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Attachment to carbohydrates of the histo-blood group type of several human Rotavirus strains ( RVA) has recently been described. Synthesis of these ligands requires a functional FUT2 enzyme, suggesting that FUT2 null homozygote (ie, nonsecretor) individuals may not be recognized by most human RVA strains. Whereas such individuals represent 20% of the control population, this retrospective study determined that none of 51 patients infected by P[8] rotavirus strains were nonsecretors. The lack of α1,2fucosylated carbohydrates in the gut surface mucosa is thus associated with resistance to symptomatic infection and virus attachment to such motifs is essential to the infection process.

Keywords. FUT2; histo-blood group antigens; ligands; rotavirus; secretor phenotype.

Group A rotaviruses (RVA) are the major etiological agents of acute gastroenteritis (GE) in young children worldwide. In 2008, prior to the release of vaccines, they were the estimated cause of over 450 000 deaths per year, mainly in countries of poor income [1]. Rotaviruses possess a segmented double-stranded RNA genome and a 3-layered capsid. The 2 outer capsid proteins VP7 and VP4 are used for classification of strains, defining the G- and P-genotypes, respectively, and as many as 27 G-genotypes, 35 P-genotypes, and 73 G/P genotype combinations of RVAs infecting humans have been described [2]. Nevertheless, most of these are of rare occurrence, and the large majority of cases are due to G1P[8], G2P[4], G3P[8], and G9P[8] strains [3].

Initial cell attachment of rotaviruses is mediated by VP8*, the distal domain of the VP4 spike protein. Until recently, it was considered that VP8* binds to either terminal, sialidase-sensitive sialic acid residues, or to internal, sialidase-insensitive sialic acid residues [4]. However, recent analyses of the carbohydrate binding properties of VP8* from various human strains of the P[4], P[6], P[8], P[9], P[11], P[14], and P[25] genotypes revealed specific recognition of neutral oligosaccharides of the histo-blood group family (HBGAs) [5–8]. HBGAs like the ABH and Lewis antigens are polymorphic glycans expressed on many cell types and particularly epithelial cells of the gut. Most VP8* variants tested so far bind to either the H type 1, Lewis b, or A antigens that have in common an α1,2-linked fucose residue (Supplementary Figure 1). The FUT2 (Secretor) gene encodes the α1,2fucosyltransferase responsible for addition of this residue, determining the secretor phenotype. Mutations of FUT2 lead to either a complete lack or a severely decreased α1,2fucosyltransferase activity and therefore an absence of α1,2fucosylated antigens in mucosal tissues and secretions, such as saliva. This determines the nonsecretor phenotype [9], which concerns about 20% of the white population. If attachment of VP8* to such α1,2fucosylated antigens represents a necessary step of the infectious process, nonsecretor patients may be expected to be resistant to RVA infection.

In this retrospective study, we investigated whether FUT2 polymorphism is associated with RVA P[8] infection, because the VP8* of strains of this genotype binds to α1,2fucosylated antigens in vitro and because they are largely dominant in Western Europe and the United States [3].

METHODS

Subjects and Samples
The study was retrospectively performed on feces samples collected and stored at −80°C from 62 RVA patients at the University Hospital of Nantes after approval by the institutional review board. Samples collections were obtained during 3 epidemics, occurring during the winters of 2011 (n = 11), 2012 (n = 39), and 2013 (n = 12). All patients presented with gastroenteritis symptoms and had a positive result for rotavirus with a rapid antigenic viral test (Rapid Strip ROTA-ADENO, Meridian Bioscience Europe, Villa Cortese, Italy). Other viral etiologies of GE, and notably norovirus (NoV), had been excluded by
standard molecular diagnosis. Patient’s age ranged from 15 days to 67 years, with a median age at 10 months, and the 3 adults patients included were immunosuppressed.

Feces samples from pediatric gastroenteritis cases of unknown etiology, excluding rotavirus and NoV infection, in the same age range as pediatric RVA patients, were used to constitute a control group with diarrheal stools.

Swabs of buccal epithelial cells were collected from 95 healthy adult individuals, constituting another group of control subjects (Institutional review board study No° BRD02/2-P).

**Nucleic Acids Extraction**

RNA and DNA were extracted from approximately 100 mg of stool sample from patients with the Nucleospin RNA virus kit (Macherey-Nagel, Düren, Germany). This kit allows efficient DNA and RNA extraction, providing proteinase K is added according to manufacturer’s instructions. Genomic DNA from swabs of buccal epithelial cells of healthy adult control subjects was extracted with the Qiagen QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

**Rotavirus Reverse-Transcriptase Polymerase Chain Reaction and VP8* Sequencing**

A 534–base pair (bp) fragment (aa 46 to 231) of the VP8* gene was amplified by reverse-transcriptase polymerase chain reaction (RT-PCR), with a procedure adapted from Liu et al [7]. Briefly, RT and PCR were performed on 5μl extract using the One Step PrimeScript kit (TaKaRa, Japan), according to the manufacturer’s recommendations. M13 universal primers were added at the 5’end of each primer (forward 5’TGACTCACGT NAATTGGRGWCAYG3’, reverse 5’YATTCTNTGATTYT GAATTGGTGG3’) for sequencing. Purified amplicons were then sequenced using the Big Dye version 1.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3130 Sequence Analyser. Sequences were analyzed by the Seqscape software and deposited in GenBank. Phylogenetic analysis was conducted using MEGA, version 5.

**FUT2 Genotyping of Control and Patients Subjects**

For all samples of the patients and control groups, a 195-bp fragment of the FUT2 gene amplified as previously described was digested with Ava II [10]. The G428A mutation abrogates this restriction site, allowing genotyping at this site that corresponds to the common FUT2 mutation in a white population. To ensure quality of the genotyping using DNA extracted from stool sample from patients with the Nucleospin RNA virus kit (Macherey-Nagel, Düren, Germany), this fragment of the FUT2 gene amplification (RT-PCR), with a procedure adapted from Liu et al [7]. The G428A mutation abrogates this restriction site, allowing genotyping at this site that corresponds to the common FUT2 mutation in a white population. To ensure quality of the genotyping using DNA extracted from stool sample from patients with the Nucleospin RNA virus kit (Macherey-Nagel, Düren, Germany), this fragment of the FUT2 gene amplification (RT-PCR), with a procedure adapted from Liu et al [7].

**RESULTS**

The VP8* sequence could be amplified from the stools of 57 patients out of 62. All cases but 1 belonged to strains of the P[8] genotype. The remaining patient, who received an allogeneic hematopoietic stem cell transplantation, presented a P[3] rotavirus of canine origin. The presence of a large majority of the P[8] genotype is concordant with recent epidemiologic data from Western Europe and France showing that they were present in 93% of cases [11]. Phylogenetic analysis of the VP8* sequences revealed that all strains belonged to the P[8]-3 clade (Figure 1). According to a very recent classification [12], most (n = 41) were classified in the P[8]-3.6 sublineage, whereas others belonged to the P[8]-3.3 subtype (7 strains) or could not be clearly classified (8 strains close to the P[8]-3.4 sublineage). There was no significant association between a particular sublineage and the age of the patient, the date of sample collection, or the FUT2 genotype. Noticeably, the 12 samples taken in 2013 were all from the P[8]-3.6 sublineage.

Of the 56 patients infected by a P[8] strain of rotavirus, 51 were successfully genotyped for FUT2, the 2 methods used giving concordant results. All were of the secretor phenotype (100%) with the following genotypes distribution: 18 (35%) SE/SE homozygous and 33 (65%) SE/se428 heterozygous. By contrast, there were 19% nonsecretors among the 95 individuals of our control healthy adult population for whom the distribution of genotypes was as expected in a white population with 21 (22%) SE/SE, 56 (59%) SE/se428, and 18 (19%) se428/se428. In the other control group composed of pediatric patients with GE of unknown etiology (nonRVA/nonNoV), 11 (26%) SE/SE, 26 (60%) SE/se428, and 6 (14%) se428/se428 were found. Distributions of the genotypes between the controls and cases groups were significantly different (P < .0007 and P = .016, respectively). By contrast, the distribution of genotypes was not significantly different between the 2 control groups (P = .79). The absence of nonsecretors (se428/se428) among patients compared to either the healthy adult control group or the nonRVA/nonNoV GE control group was also statistically significant (P = .0003 or P = .007, respectively), indicating that nonsecretors are resistant to symptomatic infection by P[8] rotavirus strains (Table 1).

**DISCUSSION**

Binding of recombinant VP8* from P[8] strains of rotavirus to α1,2fucosylated glycans (H type 1 and Lewis b antigens) was
recently reported [5]. However, no relationship with infection had been documented as yet. Binding of the VP8* from P9, P14, and P25 strains to the A blood group antigen was also recently demonstrated and its contribution to in vitro replication was shown [6, 7]. These observations suggested that infection by human strains of rotavirus could depend on the presence of polymorphic carbohydrate ligands, meaning that large numbers of individuals devoid of the appropriate histocompatibility antigens would be genetically resistant to infection. To test this hypothesis (and because synthesis of the described VP8* ligands of P8 strains require a functional FUT2 enzyme), we looked at the FUT2 genotype of patients in...
comparison with controls groups from the same location in France. Unlike in the control healthy population that comprises nearly 20% of FUT2 null subjects, there was no nonsecretor within the infected patients’ group. In addition, 14% nonsecretors were found in a second control group composed of patients with GE of unknown etiology. The latter group allowed validation of the FUT2 genotyping from diarrheal stools. The frequency of nonsecretors observed in this group was slightly lower than the well-known western European frequency. Yet, the difference was not significant (14% vs 19%; \( P = .63 \)). Overall, these results indicate that the lack of α1,2fucosylated carbohydrate motifs in the gut surface mucosa is indeed associated with resistance to symptomatic infection and that attachment of VP8* to such motifs is an essential step of the infection process. This first retrospective study performed on clinical specimens indicates the existence of a human genetic susceptibility to at least some rotavirus infection.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


Table 1. Distribution of the FUT2 Genotypes Among Patients and Controls

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<tr>
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<th>SE/SE</th>
<th>SE/se⁴²⁸</th>
<th>se⁴²⁸/se⁴²⁸</th>
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<tbody>
<tr>
<td>RVA patients</td>
<td>18 (35%)</td>
<td>33 (65%)</td>
<td>0 (0%) N = 51</td>
</tr>
<tr>
<td>nonRVA/nonNoV patients</td>
<td>18 (43%)</td>
<td>18 (43%)</td>
<td>6 (14%) N = 42</td>
</tr>
<tr>
<td>Healthy adult controls</td>
<td>21 (22%)</td>
<td>56 (59%)</td>
<td>18 (19%) N = 95</td>
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The functional wild type allele is designated SE and the mutated null allele se⁴²⁸. Homozygous se⁴²⁸/se⁴²⁸ individuals have the nonsecretor phenotype because they are unable to synthesize α1,2fucosylated glycans in saliva and gut surface epithelial cells.

Abbreviations: RVA, group A rotavirus; NoV, norovirus.