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Molecular Analysis of VP7 Gene of Rotavirus G1 Strains Isolated from North India

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Abstract Rotavirus G1 strains are the predominant cause of diarrhoea in children. Universally common rotavirus vaccines (Rotarix and RotaTeq) include G1 as the immunological component. India has recently introduced rotavirus vaccine in Universal Immunization Programme. Therefore, in the present study, VP7 gene of rotavirus G1 strains circulating in Himachal Pradesh, India is analysed to study their phylogenetic characteristics, and further comparative analysis was performed for assessment of their divergence from the vaccine strains. The rotavirus strains (JU-SOL-5, JU-SOL-58, JU-SOL-77, JU-SOL-173 and JU-SHI-14) analysed in the study were isolated from the faeces of diarrhoeic children during active surveillance for rotaviruses. The Himachal strains clustered together in G1-Lineage 1 in the phylogenetic analysis. All five isolates showed 96.4–98.8 % similarity with the other G1-Lineage 1 strains at amino acid level. However, none of them clustered in the pre-defined sublineages within lineage 1. Interestingly, all the strains were distantly related to the vaccine strains having 93.9–94.5 and 91.9–92.6 % similarities at amino acid level with Rotarix and RotaTeq strains, respectively. The comparative sequence and structural analysis of the Himachal strains with vaccine strains revealed differences in amino acids in epitope region of the protein especially at the antibody neutralization sites. The study highlights variations between the G1 strains from Himachal Pradesh, India and Rotarix and RotaTeq vaccine strains. These differences might have an impact on the neutralization efficiency of vaccine and subsequently on vaccine efficacy. This underscores further investigation to study intragenotype antigenic variability and also impact of viral evolution on vaccine effectiveness.

Introduction

Rotavirus, belonging to family *Reoviridae,* is the major causative agent of diarrhoea in children and accounts for around 0.5 million annual child deaths globally [34]. It has a genome made up of 11 segments of double-stranded RNA which encodes 6 structural (VP1, VP2, VP3, VP4, VP6 and VP7) and 6 non-structural proteins (NSP1–NSP6). The genome is encapsulated in a triple-layered capsid. The inner-most layer is made up of VP1, VP2 and VP3 and forms the core of the virus. The intermediate layer is made up of VP6 protein, whereas VP4 and VP7 form the outer most covering of the virus [8]. Based on the amino acid sequence of VP6 protein, rotaviruses are classified into eight major groups (A–H). The outer most proteins VP4 and VP7 form the basis of G/P genotyping system [23]. Till date, studies have reported 27 G genotypes and 37 P genotypes in rotaviruses [23, 37]. Out of these, the most common human infecting G and P types are G1, G2, G3, G4, G9 and P[4], P[6], P[8], respectively [7]. As far as, G and P combinations are concerned, G1P[8] (25.3 %), G2P[4] (12.5 %), G3P[8] (7.6 %), G4P[8] (6.3 %) and G9P[8] (5.7 %) are globally predominant [43]. On studying the evolutionary characteristics of the globally predominant G1 strains, it can be observed that these are classified in 11

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lineages and 17 sublineages on the basis of amino acid substitutions in the VP7 protein [30].

Rotaviruses are continuously evolving due to four major mechanisms: point mutation, interspecies transmission, reassortment events and gene rearrangement [14]. The mutations in the antigenic regions of VP7 and VP4 proteins lead to antigenic shift and drift mechanisms which give rise to antibody escape mutants. The VP7 glycoprotein contains 326 amino acids and spans across nine divergent regions (VR1–VR9) which are variable among the various genotypes [36]. Four of the divergent regions, VR5 (region A; aa 87–101), VR7 (region B; aa 141–151), VR8 (region C; aa 208–224) and VR9 (region F; aa 235–242) are considered to be the major antigen sites on the VP7 protein [10, 17, 27]. As reported by Aoki et al. [1], two regions 7-1 (includes epitopes from antigenic Regions A and C) and 7-2 (includes antigens from antigenic region C) are the sites of mutations that allow escape from neutralizing antibodies.

Currently, two rotavirus vaccines, Rotarix and RotaTeq, are globally in use to protect from rotavirus-associated complications. Rotarix is a live, attenuated vaccine having monovalent G1P[8] human rotavirus strain [40]. The pentavalent vaccine, RotaTeq contains human-bovine reassortant rotavirus strains and have G1, G2, G3, G4 and P[8] genotypes which are commonly found in humans [6]. The G1 component of the Rotarix vaccine belongs to the lineage 2, whereas RotaTeq- G1 belongs to lineage 3 [18].

Rotavirus vaccine has recently been introduced in the Universal Immunization Programme (UIP) of India and Himachal Pradesh, a northern state, is the first state to implement this project. Vaccine may exert selection pressure which could lead to emergence of new/uncommon strains. Moreover, reports are available from the pre-vaccination era documenting role of natural fluctuations/environmental factors in emergence/re-emergence of disappeared strains [41, 42]. Therefore, it becomes necessary to study the characteristics of the circulating strains in this region. In accordance with the other parts of the country, G1 is the prominent genotype circulating in Himachal Pradesh (unpublished data) but no data regarding evolutionary and antigenic characteristics are available for G1 strains prevalent in this region. Therefore, this study aims at gaining insight into the intragenotypic lineage-specific diversity of the Himachal G1 strains and their comparison with the G1 components of the vaccine strains.

Materials and Methods

Virus Strains

The rotavirus strains RVA/Human-wt/IND/JU-SOL-5/2012/G1P[6], RVA/Human-wt/IND/JU-SOL-58/2012/G1P[6], RVA/Human-wt/IND/JU-SOL-77/2012/G1P[6], RVA/Human-wt/IND/JU-SOL-173/2013/G1P[6] and RVA/Human-wt/IND/JU-SHI-14/2014/G1P[6] were isolated from diarrhoeic children <5 years of age during a pre-vaccination surveillance study in Himachal Pradesh, India during 2012–2014. The samples included in this study were from patients who were never vaccinated for rotavirus.

Elisa

Rotaviruses were detected in the stool samples using commercially available enzyme-linked immunosorbent assay kit (Premier Rotaclone; Meridian Biosciences, Inc., Cincinnati, OH) following manufacturer's instructions.

RNA Extraction, RT-PCR and Genotyping

The stool sample was diluted with phosphate buffer saline to form a 10 % faecal suspension. The RNA extraction was carried out from the faecal suspension using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Reverse transcription was performed using Verso cDNA synthesis kit (ThermoFisher scientific, Waltham, Massachusetts, USA). Genotyping was performed applying previously reported methods [13]. Briefly, first 881 bp region of VP7 gene was amplified using primers VP7-F and VP7-R. The PCR products from RT-PCR were used as a template in the multiplex hemi-nested PCR to identify the genotypes. A mixture of genotype-specific primers complementary to the variable regions of VP7 gene (G1, G2, G3, G4, G8, G9, G10) were used. The products were run on 1.5 % agarose gel and the bands were visualized on UV transilluminator.

Sequencing and Phylogenetic Analysis

The amplified 881 bp RT-PCR products were sequenced, aligned and analysed with the corresponding VP7 sequences of rotavirus G1 strains available in GenBank using ClustalW [19]. The phylogenetic analysis was carried out with MEGA software, version 6.0 [33]. The phylogenetic tree was constructed using the neighbour-joining method. The evolutionary distances were computed using the Kimura 2-parameter method. The statistical significance of the different phylogenetic groupings was estimated by bootstrap analysis with 1000 pseudoreplicates.

Nucleotide Sequence Accession Numbers

The VP7 nucleotide sequences of the rotavirus strains obtained in this study were deposited into GenBank under accession numbers KM880063 (JU-SOL-173), KP938512

(JU-SHI-14), KP938513 (JU-SOL-5), KP938514 (JU-SOL-58) and KP938515 (JU-SOL-77). The accession numbers of rotavirus reference strains used in this study were JN849114 (Rotarix-A41CB052A), GU565057 (RotaTeq-W179-9), L24165 (C95), M92651 (T449), L24164 (C60), AF426162 (SW20/21), AB018697 (AU19), U26376 (Israel-56), U26374 (Egy-8), D16343 (KU), D16323 (K2), EF079065 (7206/JP), EF079064 (7014/JP), DQ377596 (PA5/03), DQ377598 (PA2/04), AB081799 (AU007), DQ377589 (PA378), U26387 (Oh-64), U26370 (Cos-70), S83903 (1407), DQ377572 (PA78/89), DQ377587 (PA10/90), U26378 (Kor-64), AB081795 (88H249), U26373 (Egypt-7), DQ377602 (PA17c), DQ377566 (PA3c), U26366 (Ban-59), AF183857 (CH631), DQ508167 (VN-281), DQ512979 (Thai-804), AY098670 (ISO-4), AY631049 (Dhaka8-02), EF079067 (6916/JP), DQ512986 (J-4689), AF480293 (Mvd9816), AF260945 J (97S6), FJ948848 (6590), KJ870802 (KisB501), HM130926 (Seoul-045), KJ870787 (KisB104), JN258390 (2008747323), GU392988 (GER109-08), HG917358 (E88997), HG917362 (E9606), AB796443 (OH3385), AB796444 (OH3493), KF112996 (JS10-1) and JX411969 (UK-HLD).

Protein Structure Analysis

The trimer structure of VP7 protein was downloaded from Protein Data Bank by submitting PDB ID '3FMG'. The structural analysis was done with UCSF Chimera-Molecular Modelling System [29].

Results

Phylogenetic and Sequence Analysis

Phylogenetic analysis of VP7 gene sequences showed that the G1 strains isolated from Himachal Pradesh clustered into G1-Lineage 1 (Fig. 1). But within G1-Lineage 1, these strains do not cluster with any of the reported five (A–E) sublineages and lack signature sequence of any of these. These results suggest that the isolated strains may belong to a novel sublineage other than the previously reported sublineages. Moreover, none of the isolates clustered with Rotarix (A41CB052A) and RotaTeq (W-179-9) vaccine strains which belong to the G1-Lineage 2 and G1-Lineage 3, respectively.

To assess the molecular basis of rotavirus diversity, the nucleotide and amino acid sequences of VP7 protein were compared. The isolates JU-SHI-14, JU-SOL-5, JU-SOL-58, JU-SOL-77 and JU-SOL-173 were 99 % identical to each other at nucleotide level. All the five isolates showed 96.1–97.6 and 96.4–98.8 % similarity with the other lineage 1 strains on the nucleotide and amino acid levels, respectively. Within lineage 1, all the five isolates revealed the maximum identity (97.4–97.8 %) with the Uruguay strain, Mvd9816 at nucleotide level. However, on the amino acid level, the isolated strains shared maximum similarity with three strains, ISO-4 (98.2–98.8 %), VN-281 (98.2–98.8 %) and Mvd9816 (98.2–98.8 %), from India, Vietnam and Uruguay, respectively (Supplementary Table 1). This is indicative of the widespread circulation of the lineage 1 strain all around the world.

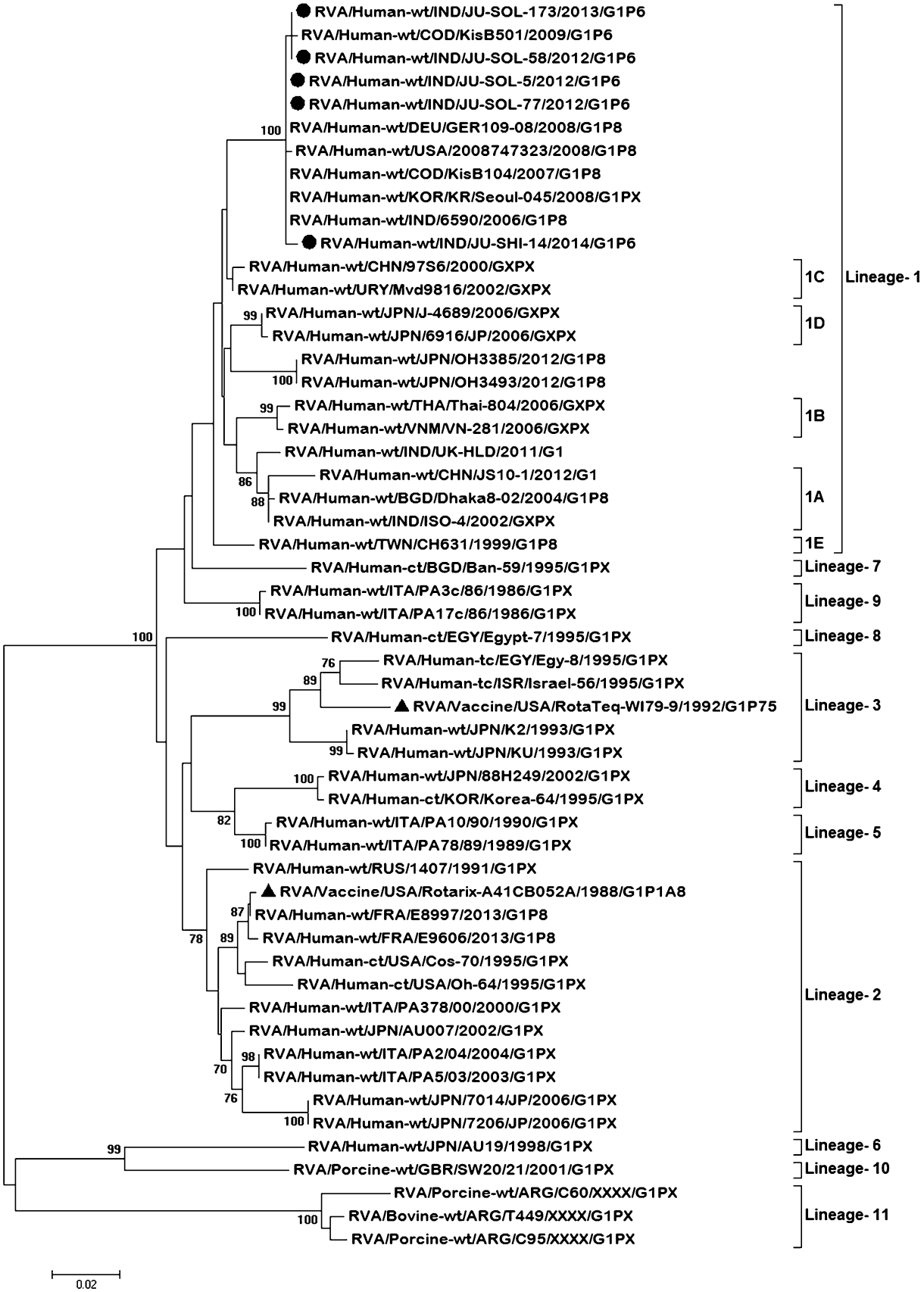
As reported by Phan et al. [30], there is a unique identification code for defining the lineages. It comprises fourteen amino acids at positions 29, 34, 35, 37, 42, 43, 50, 55, 57, 65, 68, 72, 74 and 75 that define a signature sequence. The alignment of deduced amino acid sequences of our strains revealed the signature code specific for lineage 1. Within lineage 1, the amino acid substitutions at positions 72 and 74 define sublineage 1A, 16 defines 1B, 268 defines 1C and 91 defines 1D. Sublineage 1E has no sublineage-specific mutations [30]. Interestingly, none of the isolates absolutely have the mutations specific for any of the sublineages within lineage 1 (Table 1). All the isolated strains showed a substitution of amino acid G with E at position 74 which is a characteristic feature for sublineage 1A. But there is no sublineage 1A-specific amino acid substitution at position 72. This differentiates the isolates from the sublineage 1A-specific strains. The strains JU-SHI-14 and JU-SOL-173 bear an additional substitution at positions 91 and 268, respectively, where amino acid T is substituted by A and I is replaced by V. These mutations support the fact that the Himachal isolates are diverse from the strains belonging to the five sublineages within lineage 1 and belong to a distinct sublineage.

Comparison of VP7 Sequences of Isolated G1 Strains and Vaccine Strains

The comparison of isolated strains with Rotarix vaccine strain showed 92.8–93.3 and 93.9–94.5 % similarity at nucleotide and amino acid level, respectively. Similarly, the isolated strains revealed 87–87.5 % nucleotide and 91.9–92.6 % amino acid similarity with the RotaTeq strain (Supplementary Table 2). These results demonstrate that the isolated strains are closer to the Rotarix strain on the nucleotide and amino acid levels than RotaTeq vaccine strains.

Comparison of VP7 Epitopes of Isolated G1 Strains and Vaccine Strains

The amino acid differences in the antigenic epitope region between circulating strains and vaccine strains could have a substantial effect on the vaccine efficacy [28]. Therefore, the antigenic regions of the isolated G1 strains and vaccine strains were compared to ascertain the variability between


RVA/Human-wt/IND/JU-SOL-173/2013/G1P6
RVA/Human-wt/COD/KisB501/2009/G1P6
RVA/Human-wt/IND/JU-SOL-58/2012/G1P6
RVA/Human-wt/IND/JU-SOL-5/2012/G1P6
RVA/Human-wt/IND/JU-SOL-77/2012/G1P6
RVA/Human-wt/DEU/GER109-08/2008/G1P8
RVA/Human-wt/USA/2008747323/2008/G1P8
RVA/Human-wt/COD/KisB104/2007/G1P8
RVA/Human-wt/KOR/KR/Seoul-045/2008/G1PX
RVA/Human-wt/IND/6590/2006/G1P8
RVA/Human-wt/IND/JU-SHI-14/2014/G1P6
RVA/Human-wt/CHN/97S6/2000/GXPX
RVA/Human-wt/URY/Mvd9816/2002/GXPX
RVA/Human-wt/JPN/J-4689/2006/GXPX
RVA/Human-wt/JPN/6916/JP/2006/GXPX
RVA/Human-wt/JPN/OH3385/2012/G1P8
RVA/Human-wt/JPN/OH3493/2012/G1P8
RVA/Human-wt/THA/Thai-804/2006/GXPX
RVA/Human-wt/VNM/VN-281/2006/GXPX
RVA/Human-wt/IND/UK-HLD/2011/G1
RVA/Human-wt/CHN/JS10-1/2012/G1
RVA/Human-wt/BGD/Dhaka8-02/2004/G1P8
RVA/Human-wt/IND/ISO-4/2002/GXPX
RVA/Human-wt/TWN/CH631/1999/G1P8
RVA/Human-ct/BGD/Ban-59/1995/G1PX
RVA/Human-wt/ITA/PA3c/86/1986/G1PX
RVA/Human-wt/ITA/PA17c/86/1986/G1PX
RVA/Human-ct/EGY/Egypt-7/1995/G1PX
RVA/Human-tc/EGY/Egy-8/1995/G1PX
RVA/Human-tc/ISR/Israel-56/1995/G1PX
RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P75
RVA/Human-wt/JPN/K2/1993/G1PX
RVA/Human-wt/JPN/KU/1993/G1PX
RVA/Human-wt/JPN/88H249/2002/G1PX
RVA/Human-ct/KOR/Korea-64/1995/G1PX
RVA/Human-wt/ITA/PA10/90/1990/G1PX
RVA/Human-wt/ITA/PA78/89/1989/G1PX
RVA/Human-wt/RUS/1407/1991/G1PX
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8
RVA/Human-wt/FRA/E8997/2013/G1P8
RVA/Human-wt/FRA/E9606/2013/G1P8
RVA/Human-ct/USA/Cos-70/1995/G1PX
RVA/Human-ct/USA/Oh-64/1995/G1PX
RVA/Human-wt/ITA/PA378/00/2000/G1PX
RVA/Human-wt/JPN/AU007/2002/G1PX
RVA/Human-wt/ITA/PA2/04/2004/G1PX
RVA/Human-wt/ITA/PA5/03/2003/G1PX
RVA/Human-wt/JPN/7014/JP/2006/G1PX
RVA/Human-wt/JPN/7206/JP/2006/G1PX
RVA/Human-wt/JPN/AU19/1998/G1PX
RVA/Porcine-wt/GBR/SW20/21/2001/G1PX
RVA/Porcine-wt/ARG/C60/XXXX/G1PX
RVA/Bovine-wt/ARG/T449/XXXX/G1PX
RVA/Porcine-wt/ARG/C95/XXXX/G1PX
100
100
99
100
99
86
88
100
76
89
99
99
100
82
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1C
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Lineage- 1
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Lineage- 9
Lineage- 8
Lineage- 3
Lineage- 4
Lineage- 5
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Lineage- 6
Lineage- 10
Lineage- 11
0.02

◀**Fig. 1** Phylogenetic tree of VP7 gene sequences of rotavirus G1 strains. The phylogenetic tree was constructed using neighbour-joining method. The values at the branch nodes represent the bootstrap values. The Himachal isolates are indicated with *black dots*, whereas vaccine sequences are indicated with *black triangles*. The accession numbers of the sequences used in the tree are mentioned in the text

these. The isolated strains showed five amino acid differences in the epitope region as compared with Rotarix strain, whereas RotaTeq showed a difference of seven amino acids (Table 2). A majority of the amino acid changes were found in epitope region 7-1a and it had amino acid changes at five positions. In this region, there was a transition between T → A, N → S, D (RotaTeq) → E, D → E and S → N at positions 91, 94, 97, 100 and 123, respectively. The region 7-2 has S (RotaTeq) → N and M → T/I amino acid change at positions 147 and 217, respectively. The significance of these mutations lies in the fact that they are the neutralization escape mutation sites except amino acid at position 123 [1]. As far as region 7-1b is concerned, it was found to be fully conserved between circulating strains and vaccine strains. Mapping of the amino acid variations in the antigenic regions of the isolates and vaccines on the trimeric structure of VP7 protein reveals that the differences were located inconsistently on the exposed surfaces of the protein in the epitope region (Fig. 2).

Discussion

In India, rotavirus-associated diarrhoea is the major life-threatening disease in children and accounts for around 79,000 deaths annually [16]. The lack of rotavirus vaccine

Table 1 Lineage- and sublineage-specific amino acid substitutions in VP7 protein

Sublineages under Lineage 1	Strains	Amino acid positions and substitutions																			
		16 I	29 I	34 I	35 Y	37 F	42 V	43 A	50 A	55 L	57 L	65 T	68 A	72 Q	74 G	75 I	91 T	211 N	212 V	213 D	268 I
1A	Dhaka 8-02	.	.	.	.	.	.	.	.	.	I	.	S	*R*	*E*	V	.	.	.	.	.
1B	Thai-804	*Q*	.	.	.	.	.	.	.	.	I	.	S	.	.	V	.	.	.	.	.
1C	Mvd9816	.	.	.	.	.	.	.	.	.	I	.	S	.	.	V	.	.	.	.	*V*
1D	6916	.	.	.	.	.	.	.	.	.	I	.	S	.	.	V	*N*	.	.	.	.
1E	CH631	.	.	.	.	.	.	.	.	.	I	.	S	.	.	V	.	.	.	.	.
	JU-SHI-14	.	.	.	.	.	.	.	.	.	I	.	S	.	*E*	V	*A*	.	.	.	–
	JU-SOL-5	.	.	.	.	.	.	.	.	.	I	.	S	.	*E*	V	.	.	.	.	–
	JU-SOL-58	.	.	.	.	.	.	.	.	.	I	.	S	.	*E*	V	.	–	–	–	–
	JU-SOL-77	.	.	.	.	.	.	.	.	.	I	.	S	.	*E*	V	.	.	.	.	–
	JU-SOL-173	.	.	.	.	.	.	.	.	.	I	.	S	.	*E*	V	.	.	.	.	*V*

Amino acids in *italics* represent sublineage-specific mutations

Table 2 Alignment of antigenic amino acids in VP7 protein of Himachal isolates and vaccine strains

Rotavirus Strain	Epitope 7-1a														Epitope 7-1b						Epitope 7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Rotarix-G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RotaTeq-G1	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
JU-SHI-14	T	A	S	G	E	W	K	E	Q	N	V	V	D	-	Q	N	V	D	N	-	K	D	Q	N	L	S	T	N	-
JU-SOL-5	T	T	S	G	E	W	K	E	Q	N	V	V	D	-	Q	N	V	D	-	-	K	D	Q	N	L	S	I	N	-
JU-SOL-58	T	T	S	G	E	W	K	E	Q	N	V	V	D	-	Q	-	-	-	-	-	K	D	Q	N	L	S	-	-	-
JU-SOL-77	T	T	S	G	E	W	K	E	Q	N	V	V	D	-	Q	N	V	D	N	T	K	D	Q	N	L	S	I	N	-
JU-SOL-173	T	T	S	G	E	W	K	E	Q	N	V	V	D	-	Q	N	V	D	N	T	K	D	Q	N	L	S	I	N	-

Black color represents amino acid differences between Rotarix and RotaTeq. Brown represents residues that differ from both Rotarix and Rotateq. Light grey represents amino acid residues that differ from RotaTeq.

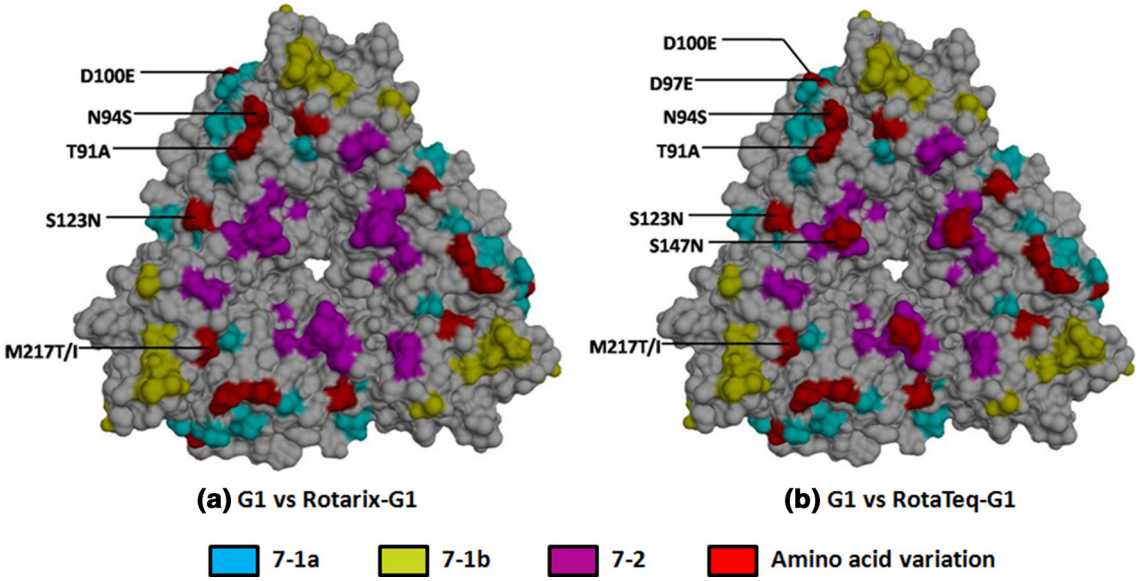

Fig. 2 Structural representation of VP7 trimer proteins. Different regions of antigenic epitopes are shown in *cyan* (7-1a), *yellow* (7-1b) and *magenta* (7-2) colours. The amino acid differences between Himachal strains and vaccine strains are indicated with *red colour*. *For interpretation of colours, refer to the online version of the article*

in the Universal Immunization Programme (UIP) was further worsening the condition. However, two vaccines, Rotarix and RotaTeq, have been licensed in the country but they are available through private sector only. Therefore, there is a big uncertainty on the vaccine coverage. Moreover, the high cost of the vaccine was also posing a problem in its widespread administration. But recently, India has introduced rotavirus vaccine in the UIP and this could prove to be very crucial in controlling the rotavirus-associated morbidity and mortality in the country.

The effectiveness of any vaccine in a particular region depends on its ability to protect against the circulating strains at that time [5, 39]. While doing a rotavirus surveillance study in Himachal Pradesh, North India, G1 was the most common VP7 genotype identified (unpublished data). In this study, we have analysed the VP7 gene of G1 strains prevalent in Himachal Pradesh and compared them with the G1 components of the Rotarix and RotaTeq vaccines to investigate the genetic and antigenic variations. The phylogenetic analysis in our study reveals that the isolated Himachal strains belong to the subgenotypic lineage 1, whereas the Rotarix and RotaTeq strains clustered in the lineages 2 and 3, respectively. In such a case, will there be any effect on vaccine efficacy? There are no robust data or evidences to prove that. But there are studies which show that intragenotypic nucleotide differences have a correlation with the antigenic differences. A study by Matthijnssens et al. [22] reported that a rotavirus-vaccinated child got infected by rotavirus G1 strain belonging to different lineages than the vaccine strain. In another study, Hoshino et al. [12] proposed G9-Lineage 1 rotavirus strains as more suitable vaccine candidate than strains belonging to lineages 2 or 3. They have constructed VP7 substitution mutants bearing VP7 gene from different G9-specific lineages to study the immunogenic potential of all the three G9 subgenotypic lineages by raising the antisera against them. The outcome of the study showed that the antibodies against lineage 1 strains were able to neutralize the lineage 2 and 3 strains very efficiently with high titer values, whereas the lineage 2- and 3- specific antibodies were not as efficient in neutralizing the lineage 1 strains. It might be possible that mutations in the lineage- and sublineage-specific amino acids belong to the antigenic region rendering antibody escaping to the strains.

In the present study, a comparative analysis between the G1 strains and vaccine (Rotarix and RotaTeq) strains revealed significant number of amino acid changes in the antigenic epitope regions 7-1 and 7-2. These amino acid differences could influence antigenicity of the strains and might give rise to neutralization antibody escape mutants [11, 28]. In 1996, Jin et al. [15] investigated a vaccine failure episode in USA where children vaccinated with the D strain of rotavirus suffered G1 rotavirus infection post immunization. The interesting part of the study was that the breakthrough strains which evaded the immunization elicited neutralizing antibodies belonged to the same serotype as the vaccine strain D and its identical Wa strains. On investigating the antigenic region of VP7 amino acid sequence, the authors found that the breakthrough strains differ from the vaccine strains at positions 97 and 147. Both these positions are very critical components of neutralization epitopes and lie in the epitope region 7-1a and

7-2, respectively. This study supports the fact that difference in antigenic region of circulating strains and vaccine strains could lead to vaccine failure or at least reduce the vaccine efficacy.

In the epitope region, Himachal G1 strains and vaccine strains exhibited variations in the amino acid composition at crucial neutralization antibody-binding sites. But these strains were found to be closer to Rotarix strain (having five amino acid substitutions) in contrast to the RotaTeq strain, where seven amino acid differences were present. As far as the antigenic epitopes of Rotarix and RotaTeq strains are concerned, they both differ at two amino acids at positions 97 and 147. In Rotarix, there is a glutamic acid and asparagine, whereas RotaTeq has aspartic acid and serine at positions 97 and 147, respectively. All of the G1 strains under investigation were found to possess glutamic acid and asparagine at these positions. These results are in accordance with other studies which showed similar results with G1 strains from other part of India [2, 3, 18, 35]. If the amino acid variations between circulating strains and vaccine strains are effective enough to reduce the vaccine efficacy, then Rotarix emerges as the favourite vaccine candidate to be administered in the region. But, it is yet to be established. Also, these amino acid substitutions in the neutralization antibody escape sites are not exclusive in this study. A number of other studies from different parts of the world have documented these mutations [4, 9, 20, 21, 24–26, 31, 44] and they seem to have no apparent adverse effect on vaccine effectiveness in their region [32, 38]. These results show that the immunization efficiency of the vaccine does not solely depend on the epitope region of VP7 protein, but there may be some other regions or other proteins which are also responsible for immunogenic potential of the virus.

To conclude, the phylogenetic and molecular analysis reveals the belonging of the isolates to a novel sublineage within lineage 1. The isolates under investigation have considerable amino acid differences as compared to the vaccine strains in the epitope region and thus might have an adverse effect on the vaccine efficacy. But this cannot be ascertained without further serological analysis and extensive genetic and molecular studies including larger proportion of circulating strains at different time intervals (pre- and post-vaccination). However, this molecular analysis gives preliminary information on the genetic diversity of the circulating strains and can navigate the further approaches to study the diversity of G1 rotaviruses circulating in Himachal Pradesh, India.

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Compliance with Ethical Standards

Conflict of interest None.

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