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(54) Title: USE OF SECRETOR, LEWIS AND SIALYL ANTIGEN LEVELS AS PREDICTORS FOR DISEASE

(57) Abstract: An individual at risk for necrotizing enterocolitis and related disorders can be identified by measuring the level of at least one secretor antigen in a biological sample from the individual and comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values. Among the secretor antigens which can be measured are: the H-1, H-2, Lewis^b and Lewis^y antigens and derivatives thereof (e.g., a sialylated form of Lewis^a, Lewis^x, Lewis^b, Lewis^y; H-1, H-2, Lewis^a, Lewis^x, Lewis^b or Lewis^y).



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USE OF SECRETOR, LEWIS AND SIALYL ANTIGEN LEVELS AS PRESICTORS FOR DISEASE

Statement as to Federally Sponsored Research

5 Funding for the work described herein was provided by the federal
government, which may have certain rights in the invention.

TECHNICAL FIELD

This disclosure relates to the field of medical diagnostics and more
particularly to materials and methods for assessing and treating inflammatory and
10 infectious disorders in infancy.

BACKGROUND

Inflammatory and infectious disorders in infancy can be associated with
serious morbidity and mortality. Inflammatory and infectious disorders that occur
perinatally, for example, necrotizing enterocolitis (NEC), sepsis, and chorioamnionitis
15 are major contributors to neonatal mortality rates. NEC, the most common
gastrointestinal medical and/or surgical emergency occurring in neonates, occurs in 7-
13% of very low birthweight infants, and is characterized by bowel injury and
intestinal necrosis. NEC mortality rates overall are in the range of 20-40%, and
mortality rates for premature infants have been reported to exceed 50%. Surgical
20 intervention is required in about 30% of cases, and surgery-associated mortality is
reported to be as high as 50%.

Survivors of inflammatory and infectious disorders of infancy can have
significant short-term and long-term morbidities, including metabolic complications,
recurrent infections, poor neurodevelopmental outcomes, and poor growth outcomes,
25 that in some instances may require repeated surgical intervention and prolonged
hospitalization.

SUMMARY

The methods described herein are based, in part, on our discovery of methods
for assessing whether an infant is at high risk of death, or likely to develop certain

inflammatory or infectious disorders, for example, necrotizing enterocolitis, sepsis, gastrointestinal infections, respiratory infections, and urinary tract infections.

Disclosed herein is a method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:

5 (a) measuring the level of at least one secretor antigen in a biological sample from the individual, and

(b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values,

10 wherein the individual is at risk for necrotizing enterocolitis if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values.

In various embodiments: the individual is an infant (e.g., infant is a neonate, a low birthweight infant, an extremely low birthweight infant or a premature infant); the at least one secretor antigen comprises an α 1,2-linked fucose antigen and/or an α 2,3 sialylated antigen; the at least one secretor antigen is selected from the group
15 consisting of: the H-1, H-2, Lewis^b and Lewis^y antigens and derivatives thereof; the derivative is a sialylated form of Lewis a, Lewis x, Lewis b or Lewis y; the derivative is a sulfated form of H-1, H-2, Lewis a, Lewis x, Lewis b or Lewis y; the biological sample is a bodily fluid or a tissue; the bodily fluid comprises saliva, blood, plasma, serum, urine, stool, amniotic fluid, hmcucus, tears or lymph; the bodily fluid comprises
20 saliva; the measuring step comprises an immunoassay; the at least one secretor antigen is selected from the group consisting of: H-1, H-2, Lewis^b and Lewis^y and derivatives thereof and wherein the individual is determined to be risk for necrotizing enterocolitis if the measured level is below the predetermined value or below the
25 predetermined range of values; the predetermined value or the predetermined range of values represents the average level of the at least one secretor antigen in a population of individuals determined to be secretors; the individual is determined to be at risk for necrotizing enterocolitis when the measured level of at least one secretor antigen is at least 10% less than the average level found in a control population of
30 secretors; the at least one secretor antigen is the H-2 antigen.

In some cases the at least one antigen is sialyl Lewis a or derivative thereof and the individual is determined to be risk for necrotizing enterocolitis if the measured level is above the predetermined value or above the predetermined range of

values. In some embodiments of this method: the infant is a neonate, a low
birthweight infant, an extremely low birthweight infant or a premature infant, the
biological sample is a bodily fluid or a tissue; the bodily fluid comprises saliva, blood,
plasma, serum, urine, stool, amniotic fluid, mucus, tears or lymph; the bodily fluid
5 comprises saliva; the measuring step comprises an immunoassay; and the
predetermined value or the predetermined range of values represents the average level
of the at least one secretor antigen in a population of individuals determined to be
secretors.

Also disclosed is a method of identifying an individual at risk for developing a
10 gastrointestinal disorder, the method comprising:

(a) measuring the level of at least one secretor antigen in a biological
sample from the individual, and

(b) comparing the measured level of the at least one secretor antigen to a
predetermined value or a predetermined range of values,

15 wherein the individual is at risk for a developing a gastrointestinal disorder if
the measured level of the at least one secretor antigen differs from the predetermined
value or is outside the predetermined range of values.

In various embodiments of this method: the individual is an infant (e.g., a
neonate, a low birthweight infant, an extremely low birthweight infant or a premature
20 infant); the measured antigens comprise an α 1,2-linked fucose antigen and/or an α 2,3
sialylated antigen; the secretor antigen is selected from the group consisting of: the
H-1, H-2, Lewis^b and Lewis^y antigens and derivatives thereof; the derivative is a
sialylated form of Lewis a, Lewis x, Lewis b or Lewis y; the derivative is a sulfated
form of Lewis a, Lewis x, Lewis b or Lewis y; the biological sample is a bodily fluid
25 or a tissue; the bodily fluid comprises saliva, blood, plasma, serum, urine, stool,
amniotic fluid, mucus, tears or lymph; the measuring step comprises an immunoassay;
the at least one secretor antigen is selected from the group consisting of: H-1, H-2,
Lewis^b and Lewis^y and derivatives thereof and wherein the individual is determined to
be risk for developing a gastrointestinal disorder if the measured level is below the
30 predetermined value or below the predetermined range of values; the predetermined
value or the predetermined range of values represents the average level of the at least
one secretor antigen in a population of individuals determined to be secretors; the
individual is determined to be at risk for necrotizing enterocolitis when the measured

level of the at least one secretor antigen is at least 10% less than average level found in control population of secretors; the at least one antigen is the H-2 antigen; the gastrointestinal disorder is gastrointestinal inflammation; the gastrointestinal disorder is gastrointestinal infection; the disorder is late onset sepsis; and the gastrointestinal infection comprises infection with one or more of *Staphylococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., *Enterobacter* spp., *Klebsiella* spp., *Bacillus* spp., *Serratia* spp., *Candida* spp, Norwalk and other Noroviruses, *Campylobacter* spp, *Vibrio cholerae*, *Bacteriodes* spp., Clostridia, *Giardia*.

10 Also disclosed is a method comprising:

(a) measuring the level of at least one secretor antigen in a biological sample from the individual, and

(b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values,

15 (c) determining that the individual is at risk for necrotizing enterocolitis if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values; and

(d) taking steps to treat or reduce the risk for necrotizing enterocolitis if the individual is determined to be at risk for necrotizing enterocolitis.

20 In this method (d) can comprise administering to the individual one or more of α 1,2 fucosyl glycans, probiotic organisms or prebiotics.

Also disclosed is a method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:

25 (a) measuring the level of FUT2 protein or mRNA encoding FUT2 in a biological sample from the patient;

(b) comparing the measured level of level of FUT2 protein or mRNA encoding FUT2 to a predetermined value or a predetermined range of values,

30 wherein the individual is at risk for necrotizing enterocolitis if the measured level of FUT2 protein or mRNA encoding FUT2 is below a predetermined value or a predetermined range of values.

Disclosed herein is a method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:

(a) providing a biological sample from the individual;

(b) determining whether the individual harbors a *FUT2* gene having genetic change that reduces the expression or activity of *FUT2*,
wherein the individual is at risk for necrotizing enterocolitis if the individual harbors a *FUT2* gene having genetic change that reduces the expression or activity of
5 *FUT2*.

Disclosed herein is a method of identifying an individual (e.g., an infant) at risk for death, the method comprising:

(a) measuring the level of at least one secretor antigen in a biological sample from the individual, and

10 (b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values,

wherein the individual is at risk for necrotizing enterocolitis if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values.

15 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and
20 other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

25 The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

30 Figure 1: Fucosyltransferase and *Fut2* mRNA expression in mouse colon. (A) α 1,2/3-fucosyltransferase in conventional [CONV], germ-free (GF) and ex-germ-free (XGF) mice; (B) α 1,2/3-fucosyltransferase activity and (C) *Fut2* mRNA in the CONV, bacteria-depleted (BD) and bacteria-repleted (XBD) mice; (D) α 1,2-

fucosylated glycan expression detected by UEA-1 lectin and corresponding Nomarski image.

Figure 2: (A) TLR4 expression is necessary for bacterial activation of intestinal fucosylation. (B) TLR4 ligands are sufficient for activation of mucosal fucosylation in bacteria-depleted mice.

Figure 3: Monocolonization by *B. fragilis* induces intestinal fucosylation.

Figure 4: Histograms indicate the distribution of H-2, Le y, and sLe a antigens measured in saliva as optical density (O.D.) values comparing 24 cases of NEC or death (top row) and 168 controls (bottom row). Samples were collected at 8-14 days (week 2). The table indicates the incidence of NEC or death, the relative risk and p-value comparing the risk groups identified by CART analysis. The triangle symbol indicates the cutpoint in continuous values of each antigen identified by CART analysis to optimally distinguish between a high and low risk group (node). This cutpoint is applied in the table for each antigen. For H-2 only: the O.D. value distribution differs significantly ($P=0.004$, Wilcoxon Mann Whitney test) comparing NEC and death cases with controls.

Figure 5: Schematic depiction of CART analysis.

Figure 6: Scatterplot of H-2 and sLe A antigen optical density (O.D.) values by EIA measured from a saliva sample collected in week 2 (days 8-14) from 192 ELBW (<1000 grams) infants in Cincinnati NICUs; 24 (12.5%) developed NEC or died. The high and low risk sets were systematically identified by CART analysis to minimize misclassification of cases and non-cases. The H-2 cut-point for high risk was identified as an O.D. < 0.627 (lowest 38% of values, including non-secretor infants). In the H-2 high risk group, 18 cases occurred in 73 infants (incidence =24.7%) compared to 6 cases in 119 infants (incidence=5.0%; relative risk [RR]=4.9, 95% confidence interval [CI]=2.0 to 11.8, $P<0.0001$). The sLe^a cutpoint for high risk was identified as an O.D. >0.318 (highest 76% of values). The infants in both high risk groups (defined by low H-2 and high sLe^a O.D. values) creates the high risk set indicated in the figure of 17 cases in 54 infants (incidence =31.5%) compared to all other infants combined, who define the low risk set, which comprises 7 cases in 138 infants (incidence =5.1%). This combined classification resulted in a highly significant split of high vs. low risk (RR = 6.2, 95% CI 2.7 to 14.1, $P<0.0001$).

Figure 7: Sequence of human "secretor" *FUT2* mRNA.

Figure 8: Sequence of human “secretor” FUT2 polypeptide.

DETAILED DESCRIPTION

The ABH and Lewis histo-blood group antigens are carbohydrates representing the terminal structures of glycan chains. The H-type histo-blood group antigens, for example, the H type 1 and 2, the Lewis^b and the Lewis^y antigens, are characterized by a fucose terminus in an α 1,2 linkage. In mammals, the H-type histo-blood group antigens are found on a wide range of tissue types including epithelial cells of organs that are in direct contact with the external environment, *e.g.*, the upper respiratory tract, the nasal epithelium and trachea, as well as the genito-urinary tract, ureter and vagina, as well as on erythrocytes, some neurons of the peripheral nervous system, thymus epithelium and the skin. In several human populations, about 80% of individuals also express H-type histo-blood group antigens in a soluble form in biological fluids, for example, saliva, breast milk, serum, tears, sweat and semen. In about the remaining 20% of individuals in these human populations, the soluble H-type histo-blood group antigens are either absent from, or found at extremely low levels, in biological fluids. These two phenotypes have been designated as “secretors” and “non-secretors” respectively and the soluble H-type histo-blood group antigens are typically referred to as secretor antigens. In other human populations the percentage of individuals who are non-secretors is lower than 20%.

The basis for the phenotypic difference between the secretor and non-secretor subpopulations stems from genetic polymorphisms in the *FUT2* gene encoding the enzyme fucosyl transferase 2 also referred to in the art as alpha(1,2)fucosyltransferase 2, EC 2.4.1.69, SE 2, SEC2, Fucosyltransferase-2 (secretor), GDP-L-fucose:beta-D-galactoside 2-alpha-L-fucosyltransferase 2, Galactoside 2-alpha-L-fucosyltransferase 2, Secretor blood group alpha-2- fucosyltransferase, Secretor factor, and transmembrane protein 2. The *FUT2* gene is also referred to in the art as the secretor gene (*Se*).

The *FUT2* gene product, FUT2, catalyzes the formation of an α -L-fucosyl-1,2- β -D-galactosyl-R structure from GDP- β -L-fucose and β -D-galactosyl-R, where R can be a glycoprotein or a glycolipid. FUT2 is expressed in many organs that generally are of endodermal origin including gut, pharynx, liver, respiratory tract, bladder, urethra and endocrine glands, although within those organs FUT2 expression is also a function of the differentiation pattern of the tissue, *e.g.*, keratinized vs. non-

keratinized squamous epithelium, ducts vs. acini of glandular tissues, as well as the particular cell type, *e.g.* secretory vs. ciliated cells in the endometrium, and mucous vs. serous cells in the salivary gland. Secretors express functional FUT2; non-secretors fail to express functional FUT2. Accordingly, soluble H-type histo-blood group antigens are not synthesized and therefore not secreted into biological fluids of non-secretors.

The experiments described in the Examples indicate that the secretor status and the expression of sialyl glycan epitopes of an infant are correlated with risk of NEC and death. More specifically, extremely low birthweight infants (ELBW) or premature infants who expressed little or no H-2 antigens in saliva, as well as infants who expressed elevated levels of sialyl Lewis a antigens in saliva, are significantly more likely to experience adverse clinical outcomes such as NEC, late onset sepsis and death compared to infants who express moderate or high levels of H-2 antigen or infants who express little or no sialyl Lewis a antigens in saliva.

Disclosed herein are materials and methods relating to the identification of individuals as risk of developing an inflammatory or infectious disorder, for example, NEC, gastrointestinal infections, or late onset sepsis. More specifically, an infant can be identified as being at risk for an inflammatory or infectious disorder, for example, NEC, gastrointestinal infection or late onset sepsis, by assessing the secretor status of the infant. In some embodiments, an infant who does not express or who expresses low levels of secretor antigens relative to a reference sample can be classified as being at risk for NEC, gastrointestinal infection or late onset sepsis. In other embodiments, an infant who expresses elevated levels of sialyl Lewis a (sLe^a) antigen relative to a reference sample can be classified as being at risk for NEC, gastrointestinal infection or late onset sepsis.

Also provided herein are methods of treatment and management of individuals at risk of developing an inflammatory or infectious disorder, for example, NEC, gastro-intestinal infections, or late onset sepsis. In some embodiments, an individual identified by secretor status as being at risk of developing NEC, gastrointestinal infection or late onset sepsis can be treated with specific therapies that include protective agents, *e.g.*, probiotic organisms or prebiotic agents, including α 1,2 fucosyl glycans. In some embodiments, the course of treatment of an individual identified by secretor status as being at risk of developing NEC, gastrointestinal infection or late

onset sepsis can be evaluated by assessing the level of secretor antigens in the mother's milk being provided to her infant and, based on the levels of secretor antigens in the food source relative to a reference sample, administering to the infant specific therapies that include protective agents, *e.g.*, α 1,2 fucosyl glycans, probiotic organisms or prebiotics.

Secretor antigens

Secretor antigens, *i.e.*, the H type 1 and 2, the Lewis^b and the Lewis^y antigens, are glycans that include a fucose terminus in an α 1,2 linkage. The term glycans as used herein refers to a compound of two or more subunit monosaccharide units joined together by a glycosidic bond, *i.e.*, a covalent bond between an anomeric hydroxyl group, in α or β configuration, of one monosaccharide and any available hydroxyl group in a second monosaccharide, regardless of additional modifications *e.g.*, linkages to other additional monosaccharide units, polypeptides, lipids or other biological or nonbiological molecules. We may refer to a saccharide polymer containing a small number, typically three to 35 or more component sugars as an oligosaccharide. All oligosaccharides described herein are described with the name or abbreviation for the non-reducing saccharide (*i.e.*, Gal), followed by the configuration of the glycosidic bond (α or β), the ring bond (1 or 2), and then the name or abbreviation of the next saccharide (*i.e.*, GlcNAc) toward the reducing end of the molecule. For a review of standard glycobiology nomenclature see, Essentials of Glycobiology, Varki *et al.*, eds., 1999, Cold Spring Harbor Laboratory Press.

The specific form of the secretor antigen can vary and depends, in part, upon the structure of the minimal disaccharide precursor, or core sequence, from which the particular antigen was assembled. The core sequence can be either the lacto type I structure, galactose (β 1-3) N-acetylglucosamine-R, which we abbreviate here as {Gal (β 1-3)GlcNAc}-R or the lacto type II structure galactose (β 1-4) N-acetylglucosamine-R, which we abbreviate here as {Gal(β 1-4)GlcNAc}-R. In the minimal, unconjugated core sequence, R can be H or other small molecule radicals. The disaccharide precursor can also be conjugated to longer glycans as oligosaccharides, or as the glycan moieties of glycolipids, peptides, proteins, mucins, or other macromolecules.

Thus, for example, the H-1 antigen is derived from the type I precursor by FUT2 (or FUT1) catalyzed addition of a fucose residue to the galactose moiety of the type I precursor in an α 1,2 linkage to generate the structure {Fucose(α 1-2) Galactose (β 1-3) N-acetylglucosamine, which we abbreviate as {Fuc (α 1-2) Gal (β 1-3) GlcNAc}. The H-1 antigen is a structural precursor to another secretor antigen, Lewis^b. The Lewis^b secretor antigen includes the H-1 structure plus a second fucose residue in a non-terminal α 1,4 linkage to the GlcNAc moiety in the configuration {Fucose(α 1-2) Galactose (β 1-3) [Fucose(α 1-4)]N-acetylglucosamine} which we abbreviate as {Fuc (α 1-2) Gal (β 1-3) [Fuc (α 1-4)] GlcNAc}.

Correspondingly, the H-2 antigen is derived from the type II precursor by FUT2 (or FUT1) catalyzed addition of a fucose residue to the galactose moiety of the type II precursor in an α 1,2 linkage to generate the structure {Fucose(α 1-2) Galactose (β 1-4) N-acetylglucosamine}, which we abbreviate as {Fuc (α 1-2) Gal (β 1-4) GlcNAc}. The H-2 antigen is a structural precursor to another secretor antigen, Lewis^y. The Lewis^y secretor antigen includes the H-2 structure plus a second fucose residue in a non-terminal α 1,3 linkage to the GlcNAc moiety in the configuration {Fucose(α 1-2) Galactose (β 1-4) [Fucose(α 1-3)]N-acetylglucosamine} which we abbreviate as {Fuc (α 1-2) Gal (β 1-4) [Fuc (α 1-3)] GlcNAc}.

A secretor antigen may comprise a single α 1,2 fucose substituted core sequence, wherein R is H or other small radicals. Alternatively, a secretor antigen can comprise a repetitive series of substituted core sequences, wherein R is another core sequence. The single core sequence or a repetitive core sequence may be present within a larger sugar. Accordingly, a secretor antigen-containing oligosaccharide can be, for example, a trisaccharide, a tetrasaccharide, a pentasaccharide, and so on. A secretor antigen can also be covalently linked to another macromolecule, *e.g.*, a polypeptide or a lipid. The single substituted core sequence can also be linked directly to a polypeptide or lipid, *e.g.*, R can be a protein or a lipid or can be present in a polysaccharide that is bound to a polypeptide or lipid. Secretor antigens may be covalently linked to polypeptides via N-linked glycosylation, that is, through an asparagine residue, or via O-linked glycosylation, for example, through serine, threonine, hydroxylproline, tyrosine or other hydroxyl containing residue. Glycoproteins that include secretor antigens include, for example without limitation, mucins, bile-salt-stimulated lipase (BSSL), and lactadherin. Examples of secretor

antigen-bearing lipids include, without limitation, H-1 glycolipid, H-2 glycolipid, Le^x glycolipid, and Le^y glycolipid .

Lewis antigens

5 The Lewis antigens can be present as any glycan containing Lewis epitopes, that is, containing H-1, H-2, Le^a, Le^b, Le^x, and/or Le^y epitopes, which are α 1,3 or α 1,4-linked fucosylated oligosaccharide moieties. This includes free oligosaccharides, glycolipids, glycoproteins, mucins, glycosaminoglycans, and glycopeptides. Individuals can express glycans that express the H-1, H-2, Le^a, Le^b, Le^x, and/or Le^y epitopes.
10

Sialyl antigens

 The sialyl antigens can be present as any glycan containing sialylated antigens, including free oligosaccharides, glycolipids (e.g., gangliosides), glycoproteins,
15 mucins, glycosaminoglycans, and glycopeptides. Individuals can express sialylated epitopes, including gangliosides and other glycans that express the sialyl Lewis a (sLe^a), sLe^b, sLe^x, and/or sLe^y epitopes.

Assaying secretor antigens

20 The level of one or more secretor antigens can be measured in any biological fluid known in the art to comprise secretor antigens. Examples of biological fluids include, without limitation, saliva, serum, blood plasma, breast milk, amniotic fluid, sweat, urine, tears, mucus, lymph, and stool. Biological fluid samples can be collected from an individual using any standard method known in the art that results
25 in the preservation of secretor antigen structure. Saliva samples may be collected using cotton swabs, wipes, suction, scraping, or by having the individual rinse the mouth and expectorate into a tube or collector. Blood samples can be obtained via venous puncture techniques. Serum samples can be prepared from whole blood using standard methods such as centrifuging blood samples that have been allowed to clot.
30 Plasma samples can be obtained by centrifuging blood samples that were treated with an anti-coagulant such as heparin. Breast milk can be collected by manual or mechanical expression. Biological fluid samples can be assayed for secretor antigens immediately following collection. Alternatively, or in addition, a biological fluid

sample can be stored for later analysis using methods known in the art that preserve secretor antigen structure, *e.g.*, freezing, drying, or freeze drying.

After determining the levels of specific secretor antigens in a biological sample, these levels can be compared with those of standard reference levels.

5 Standard reference levels typically represent the average secretor antigen levels derived from a large population of individuals. The reference population may include individuals of similar age, body size, ethnic background or general health as the individual in question. The *FUT2* genotype of the reference population may or may not be known. Thus, the secretor antigen levels in a patient's sample can be compared
10 to values derived from: 1) individuals who express wild-type *FUT2* and whose bodily fluids contain secretor antigens; 2) individuals who express variant forms of *FUT2* and have moderate to low *FUT2* activity and whose bodily fluids contain low levels of secretor antigens; or 3) individuals who have little or no *FUT2* activity and whose bodily fluids lack secretor antigens.

15 In general, an elevated level of secretor antigen can be any level of a secretor antigen that is greater than either the level of a secretor antigen found in a control sample or greater than the average level of a secretor antigen found in samples from a population of normal healthy individuals who are secretors. A reduced level of a secretor antigen can be any level of a secretor antigen that is less than either the level
20 of a secretor antigen found in a control sample or less than the average level of a secretor antigen found in samples from a population of normal healthy individuals who are secretors. Any population size can be used to determine the average level of a secretor antigen found in samples from a population of normal healthy individuals that are secretors. For example, a population of between 2 and 250, *e.g.*, 2, 3, 4, 5, 10,
25 15, 20, 25, 30, 40, 50, 100, 150, 200, 250 or more individuals can be used to determine the average level of a secretor antigen in samples from a population of normal healthy individuals, with greater accuracy in the measurement coming from larger sample populations.

A reduced level of a secretor antigen can be 10, 20, 30, 50, 60, 70, 80, 90, 100,
30 percent lower than that level found in a control sample or lower than the average level of a secretor antigen found in samples from a population of normal healthy individuals. In some cases, a reduced level of a secretor antigen can be 2, 3, 4, 5, 10, 20, 50 or more fold lower than that level found in a control sample or the average

level of a secretor antigen found in samples from a population of normal healthy individuals.

In some cases, a reference chart can be used to determine whether or not a particular level of a specific secretor antigen in a sample is low or normal relative to a control sample or a larger population. For example, a reference chart can contain the normal range of secretor antigen levels found in healthy infants of the same age, gestational age, ethnic background or general health as the individual in question. Using this reference chart, any level of a secretor antigen measured in a sample can be classified as being low, normal, or elevated relative to a control sample or relative to an average value derived from a larger population.

Alternatively, or in addition, the level of a secretor antigen in a biological sample can be “normalized” against the level of one or more additional biological markers, for example another histo-blood group antigen, such as a P or sialyl antigen, whose expression is independent of the secretor status of the individual. That is, the levels of the additional marker can be evaluated in parallel with those of the secretor antigen, either at the same time or on a separate occasion. The additional marker can serve as an internal control for sample preparation, handling and storage as well as day-to-day assay variability. The values for the level of a secretor antigen and the additional marker may be expressed as a ratio and the ratio may be compared to similar ratio obtained for a reference sample or population. Examples of useful second markers include, but are not limited to, Lewis antigens whose expression is independent of the secretor (FUT2) expression, i.e., Lewis a and Lewis x. A Lewis antigen generally includes carbohydrates having as a core sequence either the lacto type I structure or the lacto type II structure substituted with one or more fucosyl residues. Thus, for example, a useful second marker can be Lewis a {Galactose (β 1-3)[Fucose (α 1-4)]N-acetylglucosamine}, which we abbreviate as {Gal (β 1-3) Fuc (α 1-4) GlcNac}, or Lewis x {Galactose (β 1-4)[Fucose (α 1-3)]N-acetylglucosamine, which we abbreviate as {Gal (β 1-4) [Fuc (α 1-3)] GlcNac}, or the sialylated, sulfated, or sulfo-sialylated forms of these epitopes. Other commonly expressed non-Lewis blood group antigens could also be used, such as Lua (Lutheran a), P1 & P2 (the major antigens of the P blood group system), M&N, Fya &Fy b (antigens of the Duffy system), etc.

In addition, since stochastic variations in individual gene expression levels are common in biological systems, it may be desirable to normalize the level of a secretor antigen against a *panel* of two or more additional biological markers whose expression is known to be independent of the secretor status of the individual. This strategy may lead to greater accuracy in determining secretor antigen levels.

Once the relative level of a secretor antigen in an individual relative to that of a reference sample has been calculated, the individual's relative risk for gastroenteritis, necrotizing enterocolitis, late onset sepsis or death can be assessed. Any statistical method known in the art for evaluating relative risk may be used. One suitable method is Classification and Regression Tree (CART) characteristic curve analysis. CART analysis belongs to a family of nonparametric regression methods and is based on recursive partitioning to build a decision tree that optimizes the classification of individuals into high and low-risk groups. It can be applied to systematically identify cutpoints in continuous variables that maximize predictive value and minimize misclassification of cases and non-cases based on a balance between the sensitivity (*i.e.*, the number of true cases detected) and the specificity (*i.e.*, the accuracy) of a test. These two variables may also be considered positive predictive value and negative predictive value, and are correlated with diagnostic accuracy. The decision tree produced by CART analysis can be validated through Receiver Operating Curve (ROC) analysis to determine the area under the curve (AUC), which indicates the effectiveness of the relationship of the decision tree to discriminate between cases and non-cases.

In one example, CART analysis indicates that an individual classified as a low or non secretor, *i.e.*, in the 38th percentile or below of H-2 salivary expression (as measured by O.D. values), has an increased risk for NEC and death. Thus, an individual whose levels of secretor antigens are in the 38th, 33rd, 30th, 25th, 20th, 15th, 10th, 5th, or lower percentile is 4 to 5 times more likely to suffer from NEC or death than individuals whose secretor antigen levels are above the 38th percentile. Among those who express little or no secretor antigen, the highest risk group is defined by those who also express high sialyl Lewis a antigen. Infants who express little or no secretor antigen and high sialyl Lewis a antigen have more than 6-fold the risk of NEC or death relative to all other infants. Thus, the high risk group can be defined either by little or no H-2 antigen alone or by the combination of little or no H-

2 and moderate to high sialyl Lewis a antigen. Based on this comparison, as well as on other clinical indices, a clinician can predict the likelihood that a patient is at risk for NEC, and adjust treatment regimens accordingly.

The secretor status of an individual can be determined in a variety of ways.

5 The level of a secretor antigen can be measured directly in a biological fluid. For example, the level of a secretor antigen can be measured using immuno-based assays, *e.g.*, ELISA assays, radioimmunoassays, one or two dimensional gel electrophoresis coupled with immunodetection, or by using surface plasmon resonance-based biosensors, or by using chromatographic techniques, *e.g.*, high performance liquid chromatography (HPLC) or gas chromatography (GC), or by spectrometry, *e.g.*, mass spectrometry. Alternatively, or in addition, FUT2 activity can be measured in a biological sample using standard enzymology methods. In addition, *FUT2* mRNA levels may be quantified (as a surrogate for levels of FUT2 activity in a biological sample) using a variety of methods well known to the art, *e.g.* RT-PCR or quantitative RT-PCR (for example: Kroupis C, Stathopoulou A, Zygalki E, Ferekidou L, Talieri M, Lianidou ES. Clin Biochem. 2005 Jan;38(1):50-7, Dyer J, Chisenhall DM, Mores CN. J Virol Methods. 2007 Oct;145(1):9-13), or quantitative hybridization-based techniques such as cDNA or oligonucleotide microarrays (for example: Duggan D. J., Bittner M., Chen Y., Meltzer P. and Trent J. M. Nat Genet 21(1 Suppl):10-4 (1999); Cheung V. G., Morley M., Aguilar F., Massimi A., Kucherlapati R. and Childs G. Nat Genet 21(1 Suppl):15-9 (1999)).

The human secretor *FUT2* mRNA sequence is shown in Figure 7 (GenBank® Accession No NM_000511.4) and the human secretor FUT2 protein sequence in shown in Figure 8 (GenBank® Accession No. NP_000502.4).

25 Finally, the *FUT2* genotype of an individual can be determined by single nucleotide polymorphism analysis (SNP) or RT-PCR-based techniques.

Immunoassays

30 Immunoassay methods are well known to those in the art. Antibody reagents that detect specific secretor Lewis antigens, *e.g.*, H-1, H-2, Lewis^b, and Lewis^y, and other Lewis antigens. *e.g.*, Lewis^a and Lewis^x, can be generated using standard methods for antibody production or purchased from commercial sources. Antibodies can be monoclonal or polyclonal or any combination thereof. Useful antibodies can

include: monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, that are specific for the secretor or glycan epitope, and also include antibody fragments, including Fab, Fab', F(ab')₂, scFv, Fv, camelbodies, or microantibodies. In addition nucleic acid or peptide aptamer reagents capable of specifically binding to and detecting specific secretor antigens can be generated using standard published methods (e.g. The use of aptamers in large arrays for molecular diagnostics, Brody E. N., Willis M. C., Smith J. D., Jayasena S., Zichi D. and Gold L. *Mol Diagn* 4(4):381-8 (1999)).

Thus, in some embodiments, a specific anti-H-1, anti-H-2, anti-Lewis^b, or anti-Lewis^y monoclonal antibody or aptamer can have a binding affinity less than about $1 \times 10^5 K_a$ for an antigen other than H-1 and H-2, Lewis^b, and Lewis^y, respectively. In some embodiments, the anti-H-1, anti-H-2, anti-Lewis^b, or anti-Lewis^y antibody is a monoclonal antibody that binds to H-1, H-2, Lewis^b, and Lewis^y with an affinity of at least $1 \times 10^8 K_a$.

Any form of H-1 and H-2, Lewis^b, and Lewis^y can be used to generate the anti-H-1 anti-H-2, anti-Lewis^b, and anti-Lewis^y antibodies or aptamers respectively, including, minimal trisaccharide or disaccharide structures or epitope-bearing fragments thereof, and any glycan containing these epitopes. Highly suitable anti-H-1 anti-H-2, anti-Lewis^b, and anti-Lewis^y antibodies or aptamers are those of sufficient affinity and specificity to recognize and bind to their respective targets in vivo. As used herein, the term epitope refers to an antigenic determinant of a glycan.

Specific carbohydrate binding antibodies or aptamers can be molecules that 1) exhibit a threshold level of binding activity; and/or 2) do not significantly cross-react with known related glycan molecules. The binding affinity of an antibody or aptamer can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, Ann. NY Acad. Sci. 51: 660-672, 1949). In some embodiments the antibodies or aptamers can bind to their target epitopes or mimetic decoys with at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10^3 -fold, 10^4 -fold, 10^5 -fold, 10^6 -fold or greater affinity for the target glycan than to other glycans having some homology to the target glycan.

In some embodiments the antibodies or aptamers bind with high affinity to H-1 or H-2, Lewis^b, or Lewis^y of 10⁻⁴M or less, 10⁻⁷M or less, 10⁻⁹M or less or with subnanomolar affinity (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 nM or even less). In some embodiments the binding affinity of the antibodies or aptamers for H-1 or H-2, Lewis^b, or Lewis^y is at least 1 x 10⁶ Ka. In some embodiments the binding affinity of the antibodies or aptamers for H-1 or H-2, Lewis^b, or Lewis^y is at least 5 x 10⁶ Ka, at least 1 x 10⁷ Ka, at least 2 x 10⁷ Ka, at least 1 x 10⁸ Ka, or greater. Antibodies or aptamers may also be described or specified in terms of their binding affinity to H-1 and/or H-2, Lewis^a, Lewis^b, Lewis^x, and/or Lewis^y. In some embodiments binding affinities include those with a K_d less than 5 x 10⁻² M, 5 x 10⁻⁵ M to 5 x 10⁻⁷ M, 5 x 10⁻⁸ M to 5 x 10⁻¹⁰ M, 5 x 10⁻¹² M to 5 x 10⁻¹⁴ M, or less.

Antibodies or aptamers can be purified by chromatographic methods known to those of skill in the art, including ion exchange and gel filtration chromatography (for example, Caine et al., Protein Expr. Purif. (1996) 8(2):159-166). Alternatively or in addition, antibodies or aptamers can be purchased from commercial sources, for example, Abcam, Biovendor Laboratory, Calbiochem, Signet Laboratories, Accurate Chemical and Scientific Corporation, and EMD.

Levels of secretor antigens can be measured in a biological sample using any immunoassay format known to those of skill in the art. For example, in non-competitive immunoassays, the secretor antigen is “sandwiched” between two antibodies, a capture antibody and a detection antibody. Typically, the capture antibody is bound either covalently or non-covalently to a solid phase, such as a tube or well, and the detection antibody is conjugated to an enzyme in the case of ELISA assays, or is radiolabeled in the case of RIA assays. In ELISA assays, the detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Specific antibody binding is analyzed by adding an enzymatic substrate, *e.g.*, a chromogenic or fluorogenic molecule, which produces a detectable quantifiable signal upon cleavage. Specific antibody binding in RIA assays is determined by measuring the levels of radioactivity bound to the support.

In a competitive immunoassay, the antigen (analyte) in the sample competes with the labeled antigen (tracer) for a limited number of antibody binding sites. The bound antigen is separated from the excess analyte not bound to the antibody. The

amount of the analyte in the unknown sample is inversely proportional to the amount of labeled antigen, as measured in a gamma counter or spectrophotometer. Examples of competitive immunoassays include double antibody radioimmunoassays (RIAs), coated tube RIAs and coated well enzyme immunoassays (EIAs). Some examples of solid supports that can be used include plates, tubes, polystyrene beads, nylon, nitrocellulose, cellulose acetate, glass fibers and other types of porous polymers. Suitable labels include radionuclides, fluorophores, chemiluminescent labels, bioluminescent labels, enzymes, for example, as used in ELISA systems, dyes or particles such as colloidal gold or quantum dots.

Assay systems and kits designed to detect one or more specific secretor and Lewis antigens simultaneously are also within the scope of the method. The kits may be dip-stick, flow-through or migratory in design as well as other formats known to those skilled in the art. If desired, the assays can be automated to insure standardization and obtain higher throughput.

FUT2 activity assays

FUT2 activity can be measured in a biological sample using any standard method known in the art that is specific for FUT2. For example, a fucosyltransferase assay can be performed in a 20 μ l reaction volume containing 3 μ M GDP-[¹⁴C]fucose, 5 mM ATP, 25 mM sodium phosphate, pH 6.0, 40 μ g of total protein from cell extracts and phenyl β -D-galactoside or asialofetuin as acceptor substrates. Reaction mixtures are incubated at 37 °C for 2 h, and terminated by the addition of 1 ml of water. The hydrophobic fucosylated phenyl β -D-galactoside products are purified by passing the reaction products through a C₁₈ reverse-phase column. Radiolabelled asialofetuin products are purified by filtration through microfiber membranes (GF/C; Whatman) and radioactivity is measured by liquid-scintillation counting.

Nanthakumar NN, Dai D, Newburg DS, Walker WA. The role of indigenous microflora in the development of murine intestinal fucosyl- and sialyltransferases.

FASEB J (November 15, 2002) 10.1096/fj.02-0031fje (summary: FASEB J 2003;17:44-6).

FUT2 genotyping analysis

FUT2 genotyping can be performed by any standard method known in the art, for example SNP analysis or RT-PCR techniques. SNP's identified as leading to lower levels of *FUT2* activity include TRP143TER (428G-A) and ILE129PHE (385A-T). Complete deletion of the *FUT2* gene is also observed in some non-secretors. Methods are well known in the art for determining single nucleotide polymorphisms (SNPs), for example: Ahmadian A., Gharizadeh B., Gustafsson A. C., Sterky F., Nyren P., Uhlén M. and Lundberg J. *Anal Biochem* 280(1):103-10 (2000); Griffin T. J., Hall J. G., Prudent J. R. and Smith L. M. *Proc Natl Acad Sci U S A* 96(11):6301-6 (1999); Nickerson D. A., Kaiser R., Lappin S., Stewart J., Hood L. and Landegren U. *Proc Natl Acad Sci U S A* 87(22):8923-7 (1990).

Other assays

Ulex europaeus, a lectin that reacts specifically with alpha-l-fucose, can be used as the basis for detecting secretor antigens, and especially H-2. When *ulex europaeus* conjugated to colloidal gold is exposed to a sample containing secretor antigens under suitable conditions, the *ulex europaeus*-colloidal gold will aggregate thereby producing detectable color change.

Briefly, *ulex europaeus* (UEA1) (Sigma-Aldrich; St Louis, MO) is conjugated to colloidal gold after determination of the minimum amount of UEA1 and optimal pH conditions required for stabilization of the colloidal gold. On nitrocellulose strips, 2µL of the untreated test saliva is added and left to dry for 5 min; 200µL of casein solution at 1.0% in TBS are added and incubated for 3min at room temperature. The casein solution is discarded; 150µL of UEA1-colloidal gold conjugate are added at the optimal dilution in casein solution at 0.05% in TBS, and incubated for 10 min. A detectable color change indicates the presence of secretor antigens in the sample. Control samples are saliva samples known to be H-2 positive or negative.

Methods of treatment

The methods disclosed herein are also useful for the treatment of an infant at risk for infectious and inflammatory disorders. Treatment can completely or partially abolish some or all of the signs and symptoms of the infectious or inflammatory

disorder, decrease the severity of symptoms, delay their onset, or lessen the progression or severity of subsequently developed symptoms.

The methods of treatment are useful for infectious and inflammatory disorders. The methods can be used for full-term infants, premature infants or extremely low birthweight infants. Full term infants include those born between 37 and 42 weeks gestational age; premature infants are typically those born at less than 37 weeks' gestational age. Extremely low birth weight (ELBW) is generally defined as a birth weight less than 1000 g (2 lb, 3 oz). The majority of ELBW infants are also the youngest of premature newborns, usually born at 27 weeks' gestational age or younger. Nearly 1 in 10 infants with low birth weight (<2500 g) are ELBW infants.

The infectious and inflammatory disorders that may be detected using the methods described herein include necrotizing enterocolitis (NEC), gastrointestinal infections, gastrointestinal inflammation, and sepsis. These disorders may be the result of disease, injury or of unknown cause and they may be influenced by one's genetic constitution.

In NEC, portions of the bowel undergo necrosis, *i.e.*, tissue death. Although NEC affects the gastrointestinal tract it can, in severe cases, have profound systemic impact. Initial symptoms may be subtle and can include one or more of: feeding intolerance, delayed gastric emptying, abdominal distention and/or tenderness, ileus/decreased bowel sounds, and, in the advanced stages, abdominal wall erythema and hematochezia

Systemic signs are nonspecific and can include any combination of apnea, lethargy, decreased peripheral perfusion, shock (in advanced stages), cardiovascular collapse and bleeding diathesis (consumption coagulopathy). Nonspecific laboratory abnormalities can include the following: hyponatremia, metabolic acidosis, thrombocytopenia, leukopenia or leukocytosis with left shift, neutropenia, prolonged prothrombin time, and activated partial thromboplastin time, decreasing fibrinogen, and rising fibrin split products (in cases of consumption coagulopathy). Although the exact etiology is not known, the etiology may be multifactorial and involve any or all of abnormal bacterial flora, intestinal ischemia and/or reperfusion injury, and intestinal mucosal immaturity.

Gastrointestinal infections in infancy include symptoms of diarrhea; the presence of mucus or blood in stools; vomiting; dehydration; thirst; listlessness; dry

mucous membranes; sunken fontanelles; decreased skin turgor; decreased capillary filling time; tachycardia; weak pulse, reduced blood pressure; and tenting or loss of skin turgor. The infectious agents can be bacterial, fungal, viral or parasitic.

Examples of bacterial agents include, but are not limited to, *Staphylococcus* spp.,

5 *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., *Enterobacter* spp.,

Klebsiella spp., *Bacillus* spp., *Serratia* spp., *Salmonella* spp., *Shigella* spp.,

Campylobacter spp., *Yersinia* spp., and *Clostridium difficile*. Examples of fungal

agents include, but are not limited to, *Candida* spp. Examples of parasitic organisms

include, but are not limited to, *Cryptosporidium* spp., *Giardia* spp., *Entamoeba*

10 *histolytica*, *Cyclospora* spp. Examples of viral organisms include, but are not limited

to, rotavirus, cytomegalovirus, enteric adenovirus, astrovirus, adenoviruses type 40 or 41, Norwalk and other noroviruses, and saporovirus. The clinical signs of

neonatal sepsis are nonspecific and are associated with characteristics of the causative organism and the body's response to the invasion. Neonatal sepsis may be categorized

15 as early or late onset. Eighty-five percent of newborns with early-onset infection

present within 24 hours, 5% present at 24-48 hours, and a smaller percentage of

patients present between 48 hours and 6 days of life. Onset is most rapid in premature

neonates. The infant manifests overt shock with pallor, poor capillary perfusion, and edema. These signs of shock are indicative of severe compromise and are highly

20 associated with mortality. Signs of sepsis can include any or all of cardiac signs, *e.g.*,

early stage pulmonary hypertension, decreased cardiac output, hypoxemia,

progressive decreases in cardiac output with bradycardia and systemic hypotension;

metabolic signs, *e.g.*, hypoglycemia, hyperglycemia, metabolic acidosis, and jaundice;

neurologic signs, *e.g.*, meningitis, ventriculitis, arachnoiditis, vasculitis, phlebitis,

25 thrombosis, cerebral edema, infarction, stupor and irritability, impairment of

consciousness (*i.e.*, stupor with or without irritability), coma, seizures, bulging

anterior fontanel, extensor rigidity, focal cerebral signs, cranial nerve signs, nuchal

rigidity, alterations in cerebrospinal fluid (CSF), *e.g.*, an elevated white blood cell

count, an elevated protein level, a decreased CSF glucose concentration, and positive

30 culture results. Temperature instability is observed with neonatal sepsis and

meningitis, either in response to pyrogens secreted by the bacterial organisms or from sympathetic nervous system instability. The neonate is most likely to be hypothermic.

The infant may also have decreased tone, lethargy, and poor feeding. Signs of

neurologic hyperactivity are more likely when late-onset meningitis occurs. Other signs of neonatal sepsis include hematologic signs, *e.g.*, thrombocytopenia, abnormal white blood cell counts (WBC), abnormal neutrophil count (PMNs and immature forms), abnormal ratios of immature-to-total neutrophil count, disseminated intravascular coagulation (DIC), abnormalities in prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen and D-dimer levels; and gastrointestinal signs, *e.g.*, necrotizing enterocolitis.

Organisms that have been implicated in causing late-onset sepsis include coagulase-negative *Staphylococci*, *S. aureus*, *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., group B *Streptococcus*, *Serratia* spp., *Acinetobacter* spp., and *Candida* spp. The infant's skin, respiratory tract, conjunctivae, gastrointestinal tract, and umbilicus may become colonized from the environment, leading to the possibility of late-onset sepsis from invasive microorganisms. Vectors for such colonization may include vascular or urinary catheters, other indwelling lines, or contact from caregivers with bacterial colonization.

Infants identified based on their glycan phenotype (secretor, Lewis, or sialyl antigen expression) as being at risk for infectious and inflammatory disorders, *e.g.*, NEC, gastrointestinal infections, and sepsis, can be treated with therapies that include one or more protective agents. Unless the context indicates otherwise, we use the term "agent" to broadly refer to any substance that affects a target molecule or a target region of the gastrointestinal system in a clinically beneficial way (*e.g.*, to inhibit pathogens from binding to host cell surface glycans). Useful protective agents include, for example, human milk feeding, probiotic organisms, prebiotics, or α 1,2 fucosyl glycans,

The α 1,2 fucosyl glycans are saccharides that include a fucose terminus in an α 1,2 linkage and as such are homologues of secretor antigens, *i.e.*, they include a minimal disaccharide precursor, or core sequence, covalently linked to a fucose residue in an α 1,2 configuration. The core sequence can be either the lacto type I structure, galactose (β 1-3) N-acetylglucosamine-R, which we abbreviate here as {Gal (β 1-3)GlcNAc}-R or the lacto type II structure galactose (β 1-4) N-acetylglucosamine-R, which we abbreviate here as {Gal(β 1-4)GlcNAc-R}, wherein R is an H, a small radical, or another monosaccharide, disaccharide or polysaccharide or

a glycoprotein or glycolipid. These saccharides can be free oligosaccharides or conjugated and expressed as glycoproteins, glycolipids, or other structures. The conjugated and unconjugated forms of oligosaccharides are together classified as glycans. Thus, the α 1,2 fucosyl glycans can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 5 16, 18, 20, 24, 28, 32, 36 or more sugars; one or more of the sugars is covalently linked to a fucose residue in an α 1,2 configuration, so that the glycans can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 or more fucose residues. Examples of suitable α 1,2 fucosyl glycans include, without limitation, 2' -fucosyllactose (2' -FL); lacto-N-fucopentaose-I (LNF-I); lacto-N-difucohexaose I (LDFH I); 10 lactodifucotetraose (LDFT), or such fucosylglycan epitopes attached to a glycolipid, glycopeptide, glycoprotein, mucin, or other scaffold, either in the soluble form or as part of a probiotic organism. The α 1,2 fucosyl glycans can be purified from natural sources, e.g., milk, milk products or plant products, using method known to those in the art. Alternatively or in addition, glycans can be synthesized chemically either 15 from naturally occurring precursors or synthetic templates according to methods known in the art. In addition, glycans can be synthesized enzymatically, either *in vitro*, or *in vivo* using specifically engineered microorganisms such as bacteria or yeasts, using biosynthetic enzymes well known in the art

A protective agent can also be a probiotic organism, *i.e.*, a living 20 microorganism that, when ingested by the host, can modify intestinal microbial populations in a way that benefits the host. Probiotic organisms may provide an increased barrier to translocation of bacteria and bacterial products across mucosa, competitively exclude potential pathogens, modify of host response to microbial products, and enhance enteral nutrition in ways that inhibits the growth of pathogens 25 such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida albicans*.

Probiotic organisms generally include bacteria and yeast. The species of probiotic organism can vary, but suitable species for infants include *Lactobacilli*, e.g., *Lactobacillus rhamnosus* GG, *L. acidophilus*, *L. casei*, *L. plantarum*, *L. reuteri*; and *Bifidobacteria*, e.g., *Bifidobacterium infantis*, *B. bifidum*, *B. breve*, *B. animalis* subsp. 30 *lactis*, *B. longum*, as well as *Streptococcus thermophilus*. Useful yeast species include *Saccharomyces boulardii* and *Kluyveromyces lactis*. Probiotic organisms may be either naturally occurring or they may be engineered, *i.e.*, organisms may be provided with genes that enable them to acquire desirable properties such as, but not limited to,

the ability to express secretor antigens. Probiotic organisms may be administered separately or in combination. Commercially available probiotic formulations include, for example, Infloran® (Istituto Sieroterapico Berna, Como, Italy) which contains *Lactobacillus acidophilus/Bifidobacterium infantis*; ABC Dophilus (Solgar, Israel) which contains *Bifidobacterium infantis*, *B. bifidum* and *Streptococcus thermophilus*; and Dicoflor (Vitis Pharma, Warsaw, Poland) which contains *L. rhamnosus* GG. A protective agent can also be a prebiotic, *i.e.*, a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon. In contrast to a probiotic, which introduces exogenous bacteria into the colonic microbiota, a prebiotic stimulates the growth of one or a limited number of the potentially health-promoting indigenous microorganisms *e.g.*, *Bifidobacteria* or *Lactobacteria*. Examples of prebiotics include fructo-oligosaccharides, *e.g.*, inulin, xylooligosaccharides and galacto-oligosaccharides. Prebiotics can be isolated from natural sources *e.g.*, chicory roots, soybeans, Jerusalem artichokes, beans, onions, garlic, oats, wheat and barley.

One useful prebiotic is inulin, a type of fructan (polymer of fructose). Inulin-type fructans are composed of β -D-fructofuranoses attached by β -2,1 linkages. The first monomer of the chain is either a β -D-glucopyranosyl or β -D-fructopyranosyl residue. Various forms of inulin and inulin fragments are available from commercial sources, *e.g.*, inulin with a degree of polymerization (DP) from 2 to 60 is extracted from chicory roots (Raftiline; Orafiti, Tienen, Belgium); oligofructose, which is produced by partial enzymatic hydrolysis of inulin, has a DP < 10 (Raftilose; Orafiti) and the inulin from which the small-molecular-weight oligomers have been eliminated is called high-performance inulin (Raftiline HP; Orafiti). With the use of sucrose as a substrate and a 1,2- β fructan in a fructosyltransferase-catalyzed reaction, a synthetic low-molecular-weight fructan is produced that has a DP < 4 (Neosugar or Actilight; Beghin-Meji Industries, Paris).

Protective agents may be administered directly to a patient, either singly or in combination. Generally, the protective agents can be suspended in a pharmaceutically acceptable carrier (*e.g.*, physiological saline or a buffered saline solution) to facilitate their delivery. Encapsulation of the protective agents in a suitable delivery vehicle (*e.g.*, polymeric microparticles or implantable devices) may increase the efficiency of delivery. A composition can be made by combining any of the protective agents

provided herein with a pharmaceutically acceptable carrier. Such carriers can include, without limitation, sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents include mineral oil, propylene glycol, polyethylene glycol, vegetable oils, and injectable organic esters. Aqueous carriers
5 include, without limitation, water, alcohol, saline, and buffered solutions.

Preservatives, flavorings, and other additives such as, for example, antimicrobials, anti-oxidants (*e.g.*, propyl gallate), chelating agents, inert gases, and the like may also be present. It will be appreciated that any material described herein that is to be administered to a mammal can contain one or more pharmaceutically acceptable
10 carriers.

Alternatively, or in addition, protective agents may be combined with an infant's food source, *e.g.*, expressed breast milk or commercially available infant formula. Any composition described herein can be administered to any part of the host's body for subsequent delivery to the gastrointestinal tract. A composition can
15 be delivered to, for example, the mouth, nasal mucosa, blood, lungs, intestines, muscle tissues, skin, or the peritoneal cavity of a mammal. In terms of routes of delivery, a composition can be administered by intravenous, intracranial, intraperitoneal, intramuscular, subcutaneous, intramuscular, intrarectal, intravaginal, intratracheal, intradermal, or transdermal injection, by oral or nasal administration, or
20 by gradual perfusion over time. In a further example, an aerosol preparation of a composition can be given to a host by inhalation.

The dosage of protective agent that is required will depend on the nature of the agent, route of administration, the nature of the formulation, the nature of the patient's illness, the patient's size, weight, surface area, age, and sex, other drugs being
25 administered, and the judgment of the attending clinician. Wide variations in the needed dosage are to be expected in view of the variety of protective agents and the differing efficiencies of various routes of administration. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art. Administrations can be single or multiple (*e.g.*, 2- or 3-, 4-, 6-,
30 8-, 10-times, or more). Encapsulation of the protective agents in a suitable delivery vehicle (*e.g.*, polymeric microparticles) may increase the efficiency of delivery.

The duration of treatment with any composition provided herein can be any length of time from as short as one day to as long as the increased risk might be

suspected clinically, for example, through the period of neonatal intensive care unit stay or through infancy. For example, a protective agent can be administered several times per day, once a day, or once a week (for, for example, 4 weeks to several months). It is also noted that the frequency of treatment can be variable. For
5 example, the protective agents can be administered once (or twice, three times, *etc.*) daily, weekly, or monthly.

An effective amount of any composition provided herein can be administered to an individual at risk of disease or in need of treatment. The term “effective” as used herein refers to any amount that induces a desired response while not inducing
10 significant toxicity in the patient. Such an amount can be determined by assessing a patient’s response after administration of a known amount of a particular composition. In addition, the level of toxicity, if any, can be determined by assessing a patient’s clinical symptoms before and after administering a known amount of a particular composition. It is noted that the effective amount of a particular composition
15 administered to a patient can be adjusted according to a desired outcome as well as the patient’s response and level of toxicity. Significant toxicity can vary for each particular patient and depends on multiple factors including, without limitation, the patient’s disease state, age, and tolerance to side effects.

The protective agents provided herein can be administered in conjunction with other prophylactic or therapeutic modalities to an individual at risk for an infectious or inflammatory disorder, *e.g.*, NEC, a gastrointestinal infection or sepsis. The protective agents can be given prior to, simultaneously with or after treatment with other agents or regimes. Other treatments can include administration of antibiotics, for example, vancomycin, kanamycin, gentamicin, cefotaxime, clindamycin or metronidazole, enteral administration of IgG and IgA together, amino acid supplementation, the use of platelet-activating factor (PAF) antagonists or PAF-acetylhydrolase administration, polyunsaturated fatty acid administration, epidermal growth factor administration, and antenatal corticosteroids. The protective agents can also be administered along with or in addition to, other feeding regimes, including judicious administration of human milk feeding, infant formula, parenteral fluids, delayed or slow feeding.

Also provided are methods of determining the course of treatment for an infant
20 who has been identified, based on secretor status, as being at risk for NEC or a

gastrointestinal infection. The levels of secretor antigens, *e.g.*, α 1,2 fucosyl glycans, in the infant's food source can be compared with levels of the same secretor antigens in a reference sample; the levels of secretor antigens in the food source can be classified as reduced or elevated relative to those secretor antigens in a reference sample. Those infants whose secretor status indicates that they are at risk for NEC and gastrointestinal infections and whose food source also contains reduced levels of secretor antigens can be treated with one or more of α 1,2 fucosyl glycans, probiotic organisms or prebiotics.

The infant's food source can be breast milk, either from the infant's own mother or a donor source, or a commercial infant formula. The level of one or more secretor antigens can be assayed in human milk and formula *e.g.*, by ELISA, chromatography or another method and compared to those in a reference sample as described above.

Human milk oligosaccharides typically contain a lactose moiety at the reducing end and a fucose at the nonreducing end. The addition of fucose to an oligosaccharide by an α 1,2 linkage is catalyzed primarily by the fucosyltransferase produced by the secretor gene, *Se* (*FUT2*); the addition of fucose by an α 1,3 or α 1,4 linkage is catalyzed by fucosyltransferases produced by the Lewis gene, *Le* (*FUT3*) or other α 1,3 transferase genes (*FUT4*, 5, 6, 7, and 9) of this family. Variation in the activities of the 2- and 3/4-fucosyltransferases can result from inactive or partially active genetic polymorphisms. Such variation can produce milk phenotypes that vary in relative quantities of specific fucosyloligosaccharides. Women who are nonsecretors do not express measurable 2-linked fucosyloligosaccharides in their milk or other bodily fluids. However, the expression of milk fucosyloligosaccharides can vary even among secretors and the ratio of 1,2-linked fucosyloligosaccharides to those that contain only 1,3- and 1,4-linked fucose declines exponentially over the first year of lactation.

Examples of suitable α 1,2 fucosyl glycans include, without limitation, 2' - fucosyllactose (2' -FL), which is homologous to H-2; lacto-N-fucopentaose-I (LNF-I), which is homologous to H-1; lacto-N-difucohexaose I (LDFH I), which is homologous to Lewis^b; lactodifucotetraose (LDFT), which is homologous to Lewis^y, as well as lacto-N-difucohexaose II (LNF-II), which is homologous to Lewis^a; and lacto-N-difucohexaose III (LNF-III), which is homologous to Lewis^x. The core type 1

structure, lacto-*N*-tetraose (LNT), is a terminal Gal β 1,3GlcNAc on lactose. Lactose is the core for the most abundant type 2 structures in milk (2'-FL, 3-FL, and LDFT), whereas lacto-*N*-neotetraose, Gal β 1,4GlcNAc on a lactose terminus, is the core for LNF-III. In other tissues, Lewis structural moieties are based on a lactosamine backbone (Gal-GlcNAc); however, the most prevalent type 2 fucosyloligosaccharides in human milk are synthesized from lactose (Gal-Glc) and therefore are defined as the glucose analogs to the type 2 Lewis structures. The epitopes of these structures can also be found expressed in various glycoconjugates of milk, and these glycoconjugates may be used for prophylactic and/or therapeutic purposes.

Infants whose food supply contains a reduced level of secretor antigens may be supplemented enterally by feeding α 1,2 fucosyl glycans, probiotic organisms or prebiotics as described above.

EXAMPLES

Example 1. Bacterial colonization induces change in intestinal glycoylation.

Expression of fucosylated and sialylated glycans in gut change during
5 development, reminiscent of the changes seen in milk over the course of lactation. In
the mouse small intestine, *Fut2* mRNA and α 1,2/3-fucosyltransferase activity
increase abruptly at weaning, while expression of sialyltransferase activity decreases.
The inversion of these two enzyme activities coincides with an abrupt change of
mucosal glycan expression at weaning and a change in composition of gut microbiota.
10 Whether induction of these changes was through an intrinsic genetic program, or by
exogenous control by diet or adult microflora, was tested as described below.

The upregulation of fucosyltransferase that normally would occur at weaning
does not occur in germ-free mice. However, whenever post-weaning germ-free mice
are colonized, fucosyltransferase and fucosylation are induced. This suggested that
15 bacterial colonization was inducing fucosylation of the mucosal surface. This was
confirmed in mature mice that were depleted of bacteria (BD) by drinking a mixture
of antibiotics. After two weeks, *Fut2* mRNA and fucosyltransferase activity dropped
to the levels seen in germ-free mice, and fucosylglycans (*Ulex europaeus* agglutinin 1
[UEA-1] staining) were no longer expressed in the colon. Cessation of antibiotic
20 treatment and repletion with normal microbiota (XBD) caused a recovery of *Fut2*
mRNA and fucosyltransferase activity to levels of normally colonized mature gut, and
full expression of fucosylglycans on the mucosal surface. This confirmed that
fucosylation of the gut is controlled by its colonization.

It was hypothesized that the mechanism whereby extracellular colonization
25 results in the intracellular activation of the ERK and JNK pathway involves undefined
transmembrane receptors. A family of transmembrane receptors, the toll-like
receptors (TLR), were already known to sense the extracellular presence of pattern
recognition molecules unique to microbes and transmit signals to the nucleus through
transcellular signal transduction pathways. Accordingly, we tested whether one of the
30 TLR family members might be responsible for the communication between the
bacteria and the gut mucosa that results in bacterial-induced *Fut2* mRNA and
fucosyltransferase activity in the colonic mucosa. Like wild-type mice, mice with
mutations in TLR2 are able to express normal fucosylation that is lost with a loss of

colonization (Fig 12, next page). In contrast, mutants of TLR4 or its downstream mediator, MyD88, do not express the full level of fucosylation, and this fucosylation is not affected by loss of colonization. This is consistent with the TLR4 signaling pathway being necessary for bacteria-induced mucosal fucosylation. To test whether

5 activation of TLR4 per se in bacteria-depleted mice is sufficient to activate the transduction pathways that stimulate *Fut2* expression, the specific ligand for TLR4, LPS, was administered in drinking water to bacteria-depleted mice.

Fucosyltransferase activity and *Fut2* mRNA of the BD mouse colon recovers to normal adult levels when treated with ultra-pure LPS. In contrast, in BD mice treated

10 with peptidoglycan (PG), the ligand for TLR2, the level of fucosyltransferase activity and *Fut2* mRNA remains at the lower levels of BD mice. These data strongly suggest that binding and stimulation of TLR4 in bacterially depleted mice is the critical signal that is both necessary and sufficient for adult gut microbiota to signal the epithelial nuclear events that result in fucosylation of the gut.

15 If binding to fucosylated TLR4 is the essential signal for the colonization-induced mucosal fucosylation, the subset of the microbiota that bind to fucose would be expected to recapitulate this phenomenon. This hypothesis was tested with a fucose-utilizing species of the mixed microbiota. *Bacteroides fragilis* is a specific fucose-utilizing bacterium found in typical mature mammalian microbiota.

20 Monocolonization of bacterially depleted mice with *B. fragilis* induced fucosylation to the same extent as recolonization with mixed microbiota, mediated through induced *Fut2* mRNA, consistent with the fucose-utilizing bacteria of the mixed microbiota being responsible for the induction of fucose expression. If this were so, a mutant *B. fragilis* made incapable of utilizing fucose would be expected to lose the ability to

25 signal the induction of fucosylation in the mucosa, as observed in the right panel. Thus, binding to one set of fucosylated epitopes, seemingly fucosylated TLR4, in bacterially depleted mice by fucose-utilizing bacteria seems to be sufficient for the induction of the fucosylated phenotype on the intestinal mucosa.

30 **Example 2: Histo-blood Group Antigens (Glycans) in Saliva of Hospitalized Infants**

Thirty-six infants hospitalized in Cincinnati area Newborn Intensive Care Units between 24 and 42 weeks gestational age (GA) were enrolled between May and December 2005. Twelve subjects per group were stratified by gestational age into

three groups, 24 to 28 weeks, 29 to 32 weeks, and greater than or equal to 33 weeks gestational age at birth. Infants diagnosed with major congenital anomalies were excluded. Institutional Review Boards at Cincinnati Children's Hospital Medical Center, Good Samaritan Hospital, and University Hospital approved the study.

5 Informed written consent was obtained from the parents. Maternal demographic information collected included maternal age, race, obstetric history, complications encountered in the current pregnancy, and maternal medications during pregnancy. Clinical and demographic data recorded at enrollment of subjects included race, gender, gestational age, birth weight, length and head circumference. Clinical data
10 recorded on study infants during their hospital course included requirement and duration of respiratory support, initiation and type (human milk or formula) of enteral nutrition, episodes of culture-proven sepsis, occurrence of necrotizing enterocolitis (Bell's stage 2 or greater), and history of antibiotic use.

Specimen collection. Saliva specimens were obtained at enrollment and every
15 two weeks while subjects remained hospitalized. A maximum of five samples were collected from each subject. Saliva specimens were collected one to two hours after feeding by clearing the mouth of residual milk or formula with soft gauze and inserting a sterile cotton swab. Once visibly saturated with saliva, the cotton swab was transferred into a specimen container. Specimens were held briefly at 4° C then
20 transferred to -80° C. A total of 107 saliva samples were collected. Saliva-saturated cotton swabs were allowed to thaw in 1 ml phosphate buffered saline (PBS) for 5 minutes. The specimens were then centrifuged at 10,000 X g for 10 minutes and supernatants were collected. Each specimen was boiled at 100°C for 10 minutes and placed at 4° C overnight. Samples were again centrifuged at 10,000 g x for 10
25 minutes, the supernatants were collected, separated into 100 ml aliquots and placed at -80°C.

Optimal dilutions of saliva samples were determined for each antigen detection assay. Saliva specimens diluted 1:50 were used for detection of Le^a, Le^x, H-1, H-2, sialyl Le^a and sialyl Le^x antigens. Saliva specimen diluted 1:125 were used for
30 Le^y, whereas dilutions of 1:250 were used for Le^b antigen detection. For quantitative analysis of saliva samples from an individual subject, a 1:50 dilution was used for all antigens. Samples were coated onto microtiter plates (Dynex Immunlon) overnight at 4°C. After blocking with 5% Blotto, monoclonal antibodies (MAbs) specific to

Lewis and ABH antigens were used at a dilution of 1:100. The following MAbs specific to human histo-blood group antigen types were used for histo-blood group phenotype determinations. MAbs BG-4 anti-H type 1, BG-5 anti-Le^a, BG-6 anti-Le^b, BG-7 anti-Le^x, and BG-8 anti-Le^y were purchased from Signet Laboratories. MAb
5 BCR9031 anti-H type 2, BCR9010 anti-A, and BCRM 11007 anti-B were purchased from Accurate Chemical and Scientific Corporation. MAbs for Sialyl Le^a and Le^x were products of EMD, catalog number 565942 and 565953 respectively. After incubation for 1 hour at 37° C, horseradish peroxidase (HRP) conjugated goat anti-mouse IgG, IgG3 or IgM antibodies were added. After each step, the plates were
10 washed five times with PBS / Tween solution. Colorimetric reactions were detected using a TMB kit (Kirkegard & Perry Laboratories), and read at a wavelength of 450 nm using an EIA spectrum reader (Tecan).

Shown in Table 1 is the average optical density values (\pm the standard error) obtained by ELISA in study week 1 and prior to discharge for all infants ever found to
15 have a detectable level of that antigen in a saliva sample. Each antigen was considered separately. This analysis indicated that most secretor antigens (Lewis^b, Lewis^y, and H-2) were present in premature infants from birth, and that secretor antigen expression increased postpartum. As shown in Table 1, levels of secretor antigens (shown in boldface type) were higher in the samples collected at subsequent
20 time points, *i.e.*, those samples taken from infants prior to discharge. Levels of Lewis^b, Lewis^y and Lewis^b and Lewis^y showed a statistically significant increase over time; levels of the other secretor antigens, H-1 and H-2, also increased, although the rate of increase did not approach statistical significance.

Table 1: Glycan Expression in Saliva of Premature Infants

Antigen	N	Postpartum Week 1 Saliva Sample*	Pre-Discharge Saliva Sample*	Postpartum change p-value**
Secretor				
H-1	20	0.18 (0.08)	0.29 (0.06)	0.13
H-2	30	1.45 (0.20)	1.80 (0.21)	0.41
Lewis ^b	28	1.92 (0.20)	4.02 (0.45)	0.0005
Lewis ^y	31	2.78 (0.24)	3.90 (0.29)	0.024
Lewis (only)				
Lewis ^a	32	1.22 (0.46)	1.27 (0.54)	0.82
Lewis ^x	26	0.71 (0.19)	0.95 (0.32)	0.40
Sialyl				
SLe ^a	36	3.63 (0.61)	3.10 (0.89)	0.29
SLe ^x	35	1.85 (0.27)	1.61 (0.36)	0.49

* Values are expressed as the mean optical density; the standard error is in parentheses.

**p-values were determined using a Student's t-test.

5

Example 3: Antibiotic Use and glycan expression phenotype

The relationship between secretor antigen expression, postpartum age, gestational age and antibiotic use was analyzed using a Generalized Estimating Equation (GEE). Saliva samples were collected from 24 infants in the first week of age postpartum and "prior to discharge". Levels of histo-blood group antigens Lewis^a, Lewis^x, Lewis^b, Lewis^y, H-1, H-2, Sialyl-Lewis^a (SLe^a) and Sialyl-Lewis^x (SLe^x) were measured by ELISA according to the method described in Example 1. Infants were classified according to gestational age at birth into groups of 22-28 weeks, 29-34 weeks, and 35-40 weeks. For GEE analysis, histo-blood group antigen O.D. values were used as dependent variables (designated as "Model" in column 1 of Table 2) and week of age postpartum, gestational age group, and number of days of antibiotic use were included as independent variables. The resulting beta coefficients

15

for the GEE analysis are shown in Table 2. The GEE analysis indicated that the courses (defined as antibiotic treatment of the infant from 1-5 days) was associated with significantly decreased expression of secretor antigens postpartum.

Table 2 Beta coefficients (SE) from Generalized Estimating Equation

5 **(GEE) analysis:** Histoblood group antigen O.D. values (dependent variable) measured in samples from 24 infants with two saliva samples collected at Week 1 and prior to hospital discharge (3-7 weeks after birth). Week of age postpartum, gestational age group, and number of days of antibiotic use were included as independent variables

10

Model	Week of age postpartum	Gestational Age Group*	No. of courses of antibiotic use
Lewis b and y (combined O.D. values)	0.54 (.17) ***	0.005 (.39)	-0.33 (.37)
Sialyl Le X	-0.10 (.08)	-0.17 (0.21)	0.52 (.14) ***

* Gestational age groups: 1=24-28 wks, 2=29-32 wks; 3=33-40 wks at birth,

*** p≤0.001

Example 4: Extremely Low Birthweight Infant (ELBW) Outcomes Study

15

The relationship between secretor antigen expression, clinical outcome and necrotizing enterocolitis (NEC) was analyzed in a cohort of 192 extremely low birthweight (<1000 gram) infants seen in Cincinnati neonatal intensive care units, with saliva samples collected in week 1 (days 1-7) and week 2 (days 8-14) after birth.

20

This was a prospective study conducted at three hospitals that provide level III neonatal intensive care within the Cincinnati region, with infants enrolled between 2002-2004. Exclusion criteria included the presence of major chromosomal or congenital anomalies, diagnosis of cystic fibrosis, or a medical condition judged by the attending neonatologist to be incompatible with survival beyond the first week of life. After enrollment, a saliva sample was obtained along with demographic and clinical data. Infants were followed with once-weekly saliva sample collections.

25

Saliva was collected with sterile cotton-tipped swabs placed in the mouth of the infant and saturated with saliva, by nursing or research staff between 5:00 am and 10:00 am before feeding. Samples were frozen at -80 C. Saliva was extracted from the swab by

removing the cotton portion of the swab, placing it in a 1 mL syringe, and eluting the contents with 250 mL of normal saline. Figure 6 is a scatter plot of the results.

The rate of NEC in the study population was 7.8% (n=15); the rate of late onset sepsis was 34.9% (n=67); the rate of death was 9.3% (n=18). Of the 15 NEC cases, there was a 60% case fatality and the rate of death due to NEC among the 192 infants studied was 4.7% (n=9).

Levels of histo-blood group antigens Lewis^a, Lewis^x, Lewis^b, Lewis^y, H1, H2, Sialyl-Lewis^a (SLe^a) and Sialyl-Lewis^x (SLe^x) were measured by ELISA according to the method described in Example 1 in a single banked saliva sample taken from each infant. Individuals expressing one or more antigens containing an α 1,2-linked fucose were designated as secretors. Twenty percent (20%), *i.e.*, 39 infants, were classified as non-secretors (no detectable secretor antigen); 153 infants (80%) were classified as secretors.

Classification and Regression Tree (CART) analysis was used to identify the high-risk subgroups for NEC or death using the glycan (secretor, Lewis, and sialyl antigen) values of each individual. CART analysis is an established statistical method that uses tree-based partitioning to identify algorithms for diagnostic or prognostic markers of risk in clinical studies such as this. An empirical statistical technique based on recursive partitioning analysis, the method does not require parametric assumptions, and involves the segregation of different values of continuous or categorical data through a decision tree composed of progressive binary splits. Every value of each predictor variable is considered a potential split, and the optimal split is selected based on minimizing misclassification of cases and non-cases using an “impurity criterion”, which is the reduction in the residual sum of squares that would occur with a binary split of the data at that node. All seven antigens were analyzed, one at a time, using CART to generate the optimal cut-point for each variable. The categorical variables created from that step (partially shown in Figure 1) were then re-entered into a second CART model. In this step, H-2 emerged as the first split (see Figure 2), defining 73 infants into a high risk group at the lowest 38th percentile of values and below (O.D. value <0.627). This group had 18 cases of NEC or death (incidence 24.7%) compared with 6 cases of NEC or death among the 119 infants classified into the H-2 low-risk group (incidence 5.0%, P<0.0001). Among the 73 infants classified into the high-risk group by their salivary H-2 (the “low-secretors”

and non-secretors), a second split occurred in the CART model: 19 were identified as low-risk (1 case of NEC or death, incidence 5.2%) and 54 were identified as high risk (17 cases of NEC or death, incidence 31.5%). This model identified one high-risk group (the low- and non-secretors, 31.5% risk) and two low-risk groups (the 119 who
5 were high secretors and the 19 who were non- and low-secretors but also low sLe^a) with nearly identical risk approaching 5%. Lastly, these 3 nodes were re-entered into the final CART model: the result was a binary split identifying the high-risk group vs. all others (Figures 2 and 3). This single split, combining H-2 and sLe^a predictive high-risk cut-points, was found by receiver operating curve area under the curve analysis to
10 have an overall predictive value of 77. The CART analysis is summarized in Figures 5 and 6.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention.

What is claimed is:

1. A method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:
 - 5 (a) measuring the level of at least one secretor antigen in a biological sample from the individual, and
 - (b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values,
wherein the individual is at risk for necrotizing enterocolitis if the measured
10 level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values.
2. The method of claim 1, wherein the individual is an infant.
3. The method of claim 2 wherein the infant is a neonate, a low birthweight infant, an extremely low birthweight infant or a premature infant.
- 15 4. The method of claim 1, wherein the at least one secretor antigen comprises an α 1,2-linked fucose antigen and/or an α 2,3 sialylated antigen.
5. The method of claim 4 wherein the at least one secretor antigen is selected from the group consisting of: the H-1, H-2, Lewis^b and Lewis^y antigens and derivatives thereof.
- 20 6. The method of claim 5 wherein the derivative is a sialylated form of Lewis a, Lewis x, Lewis b or Lewis y.
7. The method of claim 5 wherein the derivative is a sulfated form of H-1, H-2, Lewis a, Lewis x, Lewis b or Lewis y.
8. The method of claim 1, wherein the biological sample is a bodily fluid or a
25 tissue.
9. The method of claim 8, wherein the bodily fluid comprises saliva, blood, plasma, serum, urine, stool, amniotic fluid, mucus, tears or lymph.

10. The method of claim 9, wherein the bodily fluid comprises saliva.
11. The method of claim 1, wherein the measuring step comprises an immunoassay.
12. The method of claim 1 wherein the at least one secretor antigen is selected
5 from the group consisting of: H-1, H-2, Lewis^b and Lewis^y and derivatives thereof and wherein the individual is determined to be risk for necrotizing enterocolitis if the measured level is below the predetermined value or below the predetermined range of values.
13. The method of claim 1 wherein the predetermined value or the predetermined
10 range of values represents the average level of the at least one secretor antigen in a population of individuals determined to be secretors.
14. The method of claim 1, wherein the individual is determined to be at risk for necrotizing enterocolitis when the measured level of at least one secretor antigen is at least 10% less than the average level found in a control population of secretors.
15. The method of any of the forgoing claims wherein the at least one secretor
15 antigen is the H-2 antigen.
16. The method of claim 1 wherein the at least one antigen is sialyl Lewis a or derivative thereof and wherein the individual is determined to be risk for necrotizing enterocolitis if the measured level is above the predetermined value or above the
20 predetermined range of values.
17. The method of claim 16 wherein the infant is a neonate, a low birthweight infant, an extremely low birthweight infant or a premature infant.
18. The method of claim 16, wherein the biological sample is a bodily fluid or a tissue.
19. The method of claim 18, wherein the bodily fluid comprises saliva, blood,
25 plasma, serum, urine, stool, amniotic fluid, mucus, tears or lymph.
20. The method of claim 19, wherein the bodily fluid comprises saliva.

21. The method of claim 16, wherein the measuring step comprises an immunoassay.
22. The method of claim 16 wherein the predetermined value or the predetermined range of values represents the average level of the at least one secretor antigen in a population of individuals determined to be secretors.
23. A method of identifying an individual at risk for developing a gastrointestinal disorder, the method comprising:
- (a) measuring the level of at least one secretor antigen in a biological sample from the individual, and
 - (b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values, wherein the individual is at risk for a developing a gastrointestinal disorder if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values.
24. The method of claim 23, wherein the individual is an infant.
25. The method of claim 23 wherein the infant is a neonate, a low birthweight infant, an extremely low birthweight infant or a premature infant.
26. The method of claim 23, wherein the measured antigens comprise an α 1,2-linked fucose antigen and/or an α 2,3 sialylated antigen.
27. The method of claim 26 wherein the secretor antigen is selected from the group consisting of: the H-1, H-2, Lewis^b and Lewis^y antigens and derivatives thereof.
28. The method of claim 27 wherein the derivative is a sialylated form of Lewis a, Lewis x, Lewis b or Lewis y.
29. The method of claim 27 wherein the derivative is a sulfated form of Lewis a, Lewis x, Lewis b or Lewis y.

30. The method of claim 22, wherein the biological sample is a bodily fluid or a tissue.
31. The method of claim 30, wherein the bodily fluid comprises saliva, blood, plasma, serum, urine, stool, amniotic fluid, mucus, tears or lymph.
- 5 32. The method of claim 31, wherein the bodily fluid comprises saliva.
33. The method of claim 22, wherein the measuring step comprises an immunoassay.
34. The method of claim 22 wherein the at least one secretor antigen is selected from the group consisting of: H-1, H-2, Lewis^b and Lewis^y and derivatives thereof
10 and wherein the individual is determined to be risk for developing a gastrointestinal disorder if the measured level is below the predetermined value or below the predetermined range of values.
35. The method of claim 22 wherein the predetermined value or the predetermined range of values represents the average level of the at least one secretor antigen in a
15 population of individuals determined to be secretors.
36. The method of claim 22, wherein the individual is determined to be at risk for necrotizing enterocolitis when the measured level of the at least one secretor antigen is at least 10% less than average level found in control population of secretors.
37. The method claim 22 wherein the at least one antigen is the H-2 antigen.
- 20 38. The method of any of claims 22-37 wherein the gastrointestinal disorder is gastrointestinal inflammation.
39. The method of any of claims 22-37 wherein the gastrointestinal disorder is gastrointestinal infection.
40. The method of any of claims 22-37 wherein the disorder is late onset sepsis.

41. The method of claim 39 wherein gastrointestinal infection comprises infection with one or more of *Staphylococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., *Enterobacter* spp., *Klebsiella* spp., *Bacillus* spp., *Serratia* spp., *Candida* spp, Norwalk and other Noroviruses, *Campylobacter* spp, *Vibrio cholerae*,
5 *Bacteriodes* spp., Clostridia, Giardia.

42. A method comprising:

(a) measuring the level of at least one secretor antigen in a biological sample from the individual, and

(b) comparing the measured level of the at least one secretor antigen to a
10 predetermined value or a predetermined range of values,

(c) determining that the individual is at risk for necrotizing enterocolitis if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values; and

(d) taking steps to treat or reduce the risk for necrotizing enterocolitis if
15 the individual is determined to be at risk for necrotizing enterocolitis.

43. The method of claim 42 wherein step (d) comprises administering to the individual one or more of α 1,2 fucosyl glycans, probiotic organisms or prebiotics.

44. A method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:

(a) measuring the level of FUT2 protein or mRNA encoding FUT2 in a
20 biological sample from the patient;

(b) comparing the measured level of level of FUT2 protein or mRNA encoding FUT2 to a predetermined value or a predetermined range of values,

wherein the individual is at risk for necrotizing enterocolitis if the measured
25 level of FUT2 protein or mRNA encoding FUT2 is below a predetermined value or a predetermined range of values.

45. A method of identifying an individual at risk for necrotizing enterocolitis, the method comprising:

(a) providing a biological sample from the individual;

30

(b) determining whether the individual harbors a *FUT2* gene having genetic change that reduces the expression or activity of *FUT2*,
wherein the individual is at risk for necrotizing enterocolitis if the individual harbors a *FUT2* gene having genetic change that reduces the expression or activity of
5 *FUT2*.

46. A method of identifying an individual at risk for death, the method comprising:

(a) measuring the level of at least one secretor antigen in a biological sample from the individual, and

10 (b) comparing the measured level of the at least one secretor antigen to a predetermined value or a predetermined range of values,

wherein the individual is at risk for necrotizing enterocolitis if the measured level of the at least one secretor antigen differs from the predetermined value or is outside the predetermined range of values.

15 47. The method of claim 46 wherein the individual is an infant.

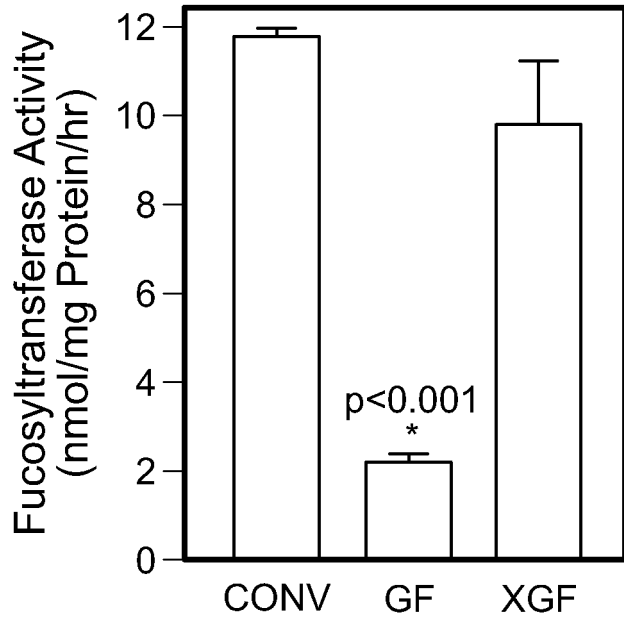


FIG. 1A

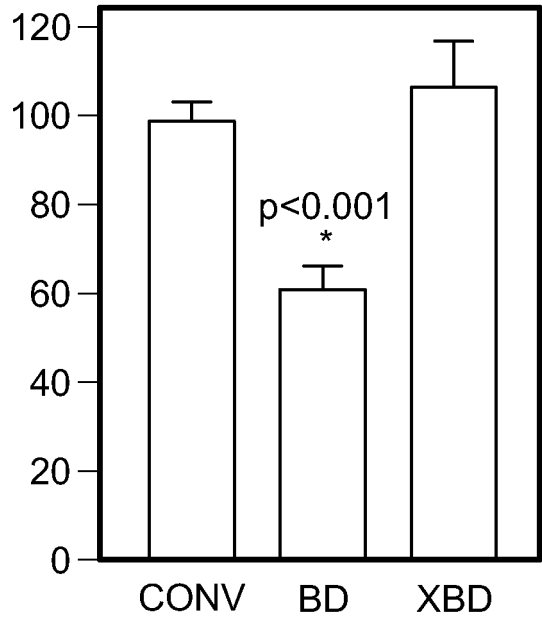


FIG. 1B

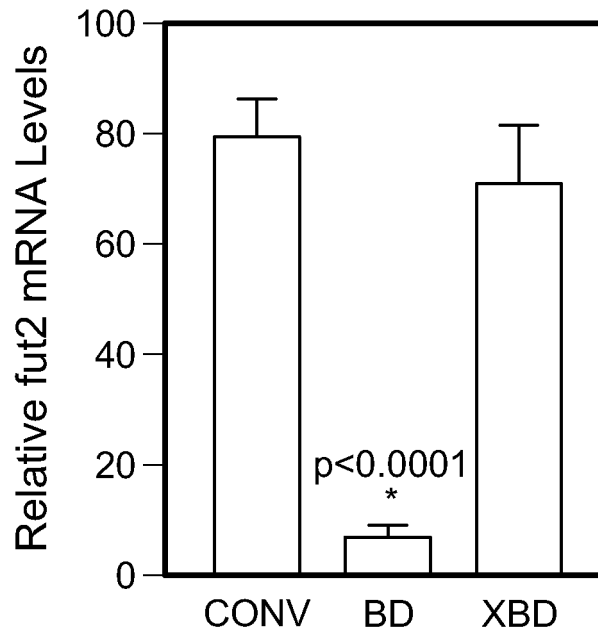


FIG. 1C

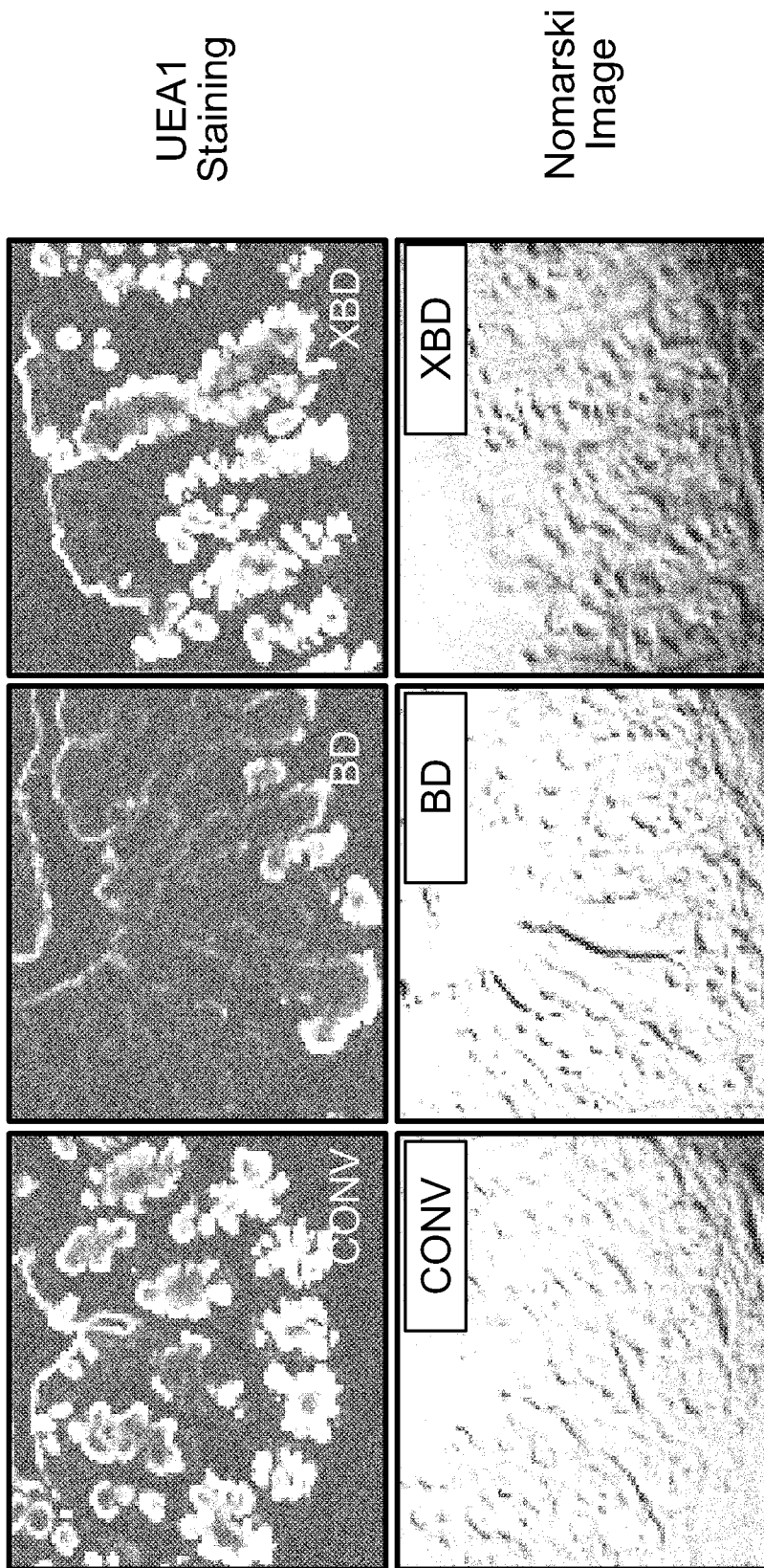


FIG. 1D

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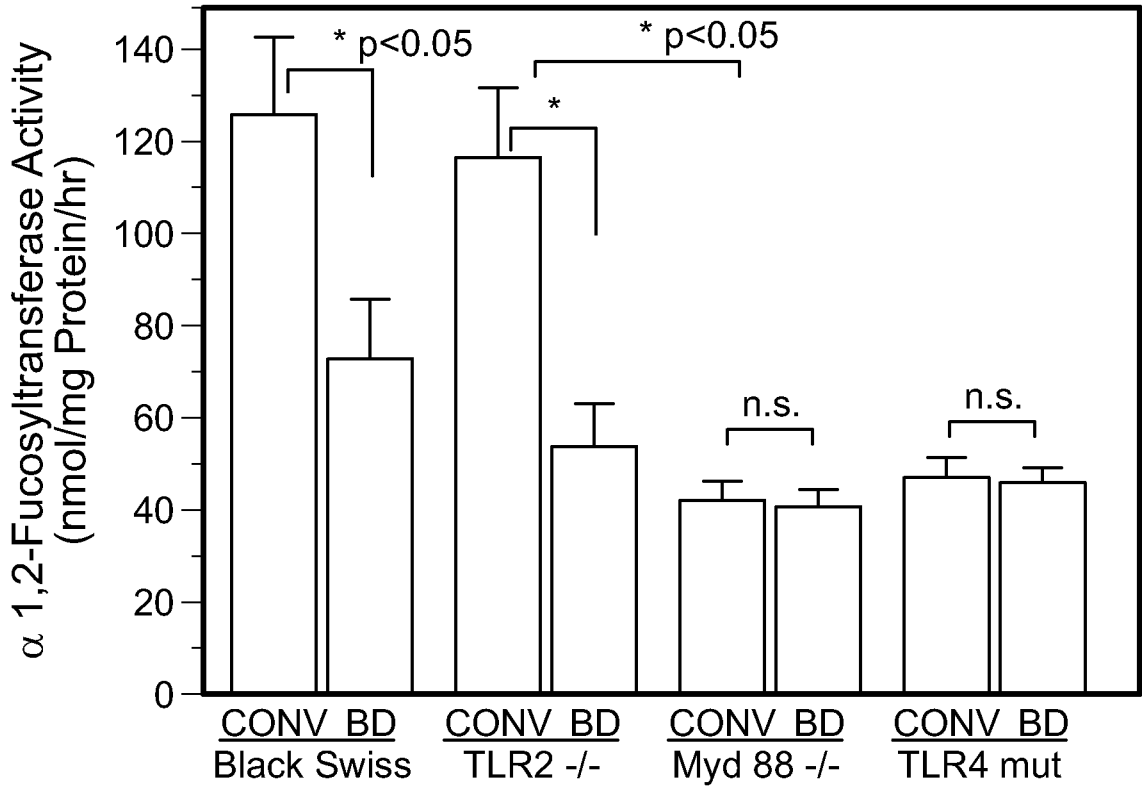


FIG. 2A

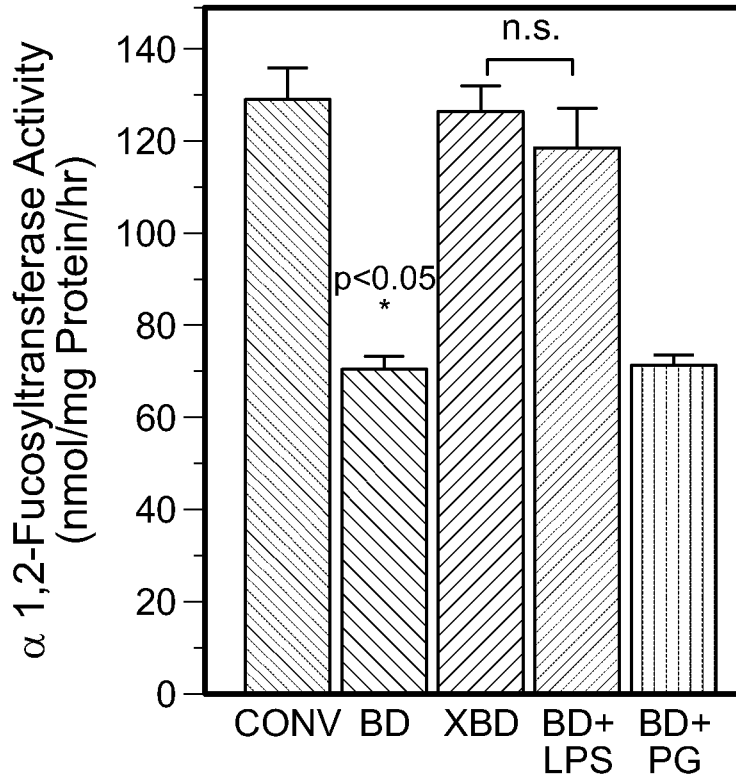


FIG. 2B

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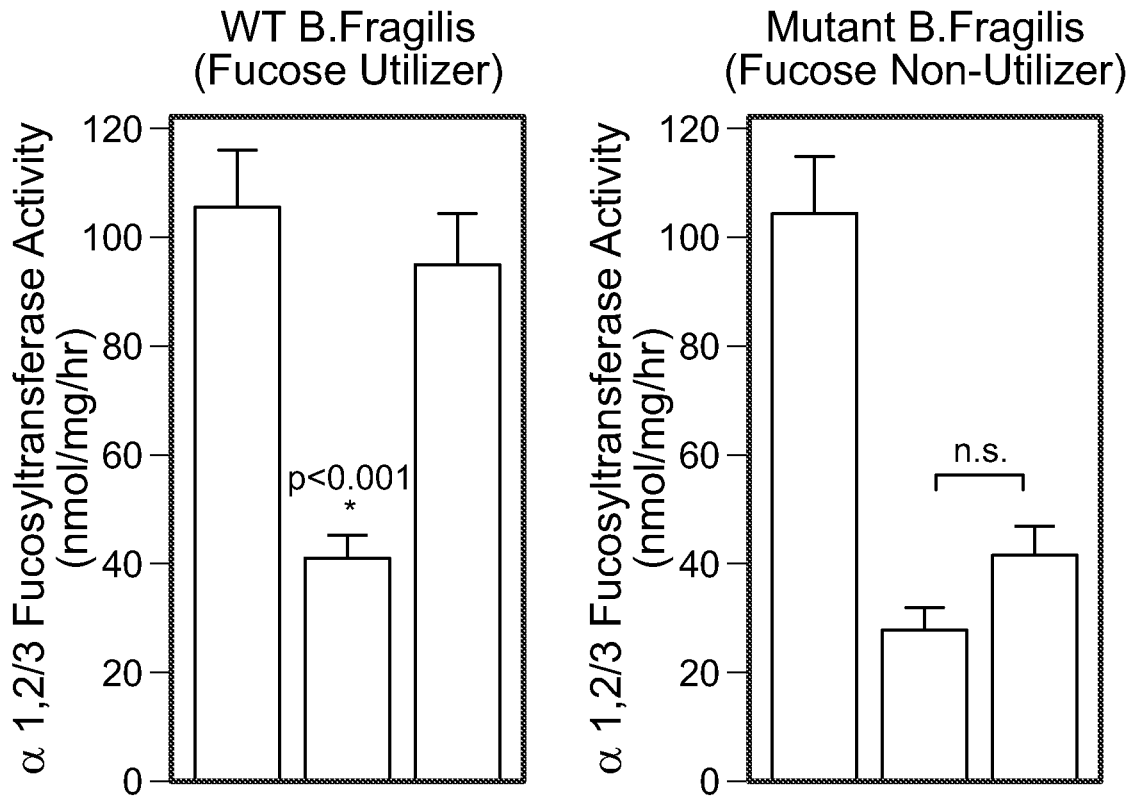


FIG. 3A

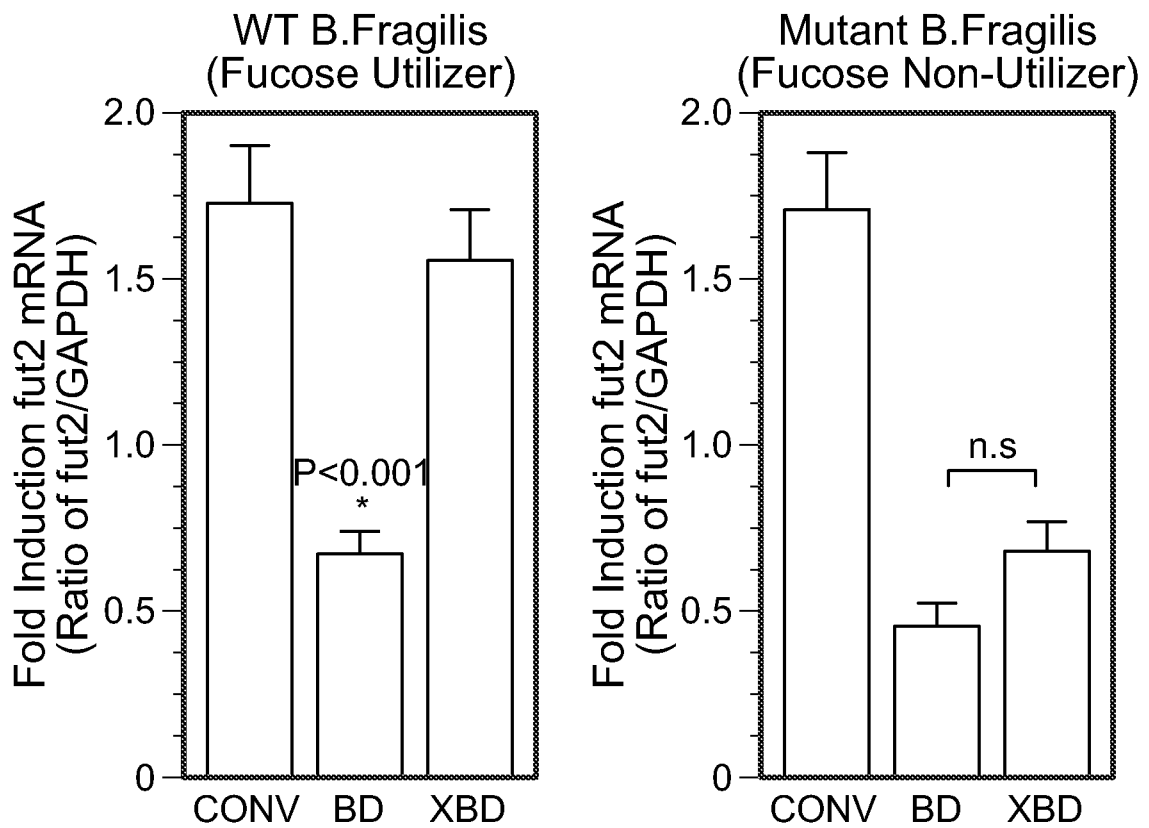
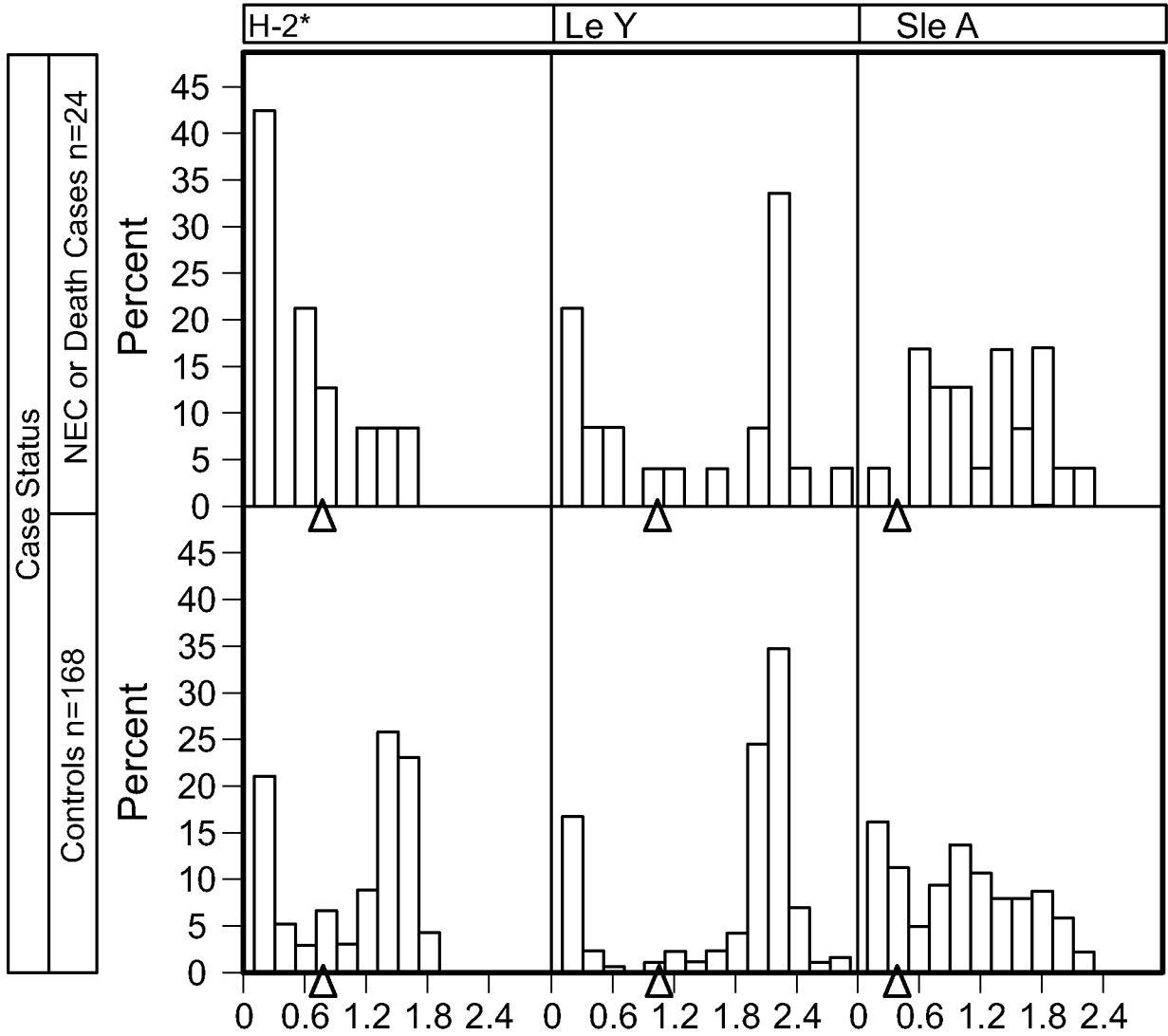


FIG. 3B

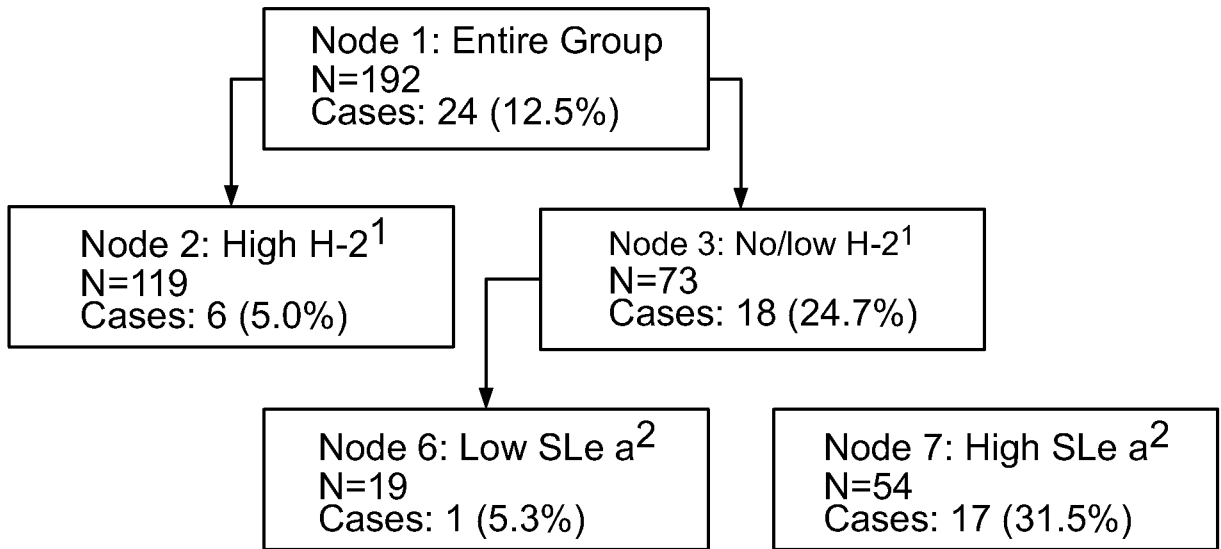


	H-2		Le Y		SLe A	
CART Cutpoint Δ	n	No. Cases (%)	n	No. Cases (%)	n	No. Cases (%)
High Risk Group	73	18 (24.7)	47	11 (23.4)	145	23 (15.9)
Low Risk Group	119	6 (5.0)	145	13 (9.0)	47	1 (2.1)
RR (95%CI) P-value	4.9 (2.0, 11.8) <0.0001		2.6 (1.3, 5.4) 0.009		7.5 (1.03, 53.7) 0.013	

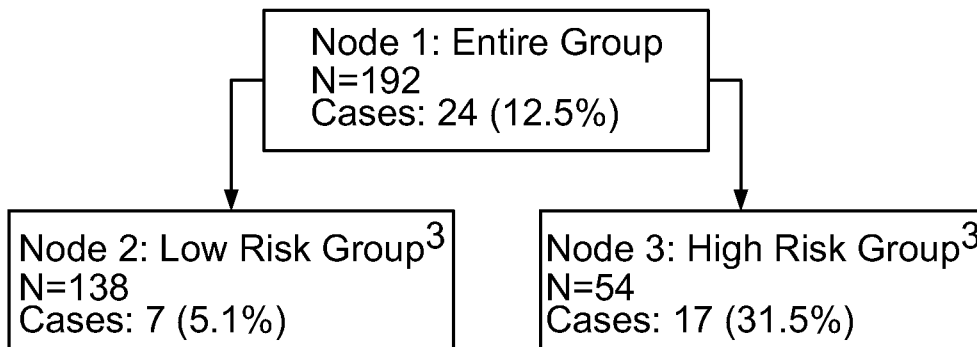
FIG. 4

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Model A. Tree Obtained from Entry of all Antigens as Categorical Variables into the Model



Model B. Tree Obtained from Entry of the 3 Final Nodes of Model A into a Single Model



¹Non-secretors and Low H-2 Secretors (Salivary H-2 O.D. ≤ 0.627) Form One Node; the High Secretors (Salivary H-2 O.D. > 0.627) Form the Other Node

²High SLe a Salivary Expression Defined as O.D.> 0.318; Low Defined as O.D. ≤ .318

³High Risk Group: Low to No H-2 (OD<0.627) and High Risk SLe a (OD>.318)

FIG. 5

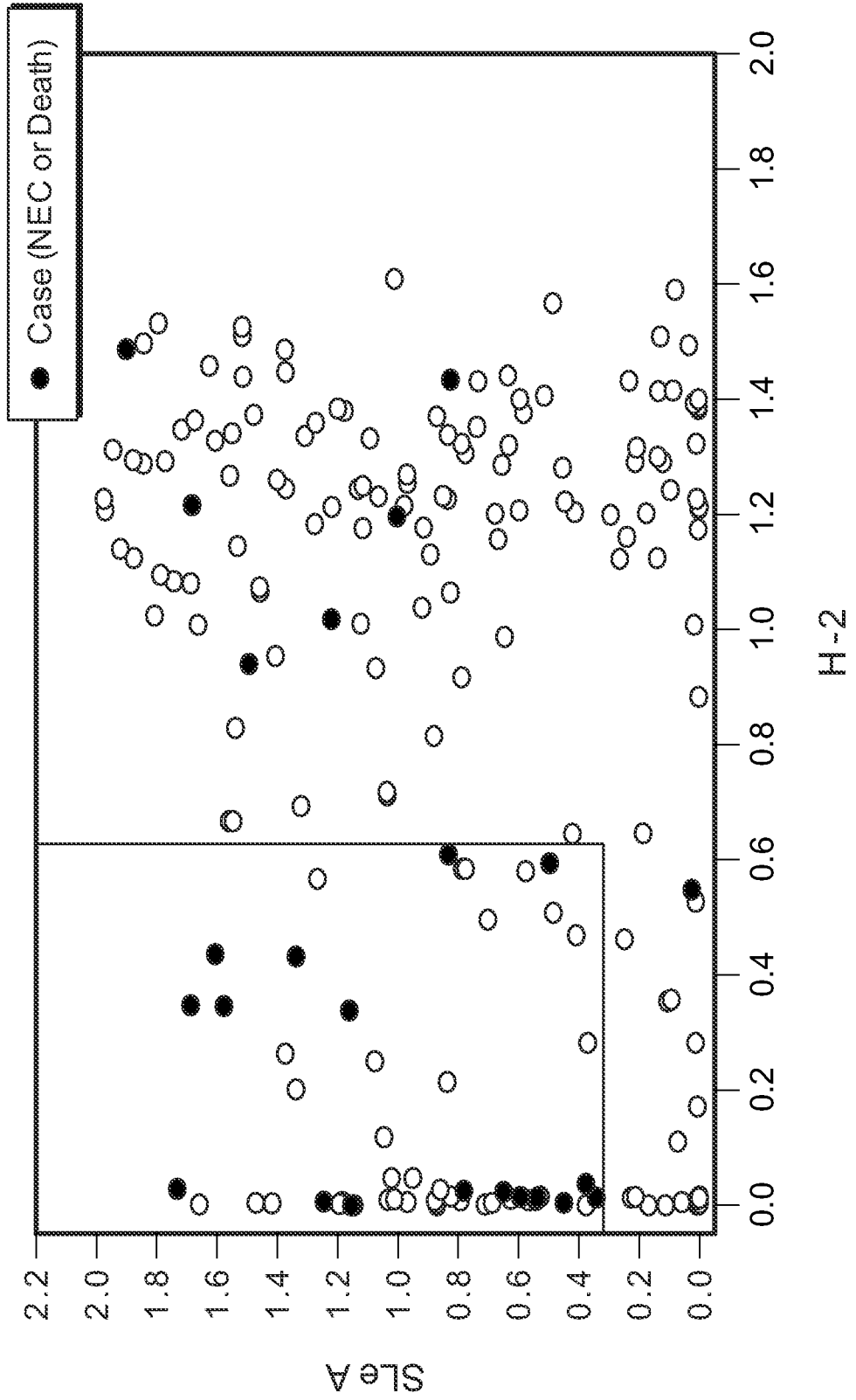


FIG. 6

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1 agtcaccgat gctggaaggg tttctttggc cctgagtgaa gagagacca gagggaacac
 61 tgaggtgcct gcccaaccac tctgtcccgg tttccttcag caggaccagg tgagagaagc
 121 catgctggtc gttcagatgc ctttctcett tcccatggcc cacttcatec tctttgtctt
 181 tacggtttcc actatatttc acgttcagca gcggctagcg aagattcaag ccatgtggga
 241 gttaccggtg cagataccag tgctagcctc aacatcaaag gcaactgggac ccagccagct
 301 caggggggatg tggacgatca atgcaatagg ccgcctgggg aaccagatgg gcgagtacgc
 361 cacactgtac gccctggcca agatgaacgg gcggcccgcc ttcaccccgg cccagatgca
 421 cagcaccctg gccccatct tcagaatcac cctgcccggg ctgcacagcg ccacggccag
 481 caggatcccc tggcagaact accacctgaa cgactggatg gaggaggaat accgccacat
 541 cccgggggag tacgtccgct tcaccggcta cccctgctcc tggaccttct accaccacct
 601 ccgccaggag atcctccagg agttcaccct gcacgaccac gtgcccggagg aggcccagaa
 661 gttcctgcgg ggcctgcagg tgaacgggag ccggcccggc acctttgtag gggtcctatgt
 721 tcgccgaggg gactatgtcc atgtcatgcc aaaagtgtgg aagggggtgg tggccgaccg
 781 gcgataccta cagcaggccc tggactggtt ccgagctcgc tacagctccc tcactctcgt
 841 ggtcaccagt aatggcatgg cctggtgtcg ggagaacatt gacacctccc acggtgatgt
 901 ggtgtttgct ggcgatggca ttgagggctc acctgccaaa gatthttgctc tactcacaca
 961 gtgtaaccac accatcatga ccattgggac gttcgggac tgggcccgat acctcacggg
 1021 cggagacacc atctacctgg ccaattacac cctccccgac tcccctttcc tcaaaatctt
 1081 taagccagag gcagccttcc tgccggagtg gacagggatt gccgcagacc tgtccccctt
 1141 actcaagcac taatgctggc ccattctttg agacctttc tcttctctg cctccctcaa
 1201 gatgagtgcc cgggcatgag aagcacatgg ttccatgagc aggaccatec tctcttctgt
 1261 gaagatgcgt tgggctgcaa gtaacagaaa tctcagtgaa cagtggcctg gcgtggtggc
 1321 tcatgcctgt aatgctcgca ctttgtgagg ccaggggtggg tggatcactt gaggtcagga
 1381 gttcaagact agcctggcca acatggtgaa accccatctc gactaaaaat acaaaaatta
 1441 gccaggcgtg gtggtgcaca cctgtaatcc cagctactcg ggaggctgag gcaagagaat
 1501 cacttgaacc caggaggcgg aggttgcagt gagccaagat ggtgccgctg cactccagtc
 1561 tgggtgacac agcaagactc catctcaaaa aaaaaaaaaa aaaaaagaaa agaaaaagaa
 1621 atgaatgggt tcaagacca taatcatgca taccacataa gaccagaagt ggcccaggtc
 1681 cagggtcagt taatttagcg gctccacaaa gtcacagtc acctgagctc catccatctt
 1741 cacatgctgt gctaccattt cttagctgta tcacccatg gtcccaaaag ggctgctaca
 1801 catccagcca tcacatgcag ataattcctt tcaaaaacag cagaaagagg ctcgttcttg
 1861 tcttggctcc ttttgaagaa tgaatgaaac cttcctaagc cttccagcaa tttcccccca
 1921 actccgatgg gtaggaattg tcacataccc atgtgaccgg ataggaggca aaagaaatga
 1981 gacttctggg attagtttag cctcagattc tgcagctgag aagttgatca gccacctctg
 2041 aaggacatgc agcttgacaga aaattagggt ggtgttacca aggtgaaaag gggaaatggc

FIG. 7A

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2101 tttagagtag acaacagaga tgccctgagg ggttgtgtag gttgttcact gcaggaagtc
2161 ccctgggtaa gaaggcaagt ggggtttaaa cagacccaca gtctactcat caaaccaggt
2221 gtccttggca ttgtgtccac ccagagagct cactgttttc ttttcttttt cttttctttt
2281 tttttttttt tttgagatgg agtcttgctg catccccag gctggagtgc agtggcatga
2341 tcttggetca ctgcagcctc cgcctcccag gttcaagcga ttctcctgcc tcagcctccc
2401 gagtagctag gattacaggt gcgtgccacc acgcccagct aatttttata tgtttagtgg
2461 aaatggagtt tcacatggt ggtcaggctg gtctcaaact cctgacctca tgatccgct
2521 tcctcggcct cccaaagtgc tgggattaca ggtgtagcc actgcgcccg gccctagagc
2581 tcaactgttt ctagttagtc catctggaag tggagccttt ttccagtttg cacaaatgtg
2641 ccatattggc ttgtagctgg catgcatcca agtccatagg tcctgcctct tcaatcctgg
2701 ctttctaggg cctgggatga tcattgctag aactgagaga ccagcctggc tcagtgaact
2761 tcagggcggt ccgttcattc tttcagtaaa tgtttgcagc acatgtgtta catgtcaggc
2821 agtgaaacc cccacagcag ccttcctct cagaggatac atttgtaacc attacacagt
2881 catcaaagga ataatttttt ttaatcacca gtgtgcatac agtcatggag ttgggtattc
2941 ccagctacca gggaggctga ggtgggagga ttgcttgatg ccaggagtta gggaatatag
3001 tgcaccgtga ttggacttgc gaatagccac tgcactgcgg cctggacgac gtagtgatac
3061 cctgactctt ataaataaat aatgaataa acacaattat gactttgcgg atggg

FIG. 7B

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1 mlvvqmpfsf pmahfilfvf tvstifhvqq rlakiqamwe lpvqipvlas tskalgpsql
61 rgmwtinaig rlgngmgeya tlyalakmng rpafipaqmh stlapifrit lpvlhsatas
121 ripwqnyhln dwmeeeyrhi pgeyvraftgy pcswtfyhh l rqeilqeftl hdhvreeaqk
181 flrglqvngs rpgtfvgvhv rrgdyvhvmp kwkqvadr rylqqaldwf rarysslifv
241 vtsngmawcr enidtshgdv vfagdiegs pakdfalltq cnhtimtigt fgiwaayltg
301 gdtiylanyt lpdspflkif kpeaaflpew tgiaadlsp lkh

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/75385

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - G01N 33/53 (2008.04) USPC - 435/7.25, 435/7.1 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) USPC - 435/7.25, 435/7.1 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 435/7.21 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WEST(DB=PGPB,USPT,USOC,EPAB,JPAB), Google Scholar(campylobacter "necrotizing enterocolitis", "necrotizing enterocolitis" "secretor antigen, "necrotizing enterocolitis" "secretory antigen", "necrotizing enterocolitis" secretor, "gastrointestinal disorder" secretor, "Helicobacter pylori" "necrotizing enterocolitis")		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BLACKWELL, The role of ABO blood groups and secretor status in host defences. FEMS Microbiology Immunology, 1989, vol 47, pp 341-350; pg 341, left col, para 1-3; right col, para 1; pg 342, Table 2; pg 345, section 5.	23-25, 34, 38-39, 41
Y		1-22, 26-33, 35-37, 40, 42-47
Y	HALLSTROM et al, Effects of mode of delivery and necrotising enterocolitis on the intestinal microflora in preterm infants. Eur J Clin Microbiol Infect Dis, 2004, vol 23, pp463-470; Abstract; pg 463, left col, para 3, ln 1-3, para 4, ln 1	1-22, 30-33, 35-37, 42-47
Y	THOMSSON et al, MUC5B glycosylation in human saliva reflects blood group and secretor status. Glycobiology 6 April 2005, vol 15, no 8, pp 791-804; Abstract; pg 791, right col, para 2, ln 22-30; pg 798, right col, para 3, ln 30-33; pg 800, left col, para 1, ln 4-7, para 2, ; right col, para 1	4-7, 11-12, 16, 18-22, 26-33, 35-37, 44
Y	SAIMAN et al, Risk factors for candidemia in Neonatal Intensive Care Unit patients. Pediatric Infectious Disease Journal, April 2000, vol 19, no 4, pp 319-324; Abstract only	40
Y	HENEGHAN et al, Effect of host Lewis and ABO blood group antigen expression on Helicobacter pylori colonisation density and the consequent inflammatory response. FEMS Immunology and Medical Microbiology 1998, vol 20, pp 257-266; pg 263, left col, para 3; right col, para 1, ln 1-4	15, 37
Y	SNELLING, Effects of probiotics on the gastrointestinal tract. Gastrointestinal infections. Current Opinion in Infectious Diseases, October 2005, vol 18, no 5, pp 420-426	43
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 3 December 2008 (03.12.2008)		Date of mailing of the international search report 29 DEC 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/75385

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IKEHARA et al, Polymorphisms of Two Fucosyltransferase Genes (Lewis and Secretor Genes) Involving Type I Lewis Antigens Are Associated with the Presence of Anti-Helicobacter pylori IgG Antibody. Cancer Epidemiology, Biomarkers & Prevention, September 2001, vol 10, pp 971-977; pg 972, left col, para 1; pg 972-973, section entitled "Determination of Se and Le Genotype."	45

专利名称(译)	使用分泌者，刘易斯和唾液酸抗原水平作为疾病的预测因子		
公开(公告)号	EP2185931A1	公开(公告)日	2010-05-19
申请号	EP2008799226	申请日	2008-09-05
[标]申请(专利权)人(译)	儿童医院医疗中心 通用医疗公司 INST国立科学城MEDICASyNUTRICION		
申请(专利权)人(译)	儿童医院医学中心 总医院CORPORATION 国家研究所科学城MEDICASyNUTRICION		
当前申请(专利权)人(译)	儿童医院医学中心 总医院CORPORATION 国家研究所科学城MEDICASyNUTRICION		
[标]发明人	MORROW ARDYTHE L RUIZ PALACIOS GUILLERMO M NEWBURG DAVID S		
发明人	MORROW, ARDYTHE L. RUIZ-PALACIOS, GUILLERMO M. NEWBURG, DAVID S.		
IPC分类号	G01N33/53 A23L33/00 G01N33/68 G01N33/80		
CPC分类号	A61K31/702 A61K31/7028 A61K31/715 A61K38/02 A61P1/00 A61P1/04 A61P31/00 A61P31/04 A61P43/00 G01N33/6893 G01N33/80 G01N2800/067 Y02A50/471 Y02A50/473 Y02A50/491 A61K35 /741 A61K35/745 A61K35/747 A61K36/06 A61K45/06		
优先权	60/970902 2007-09-07 US		
其他公开文献	EP2185931A4 EP2185931B1		
外部链接	Espacenet		

摘要(译)

可以通过测量来自个体的生物样品中至少一种分泌者抗原的水平，并将所测量的至少一种分泌者抗原的水平与预定值或预定范围进行比较，来鉴定处于坏死性小肠结肠炎和相关病症风险中的个体的值。可以测量的分泌者抗原是：H-1，H-2，Lewisb和Lewisy抗原及其衍生物（例如，唾液酸化形式的Lewis a，Lewis x，Lewis b，Lewis y；H-1，H-2，Lewis a，Lewis x，Lewis b或Lewis y）。