



# Relationship Between Rotavirus P[6] Infection in Korean Neonates and Histo-Blood Group Antigen: a Single-Center Study

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**Background:** Rotaviruses are a major cause of pediatric gastroenteritis. The rotavirus P[6] genotype is the most prevalent genotype isolated from Korean neonates but has rarely been reported in other countries. Histo-blood group antigen (HBGA) is known to play an important role in rotavirus infection. We investigated the relationship between rotavirus genotype and HBGA-Lewis blood type in Korean children and explored the reasons for the predominance of rotavirus P[6] strain in Korean neonates.

**Methods:** Blood and stool samples were collected from 16 rotavirus-infected patients. Rotavirus G (VP7) and P (VP4) genotyping was performed using reverse transcription-PCR and sequencing. Lewis antigen phenotypes (Le<sup>a</sup>/Le<sup>b</sup>) were tested, and HBGA-Lewis genotype was determined by sequencing the secretor (*FUT2*) and Lewis (*FUT3*) genes. Deduced amino acid sequences and three-dimensional structures of the VP8\* portion of the rotavirus VP4 protein were analyzed.

**Results:** All P[6] rotaviruses were isolated from neonates under one month of age, who were negative or weakly positive for the Le<sup>b</sup> antigen. However, 10 of the 11 non-P[6] rotaviruses were isolated from older children who were Le<sup>b</sup> antigen-positive. The VP8\* amino acid sequences differed among P[6], P[4], and P[8] genotypes. Korean P[6] strains showed a unique VP8\* sequence with amino acid substitutions, including Y169>L169, which differed from the sequences of P[6] strains from other countries.

**Conclusions:** The predominance of the rotavirus P[6] genotype in Korean neonates may be related to the interaction between HBGA-Lewis antigen and the VP8\* portion of the VP4 protein, and this information will be helpful in future neonatal vaccine development.

**Key Words:** Rotavirus, Neonate, Genotype, Lewis, Histo-blood group antigen, Korean; VP4 protein

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## INTRODUCTION

Rotaviruses are one of the major causes of pediatric gastroenteritis worldwide. Rotaviruses cause 5–10% of all cases of acute gastroenteritis in young children of <5 years of age, and 30% of rotavirus infections are severe enough to require treatment [1].

Rotavirus is a non-enveloped, double-stranded RNA (dsRNA) virus with a triple-layered capsid surrounding a genome of 11 dsRNA segments [1]. The outer capsid layer comprises two structural proteins, VP7 and VP4; based on these, rotaviruses are classified into at least 36 G and 51 P genotypes [2]. G1–G4 and G9 and P[4], P[6], and P[8] are the most prevalent G and P

genotypes, respectively [1, 3], and the genotypic distribution of rotavirus strains shows temporal and geographical fluctuations [1, 4].

In Korea, G1P[8] is the most prevalent genotype in children, whereas G4P[6] is the most prevalent genotype in neonates [3, 5-8]. The predominance of the G4P[6] and the G8P[6] genotype in Korean neonates has been reported from 1999 to 2019 [5-8]. Notably, the predominance of G4P[6] in neonates has not been reported in other countries. The rotavirus P[6] genotype is mainly isolated from Korean neonates, but is rarely isolated in older children [5-8].

Recently, rotavirus infection mediated by the attachment of the VP8\* domain of the VP4 spike protein to specific histo-blood group antigens (HBGAs) in the gut epithelium has been reported [9-11]. HBGA are blood-type determinants determined by an individual's secretor gene (*FUT2*, fucosyltransferase 2) and Lewis gene (*FUT3*, fucosyltransferase 3) [12].

We investigated the relationship between rotavirus genotype and HBGA-Lewis type in Korean children and explored the reasons for the predominance of rotavirus P[6] infection in Korean neonates.

## MATERIALS AND METHODS

### Patient samples

We used rotavirus-positive stool samples and corresponding blood samples, which were remnants to be discarded after rotavirus test and complete blood count test, collected from acute gastroenteritis patients at Hallym University Dongtan Sacred Heart Hospital, Hwaseong-si, Korea, between February 2019 and January 2020. The rotavirus antigen test was conducted using SD BIOLINE Rotavirus (SD BIOLINE Rotavirus; Abbott Diagnostic Korea, Yongin, Korea) or RIDASCREEN Rotavirus test kits (RIDASCREEN Rotavirus; R-Biopharm, Darmstadt, Germany), according to the manufacturer's protocol. The Lewis phenotyping test and DNA extraction were performed on day of sample collection; extracted DNA and remnant diluted stool samples were stored at  $-70^{\circ}\text{C}$  until use. During this period, 66 of the 726 stool samples (9.1%) were rotavirus-positive; adequate stool and corresponding blood samples for sequencing the four genes (the rotavirus VP4/VP7 genes and *FUT2/FUT3* genes) and Lewis antigen phenotyping were available for 16 patients. Median patient age was 3.4 years (range, 8 days–10 years), and six patients (37.5%) were under 30 days of age. The Institutional Review Board (IRB) of Hallym University Dongtan Sacred Heart Hospital (IRB No. 2017-08-007) approved the

study protocol and waived the need for informed consent.

### Rotavirus G and P genotyping

Rotavirus G (VP7) and P (VP4) genotyping was performed using reverse transcription-PCR and sequencing. Viral RNA was extracted from diluted stool samples using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and the QIAcube platform (Qiagen). The RNA was denatured and reverse-transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). The VP7 and VP4 genes were amplified from the dsRNA genome using specific primer sets, VP7-F/VP7-R and VP4-F/VP4-R, respectively [7,8,13]. PCR products were visualized by electrophoresis on agarose gels and analyzed by DNA sequencing using ABI BigDye Terminator v3.1 Cycle Sequencing kits (Applied Biosystems, Foster City, CA, USA) and an ABI 3500 XL DNA Analyzer (Applied Biosystems). Genotypes were confirmed using the Basic Local Alignment Search Tool on the National Center for Biotechnology Information (<https://blast.ncbi.nlm.nih.gov/>)

### Lewis antigen phenotyping

Lewis antigen phenotyping was performed using ID-Antigen Profile I (Bio-Rad, Cressier Switzerland) and gel suspensions containing antibodies for the P, Le<sup>a</sup>, Le<sup>b</sup>, Lu<sup>a</sup>, Lu<sup>b</sup>, and ctt antigens, according to the manufacturer's protocol. A 5% erythrocyte suspension was prepared from each EDTA-anticoagulated blood sample by mixing 50  $\mu\text{L}$  of erythrocytes in 0.5 mL of Diluent 1 solution. The cell suspension was incubated for 10 minutes at room temperature ( $18-25^{\circ}\text{C}$ ), and 12.5  $\mu\text{L}$  of the cell suspension was added to each phenotype ID-card micro-tube. The ID-cards were then centrifuged for 10 minutes in the ID-centrifuge 12 S II (Bio-Rad) and read in the range between negative to 4+, according to the agglutination strength.

### PCR and sequencing of the secretor and Lewis genes (*FUT2* and *FUT3*)

DNA was extracted from EDTA-anticoagulated blood samples using the QIAamp Blood Mini kit (Qiagen). A 1,153-bp region of the *FUT2* gene was amplified using the tk1/tk2 primer set [14], and two segments (917 and 419 bp) of the *FUT3* gene were amplified using two primer sets (N-F1/6R and 4F/8R) [15]. The forward primer sequence of N-F1 (TGATGACCGGCTATGCCTTC) was specifically designed for this study. PCR products were analyzed by DNA sequencing using ABI BigDye Terminator v3.1 Cycle Sequencing kits and an ABI 3500 XL DNA Analyzer. Variants of the *FUT2* and *FUT3* genes were detected by compari-

son with reference sequences FUT2\*01 and FUT3 (<http://erythrogene.com>). Minor allele variants at the FUT2 c.418, c.461, and c.772 and FUT3 c.59, c.202, c.314, c.508, and c.1067 sites, which are known to be related to Lewis antigen phenotypes, were examined [16, 17].

**Table 1.** Rotavirus genotype, erythrocyte Lewis antigen phenotype, and FUT2/FUT3 gene variants in patients with rotavirus infection

Rotavirus genotype	Case number	Sex/Age	Erythrocyte Lewis phenotype	Minor allele homozygosity (FUT2 c.418, c.461, c.772)	Minor allele homozygosity (FUT3 c.59, c.202, c.314, c.508, or c.1067)
G8P[6]	1 19RP-09	M/15 days	Le <sup>a</sup> (3+) Le <sup>b</sup> (-)*	c.418A>T	-
	2 19RP-11	M/8 days	Le <sup>a</sup> (1+) Le <sup>b</sup> (2+)	-	-
	3 19RP-26	M/20 days	Le <sup>a</sup> (-) Le <sup>b</sup> (-)	-	c.508G>A
	4 19RP-35	M/15 days	Le <sup>a</sup> (-) Le <sup>b</sup> (-)	c.418A>T	c.59T>G
	5 19RP-38	M/9 days	Le <sup>a</sup> (-) Le <sup>b</sup> (-)	-	c.59T>G, c.508G>A
G1P[8]	6 19RP-23	F/3 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
G8P[8]	7 19RP-04	M/6 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
	8 19RP-07	M/4 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
	9 19RP-31	F/4 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
G9P[8]	10 19RP-02	F/3 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
	11 19RP-15	F/6 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (3+)	-	-
	12 19RP-24	F/20 days	Le <sup>a</sup> (3+) Le <sup>b</sup> (3+)	-	-
G2P[4]	13 19RP-13	M/5 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
	14 19RP-17	F/10 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (3+)	-	-
	15 19RP-21	F/1 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (4+)	-	-
	16 19RP-32	M/9 yr	Le <sup>a</sup> (-) Le <sup>b</sup> (3+)	-	-

Abbreviations: M, male; F, female.

\*Agglutination strength was expressed in the range of - to 4+, according to the manufacturer's guidelines.

### Analyses of amino acid sequences and three-dimensional (3D) structure of VP8\*

The deduced amino acid sequences of the rotavirus VP8\* portion of the VP4 capsid protein were analyzed and compared with the reference VP4 sequence data from GenBank: Wa\_G1P[8]\_JX406750, DS-1\_G2P[4]\_HQ650119, RV3\_P[6]\_U16299, and P[6] strains detected in other countries (JN129097, LC095922, KF835915, and KF726067) and in Korea (MK953605 and G8P[6] from a previous study [8]).

Multiple sequence alignment and translation to amino acids were conducted using the MEGA version 7 program [18]. The 3D structures of the deduced amino acid sequences of the VP8\* portion were obtained using the Swiss-Model program [19], PDB (Protein Data Bank. <http://www.rcsb.org/>) [20], and UCSF Chimera Program [21]. Briefly, the deduced amino acid sequences of the VP8\* portion were converted to PDB format files in the Swiss-Model program, and structure comparison analyses were performed using the UCSF Chimera Program using the converted PDB files.

### Statistical analysis

The relationship between P[6] and Le<sup>b</sup> antigen negativity is expressed as a 2×2 contingency table, and *P* was calculated by Fisher's exact test using MedCalc software version 19 (MedCalc Software Ltd, Ostend, Belgium). *P*<0.05 was considered statistically significant.

## RESULTS

### Rotavirus genotype, erythrocyte Lewis antigen phenotypes, and FUT2/FUT3 gene variants in rotavirus infected patients

Table 1 shows the rotavirus genotyping, Lewis antigen phenotyping, and FUT2/FUT3 gene results. Of the 16 rotavirus-positive patients, five patients under one month of age were positive for P[6] (all G8P[6]) rotaviruses; these patients were negative (patients 1, 3, 4, and 5) or weakly positive (patient 2) for the Le<sup>b</sup> antigen. In contrast, non-P[6] rotaviruses (G2P[4]s, G8P[8]s, G9P[8]s, and G1P[8]) were isolated from older children (except

**Table 2.** Relationship between rotavirus P[6] genotype and Le<sup>b</sup> antigen negativity in patients with rotavirus infection

Rotavirus genotype	Le <sup>b</sup> (-)	Le <sup>b</sup> (+)	<i>P</i>
P[6]* (N=5)	4 (80%)	1 (20%)	0.0027
Non-P[6]* (N=11)	0 (0%)	11 (100%)	

\*All P[6] rotaviruses were isolated from neonates under one month of age, and 10 of the 11 non-P[6] rotaviruses were isolated from older children.

**Table 3.** Deduced amino acid sequences of the antigenic epitopes of the VP8\* portion of the VP4 capsid protein

Amino acid	81	91	101	111	121	130
Wa_G1P[8]_JX406750*	W I L I N S N T N G V V Y E S T N N S D F W T A V V A I E P H V N P V D R Q Y T I F G E S K Q F N V					
19RP-23_G1P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-04_G8P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-07_G8P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-31_G8P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-02_G9P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-15_G9P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
19RP-24_G9P[8]	W I L I N S N T N G V V Y E S T N N S D F W T A V I A V E P H V D P V D R Q Y N V F G E N K Q F N V					
DS-1_G2P[4]_H0650119*	W L L I S S N T N G V V Y E S T N N N D F W T A V I A V E P H V S Q T N R Q Y I L F G E N K Q F N V					
19RP-13_G2P[4]	W L L I S S N T D G V V Y E S T N N S D F W T A V I A V E P H V S P T N R Q Y V L F G E N K Q F N V					
19RP-17_G2P[4]	W L L I S S N T D G V V Y E S T N N S D F W T A V I A V E P H V S P T N R Q Y V L F G E N K Q F N V					
19RP-21_G2P[4]	W L L I S S N T D G V V Y E S T N N S D F W T A V I A V E P H V S Q T N R Q Y V L F G E N K Q F N I					
19RP-32_G2P[4]	W L L I S S N T D G V V Y E S T N N S D F W T A V I A V E P H V S P T N R Q Y V L F G E N K Q F N V					
RV3_P16_U16299*	W I L L N P T N Q Q V V L E G T N K T D I W V A L L L V E P N V T N Q S R Q Y T L F G E T K Q I T V					
19RP-09_G8P[6]	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
19RP-26_G8P[6]	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
19RP-35_G8P[6]	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
19RP-38_G8P[6]	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
RN-001_KOR_G4P[6]_MK953605	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
27_2017_KOR_G8P[6] [8]	W I L L N P T D Q Q V V L E S T N K T D I W I A L L L V E P N V T N Q S R Q Y T L F G E M R Q I T I					
P[6] RV3_U16299*	W I L L N P T N Q Q V V L E G T N R T D I W V A L L L V E P N V T N Q S R Q Y T L F G E T K Q I T V					
P[6] Nicaragua_JN129097*	W I L L N P T N Q A V V L E G T N R T D V W V A L L L I E P N T P S Q S R Q Y T L F G E I K Q I S V					
P[6] Vietnam_2007_LC095922*	W I L L S P T N Q Q V V L E G T N R T D V W V A L L L I E P N V T N Q S R Q Y T L F G E T K Q I T V					
P[6] Hungary_2002_KF835915*	W I L L N P T N Q K V V L E G T N R A D V W V A L L L I E P N T I N Q S R Q Y T L F G E T K Q I T I					
P[6] China_2013_MF726067*	W I L L S P T N Q Q V V L E G T N R T D V W V A L L L V E P N V T N Q S R Q Y T L F G E T K N I T V					

(Continued to the next page)

Table 3. Continued

Amino acid.	131	141	151	161	171	180																																												
Wa_G1P[8]_JX406750*	S	N	D	-	-	I	W	K	F	L	E	M	F	R	S	S	S	Q	N	E	F	Y	N	R	R	T	L	T	S	D	T	R	L	V	G	I	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
19RP-23_G1P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-04_G8P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-07_G8P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-31_G8P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-02_G9P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-15_G9P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
19RP-24_G9P[8]	R	N	D	-	-	I	W	K	F	L	E	M	F	R	G	S	S	Q	S	D	F	Y	N	R	R	T	L	T	S	D	T	K	L	V	G	I	L	K	Y	G	G	R	I	W	T	F	H	G	E	T
DS-1_G2P[4]_H0650119*	E	N	S	-	-	I	W	K	F	F	E	M	F	K	G	S	S	Q	G	D	F	S	N	R	R	T	L	T	S	S	N	R	L	V	G	M	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
19RP-13_G2P[4]	E	N	S	-	-	I	W	K	F	F	E	M	F	K	G	S	S	Q	S	D	F	S	N	R	R	T	L	T	S	D	N	R	L	V	G	M	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
19RP-17_G2P[4]	E	N	S	-	-	I	W	K	F	F	E	M	F	K	G	S	S	Q	S	D	F	S	N	R	R	T	L	T	S	D	N	R	L	V	G	M	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
19RP-21_G2P[4]	E	N	S	-	-	T	W	K	F	F	E	M	F	K	G	S	S	Q	S	D	F	S	N	R	R	T	L	T	S	N	N	R	L	V	G	M	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
19RP-32_G2P[4]	E	N	S	-	-	I	W	K	F	F	E	M	F	K	G	S	S	Q	S	D	F	S	N	R	R	T	L	T	S	D	N	R	L	V	G	M	L	K	Y	G	G	R	V	W	T	F	H	G	E	T
RV3_P161_U16299*	E	N	T	N	K	W	K	F	F	E	M	F	R	S	V	S	A	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	Y	N	S	V	W	T	F	H	G	E	T		
19RP-09_G8P[6]	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
19RP-26_G8P[6]	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
19RP-35_G8P[6]	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
19RP-38_G8P[6]	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
RN-001_KOR_G4P[6]_MK953605	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
27_2017_KOR_G8P[6] [8]	E	N	N	-	-	T	W	K	F	F	E	V	F	R	T	A	G	A	E	F	Q	H	K	H	T	L	T	S	D	T	K	L	A	G	F	L	K	L	Y	N	S	V	W	T	F	Y	G	E	T	
P[6] RV3_U16299*	E	N	T	N	K	W	K	F	F	E	M	F	R	S	V	S	A	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	Y	N	S	V	W	T	F	H	G	E	T		
P[6] Nicaragua_JN129097*	E	N	T	N	K	W	K	F	F	E	M	F	R	N	I	N	S	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	G	R	V	W	T	F	H	G	E	T			
P[6] Vietnam 2007_LC095922*	E	N	T	N	E	W	K	F	Y	E	M	F	R	N	S	A	N	V	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	G	R	V	W	T	F	H	G	E	T		
P[6] Hungary 2002_KF835915*	E	N	S	S	K	W	K	F	F	E	M	F	R	N	A	S	A	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	G	R	V	W	T	F	H	G	E	T			
P[6] China 2013_KF726067*	E	N	T	N	K	W	K	F	Y	E	M	F	R	N	A	S	A	E	F	Q	H	K	R	T	L	T	S	D	T	K	L	A	G	F	L	K	H	G	R	V	W	T	F	H	G	E	T			

(Continued to the next page)

Table 3. Continued

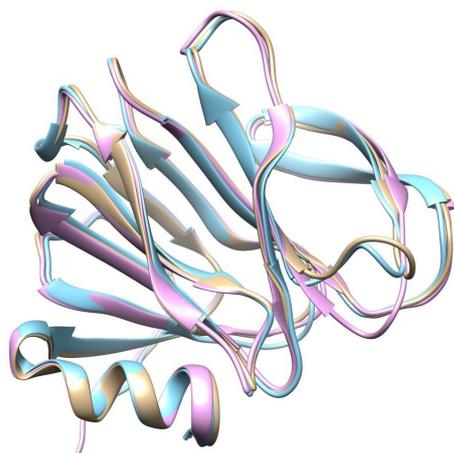
Amino acid.	181	191	201	211	221	229
Wa_G1P[8]_JX406750*	P R A T T D S S S T A N L N N I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-23_G1P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-04_G8P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-07_G8P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-31_G8P[8]	P R A T T D S S N T A N L N D I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-02_G9P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-15_G9P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-24_G9P[8]	P R A T T D S S N T A N L N G I S I T I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
DS-1_G2P[4]_H0650119*	P R A T T D S S N T A D L N N I S I I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-13_G2P[4]	P R A T T D S S S T A D L N N I S I I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-17_G2P[4]	P R A T T D S S S T A D L N N I S I I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-21_G2P[4]	P R A T T D S S N T A D L N N I S I I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
19RP-32_G2P[4]	P R A T T D S S S T A D L N N I S I I H S E F Y I I P R S Q E S K C N E Y I N N G L P P I Q N T					
RV3_P[6]_U16299*	P H A T T D Y S S T S N L S E V E T T I H V E F Y I I P R S Q E S K C V E Y I N T G L					
19RP-09_G8P[6]	P H A T T D Y L L T S N L S E V E A V I H V E F Y I I P R S Q E S K C V E Y I N N G L P P I Q N T					
19RP-26_G8P[6]	P H A T T D Y L L T S N L S E V E A V I H V E F Y I I P R S Q E S K C V D Y I N N G L P P M Q N T					
19RP-35_G8P[6]	P H A T T D Y L L T S N L S E V E A V I L V E F Y I I P R S Q E S K C V E Y I N N G L P P M Q N T					
19RP-38_G8P[6]	P H A T T D Y L L T S N L S E V E A V I L V E F Y I I P R S Q E S K C V E Y I N N G L P P M Q N T					
RN-001_KOR_G4P[6]_MK953605	P H A T T D Y L L T S N L S E V E A V I H V E F Y I I P R S Q E S K C V E Y I N T G L P P M Q N T					
27_2017_KOR_G8P[6] [8]	P H A T T D Y L L T S N L S E V E A V I H V E F Y I I P R S Q E S K C V E Y I N N G L P P M Q N T					
P[6] RV3_U16299*	P H A T T D Y S S T S N L S E V E T T I H V E F Y I I P R S Q E S K C V E Y I N T G L					
P[6] Nicaragua_JN129097*	P H A T T D Y S T S N L S E V I E T V I H A E F Y I I P R S Q E S K C N E Y I N T G L					
P[6] Vietnam 2007_LC095922*	P H A T T D Y S S N L S E V I E T V I H T E F Y I I P R S Q E S K C N E Y I N T G L					
P[6] Hungary 2002_KF835915*	P N A T T D Y S T S N L S P D I E T V I H T D F Y I I P R S Q E S K C K E Y I N T G L					
P[6] China 2013_KF726067*	P H A T T D Y S S N L S E V E T V I H T E F Y I I P R S Q E S K C N E Y I N T G L					

\*These sequences were obtained from GenBank. Brown shading indicates amino acids that differ from USA-Wa\_G1P[8], and yellow shading indicates amino acids that differ from 19RP-09\_G8P[6] strains detected in Korean neonates.

patient 12, aged 20 days); these patients were Le<sup>b</sup> antigen-positive. Table 2 shows the relationship between P[6] rotavirus and Le<sup>b</sup> antigen negativity. The patients with Le<sup>b</sup> antigen-negative phenotypes (patients 1, 3, 4, and 5) showed *FUT2* c.418A>T homozygosity, *FUT3* c.59T>G homozygosity, or *FUT3* c.508G>A homozygosity. The patients with Le<sup>b</sup> antigen-positive phenotypes did not show *FUT2* c.418A>T homozygosity, *FUT3* c.59T>G homozygosity, or *FUT3* c.508G>A homozygosity. Several other variants were also detected in the *FUT2* and *FUT3* genes; however, we focused on the *FUT2* c.418, c.461, and c.772 and *FUT3* c.59, c.202, c.314, c.508, and c.1067 sites.

#### Amino acid sequence of the VP8\* portion of the VP4 capsid protein

The amino acid sequences of the VP8\* portion of the P[8], P[4], and P[6] genotypes greatly varied according to the genotype (Table 3). The amino acid sequences of the VP8\* portion of Korean P[6] strains (19RP-09\_G8P[6], 19RP-26\_G8P[6], 19RP-35\_G8P[6], 19RP-38\_G8P[6], RN-001\_KOR\_G4P[6], and 27\_2017\_KOR\_G8P[6]) were notably different from those of P[6] strains detected in other countries (P[6] RV3, P[6] Nicaragua, P[6] Vietnam, P[6] Hungary, and P[6] China). The amino acid differences between P[6] strains from other countries and Korean P[6] strains were as follows: N88D, V103I, T125M, K126R, K136T, S145R, S146T, V147A, S148G, R155H, H169L, H178Y, and S188L S189L T198A (yellow shading in Table 3). We omitted the 19RP-11 data in Table 3, because this portion (amino acids 81–230 in Table 3) of the VP4 sequence of 19RP-11 yielded an unclear sequence chromatogram.



**Fig. 1.** Superposition of P[6] VP8\*, P[4] VP8\*, and P[8] VP8\* structures deduced from the VP4 nucleotide sequences. Sky blue, P[6]; gold, P[8]; and pink, P[4].

#### 3D structure of the VP8\* glycoprotein

Fig. 1 shows the superposition of the P[6] VP8\*, P[4] VP8\*, and P[8] VP8\* structures deduced from the VP4 amino acid sequences of P[6], P[4], and P[8] strains. Rotaviruses with similar genotypes showed nearly the same 3D structures of the VP8\* portion, although one or two amino-acid sequence differences were observed. Despite the differences in amino acid sequences at various sites between P[4], P[6], and P[8], only a slight structural difference was observed.

#### DISCUSSION

We investigated the relationship between rotavirus genotypes and HBGA-Lewis blood type in Korean children with rotavirus infections, and explored the reason for the P[6] genotype predominance in Korean neonates. Although the number of cases was small, all P[6] rotaviruses were isolated from neonates who were Le<sup>b</sup> antigen-negative or weakly positive. In contrast, non-P[6] rotaviruses were isolated from older children (except for patient 12, aged 20 days) who were Le<sup>b</sup> antigen-positive. These results suggest that neonate-specific P[6] rotaviruses easily infect neonates who are negative or weakly positive for Le<sup>b</sup> antigen, but rarely infect patients who are Le<sup>b</sup> antigen-positive or older children. Our results are supported by recent studies showing that rotavirus P[6] strains can infect Lewis-negative individuals, regardless of their secretor status [22, 23]. In the neonate gut, modification to the precursor moieties or branching of Lewis HBGA is developmentally regulated, and thus the most abundant type of Lewis HBGA in the neonate gut is the unbranched type I precursor glycan, Le(a-b-) [24]. Only a few studies in Africa have also reported the relationship between P[6] and neonates; however, the proportion of P[6] infection in neonates were at most 25% in those studies [22, 25, 26]. This phenomenon may be related to the higher proportion of Le(a-b-) in Koreans and Africans than in Caucasian or Chinese populations [14, 22, 27, 28]. Contrarily, this may be related to the noted differences in the VP8\* amino acid sequences between the Korean rotavirus P[6] strains and those of the P[6] strains detected in other countries (Table 3).

In terms of differences in the 3D structure between the rotavirus VP8\* portion and Lewis HBGA, the cleft between the beta-sheets of rotavirus P[6] has been reported to be wider than that in P[14], and rotavirus P[6] has been suggested to bind to the unbranched type I precursor glycan, Le(a-b-), rather than to the branched HBGA, Le(a-b+) [12]. However, in our study, the cleft between the beta-sheets of the P[6] rotavirus was not markedly

wider than that of P[4] or P[8] strains (Fig. 1).

A previous study suggested that a single residue of the neonatal P[6] VP8\*, specifically H169, which is well conserved in most neonate-specific P[6] genotypes, alters the glycan conformation [12]. However, all neonatal G8P[6] in this study showed a different amino acid residue, L169 instead of H169, which can cause P[6] infection in Korean neonates. Other amino acid substitutions at different sites (N88D, V103I, T125M, K126R, K136T, S145R, S146T, V147A, S148G, R155H, H169L, H178Y, S188L S189L, and T198A) may cause a small change in the 3D structure or hydrogen bond/hydrophobic interaction between the VP8\* of the rotavirus VP4 protein and HBGA molecules in patients, which may involve age- or population-related tropism of some P[6] strains [12].

The Lewis histo-blood group system consists of two major antigens, Le<sup>a</sup> and Le<sup>b</sup>, and four phenotypes, Le(a-b-), Le(a+b-), Le(a-b+), and Le(a+b+) [29]. Lewis antigen synthesis depends on the interaction of *FUT3* and *FUT2*. Although the number of cases in our study was too small to reveal the relationship between phenotype and genotype, our results are compatible with previous interpretations of Lewis genotypes [16, 17]. In terms of *FUT2*, patients harboring homozygous G428A (c.461) and G739A (c.772) variants were non-secretors, and those with a homozygous A385T (c.418) variants were weak secretors. Patients who were wild-type or harbored heterozygous variants at these sites were designated as secretors. In terms of *FUT3*, patients who harbored homozygous variants for at least T202C (c.202), G508A (c.508), or T1067A (c.1067) were designated as Le(a-b-) [16]. In another study, patients with wild-type *FUT2* and *FUT3* were classified as Le<sup>b</sup>-positive secretors [Se+, Le(a-b+)]; patients who were homozygous for the minor allele in *FUT2* at rs601338 (G428A, c.461) and/or rs602662 (G739A, c.772), with a wild-type *FUT3* genotype were classified as Le<sup>b</sup>-negative non-secretors [Se-, Le(a+b-)]; patients homozygous for the minor allele in *FUT3* at rs778986 (C314T, c.314), rs28362459 (T59G, c.59), rs3894326 (T1067A, c.1067), and/or rs3745635 (G508A, c.508), with a wild-type *FUT2* genotype were considered to have a secretor Lewis-null phenotype [Se+, Le(a-b-)]; and those homozygous for the minor allele at one or more sites in *FUT2* and *FUT3* were considered to have a non-secretor Lewis-null phenotype [Se-, Le(a-b-)] [17]. To the best of our knowledge, the relationship between all Lewis gene variants and the Lewis antigen phenotypes has not been clearly established and thus, there were slight differences in interpretation across the studies. Therefore, we focused on the *FUT2* c.418, c.461, and c.772 and the *FUT3* c.59, c.202, c.314, c.508, and c.1067

sites. Further studies will be needed to elucidate the relationship between all variants of Lewis-related genes and Lewis antigen phenotypes.

In conclusion, we found that the rotavirus P[6] genotype predominance in Korean neonates may be related to the interaction between the patient's HBGA-Lewis antigen phenotype and the VP8\* portion of the rotavirus VP4 capsid protein. Four possible explanations are suggested: (1) P[6] infection may be related to Le<sup>b</sup> antigen-negative or weakly positive status; (2) Lewis antigens are not fully developed in the neonate gut; (3) the proportion of Le(a-b-) is higher in Korea than in other countries; and (4) the antigenicity of rotavirus P[6] strains isolated in Korea is different from that of P[6] strains isolated in other countries. Although, this study is limited with the small number of patients, the results provide valuable information on future development of neonatal rotavirus vaccine.

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## AUTHOR CONTRIBUTIONS

HSK designed and performed the study and wrote the manuscript; SKL performed the experiment and helped to write the manuscript; SJO collected and analyzed the data; SHC and SHS obtained the patients' samples and clinical information; EJJ, EJC, and JH helped to analyze the data; All authors read and approved the final manuscript.

## CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this study are reported.

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