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Pédiatrie

par

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L'IMPLICATION DES STATUTS FUT2, FUT3 ET ABO DANS LA SÉVÉRITÉ DES INFECTIONS À ROTAVIRUS

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INTRODUCTION

La gastroentérite est un problème de santé publique. Dans les pays en voie de développement, elle est la troisième cause de décès, alors que dans les pays développés, la maladie est rarement fatale mais représente un important coût de santé publique (1, 2). Le rotavirus est le principal virus impliqué dans les gastroentérites aigües en pédiatrie, en particulier la souche A (RVA) (3). En France, la gastroentérite est responsable d'environ 140 000 consultations et 18 000 hospitalisations annuelles (4). La gastroentérite à rotavirus a montré être plus sévère pour les enfants âgés de plus de six mois et être rare avant l'âge d'un mois (5,6). 30 à 80% des gastroentérites dues à ce virus sont sévères, selon les études (la variabilité dépend du pays dans lequel l'étude a été réalisée, ainsi que des critères utilisés pour définir la sévérité) (7, 8). Plusieurs scores ont précédemment été proposés pour évaluer la sévérité des symptômes de la gastroentérite et sont principalement utilisés dans les études d'efficacité post-vaccinale. Le score de Vesikari (9) et le Score de Déshydratation Clinique (SDC) (10) sont les plus couramment utilisés et reflètent de manière complémentaire les conséquences cliniques des gastroentérites à rotavirus.

Le rotavirus est un virus à ARN (Acide ribonucléique), appartenant à la famille des *Reoviridae*. Deux protéines de la capside externe sont utilisées pour définir le génotype du virus : la protéine VP7 et les pics VP4. Selon la classification, la protéine VP7 détermine le génotype G (*Glycoprotéine*) et les pics VP4 déterminent le génotype P (*Protéase-sensitive*) (3). Actuellement 36 génotypes G et 51 génotypes P ont été identifiés, parmi lesquels six génotypes G (G1, G2, G3, G4, G9 et G12), ainsi que trois génotypes P (P[4], P[6], P[8]) sont globalement les plus fréquents (11). Après un contact avec le RVA, médié par transmission oro-fécale, les personnes semblent être inégales quant à la susceptibilité à développer une gastroentérite : seulement 50% des personnes développeraient des symptômes intestinaux (12, 13). Cette inégalité a aussi été retrouvée dans la réponse vaccinale : le vaccin-vivant est plus efficace dans les pays développés que dans les pays en voie de développement (14). De récentes études ont été menées pour essayer de mieux comprendre les raisons de cette disparité.

D'un côté, il a été démontré que des sucres complexes, appelés antigènes du groupe sanguin et tissulaire (HGBA), pourraient être impliqués dans l'attachement du virus aux parois digestives (15). Ces sucres sont notamment exprimés à la surface des cellules sanguines et des muqueuses orales et digestives. La présence ou l'absence de ces sucres complexes est impliquée dans la composition d'antigènes qui déterminent le phénotype

sécréteur et le phénotype Lewis. La synthèse de ces sucres dépend respectivement des gènes *FUT2* et *FUT3* qui sont sujets à un important polymorphisme génétique (**Figure 1**) (16). Dans les pays développés, où les souches P[8] et P[4] prédominent (17), on a observé que les phénotypes sécréteurs et Lewis positifs avaient un rôle « attrapeur » : le rotavirus n'est pas pathogène pour les individus ayant un phénotype non sécréteur et Lewis négatif, c'est-à-dire pour les individus avec une mutation homozygote sur les gènes *FUT2* et *FUT3*. Les individus présentant une mutation hétérozygote sur le gène *FUT2* (ayant tout de même un phénotype sécréteur) n'ont pas encore été étudiés et seront pris en compte dans ce travail. D'un autre côté, le phénotype ABO pourrait avoir une fonction protectrice : les individus du groupe O semblent être moins fréquemment infectés que ceux des autres groupes sanguins (18). Le développement d'anticorps anti-A et anti-B pourrait expliquer cette protection naturelle contre le virus. Il s'agit donc de déterminer si les personnes du groupe O, qui seraient néanmoins infectées, présenteraient des symptômes atténués comparés aux individus d'autres groupes sanguins et tissulaires (**Table 1**).

En combinant le polymorphisme *FUT2, FUT3* et *ABO,* un total de 34% des enfants pourraient être naturellement protégés contre les gastroentérites sévères à rotavirus (19). De plus, un travail indépendant sur de jeunes adultes volontaires a montré que les personnes ayant un génotype homozygote sauvage pour *FUT2* (donc un phénotype sécréteur) ont un taux d'anticorps anti-RVA plus élevés que ceux avec une mutation hétérozygote (phénotype sécréteur) ou une mutation homozygote (phénotype non sécréteur) sur ce gène. La réponse immunitaire étant plus faible chez ces patients, on a émis l'hypothèse selon laquelle elle serait le reflet d'une pathogénicité plus faible du virus (du fait d'une enzyme FUT2 moins fonctionnelle et donc d'un moindre attachement du virus à l'organisme) et ainsi, que leur gravité pourrait être moindre (20).

L'objectif principal est de rechercher une association entre le polymorphisme génétique des antigènes du groupe sanguin et tissulaire (*FUT2, FUT3* et *ABO*) et la gravité des gastroentérites à rotavirus chez les enfants de moins de cinq ans.

CONCLUSION

Cette étude démontre que la souche P[8] du rotavirus infecte presque exclusivement les enfants avec un phénotype sécréteur et Lewis positif, et préférentiellement les enfants de groupe non-O. Parmi les enfants infectés, la présence d'une mutation sur le gène *FUT2* (c'est-à-dire le génotype hétérozygote) n'est pas associée à une forme atténuée de gastroentérite. Quand ils sont infectés, les enfants du groupe O ne présentent pas non plus une forme moins sévère de la maladie.

Les enfants malades (les cas) avaient quasiment tous un statut sécréteur et Lewis positif (respectivement 99 et 98%). En Europe, environ 20% des individus sont non-sécréteurs et environ 10% sont Lewis négatifs (21), on peut donc conclure que cette partie de la population est peu à risque de développer des symptômes digestifs après un contact avec la souche P[8] du rotavirus. Parmi les phénotypes sécréteurs, il y avait plus d'enfants avec une mutation hétérozygote sur le gène *FUT2* chez les cas que chez les témoins (62,3% et 44,8% respectivement, $p<0,01$). Parmi les enfants avec une mutation hétérozygote sur le gène *FUT2*, 64,2% avaient une forme sévère alors que 58,1% avaient une forme légère à modérée ($p=0,46$).

Des études antérieures avaient prouvé que les individus avec une mutation hétérozygote sur le gène *FUT2* avaient de plus faibles taux d'anticorps anti-RVA que ceux n'ayant pas de mutation (c'est-à-dire ayant le génotype sauvage) (20, 22). Nous avions donc émis l'hypothèse selon laquelle la réponse immunitaire était plus faible du fait d'un plus faible inoculum viral et que cela pourrait être lié à des symptômes digestifs moins sévères. Mais d'après nos résultats, ce pourrait être l'opposé : à cause d'une plus faible réponse immunitaire chez les individus présentant une mutation hétérozygote sur le gène *FUT2,* ils pourraient être plus à risque de développer une infection à rotavirus, et même avoir une forme clinique plus sévère de gastroentérite (tendance retrouvée dans les analyses mais le seuil de significativité n'était pas atteint).

Par ailleurs, les résultats sur le polymorphisme ABO étaient cohérents avec ce qui a été précédemment décrit dans la littérature (8) : les enfants du groupe O sont moins représentés chez les cas que chez les témoins (21% contre 42,5%, (OR 0,37 (0,22 – 0,62)) et sont environ trois fois moins à risque de développer une gastroentérite après un contact avec le RVA que les enfants du groupe A.

En considérant les enfants avec un phénotype non-sécréteur, ceux avec un phénotype Lewis et ceux du groupe O, une partie de la population est donc naturellement protégée contre les souches virales qui circulent dans les pays développés.

Ce travail s'intègre au projet Gastrovim, qui a été mené dans deux centres hospitaliers, dans les villes de Nantes et de Cayenne. Il ne traite que des résultats nantais, un travail similaire sera effectué avec les données de Cayenne où d'autres souches virales circuleraient et où les caractéristiques génétiques seraient possiblement différentes, ce qui pourrait moduler nos conclusions.

ARTICLE

TITLE

Implication of FUT2, FUT3 and ABO statuses in rotavirus infection

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KEY WORD

Rotavirus gastroenteritis, gastroenteritis severity, HGBA, FUT2 gene, secretor phenotype, FUT3 gene, Lewis phenotype, ABO group.

ABTRACT (/300 WORDS)

Background. Rotavirus is responsible of a significant part of severe gastroenteritis. Its pathogenicity depends on histoblood group antigen sugars attachment, whose synthesis is subject of genetic polymorphism, implying mutations on FUT2, FUT3 and ABO genes. These genes are involved in synthesis of sugars expressed on oral and digestive mucosa, and sugars expression determines the secretor phenotype, the Lewis phenotype and the ABO phenotype. Susceptibility of rotavirus gastroenteritis is linked to these phenotypes. We hypothesized that genetic polymorphism could also be associated with gastroenteritis severity forms.

Objective. The objective was to evaluate the association between gastroenteritis clinical severity and FUT2 genotype, Lewis phenotype and ABO phenotype.

Methods. This prospective multicentric case-control study compares genetic characteristics and their expression between healthy and sick children, and among cases between mild and severe clinical forms. Cases were children consulting in the Emergency Department of Nantes University Hospital with gastroenteritis symptoms and controls were children without any digestive symptoms.

Results. A total of 334 children were included: 200 cases and 134 controls. Gastroenteritis was significantly associated with secretor and Lewis phenotypes: 199/200 cases had a secretor phenotype (OR 0.03 (0.00-0.16); $p<0.001$) and 198/200 cases had a Lewis phenotype (OR 0.09 (0.01 – 0.35); $p<0.01$). Rotavirus gastroenteritis was also linked to ABO phenotypes: children with O histoblood types are less likely to be infected than other blood groups (OR 0.37 (0.22 – 0.62). There were no difference in degree of severity according to FUT2 genotype ($p=0.46$) and ABO phenotype ($p=1$).

Conclusion. Children with non-secretor phenotype, Lewis negative phenotype or O histo-blood group have an innate protection against rotavirus gastroenteritis development. When infected by P[8] strain, children with O histo-blood group or with FUT2 heterozygote mutation do not have milder symptoms.

ABBREVIATION

AGE	Acute gastroenteritis
CDS	Clinical Dehydration Scale
ED	Emergency department
FUT2	Alpha (1,2) fucosyltransferase
FUT3	3(4)-L-fucosyltransferase
HBGAs	Histo-blood group antigens
RVA	Rotavirus A
RNA	Ribonucleic Acid
PCR	Polymeric Chain Reaction
RDT	Rapid Diagnosis Test

INTRODUCTION

Gastroenteritis is a worldwide public health problem. In developing countries, it's the third cause of death, whereas in higher income regions, disease is rarely fatal but represents a significant health care cost (1, 2). Rotavirus is the main virus involved in the cause of acute gastroenteritis (AGE) in children, particularly strains of rotavirus A (RVAs) (3). In France, gastroenteritis is responsible for about 140 000 consultations and 18 000 hospitalizations yearly (4). Rotavirus gastroenteritis proved to be more severe for children older than six months, and uncommon before one month (5, 6). Severe forms of rotavirus AGE represent from 30 to 80% of gastroenteritis, according to studies (variability depends on countries and criteria used to define the severity) (7, 8). Several scores have been previously proposed to assess the severity of rotavirus AGE and are mainly used in post-vaccine efficiency studies. The Vesikari score (9) and Clinical Dehydration Scale (CDS) (10) are the most commonly used and reflect complementary the clinical consequences of the rotavirus gastroenteritis.

Rotavirus is a Ribonucleic Acid (RNA) virus, belonging to *Reoviridae* family. Two outer proteins on capsid, VP7 protein and VP4 spikes, are used to define the rotavirus genotypes. According to the classification, VP7 proteins determine the G genotype *(G*lycoproteins) and VP4 spikes determine the P genotype (Protease-sensitive protein) (3). Currently, 36 G and 51 P genotypes have been identified, among which six G genotypes (G1, G2, G3, G4, G9, G12) and three P genotypes (P[4], P[6], P[8]) are globally more prevalent (11). After a contact with RVA mediated by oro-fecal transmission, individuals appear to be unequal concerning the susceptibility to develop rotavirus gastroenteritis: only 50% of people would develop intestinal symptoms (12, 13). This inequality was also found in vaccine responses: the live attenuated vaccine is more effective in developed countries than in developing ones (14). Both question the susceptibility of infection and of vaccine prevention.

Recently, research studies have been carried out for understanding this disparity. On one hand, it has been showed that complex sugars expressed on oral and digestive mucosa, named histo-blood group antigens (HBGAs), could be implied in the intestinal wall virus attachment (15). The presence or absence of these complex sugars is involved in composition of antigens that determine the secretor and the Lewis phenotypes. Synthesis of the complex sugars respectively depends on the *FUT2* and *FUT3* genes, which are subject to a significant polymorphism (**Figure 1**) (16). In developed country, where P[8] and P[4] strains are predominant (17), we observed that secretor and Lewis phenotypes have a catch-up role: rotavirus is not pathogenic for individuals with non-secretor and negative Lewis phenotypes, *i.e.* with monozygotic mutations for FUT2 and FUT3 genes. Individuals with heterozygote

mutation on FUT2 gene (*i.e.* secretor phenotype) have not been studied yet and will be considered in this work. On the other hand, ABO phenotype could have a protector function: individuals with the O histo-blood group seem to be less frequently infected than individuals with the non-O histo-blood group (18). Anti-A and anti-B antibodies development could explain this protection. It remains to determine if individuals with O histo-blood group, who are nevertheless infected, additionally present milder symptoms than individuals with non-O histo-blood group (**Table 1**).

Combining the *FUT2*, *FUT3* and *ABO* polymorphisms, a total of 34 % of infants would be naturally protected against severe rotavirus AGE (19). Moreover, an independent study on young adult volunteers showed that individuals with monozygotic wild-type for FUT2 genotypes (*i.e.* with secretor phenotype) have higher anti-RVA titers than both individuals with heterozygotic (*i.e.* secretor phenotype) and mutant homozygotic (*i.e.* non-secretor phenotype) mutations. As immune response is lower in these patients, we hypothesize that their viral inoculum could also be lower, due to less functional FUT2 enzyme, and consequently their symptoms severity might be milder for children with lower anti-RVA titer (20).

The main objective is to assess the association between the HGBA genetic polymorphism (*FUT2*, *FUT3* and *ABO*) and the rotavirus gastroenteritis severity in children less than five years.

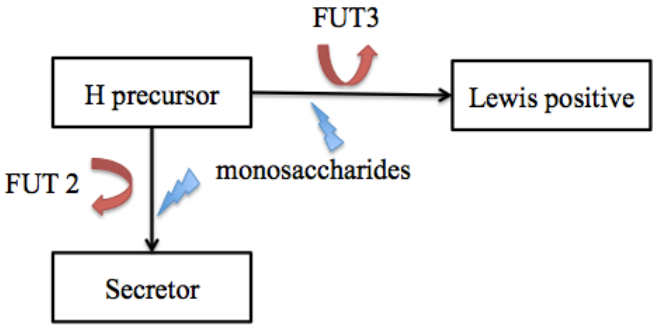

Figure 1. Simplified schema of the HGBA synthesis.

HBGA are complex sugars whose synthesis begins by the H precursor. FUT2 and FUT3 enzymes (respectively coded by FUT2 and FUT3 genes) secondly added monosaccharides. Addition of sugars leads to final sugar whose expression defines secretor and Lewis phenotypes.

Table 1. Presentation of genetic determinants of histo-blood group antigens.

Phenotype	**Genotype**	**Comment / Hypothesis**
Secretor *i.e.* monosaccharides added by **FUT2** enzyme	**FUT2 gene** - wild-type: SE/SE - heterozygote mutation: SE/se or se/SE	Good correlation between phenotype and genotype. The secretor phenotype has already been studied, interest of studying FUT2 genotype
Lewis *i.e.* monosaccharides added by **FUT3** enzyme	**FUT3 gene**	Insufficient correlation between Lewis phenotype and FUT3 genotype: Lewis phenotype will be studied
ABO antigen - A - B - O - AB	**ABO genotype** - AA or OA or OA - BB or OB or BO - OO - AB or BA	Good correlation between phenotype and genotype: phenotype will be studied

POPULATION AND METHODS

Study design, setting and participants

We conducted a case-control study nested in a prospective and multicenter study, integrated in the GASTROVIM project [reference RC15-0329]. This project was based on a prospective genetic epidemiology study conducted in parallel in Nantes and Cayenne on children hospitalized for rotavirus gastroenteritis. Our study included children from birth to 16 years old, having consulted at the pediatric Emergency Department (ED) of Nantes University Hospital, between March 2017 and June 2019. We studied the impact of genetic polymorphisms on the rotavirus gastroenteritis occurrence by comparing a group of children admitted in the ED for rotavirus gastroenteritis (cases) and children hospitalized for others reasons than rotavirus infection (controls), then on the degree of severity of rotavirus gastroenteritis among the cases only. Parent consents were obtained after giving them written information.

Inclusion criteria for cases were children aged from birth to five years old consulting in the emergency department for rotavirus gastroenteritis (cases), commonly defined by at least three soft or liquid stools or three episodes of vomiting in 24 hours, or children from birth to 16 years old, hospitalized for others reasons than gastroenteritis symptoms (controls). The diagnostic of cases was confirmed by the identification of rotavirus in stool sample. Exclusion criteria were presence of a co-infection (viral or bacterial) or history of anti-

rotavirus vaccine for cases, the presence of a positive rotavirus RDT (rapid diagnosis test) for controls, and unavailable samples for genetic or virology testing for both groups.
The ethic committee of OUEST V (December 14, 2015, n° 2016-A01047-44) has approved the study.

Data collection

For cases, pediatricians completed a hetero-questionnaire after medical examinations (**Appendix 1**). This questionnaire included information about demographic data (age, sex, parents' ethnicity), vaccine status, symptoms duration and frequency, clinical signs included in clinical dehydration scale proposed by Friedman *et al* (10) such as general appearance, eyes, mucous membranes and tears (detailed in **Appendix 2**), anterior consultations and treatments. For controls, demographic information only was collected.

Virology testing

Stool samples were collected and sent to laboratory for both cases and controls individuals. Virology laboratory confirmed or invalidated rotavirus infection with PCR testing and characterized viruses with genetic sequencing: strains were identified from the external proteins.

Gastroenteritis severity

The children were classified according to our experience in different clinical severity forms of rotavirus gastroenteritis reviewing their available medical record. In ED, children are usually considered with a severe clinical form in case of clinical signs of dehydration, profuse digestive symptoms, biological abnormalities (such as renal failure, clear serum electrolyte abnormalities), or hemodynamic disorders. These items correspond to usual clinical hospitalization criteria.

Several clinical severity scores have been proposed to assess severity of rotavirus AGE. First, the Vesikari score (**Appendix 3**) is first of all based on clinical data considered during medical evaluation without kinetic considerations: percentage of weight loss, temperature, symptoms frequency and hospitalization. It classified patients into three groups from mild to severe impairment. However, this score does not seem to be discriminative enough for patients consulting in a pediatric ED, because of its lack of specificity: most of AGE has severity criteria according to Vesikari score. Moreover, it underestimates the severity of patients with rapid clinical deterioration. Second, the CDS was based on clinical

signs only and allowed to classify infants less than 36 months from none to severe dehydration. However, this scale selects only very severe dehydration and it does not seem to be discriminative enough to predict patients at risk of aggravation without adapted medical care. Consequently, we generated a composite clinical score, combining items of those two gastroenteritis severity scores. Our score was based on the CDS, the percentage of dehydration, the maximal number per day of liquid stools and vomiting. The patients were thus classified into severe or mild to moderate forms as following: a patient will be classified into severe forms if he has a CDS equal or above 4, a percentage of dehydration equal or above 7%, a maximal number of liquid stool per day equal or above 8, or maximal number of vomiting per day equal or above 10.

Genetic polymorphisms

Saliva samples were used to determine the HGBA phenotypes in children with a panel of lectins and anti-glycan antibodies, according to the ELISA method and it was confirmed by mutations' analysis in the *FUT2*, *FUT3* and *ABO* loci, thus identifying genotypes. HGBAs are carbohydrates expressed on mucosal and enteric epithelial cells. Their synthesis depends on addition of monosaccharides to precursor disaccharides, mediated by four enzymes: α-1.2-fucosyltransferase (*FUT2* enzyme), α-1.3 fucosyltransferase (*FUT3* enzyme) and A and B enzymes. These enzymes are synthesized by three genes (respectively *FUT2*, *FUT3* and *ABO* genes), and their expression determines HGBA phenotypes: secretor or non-secretor phenotype, Lewis positive or negative phenotype, or A, B, O or AB phenotype. Our work will consider secretor, Lewis and ABO phenotypes, and FUT2 genotype only.

Statistical analysis

First, we described the characteristics and the genetic polymorphisms in cases and controls, the virology testing and the gastroenteritis symptoms in cases only. We compared the quantitative and qualitative data by using Mann-Whitney and chi-square tests (or Fisher's exact test), respectively.

Second, we described the classification of gastroenteritis clinical severity by medical record, and we compared it with the two existing clinical severity scores as well as the new composite score in terms of sensibility and specificity.

Third, we compared each genetic polymorphism (*FUT2* genotype, then Lewis, secretor, ABO phenotypes) between cases and controls. Then, we studied the impact of genetic polymorphisms according to gastroenteritis clinical severity defined by the new composite

score in the cases most at risk for rotavirus AGE (*i.e*, secretor status, positive Lewis status, heterozygotic mutation or monozygotic wild-type). We used crude logistic regression models in order to calculate odds ratios as well as their 95% CIs. For studying the combined FUT2 and ABO statuses on gastroenteritis clinical severity, we performed multiple logistic regression model by adding an interaction term between FUT2 genotypes and ABO phenotypes (**model 1**) then an interaction term between FUT2 genotypes and child age (**model 2**). P<0.05 was considered statistically significant. Statistical analyses involved using R v4.0.0 (The R Foundation).

RESULTS

Description of participants

Among the 388 eligible children aged from birth to 16 years old consulting in the emergency department, including 228 having rotavirus gastroenteritis (cases) and 160 controls, 200 cases and 134 controls were included. Children were excluded because of the absence or presence of rotavirus infection, respectively, for cases (n=22) and controls (n=6), and unavailable sample for the virology or the genetic testing (n=26) (**Figure 2**). Among cases, the principal circulating strain was P[8] strain in 199 (99.5%) children, one (0.5%) child was infected with P[4] strain, and none of the children were infected by P[6] strain.

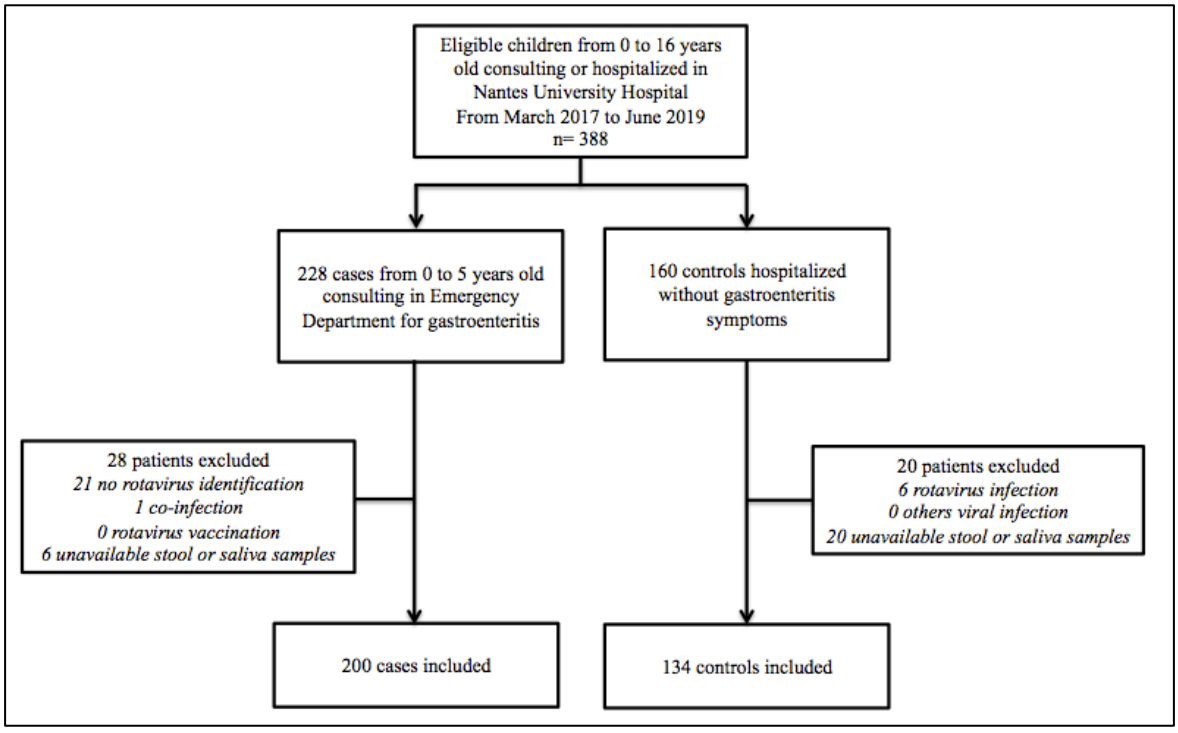

Figure 2. Flow chart of participating children.

Among the children included, the mean (SD) age was of (10.5) months (median [IQR]: 15.5 [3.5-27.6]) for cases and 17.7 (12.1) months (1.3 [0-12.1]) for controls (p<0.01). Among the entire included population, 135 cases (67.5%) and 74 controls (55.2%) were boys (p=0.03). Parents' ethnicities were principally European for cases and controls (**Table 2**).

Table 2. Description of cases and controls.

	Total (n=334)	**Cases (n=200)**	**Controls (n=134)**	**p-value**
Age (months), mean +/- SD	13.8 +/- 12,5	17.7 +/- 12.1	7,6 +/- 10.5	<0.01
Age > 6 months, n (%)	228 (68.3%)	176 (88.0%)	52 (38.8%)	<0.01
Sex (Female), n (%)	125 (37.4%)	65 (32.5%)	60 (44.8%)	0.03
Mother's ethnicity, n (%)				0.92
Europe	265 (79.3%)	157 (78.5%)	108 (80.6%)	
Sub-Saharan Africa	23 (6.9%)	12 (6.0%)	11 (8.2%)	
Middle East Africa	36 (10.8%)	23 (11.5%)	13 (9.7%)	
Others	10 (3%)	8 (4.0%)	2 (1.5%)	
Father's ethnicity, n (%)				0.80
Europe	259 (77.5%)	151 (75.5%)	108 (80.6%)	
Sub-Saharan Africa	30 (8.9%)	19 (9.5%)	11 (8.2%)	
Middle East Africa	36 (10.8%)	23 (11.5%)	13 (9.7%)	
Others	9 (26.9%)	7 (3.5%)	2 (1.5%)	

Description of gastroenteritis symptoms

Among 200 cases, 195 (97.5%) presented diarrhea and 192 (96.0%) vomiting. The mean duration of symptoms was 3.3 days (SD: 3.0, median[IQR]: 3[0-6]). A total of 100 (50.0%) of cases had a significant percentage of dehydration: 66 (33%) and 34 (17%) had lost from 5 to 10% and more than 10% of their body weight, respectively. Concerning the treatment or management, 139 (71.6%) cases required rehydration, 173 (86.5%) were hospitalized and 148 (74%) were infused. **Table 3** also describes symptoms in the two groups of severity.

Table 3. Description of gastroenteritis symptoms in cases and repartition according to severity.

	Cases (n=200)	**Mild to moderate** (n=62)	**Severe** (n=138)
Symptom duration (days), mean +/- SD	3.3 +/- 3.0	3.2 +/- 3.5	3.4 +/- 2.8
Dehydration percentage, n (%)			
< 5%	100 (55%)	41 66%)	47 (34%)
5-10%	66 (33%)	15 (24%)	51 (37%)
>10%	34 (17%)	2 (3.2%)	32 (23.2%)
Maximal temperature (°C), mean +/- SD	38.5 +/- 0.9	38.4 +/- 0.8	38.6 +/- 0.9
Friedman Score, mean +/- SD	2.3 +/- 1.9	1.1 +/- 1.3	2.9 +/- 1.9
Diarrhea, n (%)	195 (97.5%)	62 (100%)	132 (95.7%)
Diarrhea duration (days), mean +/- SD	2.8 +/- 2.6	2.5 +/- 2.1	2.9 +/- 2.8
Maximal stool number per day, mean +/- SD	8.5 +/- 6.6	5.2 +/- 3.1	10 +/- 7.2
Vomiting, n (%)	192 (96.0%)	54 (87%)	136 (98.5%)
Vomiting duration (days), mean +/- SD	2.5 +/- 1.6	2.4 +/- 1.7	2.6 +/- 1.6
Max. vomiting number per day, mean +/- SD	8.7 +/- 7.4	7.1 +/- 6.2	9.3 +/- 7.8
Oral rehydration, n (%)	139 (71.6%)	48 (77.4%)	91 (65.9%)
Hospitalization, n (%)	173 (86.5%)	39 (62.9%)	134 (97%)
Perfusion, n (%)	148 (74%)	29 (46.7%)	119 (86.2)
< 6 months, n (%)	24 (12%)	17 (70.8%)	7 (29.1%)
> 6 months, n (%)	176 (78%)	45 (25.5%)	131 (74.4%)

Gastroenteritis clinical severity scores

Of 200 cases, 138 (69%) and 62 (31%) had clinically severe and mild to moderate forms of rotavirus gastroenteritis, respectively (according to medical record). Children aged less than six months had mild to moderate form in 70.8% of cases (**Table 3**).

The three mentioned scores were compared with the medical record that our practitioners have made in a sensitivity study (**Table 4**). The Vesikari score lacks specificity (Sp=15.4%) and the CDS lacks sensitivity (Se=25.4%). With 95.7% of sensitivity and 66.1% of specificity, the composite score have better positive and negative likehood ratios.

Table 4. Sensitivity study of clinical severity scores

Tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Positive likehood ratio [IC 95%]	Negative likehood ratio [IC 95%]
Vesikari score	96.6%	15.4%	72.3%	66.7%	1.14 [1.01-1.29]	0.22 [0.07-0.69]
CDS	25.4%	96.4%	94.1%	36.1%	6.98 [1.73-28]	0.77 [0.69-0.87]
Composite score	95.7%	66.1%	86.3%	87.2%	2.82 [1.99-4.01]	0.07 [0.03-0.15]

Genetic polymorphism between cases and controls

The study of genetic polymorphisms comparison between cases and controls is presented in **Table 5.**

The gastroenteritis was significantly associated with secretor and Lewis phenotypes: 1 (0.5%) case has a negative secretor status versus 18 (13.4%) in controls (OR 0.03 (0.00-0.16); p<0.001) and two (1.0%) cases have a negative Lewis status against 13 (9.7) in controls (OR 0.09 (0.01 – 0.35); p<0.01) (**Figure 3**).

The gastroenteritis was significantly associated with FUT2 genotypes. Among cases, 75 (37.5%) and 124 (62.3%) of cases had, respectively, a monozygotic wild-type for FUT2 gene (SE/SE) or a heterozygous mutation (SE/se or se/SE) versus 56 (41.8%) and 60 (44.8%) in controls (OR [95%CI]: 1.54 (0.97 – 2.46); p<0.001).

The gastroenteritis was significantly associated with ABO phenotypes. Compared to A histo-blood group, cases with O histo-blood group had lower odds ratios than controls (OR 0.37 (0.22 – 0.62)), contrary to B histo-blood group (OR 1.25 (0.62 – 2.63)) and AB histo-blood group (OR 1.02 (0.31 – 3.92); p<0.01) (**Figure 4**).

Table 5. Crude regression analyses: impact of genetic polymorphisms between 200 cases and 134 controls

	Cases (n=200)	**Controls (n=134)**	**Crude odds ratio [95% CI]**	**p-value**
FUT2 genotype				
SE/SE (wild-type)	75 (37.5%)	56 (41.8%)	ref	<0.001
SE/se or se/SE (heterozygote)	124 (62.3%)	60 (44.8%)	1.54 (0.97 – 2.46)	
se/se (monozygote)	1 (0.5%)	18 (13.4%)	0.04 (0.00 – 0.21)	
Secretor phenotype				<0.001
Secretor	199 (99.5%)	116 (86.6%)	ref	
Non-secretor	1 (0.5%)	18 (13.4%)	0.03 (0.00 – 0.16)	
Lewis phenotype				<0.01
Positive	198 (99%)	121 (90.3%)	ref	
Negative	2 (1%)	13 (9.7%)	0.09 (0.01 – 0.35)	
ABO phenotype				<0.01
A histo-blood group	118 (59%)	60 (44.8%)	ref	
B histo-blood group	32 (16%)	13 (9.7%)	1.25 (0.62 – 2.63)	
O histo-blood group	42 (21%)	57 (42.5%)	0.37 (0.22 – 0.62)	
AB histo-blood group	8 (4%)	4 (3%)	1.02 (0.31 – 3.93)	

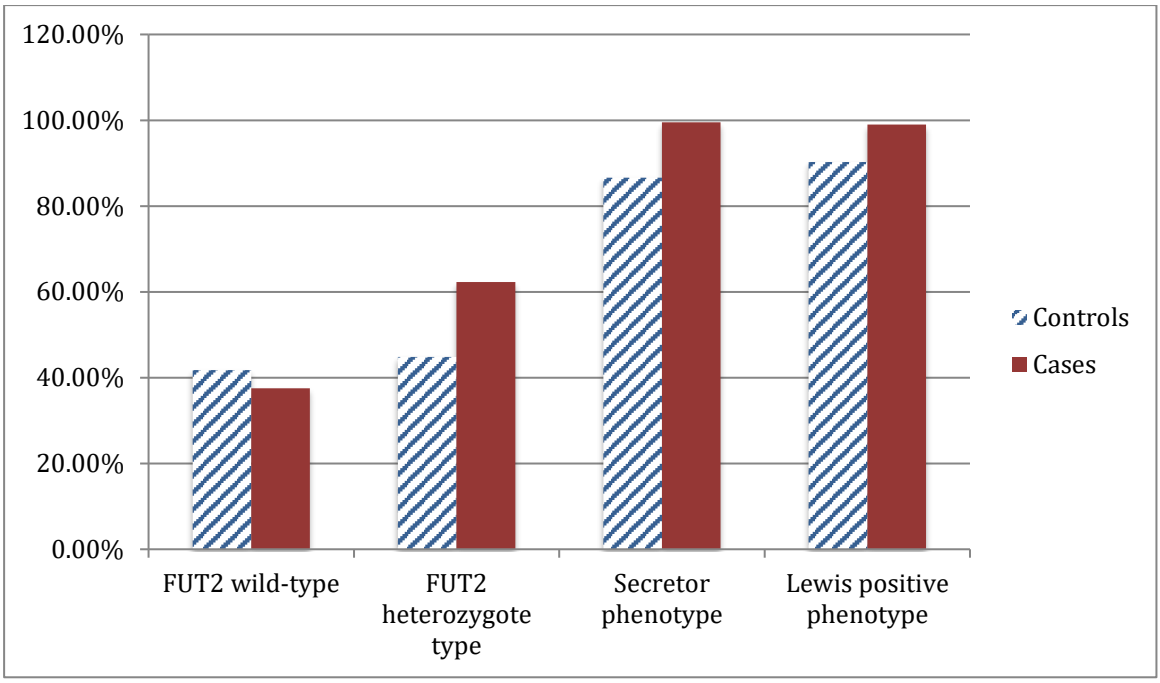

Figure 3. Comparison of FUT2, secretor and Lewis statuses between cases and controls. *Cases almost exclusively have secretor and Lewis positive phenotypes ($p<0.001$ and $p<0.01$), confirming the catch-up role of these phenotypes. There were more children with FUT2 heterozygote mutation in cases than in controls.*

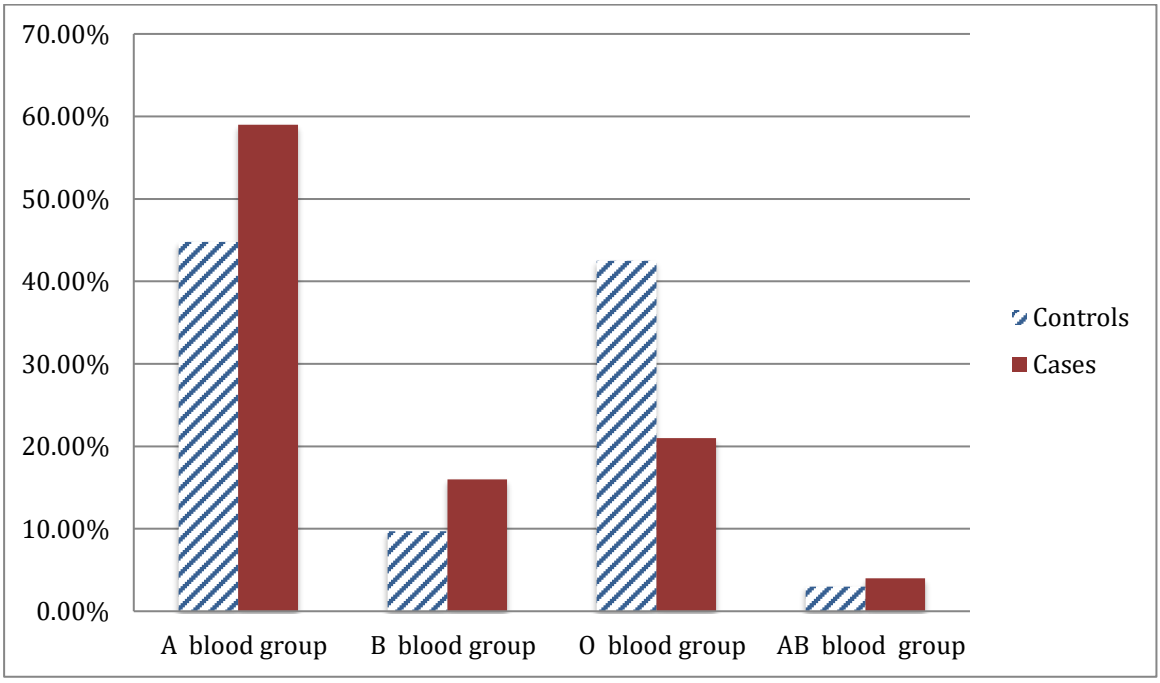

Figure 4. Comparison of ABO phenotypes between cases and controls. *Histo-blood repartition is similar in cases and controls except for the O group: cases are significantly less represented in cases than in controls ($p<0.01$), confirming the protector function of this phenotype.*

Impact of genetic polymorphisms on clinical severity of gastroenteritis in cases

The impact of genetic polymorphisms on clinical severity of gastroenteritis in the 197 cases at risk to develop rotavirus gaastroenteritis was presented in **Table 6** and **Table 7.** In crude regression analyses, the cases with severe forms and those with mild to moderate forms did not significantly differ for heterozygotic mutation for FUT2 genotype (64.2 vs. 58.1%: OR 1.30 95% CI (0.64-2.57); p=0.46), and for ABO phenotype (p=1.00) (**Table 6**). In adjusted regression analysis, the interaction term between FUT2 and ABO statuses were not significant, even after adding the interaction between FUT2 and child age (**Table 7**).

Table 6. Crude regression analyses: impact of genetic polymorphisms on AGE clinical severity in 197 cases at risk to develop rotavirus gasroenteritis.

	Severe (n=154)	**Mild to moderate (n=43)**	**Crude odds ratio [95% CI]**	**p-value**
FUT2 genotype				0.46
SE/SE (wild-type)	55 (35.7%)	18 (41.8%)	ref	
SE/se or se/SE (heterozygote)	99 (64.2%)	25 (58.1%)	1.30 (0.64 – 2.57)	
ABO phenotype				1.00
A histo-blood group	91 (59.1%)	25 (58.1%)	ref	
B histo-blood group	24 (15.6%)	7 (16.2%)	0.94 (0.38 – 2.59)	
O histo-blood group	33 (21.4%)	9 (20.9%)	1.01 (0.44 – 2.48)	
AB histo-blood group	6 (3.8%)	2 (4.5%)	0.82 (0.18 – 5.86)	

Table 7. Adjusted regression analyses: impact of genetic polymorphisms on AGE clinical severity in 197 cases at risk to develop rotavirus gastroenteritis.

	Severe (n=152)	**Mild to moderate (n=45)**	**Model 1. Adjusted odds ratio [95% CI]**	**p-value**	**Model 2. Adjusted odds ratio [95% CI]**	**p-value**
				0.94		0.85
FUT2 genotype: wild-type	52 (34.2%)	20 (44.4%)				
ABO phenotype						
A histo-blood group	36 (23.7%)	13 (28.9%)	ref		ref	
B histo-blood group	8 (5.3%)	2 (4.4%)	1.30 (0.28 – 9.38)		1.47 (0.26 – 11.90)	
O histo-blood group	7 (4.6%)	4 (8.9%)	0.86 (0.21 – 4.42)		0.56 (0.08 – 4.11)	
AB histo-blood group	2 (1.3%)	1 (2.2%)	0.65 (0.06 – 14.68)		1.33 (0.06 – 39.28)	
FUT2 genotype: heterozygote	99 (65.1%)	25 (55.6%)				
ABO phenotype						
A histo-blood group	54 (35.6%)	13 (28.9%)	ref		ref	
B histo-blood group	16 (10.5%)	5 (11.1%)	0.77 (0.25 – 2.69)		0.75 (0.24 – 2.65)	
O histo-blood group	25 (16.5%)	6 (13.3%)	1.00 (0.35 – 3.13)		0.99 (0.35 – 3.11)	
AB histo-blood group	4 (2.6%)	1 (2.2%)	0.96 (0.13 – 19.68)		0.96 (0.13 – 19.59)	

Model 1. Interaction term between FUT2 and ABO phenotypes; Model 2. Model 1 and interaction term between FUT2 and child age.

DISCUSSION

This study demonstrates that rotavirus P[8] strain infect only children with secretor and Lewis positive phenotypes, and preferentially non-O histo-blood group. Among infected children, the presence of a mutation on *FUT2* gene (heterozygote genotype) is not associated with an attenuated severity of gastroenteritis. Similary, when infected by P[8] strain, children with O histo-blood group have a comparable severity than children from others blood groups.

The cases had almost exclusively a secretor and a Lewis positive status (99 and 98% of cases). In European population, about 20% of individuals are non-secretor and 10% are Lewis negative (21) and we can conclude that this part of the population is not at risk to develop gastroenteritis symptoms after a contact with P[8] strain. Among secretor phenotype, there were more infants with heterozgote mutation on *FUT2* gene in cases than in controls (62,3% and 44,8% respectively, $p<0.01$). Among children with a heterozygote FUT2 mutation, 64.2% had a severe form and 58.1% a mild to moderate form ($p=0.46$).

Barbe and al. (22) and Gunyadin and al. (20) show that anti-RVA titer is lower in individuals with heterozygote FUT2 mutation than in individuals with monozygote wild-type. We hypothesize that immune response could be milder because of a lower viral inoculum and that it could lead to milder severity forms of rotavirus gastroenteritis. But according to this work, the right hypothesis could be the opposite: because of a lower immune response in individuals with heterozygote mutation in *FUT2* gene, they could be more susceptible to develop rotavirus infection, and even have a more severe clinical form of gastroenteritis (significant thresholds are not reach). This hypothesis could be considered in a future work.

We observed, as previously describe (18), that O histo-blood group children are less represented in cases than in controls (21% vs. 42.5%, (OR 0.37 (0.22 – 0.62)), and are three times less likely to develop rotavirus gastroenteritis than A histo-blood group (OR (0.37 (0.22 – 0.62), $p<0.01$). Characteristics of the control population is similar to those of population in developed countries, for example O histo-blood group represents 42% of the general population and 42.5% of controls in our study. So we can say that O histo-blood group is a protector factor against development of gastroenteritis but when rotavirus infected these children, their clinical severity does not differ from other histo-blood groups (OR 1.01 (0.44 – 2.48), $p=1$). Considering these results, we confirmed that a significant part of the population is naturally protected against rotavirus gastroenteritis (**Table 8**).

Table 8. Estimation of genetically determined protection against rotavirus gastroenteritis.
Values for developed countries come from literature (French blood establishment, (24))

Phenotype	Population of developed countries	Controls	Genetically determined protection
Secretor phenotype	80%	85%	15%
Lewis phenotype	90%	9.5%	10%
O histo-blood group	42%	42.5%	20%

In our study, children less than 6 months had milder forms of rotavirus gastroenteritis (70% of mild to moderate forms vs. 33% of severe forms in children less and more than 6 months respectively). Lorrot and al. (5) found same results previously by and the hypothesis that maternal anti-bodies protect this population has been retained. As maternal anti-bodies are usually considered as present during the six first months of life, we wanted to repeat analysis only considering children more than six months to avoid a confusion bias, but results weren't significant, with small group sizes. It will be interesting to repeat analysis on a larger cohort.

Our conclusions can only be applied in developed countries where the same rotavirus strains circulate and where genetic characteristics are similar, as USA, Spain and other European countries (21). It will not be true for countries where P[6] strains circulate for example; this strain infected preferentially Lewis negative individuals, whose phenotype is more prevalent in the concerned populations. (23) This is true for Sub-Saharan Africa where 20% of rotavirus strains are P[6] strains, and 33% of population is Lewis negative. The study will continue with patients of Gastrovim project included in Cayenne Hospital, where epidemiologic and genetic may differ from France and could modulate the percentage of innate protection against rotavirus gastroenteritis.

One of the limitations of our study is the place it was conducted in a Pediatrics Emergency Department, we are exposed to a selection bias with more severe forms of gastroenteritis than in a primary care place: 69% of severe form in our study, corresponding to a high value among the range described in the literature (from 30 to 80% of severe rotavirus gastroenteritis (7, 8)). The link we find between secretor, Lewis, ABO statuses and the risk to develop gastroenteritis after a contact with rotavirus could be different in the global population, including milder forms of gastroenteritis. Moreover, this selection bias is one hypothesis explaining that the validated severity scores couldn't be used. Severity scores have

been used to assess the effect of rotavirus vaccination but weren't discriminative enough to classify the AGE clinical severity in patients consulting in a hospital. The use of a non-validated score can be considered as a second bias; it has been compared to a review of medical records and was well correlated with clinical and biological signs of dehydration (24). It is based on a combination of two scores commonly used (CDS and Vesikari Score) because none of these was discriminative enough. Severity of gastroenteritis depends on two elements: digestive impairment and clinical dehydration. CDS only rallied clinical information about clinical dehydration that can be absent if adapted rehydration was conducted, even if digestive symptoms are important. The Vesikari Score rallied information about symptoms duration and degree of dehydration, those items are not efficient for patients with rapid clinical deterioration: most of infants who received a vascular filling had a low Vesikari score, whereas these patients are particularly at risk of death in absence of medical care. Sultan and al. have also find that rotavirus gastroenteritis were mainly moderate to severe, according Vesikari score (25). To select the most discriminative items to assess gastroenteritis severity, we consider frequently used hospitalization criteria. Thresholds were chosen secondarily, after reviewing medical records and classifying patients according to their gravity. This score should be tested in other hospitals to ensure its reliability.

Another limitation was the use of a questionnaire, reflecting children state at a precise moment that could have led to ignore some of severe clinical forms: for example, children who had severity criteria in hospitalization; meaning after the questionnaire had been completed. The review of medical record has reduced this risk: we changed our design study and decided to take into account the all episode of gastroenteritis to assess the severity, and not only their evaluation in ED.

The strength of this study is its originality. Indeed, previous studies also showed that in developed countries, were P[8] and P[4] are the predominant strains, rotavirus infected individuals with secretor and Lewis positives phenotypes (15, 16); but none have studied yet the impact of genetic mutations on the severity of rotavirus infection.

CONCLUSION

A part of children have a natural protection against rotavirus gastroenteritis, conferred by expression of HGBA. Children with secretor negative and Lewis negative phenotypes do not developed gastroenteritis after a contact with P[8] strain rotavirus, but heterozygotic FUT2 mutation is not associated with a milder severity gastroenteritis form. Half of the children from O histo-blood group will not develop rotavirus gastroenteritis after a contact with this strain, but when infected, children do not have milder severity gastroenteritis forms. The results from children included in Cayenne will be necessary to confirm these preliminary results.

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CONFLICT OF INTEREST

The authors have no funding or conflicts of interest to disclose.

REFERENCES

1. Boschi-Pinto, Cynthia. « Estimating Child Mortality Due to Diarrhoea in Developing Countries ». *Bulletin of the World Health Organization* 86, nº 9 (1 septembre 2008): 710-17. https://doi.org/10.2471/BLT.07.050054.

2. Tate, Jacqueline E., Anthony H. Burton, Cynthia Boschi-Pinto, et Umesh D. Parashar. « Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000–2013 ». *Clinical Infectious Diseases* 62, nº suppl 2 (1 mai 2016): S96-105. https://doi.org/10.1093/cid/civ1013.

3. Rougemont, A. de, J. Kaplon, C. Fremy, M.-C. Legrand-Guillien, A. Minoui-Tran, C. Payan, A. Vabret, et al. « Clinical Severity and Molecular Characteristics of Circulating and Emerging Rotaviruses in Young Children Attending Hospital Emergency Departments in France ». *Clinical Microbiology and Infection* 22, nº 8 (août 2016): 737.e9-737.e15. https://doi.org/10.1016/j.cmi.2016.05.025.

4. Melliez, H., P.-Y. Boelle, S. Baron, Y. Mouton, et Y. Yazdanpanah. « Morbidité et coût des infections à rotavirus en France ». *Médecine et Maladies Infectieuses* 35, nº 10 (octobre 2005): 492-99. https://doi.org/10.1016/j.medmal.2005.08.007.

5. Lorrot, M., F. Bon, M. J. El Hajje, S. Aho, M. Wolfer, H. Giraudon, J. Kaplon, et al. « Epidemiology and Clinical Features of Gastroenteritis in Hospitalised Children: Prospective Survey during a 2-Year Period in a Parisian Hospital, France ». *European Journal of Clinical Microbiology & Infectious Diseases* 30, nº 3 (mars 2011): 361-68. https://doi.org/10.1007/s10096-010-1094-9.

6. Lewnard, Joseph A., Benjamin A. Lopman, Umesh D. Parashar, Aisleen Bennett, Naor Bar-Zeev, Nigel A. Cunliffe, Prasanna Samuel, et al. « Heterogeneous Susceptibility to Rotavirus Infection and Gastroenteritis in Two Birth Cohort Studies: Parameter Estimation and Epidemiological Implications ». Édité par Cecile Viboud. *PLOS Computational Biology* 15, nº 7 (26 juillet 2019): e1007014. https://doi.org/10.1371/journal.pcbi.1007014.

7. Sultan, Muhammad Ashraf, et Zeeshan Hassan. « Assessment of Severity of Acute Gastroenteritis in the Paediatric Pakistani Population by Modified Vesikari Score ». J Pak Med Assoc 68, nº 2 (2018): 6.

8. Nakagomi, Toyoko, Kazuya Kato, Hiroyuki Tsutsumi, et Osamu Nakagomi. « The Burden of Rotavirus Gastroenteritis among Japanese Children during Its Peak Months: An Internet Survey ». Japanese Journal of Infectious Diseases 66, nº 4 (2013): 269-75. https://doi.org/10.7883/yoken.66.269.

9. Ruuska, Tarja, et Timo Vesikari. « Rotavirus Disease in Finnish Children: Use of Numerical Scores for Clinical Severity of Diarrhoeal Episodes ». Scandinavian Journal of Infectious Diseases 22, nº 3 (janvier 1990): 259-67. https://doi.org/10.3109/00365549009027046.

10. Friedman, Jeremy N., Ran D. Goldman, Rajendu Srivastava, et Patricia C. Parkin. « Development of a Clinical Dehydration Scale for Use in Children between 1 and 36 Months of Age ». *The Journal of Pediatrics* 145, nº 2 (août 2004): 201-7. https://doi.org/10.1016/j.jpeds.2004.05.035.

11. Gentsch, Jon R, Ashley R Laird, Brittany Bielfelt, Dixie D Griffin, Vivek Jain, Nigel A Cunliffe, Osamu Nakagomi, et al. « Serotype Diversity and Reassortment between Human and Animal Rotavirus Strains: Implications for Rotavirus Vaccine Programs », s. d., 14.

12. Crawford, Sue E., Sasirekha Ramani, Jacqueline E. Tate, Umesh D. Parashar, Lennart Svensson, Marie Hagbom, Manuel A. Franco, et al. « Rotavirus Infection ». *Nature Reviews Disease Primers* 3, nº 1 (décembre 2017): 17083. https://doi.org/10.1038/nrdp.2017.83.

13. Burke, Bernard, et Ulrich Desselberger. « Rotavirus Pathogenicity ». *Virology* 218, nº 2 (avril 1996): 299-305. https://doi.org/10.1006/viro.1996.0198.

14. Burnett, Eleanor, Christine L. Jonesteller, Jacqueline E. Tate, Catherine Yen, et Umesh D. Parashar. « Global Impact of Rotavirus Vaccination on Childhood Hospitalizations and Mortality From Diarrhea ». *The Journal of Infectious Diseases* 215, nº 11 (1 juin 2017): 1666-72. https://doi.org/10.1093/infdis/jix186.

15. Imbert-Marcille, Berthe-Marie, Laure Barbé, Mathilde Dupé, Béatrice Le Moullac-Vaidye, Bernard Besse, Cécile Peltier, Nathalie Ruvoën-Clouet, et Jacques Le Pendu. « A FUT2 Gene Common Polymorphism Determines Resistance to Rotavirus A of the P[8] Genotype ». *The Journal of Infectious Diseases* 209, nº 8 (15 avril 2014): 1227-30. https://doi.org/10.1093/infdis/jit655.

16. Loureiro Tonini, Marco André, Débora Maria Pires Gonçalves Barreira, Luciana Bueno de Freitas Santolin, Lays Paula Bondi Volpini, José Paulo Gagliardi Leite, Béatrice Le Moullac-Vaidye, Jacques Le Pendu, et Liliana Cruz Spano. « FUT2, Secretor Status and FUT3 Polymorphisms of Children with Acute Diarrhea Infected with Rotavirus and Norovirus in Brazil ». *Viruses* 12, nº 10 (25 septembre 2020): 1084. https://doi.org/10.3390/v12101084.

17. Iturriza-Gómara, M., T. Dallman, K. Bányai, B. Böttiger, J. Buesa, S. Diedrich, L. Fiore, et al. « Rotavirus Genotypes Co-Circulating in Europe between 2006 and 2009 as Determined by EuroRotaNet, a Pan-European Collaborative Strain Surveillance Network ». *Epidemiology and Infection* 139, nº 6 (juin 2011): 895-909. https://doi.org/10.1017/S0950268810001810.

18. Pérez-Ortín, Raúl, Susana Vila-Vicent, Noelia Carmona-Vicente, Cristina Santiso-Bellón, Jesús Rodríguez-Díaz, et Javier Buesa. « Histo-Blood Group Antigens in Children with Symptomatic Rotavirus Infection ». *Viruses* 11, nº 4 (10 avril 2019): 339. https://doi.org/10.3390/v11040339.

19. Lependu, J., Compte-Rendu Final.

20. Günaydın, Gökçe, Johan Nordgren, Sumit Sharma, et Lennart Hammarström. « Association of Elevated Rotavirus-Specific Antibody Titers with HBGA Secretor Status in Swedish Individuals: The FUT2 Gene as a Putative Susceptibility Determinant for Infection ». *Virus Research* 211 (janvier 2016): 64-68. https://doi.org/10.1016/j.virusres.2015.10.005.

21. Santos, Norma, et Yasutaka Hoshino. « Global Distribution of Rotavirus Serotypes/Genotypes and Its Implication for the Development and Implementation of an Effective Rotavirus Vaccine ». Reviews in Medical Virology 15, no 1 (janvier 2005): 29 56. https://doi.org/10.1002/rmv.448.

22. Barbé, Laure, Béatrice Le Moullac-Vaidye, Klara Echasserieau, Karine Bernardeau, Thomas Carton, Nicolai Bovin, Johan Nordgren, Lennart Svensson, Nathalie Ruvoën-Clouet, et Jacques Le Pendu. « Histo-Blood Group Antigen-Binding Specificities of Human Rotaviruses Are Associated with Gastroenteritis but Not with in Vitro Infection ». Scientific Reports 8, no 1 (décembre 2018): 12961. https://doi.org/10.1038/s41598-018-31005-4.

23. Sharma, Sumit, Marie Hagbom, Lennart Svensson, et Johan Nordgren. « The Impact of Human Genetic Polymorphisms on Rotavirus Susceptibility, Epidemiology, and Vaccine Take ». Viruses 12, no 3 (17 mars 2020): 324. https://doi.org/10.3390/v12030324.

24. Hayajneh, Wail A, Hussein Jdaitawi, Abdullah Al Shurman, et Yaseen A Hayajneh. « Comparison of Clinical Associations and Laboratory Abnormalities in Children With Moderate and Severe Dehydration »: Journal of Pediatric Gastroenterology and Nutrition 50, no 3 (mars 2010): 290 94. https://doi.org/10.1097/MPG.0b013e31819de85d.

25. Sultan, Muhammad Ashraf, et Zeeshan Hassan. « Assessment of Severity of Acute Gastroenteritis in the Paediatric Pakistani Population by Modified Vesikari Score ». J Pak Med Assoc 68, nº 2 (2018): 6.

APPENDIX

Appendix 1. Medical questionnaire.

INCLUSION GASTROVIM	
Date de consentement parental/représentants légaux : __ / __ / ____ Date de signature de l'enfant si l'âge de l'enfant est supérieur à 6 ans : __ / __ / ____ ⇒ **Inclusion le : __ / __ / ____**	**Signature investigateur :**

CRITERES D'INCLUSION	**OUI**	**NON**
Enfant de 0 à 16 ans consentant (si >6ans) à participer à l'étude GASTROVIMc	☐	☐
Admission aux Urgences Pédiatriques dans un contexte de GEA à RVA, authentifié par TDR direct	☐	☐
Consentement éclairé d'un ou des parents/représentants légaux	☐	☐
CRITERES D'EXCLUSION	**OUI**	**NON**
Situation de co-infections virales impliquant d'autres catégories de virus (norovirus, astrovirus…) confondantes dans le cadre de la recherche GASTROVIMc.	☐	☐
Tableau évocateur d'une GEA d'origine bactérienne (diarrhée invasive glairo sanglante)	☐	☐

DONNEES CAS

- **Ethnie mère :**

☐ Créole guyanais ☐ Haïtien ☐ Antillais ☐ Amérindien ☐ Brésilien ☐ Surinam ☐ Afrique subsaharien

☐ Asiatique ☐ Européen ☐ Afrique du nord/Moyen orient ☐ Guyanais ☐ Polynésien ☐ Dominicain

☐ Chinois ☐ Péruvien ☐ Autre : ……

- **Ethnie père :**

☐ Créole guyanais ☐ Haïtien ☐ Antillais ☐ Amérindien ☐ Brésilien ☐ Surinam ☐ Afrique subsaharien

☐ Asiatique ☐ Européen ☐ Afrique du nord/Moyen orient ☐ Guyanais ☐ Polynésien ☐ Dominicain

☐ Chinois ☐ Péruvien ☐ Autre : ……

- Durée des symptômes au moment de la consultation : _ _ jours
- Vaccination Rotavirus : ☐ OUI ☐ NON
  - Si oui : ○ Rotarix et année de vaccination : ○ Rotateq et année de vaccination :
- Nombre de consultations préalables à cette admission aux urgences dans le cadre de la GEA : _ _
  - Professionnel médical : _____ Date : _ _ / _ _ / _ _ _ _
  - Professionnel médical : _____ Date : _ _ / _ _ / _ _ _ _
  - Professionnel médical : _____ Date : _ _ / _ _ / _ _ _ _
- % Déshydratation évalué par le médecin : _ _ %
- Température maximum : _ _ , _ °C
- **Score de Friedman pour les enfants de 1 à 36 mois : Fait/Non Fait**
  - Si fait, calcul automatique (/8) : ☐ NA (pour les enfants supérieurs à 36 mois)

Caractéristiques	**Description**	**Points**
Apparence générale	Normal	0
	Altéré ou agité ou léthargique mais réactif au toucher	1
	Somnolent ou hypotonique ou froid ou en sueur ± comateux	2
yeux	Normaux	0
	Légèrement creux	1
	Très creux	2
Muqueuses	Humides	0
	pâteuses	1
	sèches	2
Larmes	Présence de larmes	0
	Diminution des larmes	1
	Absence de larmes	2

- Diarrhée : ☐ OUI ☐ NON
  - Durée des selles diarrhéiques à l'arrivée aux urgences : _ _ jours
  - Nombre maximum de selles par jour : _ _
- Vomissements : ☐ OUI ☐ NON
  - Durée des vomissements : _ _ jours
  - Nombre maximum de vomissements / 24 heures : _ _
- Réhydratation orale aux urgences : ☐ OUI ☐ NON
- Perfusion aux urgences : ☐ OUI ☐ NON

  Si oui : ○ Sérum physiologique ○ PG5% ○ autre : _ _ _ _ _ _ _ _
- Hospitalisation : ☐ OUI ☐ NON
- Thérapeutiques suite au passage aux urgences : ☐ OUI ☐ NON
  - Si oui :
    - _ _ _ _ _ _ _ _ _ _ _ _ _ _ Durée : _ _ jours
    - _ _ _ _ _ _ _ _ _ _ _ _ _ _ Durée : _ _ jours
    - _ _ _ _ _ _ _ _ _ _ _ _ _ _ Durée : _ _ jours
- Bilans / explorations suite au passage aux urgences : ☐ OUI ☐ NON
  - Si oui :
    - _ _ _ _____ _ _ _ Date : _____ _ _ _

Appendix 2. Clinical dehydration score (adapted from Friedman et al [10])

TABLE 1 Friedman et al's[10] CDS[a]

Characteristic	Score of 0	Score of 1	Score of 2
General appearance	Normal[b]	Thirsty, restless, or lethargic but irritable when touched	Drowsy, limp, cold, sweaty; comatose or not
Eyes	Normal	Slightly sunken	Very sunken
Mucous membranes[c]	Moist	Sticky	Dry
Tears	Tears	Decreased tears	Absent tears

Scores for the individual items are summed.

[a] Higher scores indicate more severe dehydration. Scores range from 0 to 8. A score of 0 correlates with <3% dehydration (positive likelihood ratio 2.2; 95% CI 0.9–5.3), scores of 1–4 correlate with some (3%–6%) dehydration (positive likelihood ratio 1.3, 95% CI 0.9, 1.7), and 5–8 correlates with moderate to severe (≥6%) dehydration (positive likelihood ratio 5.2; 95% CI 2.1, 12.8).[14]

[b] "Normal" includes children who may be sleeping but are easily aroused to a normal level of consciousness. This assessment takes into account the time of day and the child's usual pattern as described by the child's guardian.

[c] This is assessed on the buccal mucosa and tongue, and not the lips.

Appendix 3. Vesikari score (9).

Parameter	1	2	3
Diarrhea			
Maximum number stools per day	1–3	4–5	≥6
Diarrhea duration (day)	1–4	5	≥6
Vomiting			
Maximum number per day	1	2–4	≥5
Vomiting duration (day)	1	2	≥3
Maximum body temperature (℃)	37.1–38.4	38.5–38.9	≥39.0
Severity of dehydration (%)	N/A	1–5	≥6
Treatment	Rehydration	Hospitalization	N/A
Severity rating scales	<7 (mild)	7–10 (moderate)	≥11 (severe)

Adapted from Ruuska T and Vesikari T. Scand J Infect Dis 1990;22:259-67[4].

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Titre de Thèse : L'IMPLICATION DES STATUTS FUT2, FUT3 ET ABO DANS LA SÉVÉRITÉ DES INFECTIONS À ROTAVIRUS

RESUME

Cette étude observationnelle prospective, menée entre 2017 et 2019, évalue le risque de développer une gastroentérite à rotavirus et la gravité des symptômes en fonction de caractéristiques génétiques ayant précédemment démontré leur rôle dans la susceptibilité à la maladie. La population étudiée est composée 200 enfants atteints de gastroentérite à rotavirus, ayant été inclus après leur passage aux urgences pédiatriques du CHU de Nantes et de 134 témoins. Les 200 enfants malades avaient quasiment exclusivement un phénotype sécréteur et Lewis négatif (99 et 98%, $p<0,001$). Les enfants de phénotype sécréteur porteur d'une mutation hétérozygote sur le gène FUT2, n'ont pas une forme moins grave de la maladie. Les enfants du groupe O sont moins souvent malades que ceux des autres groupes histo-sanguins ($p<0,01$), mais quand ils sont atteints, la gravité clinique de la gastroentérite ne diffère pas de celle des autres enfants. Les résultats concernant une population d'enfants guyanais dont le profil génétique et les souches circulantes de rotavirus sont possiblement différents seront comparés à ces premiers résultats métropolitains.

MOTS-CLES

GASTROENTERITE AIGUE A ROTAVIRUS GASTROENTERITE, SEVRITE DES GASTROENTERITES, HGBA, GENE FUT2, PHENOTYPE SECRETEUR, GENE FUT3, PHENOTYPE LEWIS, GROUPE ABO