genotypes (IV, VI and VII)<sup>13</sup>. From the tree, the genetic heterogeneity of the field isolates in India was further confirmed. The recombinant (IND 170/88) was grouped with lineage B isolates, which is attributed to the presence of comparatively more genome segments (1–720 nt and 1251–2211 nt of P1 region) from genotype VI than genotype VII (721–1250 nt of P1 region)<sup>12</sup>. The alignments of the deduced amino acid sequence (737 aa) of the capsid protein genes are shown in Figure 2. The recombinant (IND 170/88) was 6.9 and 13.3% divergent at the nt level and 5.2 and 7.4% divergent at aa level from IND 490/97 and IND 17/77 respectively (Table 1). As far as the represen-



**Figure 1.** Neighbour-joining tree constructed from the nucleotide sequences of the capsid-coding region (2211 nt) of type-A FMDV. Lineages are shown in bold near the branches. Scale represents nt substitution per site. Numbers at the major nodes represent confidence values (%). (Top) Bootstrap values out of 1000 replicates. (Bottom) Quartet puzzling values measured from 10,000 iterations. The recombinant virus is shaded; \* represents the vaccine strains. The genotype designations are according to the 1D gene (639 nt) analysis (shown to the right). Branch length of the outgroup (O2 Brescia/Italy/47, GenBank AccNo. M55287) has been reduced by 75% to save space.

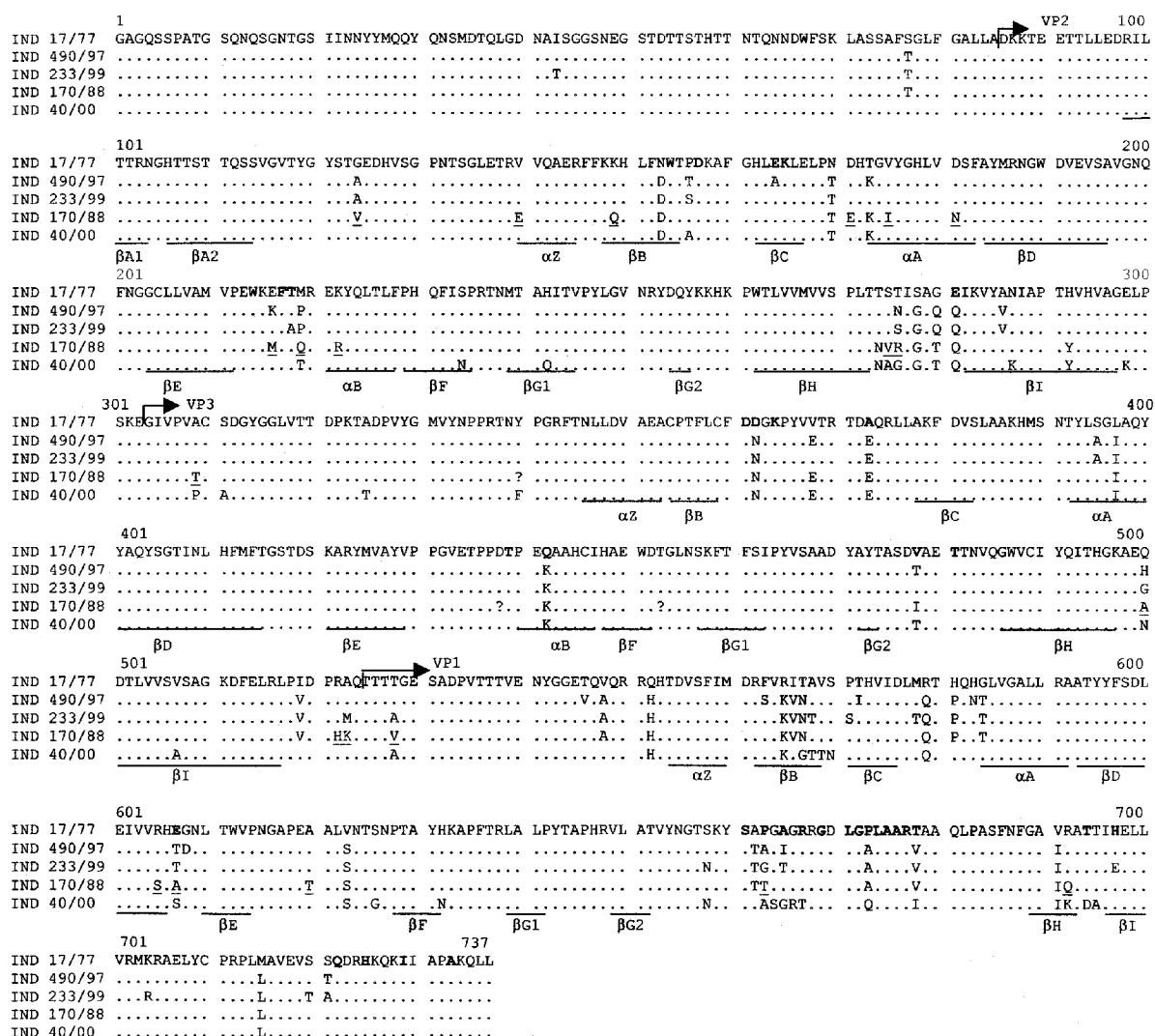
**Table 1.** Nucleotide and deduced amino acid sequence divergence among the FMDV

Virus*	IND 17/77	IND 490/97	IND 233/99	IND 40/00	IND 170/88
Nucleotide divergence (%)					
IND 17/77		12.4	14.8	13.7	13.3
IND 490/97	6.5		8.5	14.3	6.9
IND 233/99	6.7	3.8		15.7	10.6
IND 40/00	7.6	7.6	7.9		12.5
IND 170/88	7.4	5.2	6.0	7.6	
Amino acid divergence (%)					

\*Viruses belong to different genotypes based on 1D gene analysis (639 nt): IND 17/77 (genotype IV); IND 490/97 and IND 233/99 (genotype VI) and IND 40/00 (genotype VII)<sup>13</sup>.

tative isolates from the parental genotypes are concerned, IND 233/99 (genotype VI) is more close to IND 170/88 than IND 40/00 (genotype VII) at both the nt and aa level. This is due to the fact that in the recombinant larger fragments (1–720 nt and 1251–2211 nt of P1 region) were derived from the genotype VI than genotype VII (721–1250 nt of P1 region)<sup>12</sup>.

Studies on monoclonal antibody escape mutants have identified critical, neutralizable, antigenic residues in the structural proteins of the type-A FMDV. At least 34 critical amino acid residues that are involved in the virus neutralization are mapped on the VP1, VP2 and VP3 proteins<sup>23–26</sup> (see Figure 2), of which six, eight and 20 residues are present in VP2, VP3 and VP1 respectively. There are nine and 11 amino acid substitutions at the above known critical positions in the recombinant virus compared to IND 17/77 and IND 490/97. Besides, there are 20 unique amino acid substitutions in the recombinant scattered across the P1 region (Figure 2), of which three are at the known antigenic positions (VP1 83 and 139, VP3 175). Compared to the vaccine strains, substitutions at VP1-83 E/T → A, 139 P/A → T and VP3-175 V/T → I were observed in the recombinant. In addition, the recombinant shares aa residues with both the parental sequences. For example, the IND 170/88 has common residues at 28 and 26 critical positions respectively with IND 233/99 and IND 40/00. Summarizing the amino acid substitution pattern, it is evident that there are at least three major regions, namely VP2  $\beta$ H- $\beta$ I, VP1  $\beta$ B- $\beta$ C and VP1  $\beta$ G- $\beta$ H, which could play an important role in the antigenicity of the virus. In addition to the above regions, three residues at positions VP2 134, VP3 197 and VP1 83 could also be important because they lie at/near the critical residues already identified and show maximum variability in terms of frequency of amino acids. Above all, substitutions at distant places have been reported to modulate the antigenicity of the virus, that has been observed at the VP1  $\beta$ B- $\beta$ C loop which affect the binding of Mabs to the VP1  $\beta$ G- $\beta$ H loop<sup>27</sup>. Therefore, we cannot exclude the possibility of overall antigenic modulation of the virus by substitution at/near the antigenic critical



**Figure 2.** Alignment of deduced amino acid sequence of the capsid-coding (P1) region of FMDV. Amino acid positions in bold face type are antigenically critical and were identified by monoclonal antibody escape mutants<sup>23-26</sup>. Dot indicates same as IND 17/77, amino acid differences are shown in single letter code. Underlined amino acids are unique to the IND 170/88. The secondary structures (given below the alignment) are only approximations and are derived from the alignment with the serotype O1 BFS 1860 (ref. 29).

residues, as has been observed in the aa alignment (Figure 2).

In the antigenic analysis, the ratio between the antibody titres of heterologous and the homologous viruses is used to calculate the antigenic relationship (*r* value) between them. An *r* value range of 0.4–1.0 indicates that the existing vaccine strain provides enough protection, and a range of 0.2–0.4 indicates the need for a more potent vaccine. Whereas, *r* values below 0.2 indicate the need for a new vaccine strain<sup>28</sup>. The antigenic relationship obtained in the present study demonstrates divergence (*r* value of < 0.2) of the recombinant from the two vaccine strains (IND 17/77 and IND 490/97). The result also indicates the antigenic closeness (*r* value 1.00) of the recombinant with genotype VII (IND 40/00), whereas it shows antigenic divergence (*r* value 0.35) with genotype VI (IND 233/99). No correlation could be observed from

the overall nucleotide/amino acid divergence and the serological relationships among isolates.

In summary, antigenic divergence due to homologous recombination could be a concern where multiple genotypes co-circulate. This could provide an escape mechanism for the recombinant to evade the host immune defence. Even though the recombination in capsid-coding region is a rare phenomenon, in disease-endemic regions where multiple genotypes co-circulate, its role in virus evolution needs further investigation. Finally, in the recent years we have found co-circulation of multiple genotypes in India and as a result, the appearance of recombinant has been established. Hence it is wise to incorporate strains from both the genotypes in the vaccine formulation, as it will not only help to combat outbreaks due to individual genotypes, but also against the recombinants arising from these two genotypes.

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## Palaeoliquefaction evidence of prehistoric large/great earthquakes in North Bihar, India

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**The Himalayan arc, 40% of which ruptured in the last two centuries, has witnessed half a dozen large to great earthquakes including the 1833 and 1934 Bihar–Nepal earthquakes. This paper, based on palaeoliquefaction studies in the meizoseismal area of the 1934 earthquake, provides evidence for two prehistoric seismic events dated to have occurred: (i) during 1700 to 5300 years BP and (ii) earlier than 25,000 years BP, besides the well documented 1934 and 1833 seismic events. Thus the findings suggest that the study area has been continuously seismically active. However, the limited data presently precludes estimation of recurrence period of devastating earthquakes in this area.**

DURING the past two centuries, nearly half of the Himalayan arc has ruptured in half a dozen large to great earthquakes. These events have only partially released the strain out of the total slip potential of the Himalayan arc due to collision of the Indian and the Eurasian plates<sup>1</sup>. Based on the estimated slip potential given by Bilham *et al.*<sup>1</sup>, one or more large/great earthquakes may be overdue in a large part of the Himalaya. Therefore, it is of utmost importance to obtain geological evidence of earthquake recurrence at different source regions in the Himalaya. Due to lack of surface exposure of the causative faults, direct dating of these earthquakes is not possible. Paleoseismological studies<sup>2,3</sup> involving the dating constraints on the resultant secondary coseismic off fault effects like liquefaction<sup>4,5</sup> and soft sediment deformation features have been of help in providing evidence of large/great palaeo-earthquakes as well as establishing their recurrence period<sup>6,7</sup>. In the Shillong Plateau, located south of the eastern Himalayan segment in India, Sukhija *et al.*<sup>8,9</sup> have estimated a recurrence period of 400–600 years for major/great earthquakes based on the palaeoliquefaction evidences and radiocarbon dating. The objective of the present paper is to provide geological evidence of large/great earthquakes in the epicentral area of the great 1934 earthquake based on the dating of the seismically-induced liquefaction and soft sedimentary deformation features.

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