Relationship between Lactase Activity and Intestinal Flora Structure

Ying Zhang
Mingjian Huang
Jianfeng Chen
Shuyuan Wang
Jian Xu

Ying Zhang Department of Pediatrics, Zhuhai District Maternal and Child Health Hospital, Guangzhou 510220, Guangdong Province, China, Mingjian Huang Department of Pediatrics, Zhuhai District Maternal and Child Health Hospital, Guangzhou 510220, Guangdong Province, China, Jianfeng Chen Department of Pediatrics, Zhujiang Hospital of Southern Medical University, Guangzhou 510220, Guangdong Province, China, Shuyuan Wang Department of Laboratory, Guangzhou Zhuhai District Maternal and Child Health Hospital, Guangzhou 510220, Guangdong Province, China, Jian Xu* Department of General Surgery, Zhuhai District Hospital of Traditional Chinese Medicine, Guangzhou 510220, Guangdong Province, China, *Corresponding author: Jian Xu, No. 1 Nanyuan New Village, Qianjin Road, Haizhu District, Guangzhou City, Guangdong Province, China, Email: 471066426@qq.com

To analyze and explore the relationship between lactase activity and the structure of intestinal flora in infants under 1 year old. Method: Our hospital selected 60 infants and young children in the Haizhu Maternity and Child Health Hospital from August 2019 to September 2020 as the research objects. The infants in the experimental group were diagnosed as lactose intolerant infants, the control group were normal infants, and the experimental group and control group had 30 cases each. The relationship between lactase activity and intestinal flora structure. Result: The experimental group had fewer beneficial bacteria in the intestinal tract than the control group, but improved lactase activity by correcting the structure of the intestinal flora and alleviating symptoms of lactose intolerance. Conclusion: Infants and young children are prone to non-infectious diarrhea, and the causes are more complicated, but most of them are related to lactose intolerance. When infants and young children develop lactose intolerance, it is necessary to pay attention to the lack of beneficial bacteria in the intestinal tract. Targeted supplementation of probiotics (such as Lactobacillus reuteri, Clostridium butyricum, etc.) can correct the intestinal flora, improve lactase activity, and relieve symptoms of lactose intolerance.

Infants under the age of 1 mainly consume breast milk or dairy products, with lactose as the main component. Lactose is the main energy source of infants and children, which is related to the normal development of the brain. Lactose is absorbed and utilized after hydrolysis of lactase on its mucosal microvilli in the small intestine, so the digestion and absorption of intestinal lactose depends on the quantity and activity of lactase. The activity of lactase is highest in jejunum. The main component of lactose hydrolysis is lactase-phlorizin hydrolase (LPH). LPH has the activity of two enzymes: Lactase (β-D-galactosidase - galactose hydrolase), lactase-phlorizin hydrolase (glycosyl-N-ceramide- glucose hydrolase). Lactase deficiency prevents lactose in breast milk or dairy products from being decomposed and absorbed, leading to increased osmotic pressure in the intestinal cavity and osmotic diarrhea; Unhydrolyzed lactose breaks down by intestinal bacteria in the colon, produces large amounts of formic acid, lactic acid and other short-chain fatty acids and hydrogen, and leads to diarrhea, abdominal distension and abdominal pain. It is clinically called lactose intolerance. Lactose intolerance, as a common cause of chronic diarrhea in infants, can easily lead to malnutrition, anemia and insufficient energy intake in infants, and affect their physical development and intelligent development. High incidence of lactose intolerance in infants in China reaches about 47~70%. There are many methods to detect lactase deficiency, such as isotope double immunofluorescence labeling method, expiratory hydrogen test, oral sugar

tolerance test, fecal reducing sugar and pH value test, urine galactose colorimetry, etc. These methods indirectly prove the activity of disaccharide by the detection of disaccharide metabolites, and their reliability is far less than that of the direct determination of disaccharide activity in small intestinal mucosa. At present, the most reliable method for the diagnosis of lactase deficiency is cavity biopsy to determine lactase activity directly, but its invasive nature makes it difficult to be used as a routine method. Urine galactose oxidase method is used to detect and diagnose lactose intolerance in our hospital. The relationship among lactose intolerance, lactase activity and intestinal flora is the main research direction. Intestinal flora can help digestion and absorption of the intestinal tract, and the resistance to colonization and reproduction of pathogenic microorganisms; It can promote the differentiation of intestinal mucosal epithelial cells, stimulate the development of the whole-body immune system and intestinal immune system, and establish immune tolerance. The intestinal flora structure in the initial colonization stage of infancy is affected by many factors, such as delivery mode, gestational age, feeding mode, food, infection, antibiotic use, family environment and so on. Therefore, we study whether the intestinal flora affects the digestion and absorption of lactose, and whether the changes of intestinal microecology are related to the severity of gastrointestinal symptoms of lactose intolerance, understand whether the changes in the composition of intestinal flora can change the acidity and alkalinity of intestinal lactase and affect the activity of intestinal lactase.

DATA AND METHODS

General data: Sixty infants and young children in the Haizhu Maternity and Child Health Hospital from August 2019 to September 2020 were selected as the research objects. The selected subjects were infants (including newborns) taking breast milk or lactose-containing breast-feeding under 1 years of age, without digestive tract malformation, intestinal obstruction and gastrointestinal surgery history or metabolic disease. The selected subjects were divided into lactase intolerance group and normal group by the clinical manifestations of typical lactose

intolerance and the qualitative detection of urinary galactose by galactose oxidase method, among them, 30 cases were in the lactose intolerance group.

Approved by the Hospital Ethics Committee, the family members of the selected research objects have signed an informed consent.

Qualitative detection method of urine galactose adopted urine galactose detection kit (Beijing Zhongsheng Jinyu Diagnostic Technology Co., Ltd.). It was detected with urine galactose oxidase method The subjects drank breast milk or ordinary formula in proportion to 10 ml /kg, collected 2~3 h of urine, and operated according to the instructions. It was judged as lactose tolerance if the sample hole was darker or consistent; it was judged as lactose intolerance if the sample hole was lighter or showed no color.

V4-V5 Sequencing of 16S rRNA gene in intestinal flora Fresh feces samples were collected and total DNA was extracted. The V4-V5 region of 16SrRNA gene of intestinal flora was amplified by PCR. V4-V5 sequencing of 16SrRNA gene of intestinal flora was obtained using MiSeq high-throughput sequencing technology. The original sequencing data were sent to NCBI (SRA) database, and the sequences were analyzed by QIIME2, Uchime and other software.

Observation index 1) The relationship between intestinal flora and lactase activity and lactose intolerance symptoms such as diarrhea, abdominal distension and abdominal pain.

SPSS 21.0 statistical software was used. t test was used to compare the measurement data. $\chi 2$ test was used to compare the count data. Univariate analysis showed that the difference was statistically significant ($P < 0.05$).

RESULTS

Using MiSeq high-throughput sequencing technology, the total DNA was extracted from 60 stool samples, and PCR amplification was used to obtain the V4-V5 region of the intestinal flora 16SrRNA gene. The MiSeq high-throughput sequencing technology was used to obtain the sequence information of the V4-V5 region of the intestinal flora 16S rRNA gene. After sequencing, a total of 180,909 effective sequencing was obtained, and the average length of the sequencing was 648 bp.

A single sample has at least 3621 reads and a maximum of 11051 reads, with an average of 6174 per sample. Statistics of each sample OTU classification and corresponding species taxonomic pedigree, all sequences have 10 phyla, 20 categories, 57 families, 104 genera, and 143 species.

The dilution curve is completed by the Qiime2 diversity alpha-rarefaction plug-in, which calculates all specified alpha-rarefaction indicators between step 1 and maximum depth. At each sampling depth step, a sparse table is generated, and the diversity index of all samples in the table is calculated. As shown in Figure 1 below, the sample curve is close to the plateau, which proves that the sequencing depth is sufficient and most of the microbial diversity information in the data set.

Figure 1.
shows a visualization example of sparse curves using Qiime2
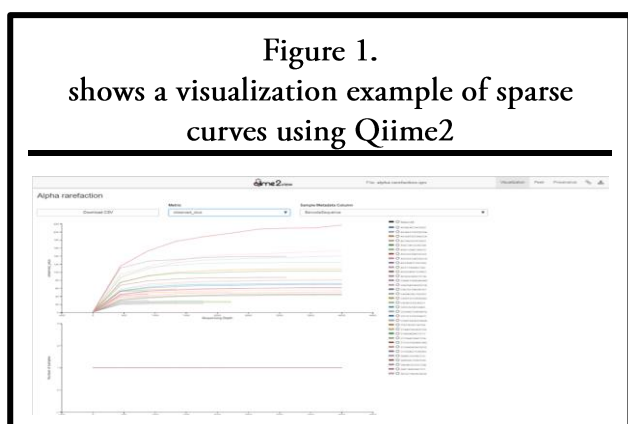
Alpha-significance analysis

Using the provided sample grouping information or metadata, use the Qiime2 diversity alpha-group-significance plugin to test the association between metadata categories and Alpha-diversity. All previously calculated Alpha-diversity indicators (section 3.1) are included in this association test. Figure 2 shows an example of the results of the correlation test. This set of significance tests allows
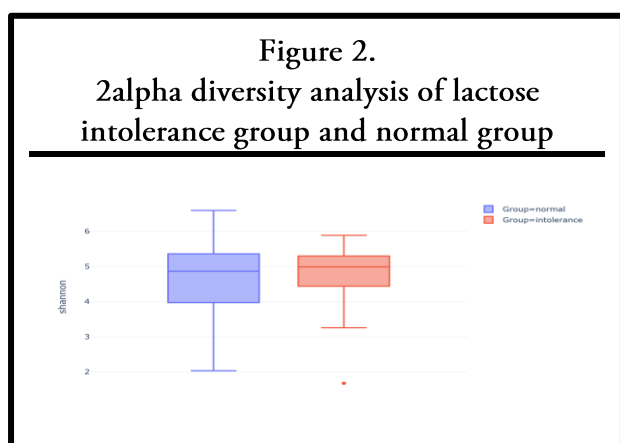
researchers to visualize which metadata categories are most strongly correlated with differences in microbial community richness and uniformity. In addition, a Kruskal-Wallis statistical test was performed to show whether the identified population differences are statistically significant.

Figure 2 showed that normal infants had higher flora richness and more dominant strains than lactose intolerant infants.

Beta-diversity index calculation

Beta-diversity is an indicator to measure the size of the difference between samples. It is usually measured by spatial distance. Common measurement indicators and characteristics are as follows:

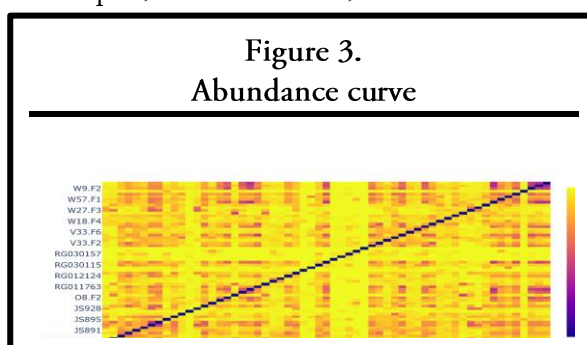Commonly used Beta-diversity indicators and their significance

1. Jaccard distance: a qualitative measure of group differences.

2. Bray-Curtis distance: a quantitative measure of group differences.

3. Unweighted UniFrac distance: a qualitative measure of community differences, including phylogenetic relationships between features/organisms.

4. Weighted UniFrac distance: a quantitative measurement method of community differences, including the phylogenetic relationship between features/organisms.

The results of the distance matrix evaluated between the samples can be found in the Beta Diversity catalog, and the results can be viewed using spreadsheet software, such as Ms Excel or Libre Office Calc. The obtained distance matrix can also be represented by different transformation or sorting techniques, such as PCoA, NMDS and UPGMA

Figure 2.
2alpha diversity analysis of lactose intolerance group and normal group

Figure 3.
Abundance curve

graphs. The results are shown in Figure 3.
PLS-DA analysis

Partial least squares - discriminant analysis (pls-da) is a supervised classification that has been widely used in the analysis of multivariate data sets to predict actual differences in sample or sample grouping. The resulting ranking graph illustrated

Figure 4.
## Partial least squares - discriminant analysis (PLS-DA) conspiracy
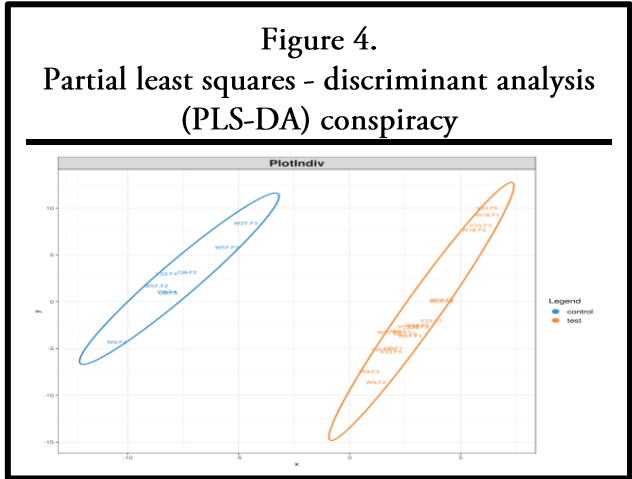
the most sample grouping, and the resulting graph was shown in Figure 4 below. The infants with lactose intolerance were in the test group and the normal infants were in the control group.

Control the levels of intestinal flora in the two groups of infants with lactose intolerance (test) and normal (control). Among them, the proportion of beneficial bacteria such as Lactobacillus reuteri, Actinomycetaceae, and Clostridium butyricum is higher than that of lactose intolerant infants. There are more lactose intolerant infants with Ruminococcaceae, Clostridium prastia, and Faeculus procuratus, and the difference is statistically significant (P<0.05), see Figure 5.

DISCUSSION

Infants under the age of 1 mainly consume breast milk or dairy products, with lactose as the main component. Lactose is the main energy source of

Figure 5.
Sex Discriminant Analysis Effect Size (LEfSe) Analysis Plot (LDA Score>2.0)

infants and children under the age of 1. Lactose can be decomposed into glucose and galactose by lactase in intestinal tract; Most galactose is converted to glucose in the liver, while untransformed galactose is excreted by the urinary tract. If the lactase activity is low, the levels of glucose and galactose in the blood decreases and the amount of galactose in urine also decreases with it. Therefore, urinary galactose determination is the main auxiliary method to judge lactase activity in infants. At the same time, intestinal flora also affects lactase activity. The intestinal flora of healthy people is maintained in a reversible range of physiological fluctuations. Intestinal dominant bacteria inhibit the growth and reproduction rate of local aerobic bacteria by consuming intestinal oxygen, in order to maintain the balance of intestinal flora. The intestinal flora imbalance leads to the decrease of lactase activity in infants, which is prone to lactose intolerance symptoms, diarrhea, abdominal distension, abdominal pain and other symptoms. Because lactose is the main intake of infants under 1 years of age, it can easily affect the absorption of trace elements, nutrition and energy, and affect the growth and development of infants, and reduce the quality of life of children. V4-V5 sequencing of 16SrRNA gene of intestinal flora was obtained using high-throughput sequencing technology. Sequencing raw data was delivered to NCBI(SRA) database and the sequence was analyzed by software. The data measured in this study have concentrated most of the microbial diversity information in the sample, and the results can reflect the true situation of the microorganism, which can provide a theoretical basis for regulating the intestinal flora in the treatment of lactose intolerance. By studying the structural characteristics of intestinal flora, it was found that the disease was accompanied by significant changes in the composition of intestinal flora. Alpha diversity of intestinal flora decreased in the infants with lactose intolerance. Intestinal flora had a certain effect on lactase activity [19]. Higher intestinal flora diversity was considered a marker of intestinal maturation. With low sensitivity to environmental factors, it was not susceptible to infection. Studies have shown that the relative abundance of Lactobacillus reuteri and Clostridium butyricum in healthy babies has increased
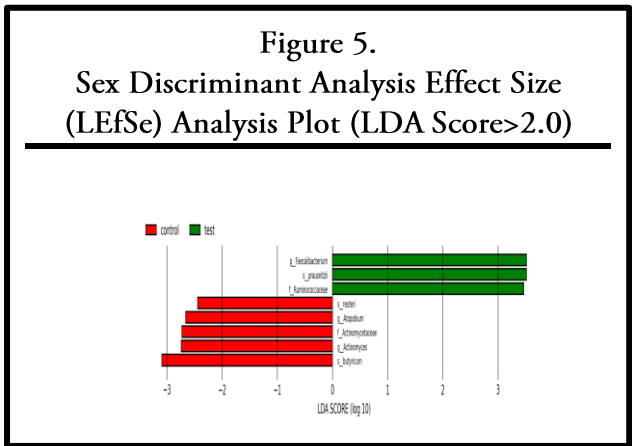
significantly. These two florae are common probiotics. With a strong adhesion ability to the intestinal mucosa, Lactobacillus reuteri can improve the distribution of intestinal flora, antagonize the colonization of harmful bacteria, and avoid intestinal diseases without inhibiting beneficial flora in gastrointestinal tract. At the same time, it can improve human function, the body's immunity and the new metabolism of the body; Clostridium butyricum can secrete tyric acid (butyric acid), an important nutrient for intestinal mucosal regeneration and repair, promote the growth of intestinal probiotics such as Bifidobacterium, inhibit the growth of intestinal harmful bacteria, maintain the balance of intestinal flora, reduce the production of intestinal toxins such as amine, ammonia, indole and poison to intestinal mucosa, and restore the immune function and normal physiological function of intestinal tract. At the same time, clostridium butyricum can produce enzymes and vitamins in the intestinal tract to promote the digestion and absorption of nutrients. Further studies showed that the relative abundance of all short-chain fatty acid-producing bacteria in lactose intolerant infants increased. Prevotella (Prevotella ruminicola) are mainly involved in the catabolism of carbohydrates and plant proteins in the intestine. The main products after decomposition are acetic acid and hydrogen. Bacteroides mainly produce succinic acid and acetic acid. Tumor coccus belongs to fermented carbohydrates that produce acetic acid and propionic acid. Short-chain fatty acids, such as acetic acid, propionic acid, butyric acid, can be absorbed by colonic epithelium and maintain the balance of intestinal microbial environment by regulating intestinal PH values. These florae are normal intestinal flora, but infants still have lactose intolerance symptoms, in a lower proportion than normal infants' probiotics. The results showed that the balance of intestinal microbial system and the increase of probiotics could increase the activity of lactase and reduce the occurrence of lactose intolerance symptoms. The core flora is shared by healthy infants and lactose intolerant infants. Lactose intolerance is less likely to occur when intestinal beneficial flora increases. Therefore, according to the lack of intestinal flora, the targeted supplement of lactose intolerance infants can alleviate the symptoms of lactose intolerance.

To sum up, when infants suffer from lactose intolerance, we need to note the lack of beneficial flora in their intestines. The targeted supplement can correct the situation of intestinal flora, increase the lactase activity and alleviate the symptoms of lactose intolerance.

ACKNOWLEDGEMENT

REFERENCES

1. HeymanMB. Lactose intolerance in infants, children, andadolescents[J]. Pediatrics 2006; 118(3): 1279 -1286.
2. Jin HZ. Lactose intolerance in infants[J]. Chinese Journal of Practical Pediatrics 2000; 15(5): 311 -312.
3. SahiT. Geneticsandepidemiologyofadult-typehypolactasia[J]. ScandJGastroenterolSuppl 1994; 202: 7-20.
4. Yan RZ, Chang YZ. Detection and analysis of rotavirus infection diarrhea and lactose intolerance in infants[J]. International Journal of Laboratory Medicine 2016; 37(10): 1426-1427.
5. Liu Y. Effect analysis of lactose free milk powder on lactose intolerant diarrhea children[J]. China Continuing Medical Education 2015; 7(33): 135-136.
6. Yang Y, He M, Cui H, Bian L, Wang Z. The prevalence of lactasc deficicncy and lactose intolerance in Chinese children of different ages. Chin Med J(Engl) 2000; 113(12): 1129-1132.
7. Imtiaz F, Savilahti E, Samesto A, et al. The T/G-13915 variant upstream of the lactase gene is the founder allele of lactase persistence in an urban Saudi population. J Med Genet 2007; 44(10): e89.
8. Ingram CJ, Elamin MF, Mulcare CA, et al. A novel polymorphism associated with lactose tolerance in Africa:multiple causes for lactase persistence?Hum gener 2007; 120(6): 779-778.
9. Sun HM,QiaoYD,Chen F,et al.The lactase gene-13910T allele cannot predict the lactase-persistence phenotype in north China.Asia Pac J Clin Nutr 2007; 16(4): 298-601.
10. Lv XY. Research trends of lactose intolerance[J]. Modern preventive medicine 2005; 32: 319-321.
11. Liu H. Effect of lactase adjuvant therapy on lactose intolerance secondary to rotavirus enteritis in infants[J]. Jiangsu medicine 2015; 41(13): 1574-1575.
12. Li XQ, Zhou F, Li YC, Guo YQ, Wang XF. Clinical significance of 13C breath test in lactose intolerance secondary to rotavirus diarrhea. Chinese Journal of Practical Diagnosis and Clinical Treatment 2011; 25: 399-400.
13. Zhang YY, Zhou LL, Yang SF. Basic study on primary lactase deficiency. International Journal of Pediatrics 2014; 41: 302-304.
14. Wang SZ, Yu JM. Clinical study on pediatrics of traditional Chinese medicine. Beijing: People's Medical Publishing House 2009: 228.

15. Lu R, Diao ZY, Xu XX. Effect of lactase on lactose intolerance secondary to acute diarrhea in infants. Journal of Pediatric Pharmacy 2013; 19: 23-25.

16. He W, Huang L, Lv B, et al. Optimization of conditions for determination of lactase activity in small intestine of BALB/c mice by disaccharidase method[J]. Journal of Zhengzhou University (Medical Sciences) 2005; 40(3): 405-408.

17. Xie J, Gong ST, Qu WJ, et al. Determination of disaccharidase activity in duodenal mucosa of children in Guangzhou area[J]. Journal of Clinical Pediatrics 2008; 3: 217-220.

18. Qiao R, Huang CY, Zeng G, et al. Application of lactose detection in feces in screening lactase deficiency patients[J]. Chinese Journal of Preventive Medicine 2006; 40(2): 112.

19. Bhale, S. P., Yadav, A. R., Pathare, P. G., Tekale, S. U., Franguelli, F. P., Kótai, L., & Pawar, R. P. SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF Transition Metal Complexes Of 4-[(2-Hydroxy-4-Methoxyphenyl) Methyleneamino]-2, 4-Dihydro-3h-1, 2, 4-Triazole-3-Thione. European Chemical Bulletin, 2020, 9(12), 430-435. http://dx.doi.org/10.17628/ecb.2020.9.430-435