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Phenotyping of Lewis and secretor HBGA from saliva and detection of new *FUT2* gene SNPs from young children from the Amazon presenting acute gastroenteritis and respiratory infection

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ABSTRACT

The Histo-blood group antigens (HBGA) are host genetic factors associated with susceptibility to rotavirus (RV) and human norovirus (HuNoV), the major etiological agents of viral acute gastroenteritis (AGE) worldwide. The *FUT2* gene expressing the alpha-1, 2-L- fucosyltransferase enzyme is important for gut HBGA expression, and also provides a composition of the phenotypic profile achieved through mutations occurring in populations with different evolutionary histories; as such, it can be considered a genetic population marker. In this study, Lewis and secretor HBGA phenotyping was performed using 352 saliva samples collected from children between three months and five years old born in the Amazon (Brazil, Venezuela and English Guyana) presenting AGE or acute respiratory infection (ARI), the latter considered as control samples. The total of children phenotyped as secretors was 323, corresponding to 91.80%. From these, 207 (58.80%) had a Le (a + b+) profile. The HBGA profiles were equally found in children with AGE as well as with ARI. The rs1047781 of the *FUT2* gene was not detected in DNA from saliva cells with a Le (a+b+) profile. However, mutations not yet described in the *FUT2* gene were observed: missense 325A > T, 501C > T, 585C > T, 855A > T and missense substitutions 327C > T [S (Ser) > C (Cys)], 446 T > C [L(Leu) > P(Pro)], 723C > A [N(Asn) > K(Lys)], 724A > T [I(Ile) > F(Phe)], 736C > A [H(His) > N(Asn)]. The SNP distribution in the *FUT2* gene of the analyzed samples was very similar to that described in Asian populations, including indigenous tribes.

1. Introduction

According to the World Health Organization (WHO), acute gastroenteritis (AGE) is the second leading cause of death in children under five years, provoking around 525,000 deaths each year (WHO, 2017). Pathogens including parasites and bacteria are responsible for AGE, but viruses such as rotavirus A (RVA) and human norovirus (HuNoV), are also important etiological agents (Monedero et al., 2018; Munnink and van der Hoek, 2016). The Histo-blood group antigens (HBGA) comprising AB0, Hh, secretor and Lewis systems are important factors in host susceptibility to enteropathogenic infections (Cooling, 2015; Almand et al., 2017; Ramani et al., 2016). The HBGAs are not only expressed in red blood cells (RBC), but are also present in many tissues, such as the intestine, and bodily secretions such as saliva (Daniels, 2013). The presence of H antigens (secretion) in saliva is governed by the *FUT2* gene encoding an alpha-1, 2-L- fucosyltransferase enzyme.

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Individuals who secrete A or B antigens also secrete H antigens. Non-secretors have a non-functional FUT enzyme, and so do not secrete those antigens even though they are expressed in their RBC.

The Lewis system consists of two major antigens, and three common phenotypes: Le(a − b−), Le(a + b−), and Le(a − b+). The Lewis determinants are oligosaccharides which are synthesized by the sequential addition of sugar units to oligosaccharide chains by fucosyltransferases previously described and encoded by *FUT1* and *FUT2* genes; and by alpha-(1,3/1,4)-fucosyltransferases encoded by the *FUT3* (Lewis) gene. The type 2 oligosaccharide chains are expressed mainly in erythrocytes and in vascular endothelial cells, while the type 1 oligosaccharide chains are expressed in the digestive and respiratory tracts and in secretions (Koda et al., 2001). The *FUT2* gene has significant polymorphism with ethnic specificity and several single nucleotide polymorphisms (SNPs) have been reported (Ferrer-Admetlla et al., 2009). The main rs601338 was described by Kelly et al. (1995) conferring a non-secretory phenotype for the individuals carrying the nonsense mutation. The rs1047781SNP corresponds to a missense mutation commonly found in Asian populations (Cooling, 2015) and homozygous carriers of this mutation are considered weak secretors, leading to low levels of alpha-1, 2-L- fucosyltransferase enzymes and the Le(a + b+) phenotype. A multiplicity of research approaches including genetic analyses have been used to study the ancient settlement of individuals in the American continent (Ruiz-Linares, 2015). The major question is the route of entry of the initial settlers. This central question has been approached with variable degrees of success using various types of genetic markers examined in native populations. The first studies used information from blood groups and proteins (Cavalli-Sforza et al., 1994), after Chang et al. (2002) proposed that the mutations or polymorphisms of the *FUT2* gene would be important markers and could be useful for investigating population genetics. There are few studies in Brazil which highlight the *FUT2* gene as a genetic marker, despite being a country of great proportions and with a high ethnical diversity; one of these few was presented by Vicentini et al., 2013. This study involved a black population from Southeastern Brazil, descendants of former African slaves, who were infected with HuNoV; living in semi-isolated communities called “Quilombola Communities”.

The aim of this study was to characterize the Lewis and secretor HBGA from saliva obtained from young children with AGE and ARI (control population) living in the Amazon. We also want to elucidate the hypothesis that the population of indigenous ethnicity presents genetic HBGA markers related with the Asian population by comparing our data from *FUT2* gene SNPs found in the saliva of these young children from the Amazon with those already detected in Asian populations.

2. Material and methods

2.1. Ethics statement and study population

This study was approved by the Federal University of Roraima Ethical Research Committee (CEP N°: 1.333.480 from November 23, 2015), whose main objective is to identify enteropathogens causing gastroenteritis in children living in the Amazon. The Amazon is comprised of the International Amazon (Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, English Guyana, French Guyana and Suriname), the Northern region and the Brazilian Legal Amazon or Brazilian Amazon (Fig. 1). All children included here received emergency medical assistance when attended at Santo Antonio Hospital in the city of Roraima. The Santo Antonio Hospital exclusively attended children up to 12 years of age from the city of Boa Vista (RR state) or from demarcated indigenous areas, including the Amazon rainforest. Different ethnic groups live in the demarcated indigenous areas, but the ethnic group in which the children from this study live, declared by their parents, does not necessarily represent their pure ethnicity. However, there is a real ancestrality denoted by physical and cultural aspects. The clinical samples were collected after an informed consent form was signed by the parents or guardians of the children attended. Two groups of children up to five years old presenting acute gastroenteritis (AGE) or acute respiratory infection (ARI) were included in this study, the latter as a control population.

2.2. Collection and processing of saliva samples

From January 2016 to April 2017, saliva samples and epithelial cells were collected from each child using a CHEMBIO® saliva oral swab device, reference number 25–1616 (Medford, NY, USA). The tubes containing collected saliva samples were kept at −20 °C until the moment of processing. For processing, 1.2 mL of phosphate buffered saline (PBS), pH 7.2, were added to each tube containing the collected saliva, followed by a vigorous vortex, and the total volume (about 1 mL) was then transferred to a new tube. The tubes containing the 1:5 diluted saliva were maintained at -20 °C and used in Enzyme Immunoassay tests (EIA) and total genomic DNA extraction.

2.3. Enzyme immunoassay test to detect A, B, Le^a and Le^b antigens

This EIA was performed as previously described by Nordgren et al. (2014) with adjustments. Each saliva sample previously processed was diluted 1:100 in 100 μL of 0.1 M pH 9,6 carbonate bicarbonate buffer and used to coat each well of a NUNC plate 96F (Maxisorp®; Thermo Fisher Scientific, Roskilde, Denmark). The plate was initially incubated for 2 h at 37 °C, followed by overnight incubation at 4 °C, and the next day the plate was washed 4 times with PBST (PBS containing 0.05% of tween 20). For each group of four wells coated with the same saliva sample, 100 μL of one of the four mAb were applied, a different one in each well, those being: 1) -anti-A (ABO1 clone 9113D10); 2) -anti-B (ABO2 clone 9621A8), both from Diagast®, Loos Cedex, France; 3) -anti-Le^a (LE1 clone LEA2, Seraclone); 4) -anti-Le^b (LE2 clone LM129/181, DiaClon), both from Bio-Rad Laboratories®, Hercules, CA. The anti-A, anti-Le^a and anti-Le^b mAbs were diluted 1:5000 and the anti-B mAb were diluted 1:1000 in PBST containing 5% of fetal bovine serum (FBS). The plate was incubated for 1 h and 30 min at 37 °C, washed as described previously, then a 1:3000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (heavy plus light chain) (Promega Corporation®, Madison, USA) was added, and the plate was incubated for another 1 h and 30 min at 37 °C. With the plate washed again four times, the reaction was developed using 3,3′,5,5′-Tetramethylbenzidine (TMB) Substrate Systems (Sigma-Aldrich®, St.Louis, USA) and stopped by addition of 2 M H_2SO_4. For each plate containing saliva samples, the following control saliva samples were included: *profile 1* = Le (a-b-) *profile 2* = Le (a-b+) and *profile 3* = Le (a + b-). These control saliva samples were obtained from adult donors born in the state of Rio de Janeiro, Brazil (The Oswaldo Cruz Foundation Ethical Research Committee - CEP/Fiocruz number 311/2006). All the saliva samples were evaluated in duplicate and the plate was read at 450 nm in a spectrophotometer. The cutoff value was twice the mean level of blank control (PBST containing 5% of FBS) and conjugate control (horseradish peroxidase-conjugated goat anti-mouse IgG diluted in PBST containing 5% of FBS).

2.4. Enzyme immunoassay to detect *Fucα1-2Gal-R*

Le (a-b-) saliva samples (did not react to either mAb anti-Le^a or Le^b) were tested for detection of *Fucα1-2Gal-R*, using a lectin based EIA, according to Nordgren et al. (2014) and using saliva samples diluted 1:500. All the saliva samples were evaluated in duplicate, using control saliva samples and cut-off values as described.

2.5. Titration of Le^a Seraclone antibody

A titration of LE1 Le^a mAb was performed using 1:500, 1:1000,

Fig. 1. Map of the Amazon obtained from http://pportalparamazonia.blogspot.com/2016/01/amazonia-legal-e-internacional.html with minor modifications. -Surrounded by a yellow line: Northern region; -Light green: Brazilian Legal Amazon or Brazilian Amazon including the Brazilian states of Mato Grosso (MT), Tocantins (TO) and half of Maranhão (MA); −Dark green: International Amazon, including Brazil (60% of this area), Bolivia, Peru, Ecuador, Colombia, Venezuela, English Guyana, French Guyana and Suriname. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1:2000, 1:3000, 1:4000, 1:5000 and 1:10000 dilutions. The Le^b, Le^a, and control saliva samples as described were used, as well as six samples from children from the Amazon. All the saliva samples were evaluated in duplicate and the plate was read at 450 nm in a spectrophotometer (BioTek, Vermont, USA). The cutoff value was twice the mean level of the blank and conjugate control samples.

2.6. Total genomic DNA extraction from saliva

Total genomic DNA was extracted from a volume of 200 µL from each saliva sample collected from children phenotyped as Le(a + b+) and control saliva samples as described in item 2.3. The original Boom et al. (1990) method was used with minor modifications (da Silva et al., 2015). Five microliters of the total genomic DNA eluted with 50 µL of TE buffer (10 mM tris, 1 mM EDTA, pH 8.0) were used in the touchdown PCR (TD-PCR).

2.7. Touchdown PCR and nucleotide sequencing

The PCR amplicons were obtained by TD-PCR from DNA extracted from children phenotyped as Le(a + b+) and control saliva samples, using primers as described by Bucardo et al. (2009) for the coding and 3′ untranslated regions of the *FUT2* gene. The Platinum Taq DNA Polymerase High Fidelity enzyme was used for amplification according to the manufacturer's recommendations (Thermo Fisher Scientific®, Waltham, MA, USA). TD-PCR was carried out according to the touchdown parameters: 65 °C -55 °C (annealing), 26 + 19 (cycles) and 68 °C (elongation). The primers were also used to detect the 385A > T SNP and other SNPs by nucleotide sequencing. After the TD-PCR, the amplicons were purified using Wizard PCR Prep Columns® according to the manufacturer's recommendations (Promega®, Madison, WA, USA) and sequenced using an ABI Prism BigDye Terminator 3.1 Cycle Sequencing Ready Reaction Kit® and ABI Prism 3730 Genetic Analyser® (Applied Biosystems®, Foster City, CA, USA). The chromatograms and *FUT2* gene encoding nucleotide sequences were analyzed using the free tracer viewer Chromas 2.4® (Technelysium Pty Ltd.®, South Brisbane, QLD, AUS). Nucleotide and amino acid multiple alignments were done using the Mega -Molecular Evolutionary Genetic Analysis version 7.0 software (Kumar et al., 2016) on results from saliva samples, control samples and the U17894.1 reference sequence described by Kelly et al. (1995), obtained from the GenBank database of the National Center for Biotechnology Information (NCBI). The *FUT2* gene SNPs identified so far were accessed through the NCBI database of SNPs (https://www.ncbi.nlm.nih.gov/snp).

2.8. Statistical analysis of data

We considered the 2010 census of the population of infants aged up to five years in the state of Roraima performed by the Brazilian Institute of Geography and Statistics (IBGE), which corresponded to 47,603 children under the age of five. The sample estimate for this population size, with a margin of error (ME) of 5.2%, was approximately 353 children, from which 352 saliva samples were analyzed in this study. A 95% confidence level was assumed. Bioestat software version 5.3 (www.mamiraua.org.br) was used for statistical analysis.

Table 1
Data from the study population and number of saliva samples collected: 1. Municipality name is the region of origin of each child attended; 2. N = Total number of saliva samples collected; (%) = per cent of the total number of total saliva samples collected; 3. Ethnic group corresponds to the group of individuals that lives in that particular region to which the children belong to.

Municipality name[a]	N (%)	Ethnic group
Alto Alegre	40(11.36)	Macuxi, Wapixana and Yanomami
Amajari	20(5.68)	Macuxi, Wapixana and Yanomami
Amazonas (AM)	7(1.99)	Yanomami
Boa Vista	179(50.85)	Macuxi, Wapixana and Taurepangue
Bonfim	10(2.84)	Macuxi and Wapixana
Cantá	10(2.84)	Wapixana
Caracaraí	4(1.14)	Yanomami
Caroebe	1(0.28)	Wai-Wai
English Guyana	4(1.14)	Macuxi and Wapixana
Iracema	4(1.14)	Yanomami
Mucajaí	8(2.27)	Yanomami
Normandia	7(1.99)	Macuxi,Wapixana and Ingarincó
Pacaraima	8(2.27)	Macuxi,Wapixana and Taurepangue
Rorainópolis	6(1.70)	Waimirim and Afroari
São Luiz do Anauá	1(0.28)	Wai-Wai
Uiramutã	29(8.23)	Macuxi,Wapixana and Ingarincó
Venezuela	14(4.00)	Taurepangue (Pemon[b]) and Macuxi
Total	352(100.00)	

[a] All the municipalities are located in the state of Roraima(RR), except Amazonas that corresponds to the state of Amazonas (AM) and the countries English Guyana and Venezuela.
[b] The Taurepang self-designate Pemon, a term that means "people".

3. Results

Saliva samples were collected from 352 children (one sample/child) aged between three months and five years old, these being 192 boys and 160 girls. Two hundred and thirty-two children (66%) presented AGE and 120 (34%) presented ARI. Table 1 shows the region of origin of each child attended, number of samples collected and their ethnic group.

3.1. HBGA phenotype detected in saliva samples from young Amazonian children

A quality verification step of the mAb used to determine the HBGA phenotype was performed with a small number of saliva samples (n = 60), evaluated by EIA to detect A, B, Le^a and Le^b antigens exactly as described by Nordgren et al. (2014), including the dilution of mAb as defined by those authors. The positivity profile for the A, B, AB or 0 antigens in the saliva from the children in this study was as follows: 0 = 221 (63%); A = 65 (18%); B = 15 (4%) and AB = 51 (15%). Most of the 207 samples phenotyped as Le (a + b+) were phenotyped as "0". > 50% of the saliva samples presented high absorbance values for anti-Le^a and anti-Le^b mAbs simultaneously. To check for the possibility of cross reactivity between the anti-Le^a and anti-Le^b mAb, a titration of the anti-Le^a mAb was performed (Seraclone®). Saliva controls (profile 1, 2 and 3) and anti-Le^b mAb were included in the titration. The endpoint titer was 1:5000 (Fig. 2).

Three hundred fifty-two saliva samples were evaluated to define the Lewis and secretor HBGA profile as shown in Table 2. Remarkably, 207 saliva samples (58.80%) were phenotyped as Le(a + b+). Seventy-three saliva samples (20.60%) were phenotyped as Le(a-b+) and eighteen (5.0%) as Le(a + b-). The saliva samples collected from fifty-five (15.60%) children did not react with anti-Le^a or Le^b (a-b-) mAb and were considered undetermined concerning the secretor phenotype and Lewis negative.

To define the total of children with a secretor profile, we performed the EIA test to detect *Fucα1-2Gal-R* with the fifty-five undetermined saliva samples (Table 2. Eleven saliva samples presented absorbance value below cutoff values (negative samples) and 26 samples above

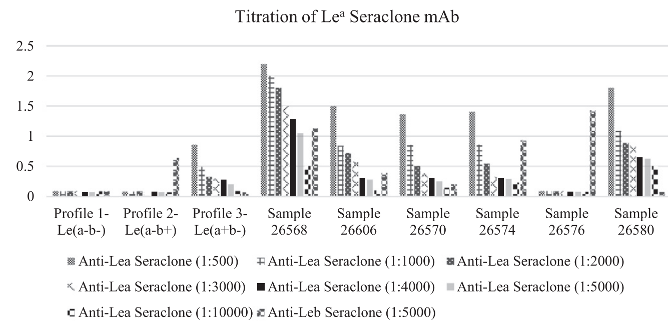

Fig. 2. Graphic of the anti-Le mAb titration(a): The graphic demonstrates the titration result of the LE1 anti- Le(a) mAb from Seraclone (Bio-Rad Laboratories, Hercules, CA.). The "y" axis corresponds to absorbance values for each sample evaluated with a different dilution of anti-Le(a) mAb and anti-Le (b) mAb (the anti-Le(a) mAb was included as a control antibody). Positive samples are presenting absorbance values above 0,190, according to the cutoff test.

Table 2
Final result of the HBGA phenotyping: Secretor phenotype results defined by detection of the *Fucα1-2Gal-R* in the 352 saliva samples collected from children from the Amazon. This Table also shows the secretor and Lewis HBGA phenotypes for each group of saliva samples collected from children with AGE or ARI. A, B or 0 antigens were detected by EIA in saliva.

Secretor and non-secretor profile	N total (%) samples collected from children with		N total (%) profile
	AGE	ARI	
Le(a + b +) secretor	135(58.10)	72(60.00)	207 (58.80)
Le(a-b +) secretor	49(21.10)	23 (19,20)	72 (20.50)
Le(a-b-) secretor	31(13.30)	13(10,80)	44(12.50)
Le(a + b-) non-secretor	9(4.00)	9(7,50)	18(5.10)
Le(a-b-) non-secretor	8 (3.50)	3(2,50)	11(3.10)
Total	nnnnn(100.00)	120 (100.00)	352(100.00)

(positive samples). Samples with absorbance values considered borderline (10% below or above cut off values) were re-tested. At the end, a total of 323 (91.80%) secretor children (including samples phenotyped as Se^w) from the Amazon and 29 (8.2%) non-secretors were found.

3.2. FUT2 SNPs detected in young Amazonian children

From the 207 saliva samples collected from children phenotyped as Le(a + b+), 166 continuous sequences (contigs) of the coding region and the 3′ untranslated regions of the *FUT2* gene were obtained by TD-PCR (80.20%). Table 3 shows all the SNPs detected including some not yet described for the *FUT2* gene (written in bold). The SNPs were classified by frequency of detected SNPs in low, medium and high; according, respectively, to the occurrence of the SNPs in ≥1 to ≤10%, ≥11 to ≤60% and ≥ 61 to ≤93% of DNA sequences from Le (a + b+) children. This criterion shows that the rs281377 was detected in DNA from all children phenotyped as Le (a + b+), except in children from the Iracema Municipality. The Le (a + b+) phenotype and *FUT2* gene SNPs were distributed amongst the municipalities together with other Lewis and secretor HBGA phenotypes, except for Iracema, from which all four saliva samples analyzed were phenotyped as Le (a + b+) (Table 1). Two SNPs in the 3′ untranslated region of the *FUT2* gene were detected with high frequency in the DNA sequences analyzed but not in the rs485073 and rs603985 control samples. However, the Iracema municipality did not present the rs485073 (Table 3). The rs1047781 SNP was not detected in any of the DNA sequences analyzed from children phenotyped as Le (a + b+).

Table 3
SNPs detected in the DNA sequences from children phenotyped as Le(a+b+): The municipality of the children is indicated, as well as the substitution of the nucleotide (SNP), amino acid (AA) or no AA (None) for the indicated position. Letters in bold highlight the SNPs not yet described (new) in the *FUT2* gene. The table presents the Blood group antigen Gene MUTation (BGMUT) database (*www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut*) and NCBI-SNP (within parentheses) nomenclatures for the SNP position. The substitutions are indicated using the NCBI-SNP nomenclature.

Position and substitution		Frequency detected in DNA sequences from Le(a + b+) secretor children from different municipalities (high, medium and low)
SNP	AA	
40 A > G (rs1800021)	I(Ile) > V(Val)	(Low) Boa Vista
113 C > T (rs114018037)	A(Ala) > V(Val)	(Low) Boa Vista
216 C > T (rs681343)	None	(High) Boa Vista, Iracema
325 A > T (new)	**None**	**(High) Boa Vista Iracema, English Guyana and Venezuela**
327 C > T (new)	**S(Ser) > C(Cys)**	**(High) Boa Vista Iracema, English Guyana and Venezuela**
357 C > T (rs281377)	None	(High) All municipalities, Venezuela and English Guyana, except from Iracema
446 T > C (new)	**L(Leu) > P(Pro)**	**(Low) Boa Vista, Normandia**
480 C > T (rs1800027)	None	(Low) Boa Vista
501 C > T (new)	**None**	**(Low) Normandia**
519 C > A (rs748593261)	**None**	**(Low) Boa Vista**
585 C > T (new)	**None**	**(Low) Boa Vista**
685 G > T (rs375360260)	V(Val) > L(Leu)	(High) Boa Vista
723 C > A (new)	**N(Asn) > K(Lys)**	**(High) Iracema and Boa Vista**
724 A > T (new)	**I(Ile) > F(Phe)**	**(High) Iracema and Boa Vista**
736 C > A (new)	**H(His) > N(Asn)**	**(Low) Alto Alegre**
739 G > A (rs602662)	G(Gly) > S(Ser)	(Low) Iracema
855 A > T (new)	**None**	**(Medium) Venezuela**
960 A > G (rs485186)	None	(Medium) Boa Vista, Normandia
1009 A > G (rs485073)	None	(High) All municipalities except from Iracema
1011 T > C (rs603985)	None	(High) All municipalities

3.3. *FUT2 gene SNPs from the saliva of young Amazonian children as population markers*

Comparing Quilombola descendants with the children studied, only the rs281377 was common to both groups. On the other hand, the rs281377, rs1800027, rs375360260 and rs602662 located in the coding region and the rs485073 and rs603985 located in the 3′ untranslated region of the *FUT2* gene, were both common to Asian individuals and children from the Amazon.

4. Discussion

HBGA can play a direct role in infection by serving as receptors and/or coreceptors for microorganisms, parasites, and viruses. Therefore, it is important to study the HBGA profiles in populations (Monedero et al., 2018; Cooling, 2015). The reasons why the differential distributions of HBGA phenotypes occurs at a populational level are not fully understood but it is believed that selective pressure imposed by disease-causing microorganisms contributed to this process (De Mattos, 2016). Children from the Amazon presenting AGE between 3 months and 5 years old were enrolled in this phenotyping and children presenting ARI were considered as control. The HBGA profile detected was similar in both groups. AGE is caused by a variety of pathogens including parasites, bacteria, and enteric viruses. Lewis and secretor HBGA antigens are host susceptibility factors for infection by HuNoVs and RVA since these antigens serve as a viral attachment factor in the gastrointestinal tract. The association of HBGA with ARI is restricted to some initial reports as reviewed by Cooling (2015). So far, there have been no reports strongly correlating HBGA to a viral agent for ARI. However, it is important to consider the high percentage of young children presenting an ARI diagnosis and a Le (a + b+) profile. The similarity in the HBGA profile detected between the groups show the importance of defining the specific pathogen agents responsible for each one of the infectious diseases (Thorne et al., 2018).

The Le(a + b+) HBGA profile was prevalent in younger children from the Amazon. The Le(a) mAb reaction was very strong, similarly as seen with the Asian population (Cutbush et al., 1956; Fung et al., 2014). We considered that the LE1 Le(a) mAb have detected the Le(a) antigen in 207 saliva samples, which corresponds to 58.80% of the total samples, and thus phenotyped them as Le(a + b+). The confidence on this was based on the results of LE1 Le(a) mAb titration which clearly shows that using the 1:5000 dilution indicated by Nordgren et al. (2014), presented no cross-reactivity using the control saliva samples. As described in Fig. 2, the control samples identified here as 26,580 and 26,576 were easily phenotyped as Le (a + b-) and Le (a-b+) respectively, whereas samples 26,606, 26,574 and mainly 26,568 showed reactivity for both Le(a) and Le(b) mAb. Both absorbance values for positivity to Le(a) and Le(b) were well above the cutoff values: three and ten times the value of the calculated cutoff, respectively (data not shown). As to explain and/or verify the Le(a + b+) phenotyping, the coding and the 3′ untranslated regions of the *FUT2* gene were amplified by PCR (Korbie and Mattick, 2008). The amplicons were sequenced to detect the known rs1047781 weak secretor genotype. The rs1047781 SNP was not detected in any of the samples, nor the principal SNP responsible for the non-secretor phenotype (rs601338) that has been observed in different populations (Kelly et al., 1995; Ferrer-Admetlla et al., 2009). Not all H-type 1 precursors are converted to H-type 1 antigens, depending on the efficacy of the FUT2 enzyme, which may be regulated in several ways, including as of yet undescribed genetic polymorphisms, which can explain why both Lewis a and Lewis b can be present at the same time (De Mattos, 2016).

In different populations the non-secretor phenotype ranges from 10 to 20% (Ferrer-Admetlla et al., 2009). In the Brazilian population, the occurrence of this phenotype is also low (Vicentini et al., 2013; Bernando et al., 2016), despite very few studies having been presented. The 8.2% of non-secretors found in children in the Amazon were thus consistent with previous results. The correlation between the SNPs detected in children from a Quilombola community of slave descendants, who live in Espírito Santo, Southeastern Brazil (Vicentini et al., 2013) with the SNPs detected in this study was low. Only the rs281377 was common between these two groups. Instead, younger children from the Amazon also have the rs1800027, rs375360260, rs602662, rs485073 and rs603985 in common with Asian populations including indigenous tribes phenotyped as Le (a + b+). These common SNPs were identified in Chinese and Thai populations (Chang et al., 1999; Yu et al., 1995), Taiwanese indigenous groups and Taiwanese populations (Yu et al., 1996, 1999, 2001), Native Chinese, Nepalese and Indonesian populations (Pang et al., 2001), and Taiwan aborigines (Chang et al., 2002). We investigated the hypothesis that the population of indigenous ethnicity presents genetic HBGA markers related with the Asian population, by comparing the SNPs found in the *FUT2* gene from the saliva of these young children from the Amazon with SNPs already detected in the Asian population. One hundred and seventy-nine (50.85%) saliva samples were collected from the Boa Vista Municipality. This number is representative enough to consider some genetic proximity between native Asian and Amazonian individuals.

5. Conclusion

The population of young children in this study of the Amazonian region presented a phenotypic profile of the secretor and Lewis HBGA, and of *FUT2* gene SNPs, similar to those described in the Asian population, mainly of indigenous tribes. There was no difference to the profile found in children with AGE and ARI.

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Conflict of interest

The authors are unaware of any conflict of interest.

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