
1Kshitija S Rane-Yadav, 2Divashree Jhurani, 3Dattatraya S Joshi, 4Niman C Mohanty, 5Nitin N Kadam

**ABSTRACT**

Rotavirus is a segmented double stranded ribonucleic acid virus with typical surface antigens, viral protein 7 and viral protein 4. Almost 5 million children all over world are reported to be infected with this virus. In vitro studies have shown binding of rhesus rotavirus surface protein with ‘Sia’ groups of a histo blood group antigens (HBGAs). In case of humans, rotavirus type A is more prevalent. The precise genomic diversity of ABO (H) and Lewis Blood Group System in various ethnic populations may provide plausible explanation for prevalence of specific P genotypes. Present study aims to identify role of Single Nucleotide Polymorphisms in the host *FUT2* gene in the host susceptibility to risk of rotavirus infection of P genotypes. The study indicates that null allele at certain loci of fucosyltransferase-2 (*FUT2*) gene, also known as secretor (Se) gene leads to lack of functionally active enzyme. This results in absence of α-1/2 fucosylated glycan and may protect the child against rotavirus infection of specific strain. *FUT2* gene alleles at loci 428 (AA) and 302 (TT) are found to be associated with group A rotaviruses. Both these alleles were frequent in population under study. Presence of any of these allele in children of Indian origin leads to non-secretor phenotype and hence if exposed to P[4] and P[8] genotypes of rotavirus, can resist the infection.

**Keywords:** Fucosyltransferase-2, Genetic predisposition, Histo-blood group antigens, Rotavirus, Single-nucleotide polymorphism.


**Source of support:** MGMIHS and DST India.

**Conflict of interest:** None

**INTRODUCTION**

Genetics of secretor status has attracted considerable attention in recent times as it appears to have significant association with predisposition to many infectious diseases, such as acquired immunodeficiency syndrome, gastric cancer, etc. Identification of secretor status is carried out based on Lewis Blood Group System. In this system, individuals are classified based on expression of Lewis glycoproteins on the surface of RBCs, endothelial tissues, kidney cells, genitourinary and gastrointestinal epithelial cells as well as in body secretions. There are two main Lewis HBGAs, Lea and Leb, expressing three common phenotypes: Le(a+b−), Le(a−b+), and Le(a−b−). The HBGA can serve as a ligands for pathogen initiating infection via cell attachment.

The major allantogen antigen in blood tissue is ABO, but even Lewis antigens have acquired attention in the past few years because of their expression on histological tissue cells and in mucous secretions. The expression of Lewis paratopes on the erythrocytes is dependent on adsorption of secreted Lewis antigen present in serum by RBC membranes. The Lewis blood group antigen system is associated with the ABO system. It depends on two different types of fucosyltransferases. Genes for enzymes are mapped to chromosome 19p13.3 (*FUT3* or Lewis gene) and 19q13.3 (*FUT2* or secretor gene).

The *FUT2* gene encodes for an enzyme α-1,2 fucosyltransferase, which produces type I and 3 H antigens. Another enzyme encoded by gene *FUT3*, α-1,4 fucosyltransferase, regulates fucosylation of H type I antigen substrate to produce Leα or Leβ antigens (Fig. 1). The gene *FUT2* is conserved through evolution and is composed of two exons and one intron. Exon 2 is an ORF for transcription and protein expression. Approximately 20% of Europeans and Africans are found to present with null allele of *FUT2* gene. Any mutation or polymorphism causing disruption of the active site may compromise the expression of fucosyltransferase-2 enzyme. In this case, as there will not be α-1,2 fucosylation, subsequently there will be lack of expression of Lewis antigen.
mutation at the allele leads to less expression of active enzyme; hence, partially Leα is converted to Leβ. About 30 to 40% of Asians typically are found to show such a phenotype. The FUT3 gene regulates expression of Lewis antigens, but FUT2 gene regulates secretion of these antigens. Subcellular localization of enzyme is in the Golgi apparatus.11 In the absence of FUT3 gene product, the individual is not able to produce any of the Lewis antigens.

In recent times, HBGAs in its various forms are found to be associated with various diseases including viral diarrhea. Diarrhea has consistently been described as the second leading cause of mortality among children under 5 years of age worldwide. The RV, followed by norovirus, is the most lethal agent of acute diarrhea associated with mortality in this age group. Worldwide, nearly 453,000 children die each year due to RV infection, of which about 98,621 die in India each year.12 These are segmented RNA viruses undergoing genetic reassortment. There are 27 G and 35 P genotypes known so far.13 The P[4], P[6], and P[8] are major P-genotypes globally. The RV causes acute or sometimes severe diarrhea.

In 2012, two different groups in the Baylor College of Medicine, located in the Texas Medical Center in Houston, Texas, USA, and University of Cincinnati, Ohio, USA, showed that the distal portion of RV VP4 spike protein (VP8*) is implicated in binding to cellular receptors, thereby facilitating viral attachment and entry. A-type HBGAs binds to the HR VP8* at the same location as the Sia in the VP8* of animal RV, and suggests how subtle changes within the same structural framework allow for such receptor switching.14,15 A few studies have been conducted on European, American, African, as well as Asian population.16-20 Scientists reported that binding to these host ligands is shown to be strain-specific, but no reports have been recorded so far to support involvement of host genetics in this receptor recognition and pathogenesis. The ABO(H) and Lewis blood group system is synthesized by the fucosyltransferase family of enzymes, the gene pool for which is highly polymorphic and ethnicity specific.

It has been reported worldwide that genetic polymorphisms of FUT-2(Se) and FUT-3(Le) gene determine presence of HBGAs on tissues other than RBCs and body secretions. Particularly secretor and Lewis phenotypes exhibit differences in diverse human populations. Expression of Lewis b (Leb) antigens which are reported to interact with RV is regulated by FUT-2 gene. The risk of RV infection thus depends on successful recognition of host ligand by the viral surface protein (VP8). Any change in the ligand could lead to fulfilling of such interaction and subsequent infection. Such change in host ligand could result from the functional deficiency of fucosyltransferase-2 enzyme. This has been recognized in a few recent reports. The polymorphisms in the FUT2 gene seem to be responsible for induction of these changes and, hence, the present study is focused on finding such SNPs in the population under study. Single-nucleotide polymorphisms data available for Europeans, Caucasians, Americans, or Japanese population cannot be extrapolated for the Indian population as ethnic diversity is larger. To the best of our knowledge, no report has appeared which comments on polymorphisms of the FUT2 gene and susceptibility to RV in Indian population. The current study is possibly the first of its kind carried out in India.
MATERIALS AND METHODS

This study has been performed at the MGM Institute of Health Sciences, Navi Mumbai, India, from December 2014 to November 2015. It has a well-equipped, state-of-the-art Pediatric Department for diagnosis and treatment of RV infection. Stool sample in sterile container and ethylenediaminetetraacetic acid (EDTA) blood was obtained from the patient on the very first day of admission. The project and the protocol were followed by the MGM Institutional Ethics Review Committee.

Inclusion Criteria

The study included children below 60 months (5 years) of age, having acute diarrhea as defined by the World Health Organization.21 Informed consent was taken from parents who were willing to participate in the study.

Exclusion Criteria

Any child having bacterial diarrhea, mucus, or blood after clinical and lab evaluation was excluded. Also, the child with small and frequent stools >15 days with or without tenesmus was not considered.

The day of initiation of loose stool was considered. Patients negative for RV in order to show similar symptoms were asked for another fresh sample daily until the symptoms subsided or discharged. The blood sample of the same patient has been used for Lewis typing and extraction of host DNA. Informed consent was duly signed by the guardian or parent of the child patient.

Electropherotyping and RT-PCR from Stool Samples

Generally, for screening purposes, the enzyme-linked immunosorbent assay is in use, but as this technique lacks sensitivity, electropherotyping22 was chosen. About 10% of stool suspension was prepared and viral nucleic acid extracted using QIAamp viral RNA mini kit (Qiagen). Sample was maintained at 4°C during centrifugation and all other steps. Pellets were resuspended in 25 µL diethyl pyrocarbonate water. Sample was stored at −20°C until use. A 7% RNA polyacrylamide gel electrophoresis (PAGE) was used for RNA electrophoresis. A 30% acrylamide (Merck), 10% ammonium per sulfate (HiMedia), and tetramethylethylenediamine (HiMedia) were used as standard protocol.22 A 1.5M Tris base (HiMedia) at pH 8.8 was used as resolving gel buffer and Tris glycine as tank buffer. The gel was stained with standard protocol of silver staining (Sigma) after methanol fixation and developed by chilled 3% sodium hydroxide (HiMedia) and immediate addition of formaldehyde (Merck) to catalyze reaction.22 P-genotyping was performed by RT-PCR mentioned elsewhere (Qiagen).22

Leb Phenotyping of RBC by Hemagglutination Test

All acute gastroenteritis cases were typed for Lewis antigens to check for the presence of Leb on RBCs. This confirms exocrine secretion of ABO antigens as RBCs adsorb this molecule from plasma. Lewis antisera by Immucore Pvt. Ltd. were used for agglutination test. Agglutination with any of the tubes confirms presence of the corresponding antigen; only Leb positive sample is supposed to be a nonsecretor, Leb is a secretor, whereas agglutination in both the tubes confirms weak secretor status. Lewis antigen-negative patients were not considered in this study (Fig. 2).

Scoring Diarrhea Symptoms on Vesikari System

In 1990, Ruuska and Vesikari23 described a numerical scale to assess severity of gastroenteritis based on duration and frequency of diarrhea, vomiting, fever, dehydration, and type of treatment required (Table 1). These have been used...
Kshitija S Rane-Yadav et al

Table 1: Vesikari scoring\textsuperscript{23} to determine severity of RV diarrhea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>1–3</td>
<td>4–5</td>
<td>≥6</td>
</tr>
<tr>
<td>Maximum no stools/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea duration (days)</td>
<td>1–4</td>
<td>5</td>
<td>≥6</td>
</tr>
<tr>
<td>Maximum no vomiting/day</td>
<td>1</td>
<td>2–14</td>
<td>≥5</td>
</tr>
<tr>
<td>Vomiting duration (days)</td>
<td>1</td>
<td>2</td>
<td>≥3</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.1–38.4</td>
<td>38.5–38.9</td>
<td>≥39.0</td>
</tr>
<tr>
<td>Dehydration</td>
<td>N/A</td>
<td>1–5 %</td>
<td>≥6%</td>
</tr>
<tr>
<td>Temperature</td>
<td>N/A</td>
<td>1–5 %</td>
<td>≥6%</td>
</tr>
<tr>
<td>Treatment</td>
<td>Rehydration</td>
<td>Hospitalization</td>
<td>Hospitalization</td>
</tr>
</tbody>
</table>

Table 2: Severity category\textsuperscript{23} on basis of Vesikari scoring

<table>
<thead>
<tr>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Maximum score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7</td>
<td>7–10</td>
<td>≥11</td>
<td>20</td>
</tr>
</tbody>
</table>

Direct Sequencing of FUT2 Gene to Monitor SNPs

The partial sequencing of exon 2 of FUT2 gene was carried out by Sanger’s method to identify polymorphisms or mutations, which can be correlated to expression of fucosyltransferase-2 enzyme. The ORF of the gene spanning 1272 bp of exon 2 is amplified with flanking sense and antisense primers given below. Products were checked on agarose gel with DNA marker. Primers\textsuperscript{24} (Table 3) used for the reaction were confirmed using the National Centre for Biotechnology Information Primer Blast and were supplied by Sigma.

The PCR products were purified by slicing the band in gel using SV wizard gel purification kit (Promega). Nanodrop reading of purified product was taken. Next protocol was performed at Scigenome labs, Kochi. Around 50 to 70 ng DNA was used with primer 1.5 pmol/µL. About 7 µL of sequencing reaction was set up in thermal cycler containing 5× sequencing buffer (ABI\textsuperscript{TM}), purified PCR product, primers, and milliQ water. Postcycling cleanup of extension products was done by BigDyeX terminator kit. Primary data analysis software processes raw sequence data in an *ab1 file using algorithms. Then KB\textsuperscript{TM} basecaller is used that processes the fluorescence signals followed by Mobility Shift Correction. Electropherograms obtained were checked for signal-to-noise ratio. Good quality results were accepted. Secondary data analysis was performed using FASTA format sequence by multiple sequence alignment with wild-type sequence using basic local alignment search tool. Every electropherogram was assessed manually for overlapping peaks at any base call, conclusive of heterozygous allele.

RESULTS

P[4] and P[8] Genotypes were Prevalent in Study Population

A total of 150 samples were collected from the pediatric ward and outpatient department of MGM Hospital, Navi Mumbai, India, from Nov 2014 to Dec 2015. A total of 17 samples (11.33\%) were found to be positive for RV RNA. Short and long electropherotypic strains were observed (Fig. 3). All genotypes were of the P[4] (n = 2, 11.76\%) or P[8] (n = 15, 88.24\%) type.

Expression of Le\textsuperscript{b} in Children causes Severe Diarrhea and is significantly associated with RV Infection

A total of 200 samples (100 healthy controls and 100 diarrhea patients) were screened by Lewis hemagglutination test to determine secretor phenotype using Le\textsuperscript{a} and Le\textsuperscript{b} antisera. About 11 diarrhea samples could not be typed as samples were hemolyzed. About 89 were analyzed, which included 17 rota-positive and 72 rota-negative cases. Vesikari score for each case was calculated, and for the purpose of comparison.

Table 3: Primers used for amplification of FUT2 gene for sequencing. These are flanking sequences of exon 2 which is ORF

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUT F</td>
<td>TGCCAAAGATTATCACAACCTGAAG</td>
</tr>
<tr>
<td>FUT R</td>
<td>GATTTCGTGTTACCTGCAGCCCA</td>
</tr>
</tbody>
</table>

Fig. 3: Electropherotyping. RNA extracts from pediatric stool samples analyzed by electropherotyping RNA PAGE gel of selective RV-positive cases stained by silver nitrate showing three long (lane 1, 2 and 4) and one short (lane 3) phenotype
between various groups, MVS was calculated (Graph 1). Diarrhea in case of RV infection was severe (MVS = 12.66) and all cases (n = 17) were Leb hemagglutination positive. Non-RV diarrhea cases (n = 72) were moderate (MVS = 10.05), perhaps for Leb-positive cases (n = 11) and was milder (MVS = 8.50) than secretors (n = 55) (MVS = 9.11).

Observed frequency of nonsecretors and weak secretors in healthy vs infected individuals, when plotted, showed significant difference in expression of Leb antigen (Graph 2). None of the RV-positive samples were found to lack α-1/2 fucosylated glycan expression. Single sample showed weak expression of Leb. Frequency of weak secretors and nonsecretors was found to be high in healthy population as well as in cases with NRV diarrhea. The RV has been found to cause diarrhea in secretors or weak secretor cases.

Graph 1: Disease severity according to Vesikari score. Lewis b positive from RV (RV Le B pos) infected and noninfected (NRV Le B pos) shows significant difference in severity as per Vesikari scoring system. Rotavirus-positive cases, all secretors had altogether severe (mean = 12) diarrhea. NRV cases had moderate (mean = 10.05) diarrhea. Nonsecretors had milder symptoms (MVS = 8.5) compared with secretors (MVS = 9.11)

Graph 2: Distribution of Lea and Leb antigen. Phenotypic status among 172 individuals divided into three groups of healthy individuals and diarrhea patients positive and negative for RV infection, Navi Mumbai, Maharashtra, India; December 2014 to November 2015. Frequency of nonsecretors and Lewis-negative phenotypes is higher among healthy cohort and NRV cases


The partial sequencing of the gene was carried out to monitor various polymorphisms in FUT2, which might be important for expression. The FUT2 gene is 9980 bp (48695971 to 48705950 from pter) composed of two exons, 118 bp and 2995 bp. First exon is the untranslated region and the second exon codes for fucosyltransferase-2 enzyme, 343 amino acids. Using a pair of primers flanking the ORF, target region of 1272 bp was amplified (Fig. 4). Bidirectional Sanger’s sequencing of target region using the same primers was carried out (SciGenome Labs Private Limited, Kakkanad, Kerala,

Fig. 4: A 1.5% agarose gel showing 1272 bp amplified PCR product of FUT2 gene. Lanes 1 to 7 and lane 9 to 12 are samples from various study groups. Lane 8: 100 bp ladder
India). A 1272 bp target region was performed. Totally, 36 samples were sequenced for \textit{FUT2} gene flanking ORF, viz., 12 RV-positive (9 secretor + 3 weak secretor), 14 RV-negative (3 secretor + 3 weak secretor + 8 nonsecretor), and 10 healthy nonsecretors. All stool samples from the RV-infected patients were found to belong to \textit{Le}^{b+}-positive antigen type (secretor/weak secretor). The SNPs observed in nonsecretors are listed in Table 4. The 428 \textit{G} → \textit{A} and 302 \textit{C} → \textit{T} are critical as they are associated with compromised fucosyltransferase-2 activity.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Allele</th>
<th>Reading frame</th>
<th>Amino acid</th>
<th>H-NS n = 10</th>
<th>NRV n = 14</th>
<th>RV n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>171 A→G</td>
<td>GCA→GCG</td>
<td>No change</td>
<td>0 AG</td>
<td>7 AG</td>
<td>3 AG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 GG</td>
<td>1 GG</td>
<td>0 GG</td>
</tr>
<tr>
<td>2</td>
<td>216 C→T</td>
<td>TAC→TAT</td>
<td>No change</td>
<td>0 CT</td>
<td>6 CT</td>
<td>2 CT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 TT</td>
<td>2 TT</td>
<td>1 TT</td>
</tr>
<tr>
<td>3</td>
<td>302 C→T</td>
<td>CCG→CTG</td>
<td>P→L</td>
<td>0 CT</td>
<td>6 CT</td>
<td>4 CT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 TT</td>
<td>2 TT</td>
<td>0 TT</td>
</tr>
<tr>
<td>4</td>
<td>357 C→T</td>
<td>AAC→AAT</td>
<td>No change</td>
<td>0 CT</td>
<td>6 CT</td>
<td>6 CT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 TT</td>
<td>1 TT</td>
<td>3 TT</td>
</tr>
<tr>
<td>5</td>
<td>428 G→A</td>
<td>TGG→TAG</td>
<td>W→X</td>
<td>0 GA</td>
<td>7 GA</td>
<td>3 GA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 AA</td>
<td>1 AA</td>
<td>0 AA</td>
</tr>
<tr>
<td>6</td>
<td>739 G→A</td>
<td>GGT→AGT</td>
<td>G→S</td>
<td>1 GA</td>
<td>3 GA</td>
<td>2 GA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 AA</td>
<td>0 AA</td>
<td>0 AA</td>
</tr>
</tbody>
</table>

Data show presence (+) or absence (−) of homozygous SNPs in healthy nonsecretors (n = 10), NRV (n = 14), and RV (n = 12). Number of individuals with homozygous or heterozygous loci has been mentioned in each column. No mutant homogyzote for loci 302, 428, and 739 was found in RV-positive group (n = 12). Mutant allele at loci 739 is not restricted to RV infection and NRV group resembles same trend.

Graph 3A to F: Electropherograms of 302 bp and 428 bp. Samples of secretors, weak secretors, and nonsecretors were processed for sequencing. Partial electropherograms of \textit{FUT2} gene showing loci 302 bp and 428 bp in heterozygous and homozygous (wild type and mutant) forms. Shaded region indicates position of SNP 302/428. (A) 302 C/C (homozygous wild type allele observed in all groups); (B) 302 C/T (heterozygous allele observed in all groups); (C) 302 T/T (homozygous mutant allele restricted to nonsecretors); (D) 428 G/G (homozygous wild type allele observed in all groups); (E) 428 G/A (heterozygous allele observed in all groups); and (F) 428 A/A (homozygous mutant allele restricted to nonsecretors).
ase enzyme and, hence, lack of expression of Leb\(^b\) antigen in secretion on RBCs. Sequence analysis of FUT-2 gene in patients with positive RV infection showed concordant results with hemagglutination test based on Lewis b antisera. None of the patients with RV infection showed presence of null allele in FUT2 gene ORF. Four weak secretor patients with RV infection were heterozygous at position 428 bp (n = 3) or at 302 bp (n=1) of the FUT2 gene. This correlates with low expression of Leb\(^b\) on the RBCs, Le\(^b^+\)/Le\(^b^-\).

**Statistical Analysis**

Nonsecretor phenotype was not found to be associated with any of the RV-positive cases. About 16 (92.30%) were SE/SE homozygous and 1 (7.69%) was SE/se heterozygous. By contrast, there were 16 (16%) confirmed nonsecretors among the 100 individuals of our control healthy adult population. Distributions of the genotypes between the controls and rota-positive cases were significantly different (p-value = 0.04). In the other control group including U5C with gastroenteritis of unknown etiology (non-RVA), 55 (76.38%) SE/SE (Leb\(^+\)/+), 6 (8.33%) SE/se (Leb\(^+\)/−), and 11 (15.27%) se/se (Leb\(^-\)/−) were found. No significant difference was found in healthy and NRV group with p-value 0.24. The absence of null allele among patients compared with either the healthy adult control group or the NRV control group was significant, indicating that nonsecretors are resistant to symptomatic infection by RV, but they can be susceptible to other viruses causing diarrhea.

Presence of null allele at loci 302 (302 CT/TT) and 428 (428 GA/AA) was found to be significantly associated with nonsecretors. The RV-infected cases in the present study were associated with wild type alleles at nucleotide positions 302 bp and 428 bp (Table 5). Counts of heterozygotes and homozygous mutants in each group were statistically evaluated by Fischer’s exact test (level of significance = 0.1) for probability calculation (2 x 2 contingency table). There was significant difference between nonsecretor cohort and RV-infected test cohort in context with nucleotide 302 bp and 428 bp of ORF at exon 2 of FUT2 gene.

**DISCUSSION**

In cases of viral infection, host susceptibility is dependent on the successful recognition of host receptor by the ligand on the pathogen. Risk of infection, thus, could genetically predispose the host to infection from that specific pathogen. Any structural alteration mediated through mutations or polymorphisms at critical sites in the respective gene (of the host or pathogen) could result in failure of recognition of the host, leading to the development of resistance to that infection. As Collins\(^{25}\) states, “most misspelled genes cause a predisposition and not a predetermination of a disease”; such misspelling could occur due to mutations or polymorphisms in the respective genes of the host or the pathogen. Various loci in the human genome have been mapped, which predispose individuals to infectious diseases, such as malaria, tuberculosis, human immunodeficiency virus, etc.\(^{26}\) In recent years, there is considerable interest in the role of the secretor status and Leb blood group antigens of the individual that serve as attachment targets for pathogens.\(^{27}\) It was, therefore, of interest to study if susceptibility or resistance to RV infection is associated with secretor or nonsecretor status. The study focused on examining, such association, if any, for RV.

About 150 cases of diarrhea were screened for infection to RV, of which 17 found to be positive. P-genotyping data indicated that P[4] and P[8] are predominant in Navi Mumbai region. Studies undertaken in various parts of India showed similar results.\(^{28,29}\) A few cases of P[6] have been reported in this region unlike the African population.\(^{17}\) About 172 EDTA blood samples were tested for Leb\(^b\) antisera-based hemagglutination test, of which 100 were from random healthy individuals. The results could be confirmed for 90 samples, viz., 66 secretors (Le\(^a^-\)/Le\(^b^+\)), 16 nonsecretors (Le\(^a^-\)/Le\(^b^-\)), and 8 weak secretors (Le\(^a^-\)/Le\(^b^-\)). About 10 samples were Lewis negative (Le\(^a^-\)/Le\(^b^-\)); hence, they were inconclusive. No reports are available in India for viral diarrhea cases and their association with RV infection. Parallel blood examination of viral diarrhea cases was carried out for 89 cases. All 17 RV-positive cases were secretors, one of which was weak secretor (p-value = 0.04) (Graph 2). Another 72 NRV cases had frequencies similar to control cases with minor deviation in frequency of nonsecretors and Lewis negative (p-value = 0.24). Attachment of RV to glycan has been previously reported by few groups considering murine, bovine, or other animal strains.\(^{15,16,19}\) There are some reports suggesting that RV VP8 surface protein interacts with Lewis antigen, Le\(^b\) specifically, which is adsorbed on mucosal epithelial cells, such as enterocytes of secretor or weak secretor individuals. There are reports which indicate that human RVs recognize HBGA.\(^{30,32}\) Thus, presence of HBGA on human cells could predispose the host to

---

**Table 5:** Probability value calculation using Fisher exact test for 2 x 2 contingency table (level of significance 0.1)

<table>
<thead>
<tr>
<th></th>
<th>Healthy NS n = 10</th>
<th>RV n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AA-FUT2</strong>&lt;sup&gt;302−/−&lt;/sup&gt;</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>GA-FUT2</strong>&lt;sup&gt;302−/−&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.018 S</td>
<td></td>
</tr>
<tr>
<td><strong>TT-FUT2</strong>&lt;sup&gt;428−/−&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>CT-FUT2</strong>&lt;sup&gt;428−/−&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.067 S</td>
<td></td>
</tr>
</tbody>
</table>

Significant difference was found in allelic counts for homozygous and heterozygous mutation at 302 nt and 428 nt of FUT2 gene between two groups of nonsecretors and RV. It proves genetic difference in two groups.
RV infection. The HBGA are secretory ABO(H) antigens, which classify the population into two groups—secretors and nonsecretors. Secretor shows presence of ABO antigens in saliva, tears, mucosa as well as on the surface of epithelial tissue cells, whereas nonsecretors do not. About 20% of Caucasian population was found to be nonsecretors.33 In India, various studies have given frequencies of nonsecretors at around 70 to 100%.34,35 Surprisingly, of the remaining population, not all are found to be secretors, but some of them can be clubbed together to form another classification of weak secretors.36 The larger part of Asian population reportedly show weak activity of fucosyltransferase-2 enzyme; hence, the marker Le$^b$ shows “dim” expression. The population is defined by term weak secretor and at genome level, they are heterozygous for null allele.37

A major part of blood group systems is generated by a family of homologous proteins (enzymes) fucosyltransferases. Secretory or nonsecretory nature of these ABO(H) antigens depends on functional status of some of these enzymes. Specific enzyme fucosyltransferase-2 coded by gene FUT-2 or Se plays a major role by adding α-1→2 fucose to precursor molecule. Another enzyme fucosyltransferase-3 coded by gene FUT-3 or Le regulates expression of Lewis antigens by α-1→3 fucosylation. Though both enzymes are critical for Lewis blood group system, only FUT2 is associated with expression of Le$^b$ antigens on epithelial cells. Role of FUT3 is required to determine secretor status of Lewis-negative (Lea$^-$ Leb$^-$) individuals. Secretor individual expresses fucosyltransferase-2 enzyme; hence, they show presence of Le$^b$ antigen in saliva, tears, mucosa as well as on the surface of epithelial tissue cells. Any such mutation or variant polymorphism in FUT-2, which can change the active site or translation of inactive protein, ceases α-1→2 fucosylation. These individuals are nonsecretors due to absence of ABO antigens in secretions, on epithelial cells, and even on RBCs. Vesikari scoring for study groups showed that RV diarrhea was more severe (MVS = 12) in the maximum number of cases compared with NRV diarrhea (MVS = 10.05). The interesting trend was observed that among the NRV cases, individuals with secretor status (MVS = 9.11) had comparatively severe diarrhea symptoms than nonsecretors (MVS = 8.5). This indicates that other pathogens might involve Lewis antigen although to a lower extent compared with the RV. Association of HBGA with norovirus has been previously reported. It is required to perform broader studies in association with HBGA to target pediatric viral diarrhea in terms of vaccines and therapy.

There are 55 known polymorphisms of FUT2 gene as per a recent study.38 Some of them are nonsynonymous and, if homozygous, can confer nonsecretor status to the individual. Present study elucidates prevalence of six SNPs—171 bp, 216 bp, 302 bp, 357 bp, 428 bp, and 739 bp—for population in this region. There is a single study from France18 carried out in year 2016, which has stated the association of FUT2 polymorphism with resistance to RV infection. The group has performed restriction fragment length polymorphism assay to determine alleles GG/GA/AA at loci 428.27 The ORF of FUT2 gene was scanned to elucidate various other polymorphisms and mutations, which could be associated with RV pathogenesis. We have found G428A polymorphism in 3 out of 17 RV-positive samples. All three were heterozygous mutations Se/se$^{428}$ and have shown weak secretor status (Le$^a$ Le$^b$) by RBC hemagglutination test. The NRV cases showed higher frequency (8 out of 14) for presence of this polymorphic locus and a single sample was heterozygous. The rest all were homozygous mutants (se$^{428}$/se$^{428}$).

The study reports another SNP C302T, which could show possible predisposition to RV P[4], P[8] for children of Indian origin. Nonsecretors from healthy as well as NRV group showed se$^{302}$/se$^{302}$ allele, whereas RV-positive cases were heterozygous mutant Se/se$^{302}$. This leads to a change in codon (CCG→CTG). Subsequently at position 101, proline is replaced by leucine. There could be possibility that conformational changes happened in the enzyme making the active site incompatible for α-1/2 fucosylation of H precursor antigen. The se$^{302}$ allele was reported to be restricted to Thai and Bangladeshi populations.39 We report its presence in Indian population as well. Frequency of 302T and 428A allele was found the lowest among RV (RV-positive) group (Table 6). The prevalence of these mutant alleles was predominant in NRV (N RV) cases but compared with nonsecretor cases, RV cases showed almost similar (302T—16.66 vs. 20%) or even less prevalence (428A—20.83% vs. 50%) for homozygous mutant alleles for both these loci, viz., the se$^{302}$/se$^{302}$ and se$^{428}$/se$^{428}$ were restricted only to nonsecretors and not observed in any case of RV-positive patient. Association of G428A and C302T with RV infection is statistically significant (p-value = 0.018 and 0.067 respectively, level of confidence = 0.1) (Table 5).

The strains circulating in Europe, America, and other White populations are different compared with India. The reason for this can be ethnicity-based host genetics.

### Table 6: Distribution of alleles 302 and 428 among NRV and RV group compared with nonsecretors

<table>
<thead>
<tr>
<th>Allele</th>
<th>NS n = 10</th>
<th>NRV n = 14</th>
<th>RV n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chromosome</td>
<td>Chromosome</td>
<td>Chromosome</td>
</tr>
<tr>
<td></td>
<td>no = 20</td>
<td>no = 28</td>
<td>no = 24</td>
</tr>
<tr>
<td>302 C</td>
<td>16 (80%)</td>
<td>18 (64.28%)</td>
<td>20 (83.33%)</td>
</tr>
<tr>
<td>302 T</td>
<td>4 (20%)</td>
<td>10 (35.71%)</td>
<td>4 (16.66%)</td>
</tr>
<tr>
<td>428 G</td>
<td>10 (50%)</td>
<td>19 (67.85%)</td>
<td>19 (79.16%)</td>
</tr>
<tr>
<td>428 A</td>
<td>10 (50%)</td>
<td>9 (32.14%)</td>
<td>5 (20.83%)</td>
</tr>
</tbody>
</table>
The present study gives plausible explanation based on host genetics for discrepant vaccine efficacy in different continent and suggests that specific population can be genetically predisposed precisely to specific strains of RV based on fucosyltransferase-2 gene.

CONCLUSION

The study shows that the risk for infection from RV P[4] and P[8] genotypes seems to be associated with secretor or nonsecretor status of the individual. The FUT2 gene and its polymorphisms play an important role in the determination of secretor status. The FUT2 gene alleles are at loci 428 bp and 302 bp and are found to be associated with group A RVs. Presence of 428 (AA) and 302 (TT) allele in children of Indian origin leads to nonsecretor phenotype. Both these alleles were less frequent (<20%) in the population under study. Individuals with secretor status seem to have higher risk for infection from P[4] and P[8] RVs. Our studies also indicate that alteration at 428 bp or 302 bp polymorphisms could confer resistance or low risk from P[4] and P[8] RV infection.

ACKNOWLEDGMENTS

Authors would like to thank MGM Hospital for Women’s and Children, Kalamboli, Navi Mumbai, Maharashtra, India, and Women Scientist Scheme-A, Department of Science and Technology (DST), Government of India, for financial support for undertaking the research project. The authors extend their gratitude to Staff of Central Research Laboratory and Mr. Kishor Raut, Professor, Department of Community Medicine, MGM Medical College, Navi Mumbai, Maharashtra, India for their guidance and support.

REFERENCES