Journal of the Pediatric Infectious Diseases Society

ORIGINAL ARTICLE

Fucosyltransferase Gene Polymorphisms and Lewis^b-Negative Status Are Frequent in Swedish Newborns, With Implications for Infectious Disease Susceptibility and Personalized Medicine

Jovanka R. King,[1,2] Jezabel Varadé,[1] and Lennart Hammarström[1]

[1]Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden; [2]Department of Immunopathology, SA Pathology, Women's and Children's Hospital Campus, and Robinson Research Institute and Discipline of Paediatrics, School of Medicine, University of Adelaide, North Adelaide, South Australia

Background. Single-nucleotide polymorphisms (SNPs) in the fucosyltransferase genes *FUT2* and *FUT3* have been associated with susceptibility to various infectious and inflammatory disorders. *FUT* variations influence the expression of human histo-blood group antigens (HBGAs) (H-type 1 and Lewis), which are highly expressed in the gut and play an important role in microbial attachment, metabolism, colonization, and shaping of the microbiome. In particular, *FUT* polymorphisms confer susceptibility to specific rotavirus and norovirus genotypes, which has important global health implications.

Methods. We designed a genotyping method using a nested polymerase chain reaction approach to determine the frequency of SNPs in *FUT2* and *FUT3*, thereby inferring the prevalence of Lewis^b-positive, Lewis^b-negative, secretor, and nonsecretor phenotypes in 520 Swedish newborns.

Results. There was an increased frequency of homozygotes for the minor allele for 1 SNP in *FUT2* and 4 SNPs in *FUT3*. Overall, 37.3% of newborns were found to have Lewis b negative phenotypes (Le (a^+b^-) or Le (a^-b^-). Using our new, sensitive genotyping method, we were able to genetically define the Le (a^-b^-) individuals based on their secretor status and found that the frequency of Lewis b negative newborns in our cohort was 28%.

Conclusions. Given the high frequency of fucosyltransferase polymorphisms observed in our newborn cohort and the implications for disease susceptibility, *FUT* genotyping might play a future role in personalized health care, including recommendations for disease screening, therapy, and vaccination.

Keywords. fucosyltransferase; *FUT2*; *FUT3*; Lewis^b; secretor; nonsecretor; rotavirus; norovirus.

The fucosyltransferase 2 (*FUT2*) and fucosyltransferase 3 (*FUT3*) genes give rise to H-type 1 and Lewis human histo-blood group antigens (HBGAs). Single-nucleotide polymorphisms (SNPs) located in these genes are associated with susceptibility or resistance to various infectious and inflammatory diseases and play a role in shaping the microbiome as a result of the influence of HBGAs on colonization patterns of the commensal intestinal flora. These antigens are highly expressed in the gut mucosa and secretions and are implicated in susceptibility to a range of microorganisms and other environmental stimuli [1]. HBGAs are receptors for various pathogens, including rotavirus, norovirus, *Helicobacter pylori*, and *Campylobacter jejuni*. The pattern of antigen expression by each individual, therefore, determines his or her susceptibility to infection [2–5]. In the case of *Escherichia coli*, HBGAs have been shown to contribute to microbial metabolism by providing a carbon source [6] and also to provide nutrition for other bacteria, including commensal flora [1]. As a result of these mechanisms, *FUT* polymorphisms play an important role in infectivity by pathogenic microorganisms, intestinal colonization with commensal flora, and shaping of the microbiome [7], and there are implications for host defense, intestinal homeostasis, and disease susceptibility.

The secretor (*FUT2*) gene is located on chromosome 19q13.3 and encodes the enzyme α-1,2-fucosyltransferase, which converts the type 1 chain precursor to H-type 1 antigen. The Lewis (*FUT3*) gene is located on chromosome 19p13.3 and encodes α-1,3-fucosyltransferase, which converts the H-type 1 antigen to Lewis^b and the type 1 chain precursor to Lewis^a [8, 9] (Figure 1). The presence of specific alleles in *FUT2* and *FUT3* result in differential gene expression, protein production, and enzyme activity [10, 11]. The prevalence of each haplotype and ensuing Lewis^b and secretor phenotype markedly differs between populations (Table 1) [12, 13]. A previous study in a Swedish population (of 207 healthy individuals) found 55% of the participants to

Received 1 April 2018; editorial decision 7 August 2018; accepted 26 October 2018; Published online December 9, 2018.

Correspondence: J. Varadé, Division of Clinical Immunology, Department of Laboratory Medicine, Alfred Nobels Allé 8, Huddinge F79 14186 Stockholm, Sweden (jezabel.varade@ki.se).

Journal of the Pediatric Infectious Diseases Society 2019;8(6):507–518

DOI: 10.1093/jpids/piy085

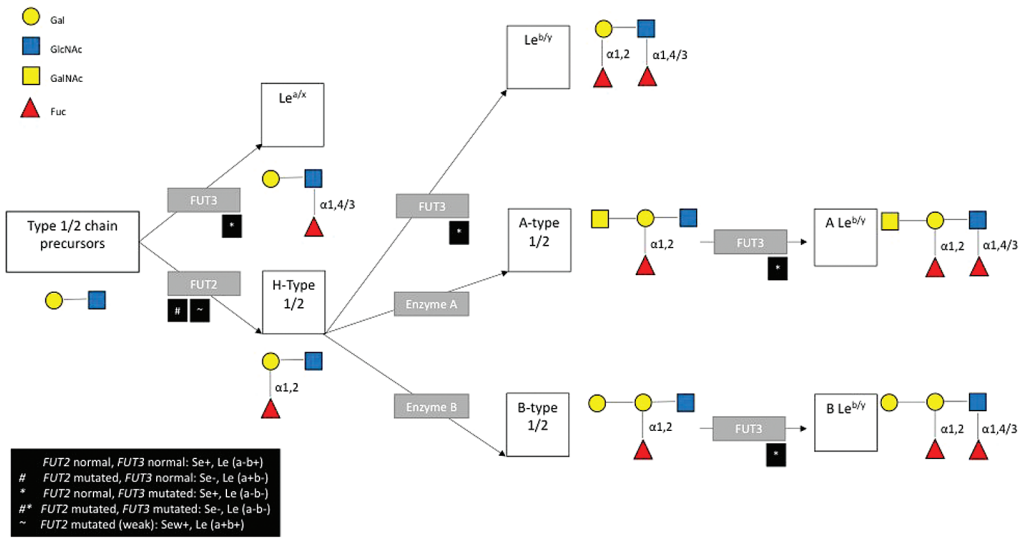


Figure 1. Histo-blood group antigen biosynthetic pathways from Type 1 and Type 2 precursors. *FUT2* generally encodes for Lewis and blood group antigen expression on Type 1 glycans, and *FUT1* generally encodes for Lewis expression on Type 2 glycans. Antigens are given in white boxes, key genes encoding enzymes are given in grey boxes. Effects of *FUT2* and *FUT3* mutations on enzyme expression and ensuing secretor, Lewis a and b phenotypes are given in black boxes. *FUT2* = α(1,2)fucosyltransferase, *FUT3* = α (1,3-4)fucosyltransferase 3, Enzyme A = Nacetylgalactosaminetransferase, Enzyme B = α-galactosyltransferase, Gal = D-galactose, GlcNAc = N-acetylglucosamine, GalNAc = N-acetylgalactosamine, Fuc = L-fucose.

be Lewis^b positive with a secretor phenotype [Se^+, $Le(a^-b^+)$], 31% were Lewis^b-negative nonsecretors [Se^-, $Le(a^+b^-)$], 11% were secretors with a Lewis-null phenotype [Se^+, $Le(a^-b^-)$], and 3% were nonsecretors and Lewis null [Se^-, $Le(a^-b^-)$] [14].

It was noted recently that H-type 1 and Lewis HBGAs are putative receptors for norovirus and rotavirus VP8* and thus play a role in viral attachment and entry into enterocytes [2, 15, 16]. Interindividual genetic variations (SNPs) in HBGAs have been found to confer either susceptibility or resistance to infection with specific norovirus and rotavirus genotypes [17]. Rotavirus infection is an important global health issue; it is a major cause of infectious gastroenteritis worldwide and accounts for approximately 215 000 child deaths annually, predominantly in developing countries [18]. Rotavirus is a nonenveloped double-stranded RNA virus that belongs to the *Reoviridae* family. Rotavirus strains P[4] and P[8] bind to Lewis^b and H-type 1 HBGAs, and strain P[6] binds to the H-type 1 HBGA alone.

Table 1. Reported Prevalence of Secretor/Lewis^b Status in Different Populations[a]

	Population Prevalence (%)			
Status	Caucasian	African	Japanese	Chinese
Se^+, $Le(a^-b^+)$, secretor phenotype, Lewis^b positive	72	55	73	62
Se^-, $Le(a^+b^-)$, nonsecretor phenotype, Lewis^b negative	22	20	0.2	0
Se^+ or Se^-, $Le(a^-b^-)$, any secretor phenotype, Lewis-null phenotype	6	25	10	11
Sew^+, $Le(a^+b^+)$ (rare), Lewis^b-positive "weak" secretor	Rare	Rare	16.8	27

[a]Adapted from Reid et al [12] and Daniels and Bromilow [13].

Hence, individuals who have a secretor phenotype (ie, express Lewis^b antigen) are more prone to rotavirus P[8] infection [11, 17], whereas nonsecretors have an intrinsic resistance to infection by these strains [11]. This finding is supported also by the observation that secretors produce higher levels of anti-rotavirus antibodies than nonsecretors [14]. Two safe and efficacious live attenuated rotavirus vaccines are available. Either monovalent Rotarix (GSK Biologicals, Brentford, United Kingdom) or multivalent RotaTeq (Merck & Co, New York, NY) is included in routine immunization schedules in many countries. However, it is not yet included in the Swedish childhood immunization program.

Norovirus, an RNA virus in the family *Caliciviridae*, accounts for approximately 20% of all cases of acute gastroenteritis globally and represents an important public health issue because of its high rate of transmissibility [19]. Six genogroups of norovirus exist, and groups GI and GII account for the majority of infections [20]. A recent meta-analysis revealed that secretors were 4.2 times more likely to be infected with norovirus than were nonsecretors and had a 9.9 times greater risk of GII.4 genotype infection [20]. Secretors were found to have a 26.6 times greater risk of rotavirus infection than were nonsecretors [20]; 1 included study revealed that nonsecretor status was protective against severe rotavirus infection [21]. The authors of this study further highlighted population-specific differences in the frequency of *FUT2* polymorphisms, showing that the prevalence of nonsecretors was significantly lower in Hispanic children [21].

Given the implications of fucosyltransferase gene polymorphisms and ensuing Lewis^b and secretor phenotypes in

determining disease susceptibility, we genotyped a cohort of Swedish neonates to determine the frequencies of 4 SNPs in *FUT3* and 2 in *FUT2* and the prevalence of secretors, nonsecretors, Lewis^b-negative, and Lewis^b-positive neonates.

MATERIALS AND METHODS

Sample Recruitment

This study was carried out in accordance with the standing regional ethical committee and Karolinska Institutet policies, which permit the use of anonymized biological samples for research purposes. As part of the routine neonatal screening program, a Guthrie card specimen is collected from each Swedish newborn; blood from a heel punch is blotted onto filter paper and tested for a range of diseases in the first few days of life. A punch measuring 3.2 mm in diameter was taken from a dried blood spot from each of 520 anonymized newborn Guthrie cards from the Centre for Inherited Metabolic Diseases (Karolinska University Hospital Solna, Stockholm, Sweden).

Selection of *FUT2* and *FUT3* SNPs for Analysis

Two SNPs in *FUT2* (rs601338 and rs602662) and 4 in *FUT3* (rs778986, rs28362459, rs3894326, and rs3745635) were selected for analysis on the basis of previous publications that suggested that polymorphisms at these sites, either alone or in combination with other polymorphisms, confer abnormal fucosyltransferase enzyme activity and therefore are associated with secretor or nonsecretor Lewis^b-negative status (Supplementary Table 1).

In silico Analysis

Because *FUT2* and *FUT3* are partially duplicated in the genome, particularly in the genes *FUT1,* and *FUT5* and/or *FUT6* respectively, we assessed the specificity of the primers used in different publications for genotyping the SNPs rs601338 and rs602662 in *FUT2* and rs778986, rs28362459, rs3894326, and rs3745635 in *FUT3.* To establish whether the different primers described in the publications identified in the systematic review were specific, we checked the annealing region for each pair of primers using the Ensembl BLAST tool (see http://www.ensembl.org/index.html, last accessed: July 19, 2017). Primer pairs that annealed in more than 1 chromosomal location and produced an amplicon of less than 100 base pairs were classified as nonspecific.

Genotyping for SNPs in the *FUT2* and *FUT3* Genes

Genomic DNA was extracted from the neonatal dried blood spots using a DNA-extraction-kit method (Qiagen, Dusseldorf, Germany) according to manufacturer instructions. Genomic DNA was preamplified by polymerase chain reaction (PCR) using primers specific for the *FUT2* and *FUT3* gene regions containing SNPs of interest (Supplementary Table 2). Thirty nanograms of genomic DNA, 10 µL of 1× GoTaq colorless buffer (Promega, Madison, Wisconsin), 3 µL of 10 mM deoxyribonucleotide triphosphate (dNTP) (Invitrogen, Carlsbad, California), 3 µg of 1.5 mM/µL $MgCl_2$ (Promega), 2 µL of each forward and reverse amplification primer at 10 nM (Eurofins Scientific, Brussels, Belgium), 0.25 µL of GoTaq DNA polymerase (Promega), and 26.75 µL of distilled water were combined to result in a total reaction volume of 50 µL. Thermal cycler conditions were as follows: initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturalization at 95°C for 30 seconds, annealing at 68°C (*FUT2*) or 60°C (*FUT3*) for 30 seconds, and extension at 72°C for 1 minute and then final extension at 72°C for 10 minutes. PCR products were visualized in 1% agarose gel. TaqMan chemistry (Life Technologies, Carlsbad, California) was used to genotype 2 SNPs in *FUT2* (rs601338 [C_2405292_10] and rs602662 [C_2405293_10]) and 2 SNPs in *FUT3* (rs3894326 [C_801690_10] and rs778986 [C_11458475_20]). Nontemplate negative controls and samples confirmed (by Sanger sequencing) to have mutant alleles were used as positive controls in the assays. Four microliters of amplified PCR product diluted 1:200 were used in the TaqMan reaction according to manufacturer conditions in a final volume of 20 µL (Life Technologies) and analyzed using a real-time PCR system under conditions recommended by the manufacturer (Applied Biosystems, Foster City, California). The *FUT3* SNPs rs3745635 and rs28362459 were genotyped using 4 µL of amplified PCR product diluted 1:80 as a template for Sanger sequencing.

Inferring Secretor and Lewis^b Status

Neonates found to carry the ancestral (wild-type) genotype at all evaluated *FUT2* and *FUT3* SNP sites were considered to have normal *FUT2* and *FUT3* expression and were classified as Lewis^b-positive secretors [Se^+, $Le(a^-b^+)$]. Neonates who were homozygous for the minor allele in *FUT2* at rs601338 and/or rs602662 with a wild-type *FUT3* genotype were classified as Lewis^b-negative nonsecretors [Se^-, $Le(a^+b^-)$]. Neonates homozygous for the minor allele in *FUT3* at rs778986, rs28362459, rs3894326, and/or rs3745635 with a wild-type *FUT2* genotype were considered to have a secretor Lewis-null phenotype [Se^+, $Le(a^-b^-)$], and those who were homozygous for the minor allele at 1 or more sites in *FUT2* and *FUT3* were considered to have a nonsecretor Lewis-null phenotype [Se^-, $Le(a^-b^-)$].

Statistical Analysis

The frequency of each of the 6 SNPs analyzed in our study population was determined and compared with data obtained from the 1000 Genomes Project European Caucasian (EUR) population (see http://www.internationalgenome.org, last accessed: July 3, 2017). As previously described, the frequencies of the secretor, nonsecretor, Lewis^b-negative, and Lewis-null phenotypes were determined on the basis of analysis of the pattern of SNPs present in each neonate. Statistical analyses were performed using SPSS Statistics 23 (IBM, Armonk, New York).

Systematic Review and Meta-analysis

To compare our genotyping results with those in other published cohorts in different populations, we conducted a systematic review and meta-analysis using PubMed (see http://www.ncbi.nlm.nih.gov/pubmed, last accessed: July 3, 2017), Medline Ovid (see http://www.ovid.com, last accessed: July 3, 2017) and the Cochrane Library (see http://www.cochranelibrary.com, last accessed: July 3, 2017) databases by applying the Medical Subject Heading terms "*FUT2*," "*FUT3*," "single nucleotide polymorphism," "secretor," "nonsecretor," "Lewis^b," and "Lewis^b negative." We also evaluated minor genotype frequencies for each site in the published studies and the 1000 Genomes Project for selected populations. Review Manager 5.0 (2008 Cochrane Collaboration, Oxford, United Kingdom) was used to carry out the statistical analysis. The stages of the systematic review and meta-analysis, including applied inclusion and exclusion criteria are shown in Figure 2.

RESULTS

Systematic Review and Meta-analysis

Of the 126 potentially eligible studies identified after initial exclusion of duplicates, errata, and meeting abstract communications, 96 were evaluated in more detail, and 17 were included in

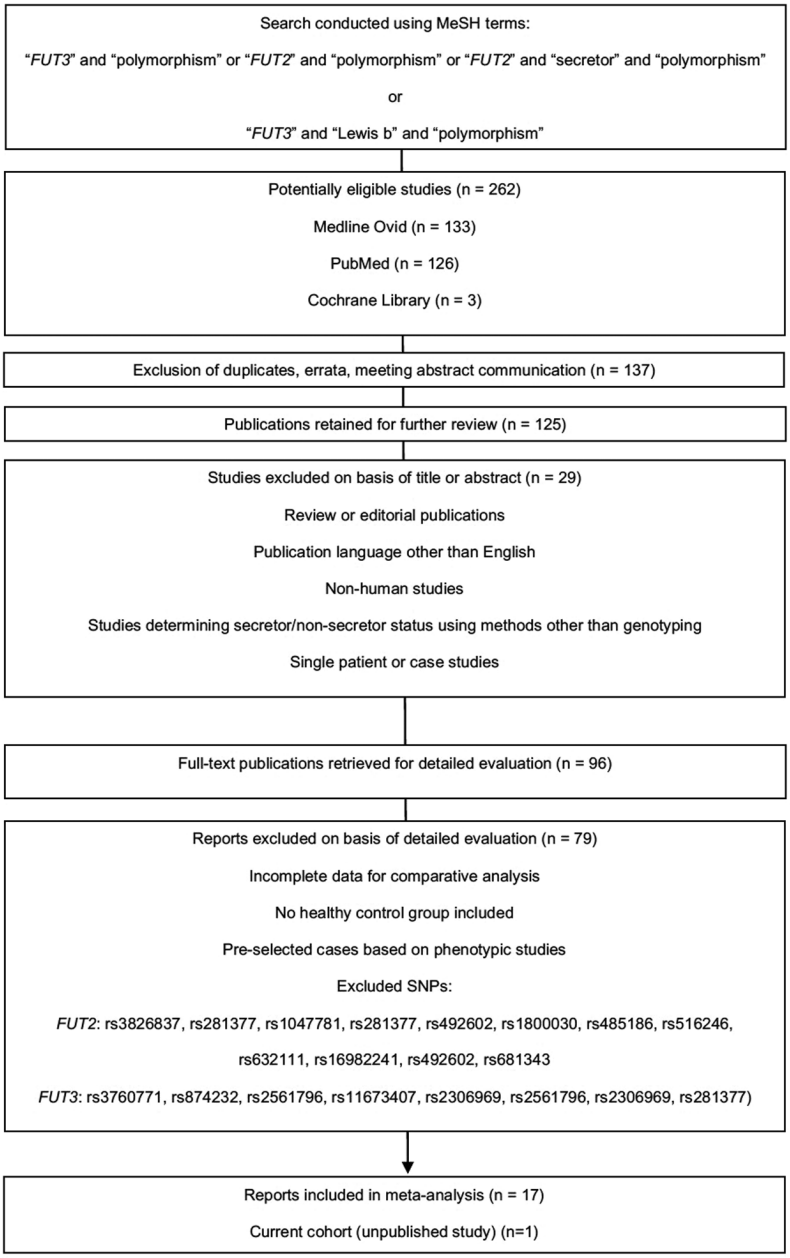
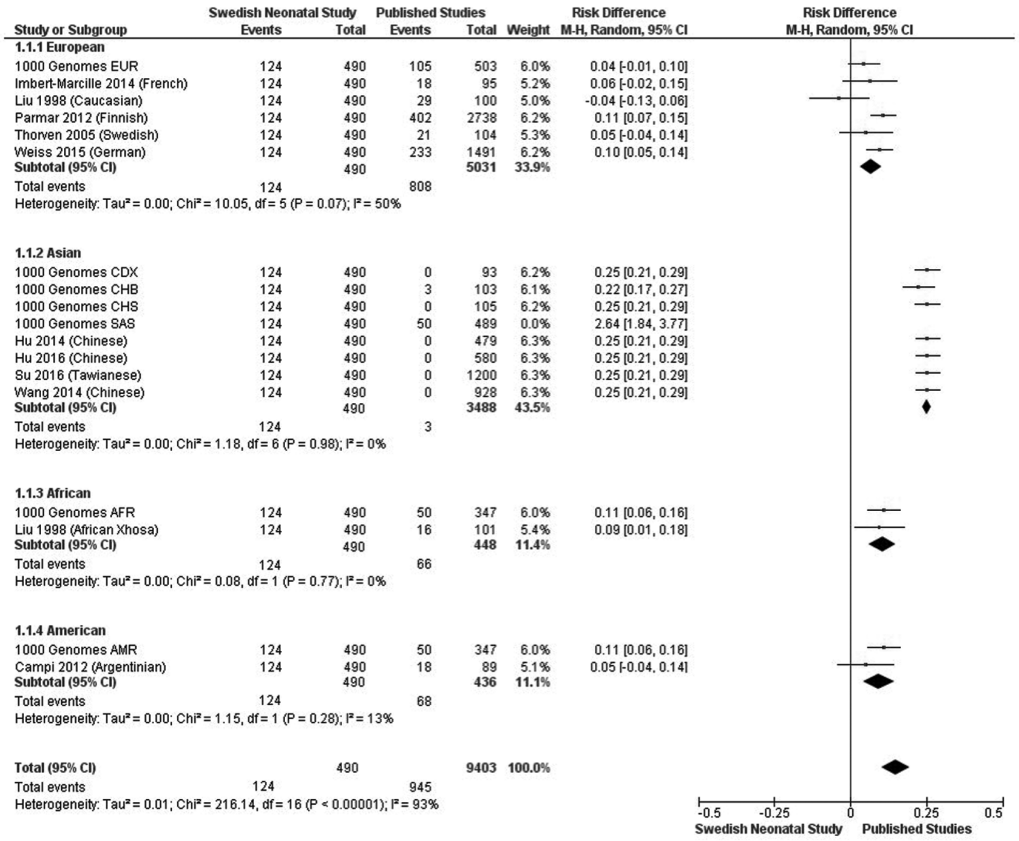


Figure 2. Flow diagram of systematic review and meta-analysis. Abbreviation: MeSH, Medical Subject Heading.

A **rs601338**



rs602662

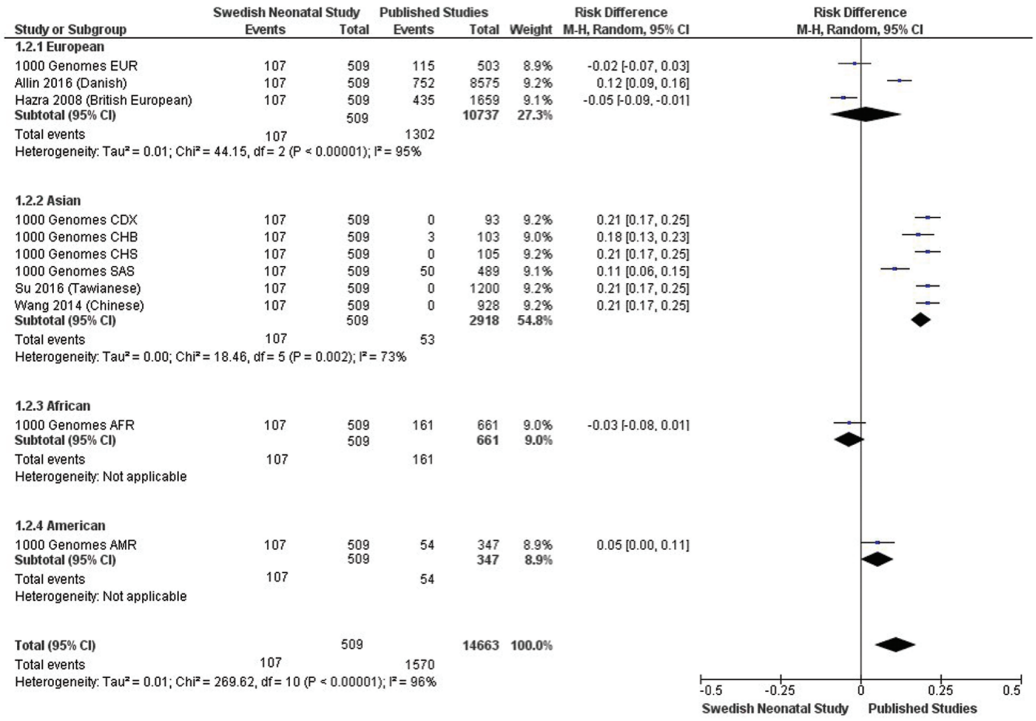
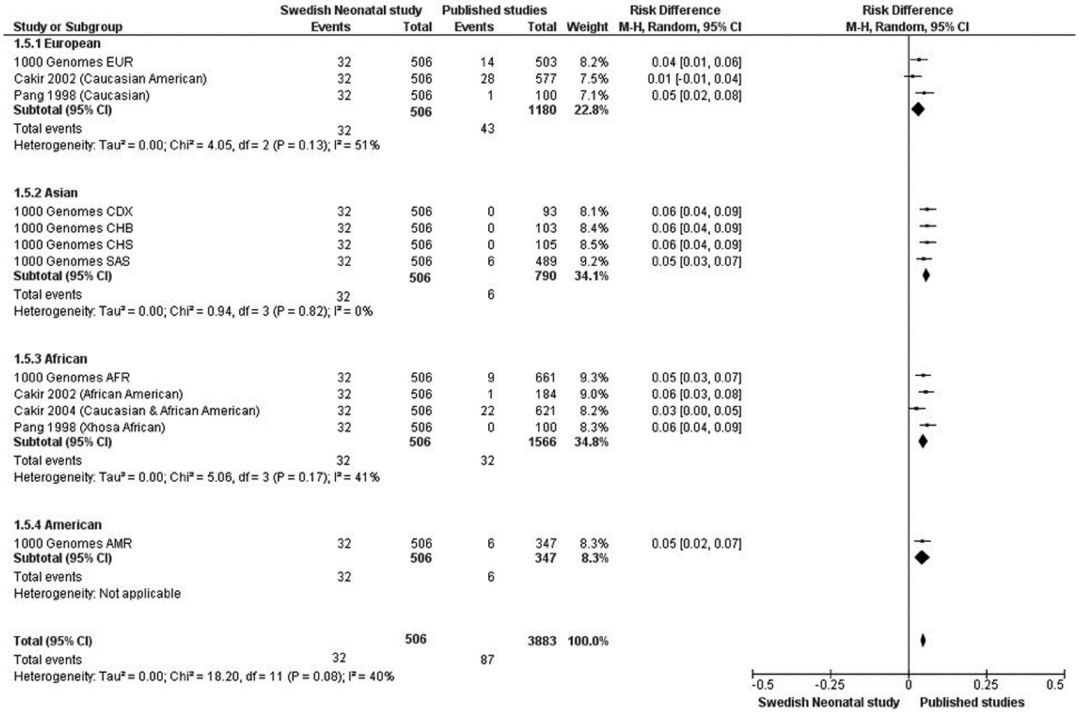


Figure 3. Forrest plots, minor genotype frequency for evaluated *FUT2* SNPs (A) and *FUT3* SNPs (B) in published studies, 1000 Genomes Project for selected populations and the current study. Abbreviation: AFR, African; AMR, American; CDX, Chinese Dai in Xishuangbanna China; CHB, Han Chinese in Beijing, China; CHS, Southern Han Chinese, China; CLM, Colombian in Medellin, Colombia; GBR, British in England and Scotland; EUR, European; FIN, Finnish in Finland; SAS, South Asian.

B **rs778986**



rs3894326

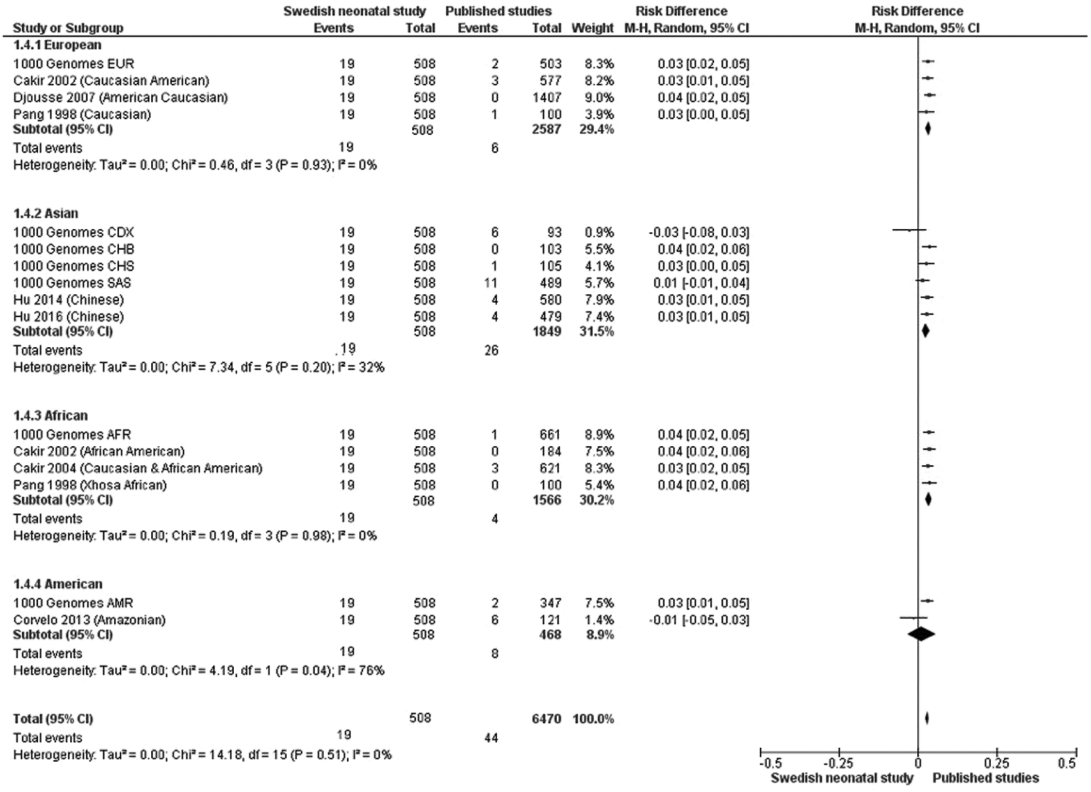
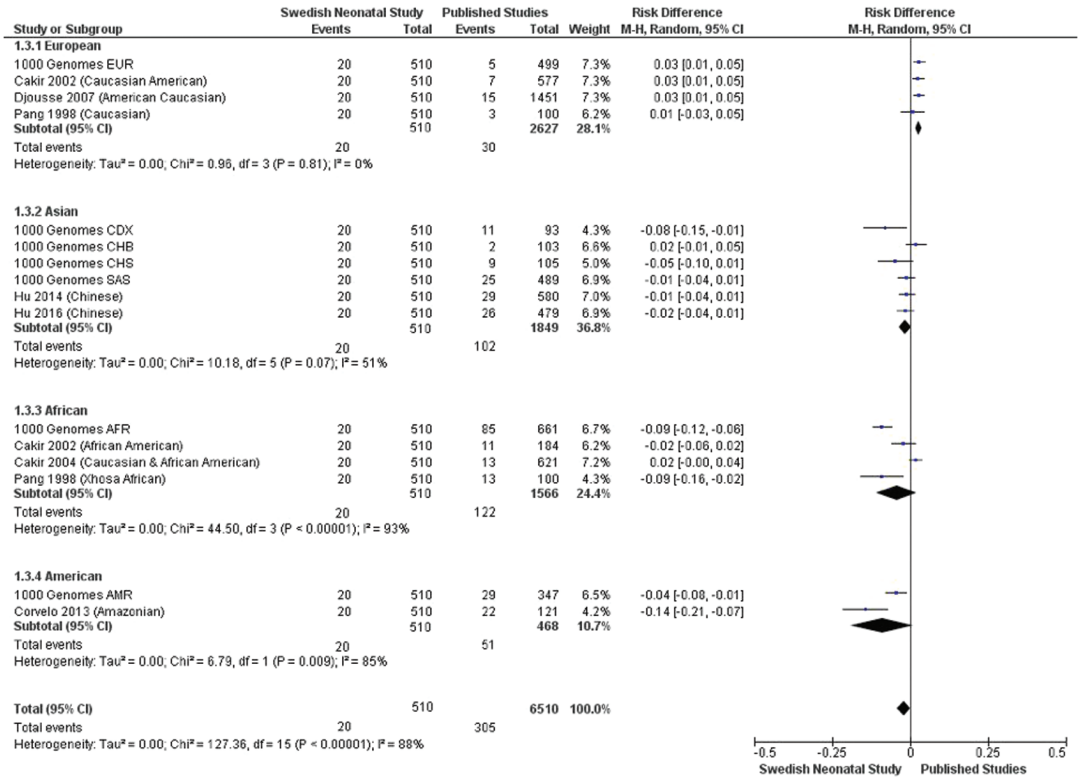


Figure 3. Continued.

rs28362459



rs3745635

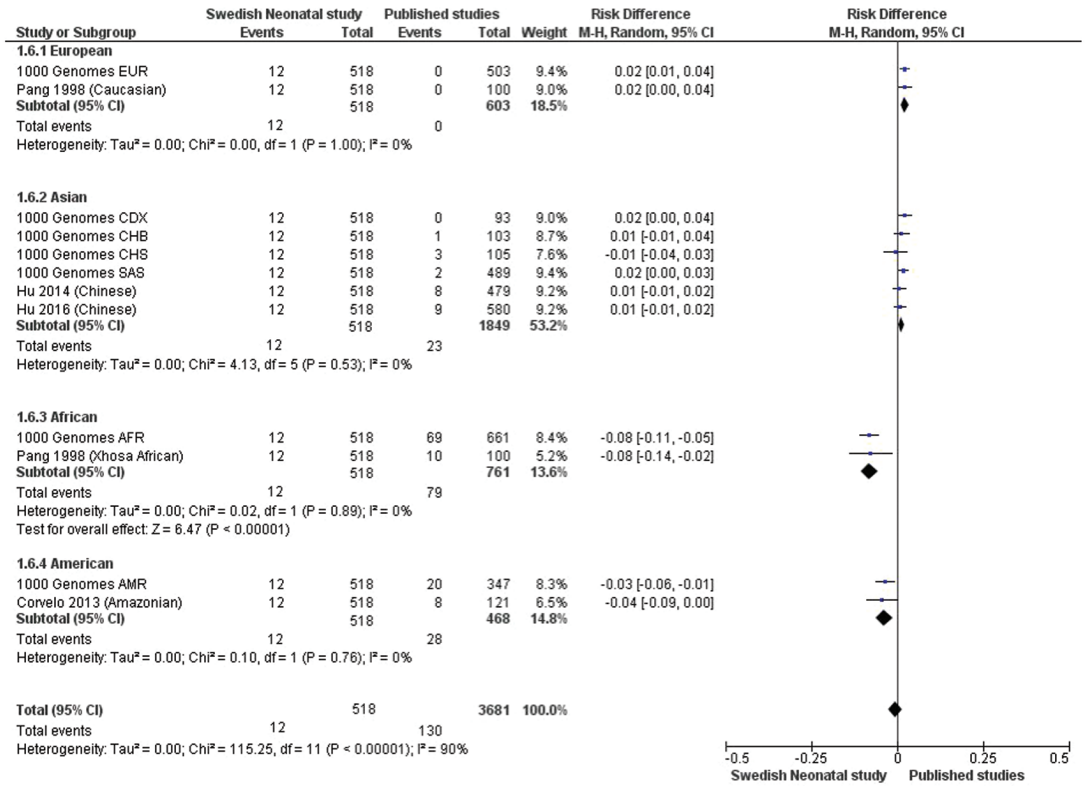


Figure 3. Continued.

Table 2. Genotype and Minor Allele Frequencies of Evaluated SNPs in *FUT2* and *FUT3*

SNP	Gene	Genotype Allele	Swedish Neonate Cohort (n [%])	1000 Genomes CEU Cohort (n [%])	$P^{a,b}$ (Homozygotes for Minor Allele)	OR (95% CI)[b]
rs601338	*FUT2*	AA	124 (25)	105 (21)	.097	1.28 (0.96–1.73)
		AG	161 (33)	234 (47)		
		GG	205 (42)	164 (33)		
rs602662	*FUT2*	AA	107 (21)	115 (23)	.479	0.90 (0.67–1.21)
		AG	228 (45)	241 (48)		
		GG	174 (34)	147 (29)		
rs778986	*FUT3*	TT	32 (6)	14 (3)	**.007**	**2.36 (1.24–4.48)**
		CT	132 (26)	153 (30)		
		CC	342 (68)	336 (67)		
rs3894326	*FUT3*	TT	19 (4)	2 (0.4)	**<.001**	**0.006 (0.004–0.01)**
		AT	99 (19)	69 (14)		
		AA	390 (77)	432 (86)		
rs28362459	*FUT3*	GG	20 (4)	5 (1)	**.003**	**4.07 (1.51–10.92)**
		GT	72 (14)	89 (18)		
		TT	418 (82)	409 (81)		
rs3745635	*FUT3*	AA	12 (2)	0 (0)	**.001**	**NA**
		AG	26 (5)	16 (3)		
		GG	480 (93)	487 (97)		

Abbreviations: CEU, Northern Europeans from Utah; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]χ^2 test.
[b]Bold values indicates statistically significant results.

the meta-analysis (Figure 2; Supplementary Table 3). The frequencies of the minor allele genotype for homozygotes for each evaluated SNP in our cohort were compared with those in other published studies and the 1000 Genomes Project for different populations (Figure 3). As expected, differences were noted in minor allele genotype frequencies in different populations.

SNP Genotyping Results

Statistically significant differences between the frequencies of the minor allele genotype for homozygotes for rs778986*T ($P = .007$), rs3894326*A ($P \leq 0.001$), rs28362459*G ($P = .003$), and rs3745635*A ($P = .001$) in *FUT3* in our cohort and those in the 1000 Genomes Project cohort were found (Table 2). We found no statistically significant difference in the frequencies of the minor allele genotype for homozygotes for the *FUT2* SNPs rs601338*A or rs602662*A. All except 1 SNP (rs602662) had a departure from Hardy–Weinberg equilibrium. We successfully genotyped 490 infants for rs601338, 509 for rs602662, 506 for rs778986, 508 for rs3894326, 510 for rs28362459, and 518 for rs3745635 (overall genotyping success rate for SNPs in this study, >95%).

Identification of a New SNP in FUT3

We identified 1 new SNP (G/T) at position 5844777 in the *FUT3* gene, 4 base pairs upstream of rs28362459 in 3 samples (National Center for Biotechnology Information submitted SNP number (ss) = 2137543878, rs1391064014). This SNP results in a synonymous change (Leu > Leu). One heterozygous (GT) and 2 homozygous (GG) neonates were identified (minor allele frequency of 0.01).

Inferred Prevalence of Secretor, Nonsecretor, Lewis[b]-Negative, and Lewis-Null Phenotypes

The most prevalent minor alleles in our cohort were rs601338*A in *FUT2* (124 infants [25%]) and rs778986*T in *FUT3* (32 infants [6%]). To calculate the prevalence of each inferred phenotype correlating with the *FUT2*/*FUT3* haplotypes in the Swedish population, we included all neonates genotyped for all 6 SNPs in this study, and 457 neonates were considered. In our cohort, 62.7% of the neonates were classified as Lewis[b] positive with a secretor phenotype [Se^+, $Le(a^-b^+)$], 23.5% were Lewis[b]-negative nonsecretors [Se^-, $Le(a^+b^-)$], 9.6% were considered to have a secretor Lewis-null phenotype [Se^+, $Le(a^-b^-)$], and 4.2% had a nonsecretor Lewis-null phenotype [Se^-, $Le(a^-b^-)$] (Table 3). In total, on the basis of SNP genotyping, 37.3% of the Swedish newborns were found to have Lewis b negative

Table 3. Prevalence of Inferred Secretor/Lewis[b] Phenotypes in a Swedish Neonatal Cohort

Secretor/Lewis[b] Phenotype	Prevalence (n [%])
Se^+, $Le(a^-b^+)$, secretor Lewis[b]-positive phenotype, normal *FUT2* expression, normal *FUT3* expression	286 (62.7)
Se^-, $Le(a^+b^-)$, nonsecretor Lewis[b]-negative phenotype, *FUT2* mutation, normal *FUT3* expression	107 (23.5)
Se^+, $Le(a^-b^-)$, secretor Lewis-null phenotype, normal *FUT2* expression, *FUT3* mutation	44 (9.6)
Se^-, $Le(a^-b^-)$, nonsecretor Lewis-null phenotype, *FUT2* mutation, *FUT3* mutation	19 (4.2)
Sew, $Le(a^+b^+)$ (rare), "weak" secretor Lewis[b]-positive phenotype, *FUT2* mutation (could not be assessed in this study)	NA

Abbreviation: NA, not available.

phenotypes (Le (a^+b^-) or Le (a^-b^-). However, using our new sensitive genotyping method, we were able to genetically define the Le(a^-b^-) individuals based on their secretor status. Overall, the frequency of Lewis b negative newborns was 28%, in keeping with the expected frequency in Caucasian populations [12, 13].

DISCUSSION

We noted statistically significant higher-than-expected frequencies of the minor allele genotype for homozygotes in all 4 SNPs in *FUT3* (rs778986*T, rs3894326*A, rs28362459*G, and rs3745635*A). The frequencies of the *FUT2* SNPs rs601338*A and rs602662*A were not significantly different than those in the 1000 Genomes Project cohort. We found an ensuing higher prevalence of secretors and Lewis^b-negative neonates in our cohort. Along with differences in methodology and genotyping techniques by which SNPs were identified in other studies and the variability of SNPs selected for analysis, other possible explanations for our findings include population heterogeneity and the presence of "weak" secretors (*Sew*) or compensatory transferases in our cohort. Weak-secretor phenotypes have been described for various populations and are associated with homozygosity for the minor allele genotype in *FUT2* SNPs A385T (rs1047781) in East Asian populations [22] and G739A (rs602662) and T839C in Portuguese populations [23].

FUT2 and *FUT3* gene segments are partially duplicated in the genome, particularly in *FUT1* and *FUT5* and/or *FUT6*. Hence, we designed a nested-PCR approach to ensure binding specificity of our secondary primers and probes to facilitate acquisition of the most accurate genotyping data. Depending on the genotyping technique used, the replication of *FUT2* and *FUT3* gene segments elsewhere in the genome indicates that there is a risk of nonspecific binding to irrelevant genomic segments and inaccurate genotyping results. However, the reliability of genotyping is improved markedly if a preamplification step is applied to genomic DNA using highly specific primers to isolate the areas of interest in *FUT2* and *FUT3*.

In addition to methodologic differences, it is likely that the number and combination of SNPs analyzed will affect the observed frequencies of various secretor and Lewis^b phenotypes in a population. Many *FUT2* and *FUT3* polymorphisms have been identified, each with variable frequencies in different populations, and evidence of functional effects on enzyme function for many, but not all, SNPs has been documented. Comparison between studies is difficult, given the heterogeneity in the assignment of Lewis^b and secretor statuses based on genotyping alone and variable combinations of evaluated SNPs. It is possible that the true prevalence is even higher because of the presence of additional as-yet-unidentified SNPs with functional effects. For example, we identified a new SNP in *FUT3* that might give rise to altered Lewis antigen expression, although the functional consequence of this SNP requires further exploration. The influences of heterozygous and compound heterozygous genotypes on enzyme activity have also been described; however, in the majority of studies, only homozygosity for the minor allele is considered when assigning Lewis^b-negative status. Hence, the lack of uniformity in SNPs selected for evaluation, and consideration of homozygous and heterozygous genotypes might explain discrepancies in the reported frequencies between the studies. Therefore, the true frequency of Lewis^b-negative individuals might be underestimated. Furthermore, the effect of copy-number variations, deletions, and fusions (particularly in *FUT2*) in some populations have been described, but they require further investigation [24, 25].

Population heterogeneity also might contribute to the higher minor allele genotype frequencies in the SNPs analyzed in our cohort. It is likely that a percentage of our cohort were of non-Caucasian ethnicity, which explains the deviation from Hardy–Weinberg equilibrium observed in all but 1 evaluated SNP. A small percentage of individuals have a *FUT2* mutation with a *Le*(a^+b^+) phenotype and are weak secretors, but it is a rare phenotype in Caucasians. However, the prevalence is higher in other populations (Table 1) [12, 13, 26] and is indistinguishable from a nonsecretor phenotype on the basis of genotyping methods alone. As such, a small number of neonates in our cohort might have been weak secretors. Furthermore, there might be a role for other compensatory transferases in those with *FUT2* polymorphisms such that they were phenotypically and functionally secretors, which could have inflated our nonsecretor frequency artificially.

In some studies, secretor and Lewis antigen status is classified phenotypically using antigen expression assays alone, without genotyping. It should be noted that the expression of HBGAs can change in people with an altered physiological state such as pregnancy and in those with malignancy [13, 27]. Although Lewis antigens are reported to be fully developed and detectable in saliva at birth, the presence of various red cell antigens is variable in the first weeks of life and does not reach full maturity until approximately 2 years of age [27, 28]. As such, in early childhood, genotyping-based assignment of secretor and Lewis^b status is more reliable than the detection of red cell antigens, but results are comparable to those in salivary antigen studies.

The Lewis antigen system is complex, and although our knowledge of the precise physiological role of these HBGAs remains incomplete, it has become apparent that fucosyltransferase polymorphisms and ensuing antigen expression are associated with predisposition to certain infections and diseases. As such, determining an individual's Lewis^b and secretor statuses is likely to have important implications for the provision of personalized medicine, including guidance of specific advice for prevention, screening, and management of disease. On a population level, understanding the prevalence of these polymorphisms has implications for public health and vaccination strategies.

FUT2 secretor status is associated with increased susceptibility to some genotypes of norovirus and rotavirus [11, 17], whereas nonsecretor status protects against infection [21]. Therefore, from a mechanistic perspective, vaccinating nonsecretor individuals who lack putative rotavirus P[8] receptors with a monovalent vaccine for this rotavirus genotype would be expected to elicit a poorer immune response and be less effective in these individuals. Vaccinating nonsecretors with a monovalent vaccine might be an explanation for the high rates of vaccine failure in countries such as Africa, where the frequency of Lewis^b-negative nonsecretor individuals is high. However, studies that compared the vaccine efficacy of monovalent and multivalent vaccines found that efficacies differed by only 1% in both moderate-to-high-income and developing-world settings [29, 30]. The immune response to rotavirus infection and mechanisms by which previous natural infection and immunization confer protection against subsequent infection are multifaceted, and the roles of neutralizing antibodies (both homotypic and heterotypic), interferon-mediated innate immune responses, and cell-mediated mechanisms have been investigated [31, 32]. The induction of serotype-specific neutralizing antibodies was thought to be critical for protection against rotavirus infection, resulting in the development of multivalent vaccines; however, the demonstrable efficacy of monovalent vaccines has challenged this premise [31]. A recent study found that heterotypic protective immunity to rotavirus is mediated by the production of heterotypic antibodies directed primarily toward the stalk (VP5*) of the viral attachment protein VP4, along with VP8* and surface glycoprotein VP7 [33]. Heterotypic antibody responses have been shown to confer protection against multiple rotavirus types despite monovalent vaccination [20, 34], although it remains unknown if the heterotypic antibody titers achieved for other viral strains are sufficient to confer adequate long-lasting protection.

Given the safety and efficacy of rotavirus vaccination and its demonstrated ability to reduce rotavirus-associated morbidity and death, its inclusion in routine immunization programs worldwide should be considered. In regions where rotavirus vaccination is not offered as a standard practice for all infants, evaluation of *FUT* polymorphisms and ensuing secretor and Lewis^b statuses could identify those infants who would derive the greatest benefit from rotavirus vaccination. From a mechanistic perspective, given the high prevalence of *FUT* polymorphisms in our population, a multivalent vaccine would be favorable.

Secretor status has been shown to play a role in the infectivity of other infectious diseases, including those caused by *C jejuni* [3], *H pylori* [4, 35], and human immunodeficiency virus (HIV) [36–38]. Blood group antigens facilitate *H pylori* binding to the gastric epithelium, and individuals who are secretors [4] and blood group O positive [35] are at an increased risk of infection and subsequent gastric ulceration. Nonsecretors seem to have a reduced risk of HIV infection, possibly as a result of the modification of mucosal surface carbohydrates [36], and reduced HIV-1 disease progression was found in some series [37, 38]. HBGAs provide a carbon source essential for the metabolism of bacteria such as *E coli*, thereby contributing to the virulence of these pathogens [6]. *FUT2* polymorphisms also have been associated with poor outcome and complications in premature infants, including Gram-negative sepsis and necrotizing enterocolitis [39], although these results were not reproduced in a subsequent cohort [40].

It has been found that *FUT* polymorphisms play a significant role in the shaping of the microbiome in terms of colonization with commensal microbiota. Intestinal dysbiosis is implicated in the pathogenesis of various autoimmune and inflammatory diseases [1]. Secretors likely have a better ability to stabilize their microbiome; secretion of HBGAs into body fluids confers an improved first line of defense against pathogens and other environmental elements, and beneficial bacteria thrive on glycosylation products [1]. *FUT2* polymorphisms have been implicated in some populations as a risk factor for Crohn disease [41–43], ulcerative colitis [43, 44], Behçet disease [45], celiac disease [43], and diabetes mellitus. In addition to being a risk factor for primary sclerosing cholangitis (PSC) [46], *FUT2* polymorphisms have been shown to influence biochemical parameters in patients with PSC, which affects interpretation of the carcinoembryonic antigen test that is used for malignancy screening [47]. These findings have important implications for surveillance and investigation in this patient group and might be applicable to screening for other gastrointestinal malignancies [47]. Serum lipase levels and the risk of chronic pancreatitis are elevated in nonsecretors [1]. Fucosyltransferase polymorphisms have been implicated also as risk factors for cardiovascular disease [10, 48] and various malignancies [49–51]. They have also been shown to influence serum metabolite pathways, including vitamin B_{12} levels [52, 53].

Given the implications of fucosyltransferase polymorphisms for disease susceptibility, screening of individuals might provide useful information to assist clinicians in providing personalized healthcare. Early identification of interindividual genetic differences in the newborn period would enable information to be readily available and actionable as needed, enabling accurate prediction of disease predisposition and facilitating appropriately informed decisions regarding screening, treatment, and preventative therapies such as vaccination. Furthermore, the role of HBGAs in disease pathogenesis should be considered in the development of novel treatment modalities and in vaccine development.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

Acknowledgments. J. R. K. is the recipient of the Mike and Carole Ralston Travelling Fellowship 2016 from the RCPA Foundation. We acknowledge Lennart Svensson, Thomas Boren, and Johan Nordgren for their critical review of the manuscript. We sincerely thank Ulrika von Döbeln for provision of the DBS specimens and review of the manuscript.

Financial support. This work was supported by grants from Vetenskapsrådet and the Stockholm County Council (ALF project) to L. H.

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

1. Weiss FU, Schurmann C, Guenther A, et al. Fucosyltransferase 2 (FUT2) non-secretor status and blood group B are associated with elevated serum lipase activity in asymptomatic subjects, and an increased risk for chronic pancreatitis: a genetic association study. Gut **2015**; 64:646–56.
2. Hu L, Crawford SE, Czako R, et al. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. Nature **2012**; 485:256–9.
3. Ruiz-Palacios GM, Cervantes LE, Ramos P, et al. *Campylobacter jejuni* binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. J Biol Chem **2003**; 278:14112–20.
4. Borén T, Falk P, Roth KA, et al. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science **1993**; 262:1892–5.
5. Marionneau S, Airaud F, Bovin NV, et al. Influence of the combined ABO, FUT2, and FUT3 polymorphism on susceptibility to Norwalk virus attachment. J Infect Dis **2005**; 192:1071–7.
6. Pacheco AR, Curtis MM, Ritchie JM, et al. Fucose sensing regulates bacterial intestinal colonization. Nature **2012**; 492:113–7.
7. Wacklin P, Tuimala J, Nikkilä J, et al. Faecal microbiota composition in adults is associated with the FUT2 gene determining the secretor status. PLoS One **2014**; 9:e94863.
8. Taube S, Jiang M, Wobus CE. Glycosphingolipids as receptors for non-enveloped viruses. Viruses **2010**; 2:1011–49.
9. Orntoft TF, Holmes EH, Johnson P, et al. Differential tissue expression of the Lewis blood group antigens: enzymatic, immunohistologic, and immunochemical evidence for Lewis a and b antigen expression in *Le*(*a-b-*) individuals. Blood **1991**; 77:1389–96.
10. Salomaa V, Pankow J, Heiss G, et al. Genetic background of Lewis negative blood group phenotype and its association with atherosclerotic disease in the NHLBI family heart study. J Intern Med **2000**; 247:689–98.
11. CI, CDX, CHB, CHS, SAS, AFR, -Marcille BM, Barbé L, Dupé M, et al. A *FUT2* gene common polymorphism determines resistance to rotavirus A of the P[8] genotype. J Infect Dis **2014**; 209:1227–30.
12. Reid C, Lomas-Francis C, Olsson M. Lewis blood group system. In: Reid M, Olsson LFM, ed. The Blood Group Antigen Factsbook. 3rd ed. Elsevier; **2012**.
13. Daniels G, Bromilow I. Essential Guide to Blood Groups. 3rd ed. John Wiley & Sons, Ltd; **2014**.
14. Günaydın G, Nordgren J, Sharma S, Hammarström L. Association of elevated rotavirus-specific antibody titers with HBGA secretor status in Swedish individuals: the *FUT2* gene as a putative susceptibility determinant for infection. Virus Res **2016**; 211:64–8.
15. Huang P, Xia M, Tan M, et al. Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. J Virol **2012**; 86:4833–43.
16. Böhm R, Fleming FE, Maggioni A, et al. Revisiting the role of histo-blood group antigens in rotavirus host-cell invasion. Nat Commun **2015**; 6:5907.
17. Nordgren J, Sharma S, Bucardo F, et al. Both Lewis and secretor status mediate susceptibility to rotavirus infections in a rotavirus genotype-dependent manner. Clin Infect Dis **2014**; 59:1567–73.
18. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD; World Health Organization–Coordinated Global Rotavirus Surveillance Network. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000–2013. Clin Infect Dis **2016**; 62(Suppl 2):S96–105.
19. Currier RL, Payne DC, Staat MA, et al. Innate susceptibility to norovirus infections influenced by *FUT2* genotype in a United States pediatric population. Clin Infect Dis **2015**; 60:1631–8.
20. Kambhampati A, Payne DC, Costantini V, Lopman BA. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. Clin Infect Dis **2016**; 62:11–8.
21. Payne DC, Currier RL, Staat MA, et al. Epidemiologic association between *FUT2* secretor status and severe rotavirus gastroenteritis in children in the United States. JAMA Pediatr **2015**; 169:1040–5.
22. Silva LM, Carvalho AS, Guillon P, et al. Infection-associated *FUT2* (fucosyltransferase 2) genetic variation and impact on functionality assessed by in vivo studies. Glycoconj J **2010**; 27:61–8.
23. Serpa J, Mendes N, Reis CA, et al. Two new *FUT2* (fucosyltransferase 2 gene) missense polymorphisms, 739G–>A and 839T–>C, are partly responsible for non-secretor status in a Caucasian population from northern Portugal. Biochem J **2004**; 383:469–74.
24. Park KU, Song J, Han KS, Kim JQ. The fusion allele of the *FUT2* (secretor type alpha(1,2)-fucosyltransferase) gene at a high frequency and a new *se385* allele in a Korean population. Ann Hematol **2005**; 84:656–60.
25. Soejima M, Koda Y. TaqMan-based real-time polymerase chain reaction for detection of *FUT2* copy number variations: identification of novel Alu-mediated deletion. Transfusion **2011**; 51:762–9.
26. Henry SM, Benny AG, Woodfield DG. Investigation of Lewis phenotypes in Polynesians: evidence of a weak secretor phenotype. Vox Sang **1990**; 58:61–6.
27. Assali NS. Pathophysiology of Gestation. New York: Academic Press; **1972**.
28. Henry S, Oriol R, Samuelsson B. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang **1995**; 69:166–82.
29. Shah MP, Tate JE, Mwenda JM, et al. Estimated reductions in hospitalizations and deaths from childhood diarrhea following implementation of rotavirus vaccination in Africa. Expert Rev Vaccines **2017**; 16:987–95.
30. Cortese MM, Immergluck LC, Held M, et al. Effectiveness of monovalent and pentavalent rotavirus vaccine. Pediatrics **2013**; 132:e25–33.
31. Ward RL, Clark HF, Offit PA. Influence of potential protective mechanisms on the development of live rotavirus vaccines. J Infect Dis **2010**; 202(Suppl):S72–9.
32. Angel J, Franco MA, Greenberg HB. Rotavirus immune responses and correlates of protection. Curr Opin Virol **2012**; 2:419–25.
33. Nair N, Feng N, Blum LK, et al. VP4- and VP7-specific antibodies mediate heterotypic immunity to rotavirus in humans. Sci Transl Med **2017**; 9:eaam5434.
34. Leshem E, Lopman B, Glass R, et al. Distribution of rotavirus strains and strain-specific effectiveness of the rotavirus vaccine after its introduction: a systematic review and meta-analysis. Lancet Infect Dis **2014**; 14:847–56.
35. Azevedo M, Eriksson S, Mendes N, et al. Infection by *Helicobacter pylori* expressing the BabA adhesin is influenced by the secretor phenotype. J Pathol **2008**; 215:308–16.
36. Ali S, Niang MA, N'doye I, et al. Secretor polymorphism and human immunodeficiency virus infection in Senegalese women. J Infect Dis **2000**; 181:737–9.
37. Bigham AW, Mackelprang RD, Celum C, et al. Variants in host viral replication cycle genes are associated with heterosexual HIV-1 acquisition in Africans. J Acquir Immune Defic Syndr **2014**; 66:127–34.
38. Kindberg E, Hejdeman B, Bratt G, et al. A nonsense mutation (428G–>A) in the fucosyltransferase *FUT2* gene affects the progression of HIV-1 infection. AIDS **2006**; 20:685–9.
39. Morrow AL, Meinzen-Derr J, Huang P, et al. Fucosyltransferase 2 non-secretor and low secretor status predicts severe outcomes in premature infants. J Pediatr **2011**; 158:745–51.
40. Demmert M, Schaper A, Pagel J, et al. *FUT 2* polymorphism and outcome in very-low-birth-weight infants. Pediatr Res **2015**; 77:586–90.
41. McGovern DP, Jones MR, Taylor KD, et al.; International IBD Genetics Consortium. Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. Hum Mol Genet **2010**; 19:3468–76.
42. Hu DY, Shao XX, Xu CL, et al. Associations of *FUT2* and *FUT3* gene polymorphisms with Crohn's disease in Chinese patients. J Gastroenterol Hepatol **2014**; 29:1778–85.
43. Parmar AS, Alakulppi N, Paavola-Sakki P, et al. Association study of *FUT2* (rs601338) with celiac disease and inflammatory bowel disease in the Finnish population. Tissue Antigens **2012**; 80:488–93.
44. Aheman A, Luo HS, Gao F. Association of fucosyltransferase 2 gene variants with ulcerative colitis in Han and Uyghur patients in China. World J Gastroenterol **2012**; 18:4758–64.
45. Xavier JM, Shahram F, Sousa I, et al. *FUT2*: filling the gap between genes and environment in Behçet's disease? Ann Rheum Dis **2015**; 74:618–24.
46. Folseraas T, Melum E, Rausch P, et al. Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. J Hepatol **2012**; 57:366–75.
47. Wannhoff A, Folseraas T, Brune M, et al. A common genetic variant of fucosyltransferase 2 correlates with serum carcinoembryonic antigen levels and affects cancer screening in patients with primary sclerosing cholangitis. United European Gastroenterol J **2016**; 4:84–91.

48. Cakir B, Heiss G, Pankow JS, et al. Association of the Lewis genotype with cardiovascular risk factors and subclinical carotid atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) study. J Intern Med **2004**; 255:40–51.
49. Duell EJ, Bonet C, Muñoz X, et al. Variation at ABO histo-blood group and *FUT* loci and diffuse and intestinal gastric cancer risk in a European population. Int J Cancer **2015**; 136:880–93.
50. Teresa DB, Santos RA, Takahashi CS, et al. Polymorphisms of Lewis and secretor genes are related to breast cancer and metastasis in axillary lymph nodes. Tumour Biol **2010**; 31:401–9.
51. Padró M, Cobler L, Garrido M, de Bolós C. Down-regulation of *FUT3* and *FUT5* by shRNA alters Lewis antigens expression and reduces the adhesion capacities of gastric cancer cells. Biochim Biophys Acta **2011**; 1810:1141–9.
52. Zinck JW, de Groh M, MacFarlane AJ. Genetic modifiers of folate, vitamin B-12, and homocysteine status in a cross-sectional study of the Canadian population. Am J Clin Nutr **2015**; 101:1295–304.
53. Tanwar VS, Chand MP, Kumar J, et al. Common variant in *FUT2* gene is associated with levels of vitamin B(12) in Indian population. Gene **2013**; 515:224–8.