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Gut microbiota dysbiosis is associated with Henoch-Schönlein Purpura in children

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ABSTRACT

Background: Alterations in the intestinal microbiota have been associated with the development of allergic diseases, such as asthma and food allergies. However, there is no report detailing the role of microbiota alterations in Henoch-Schönlein Purpura (HSP) development.

Method: A total of 85 children with HSP and 70 healthy children were recruited for this study. Intestinal microbiota composition was analyzed by 16S rRNA gene-based pyrosequencing. Fecal microbial diversity and composition were compared.

Result: We compared the gut microbiota of 155 subjects and found that children with HSP exhibited gut microbial dysbiosis. Lower microbial diversity and richness were found in HSP patients when compared to the control group. Based on an analysis of similarities, the composition of the microbiota in HSP patients was also different from that of the control group ($r = 0.306$, $P = 0.001$). The relative abundance of the bacterial genera *Dialister* ($P < 0.0001$), *Roseburia* ($P < 0.0001$), and *Parasutterella* ($P < 0.0001$) was significantly decreased in HSP children, while the relative abundance of *Parabacteroides* ($P < 0.006$) and *Enterococcus* ($P < 0.0001$) in these children was significantly increased. Based on Spearman correlation analysis, the LOS showed a significant negative ($P < 0.05$) correlation with the genera *Paraprevotella* and *Roseburia*. Meanwhile, IgA levels exhibited a significant negative ($P < 0.01$) correlation with the genus *Bifidobacterium*.

Conclusions: Our results indicate that HSP is associated with significant compositional and structural changes in the gut microbiota. These results enhance the potential for future microbial-based therapies to improve the clinical outcome of HSP in children.

1. Introduction

Henoch-Schonlein purpura (HSP) is an IgA-mediated vascular allergic hemorrhagic disease that annually affects 10 to 20 children per 100,000 and exhibits peak incidence at 4 to 6 years of age [1]. It is the most common vasculitis that occurs during childhood. The most common clinical manifestations usually include palpable purpura or petechiae, (poly)arthralgia, gastrointestinal disturbances and glomerulonephritis [2]. It has been established that HSP patients usually have more than two clinical manifestation of the disease, referred to as mixed-type HSP. Though the pathogenesis of HSP is still unknown, studies have shown that it may be correlated with allergies caused by infections, vaccinations, pollen, food, drugs, and/or other factors [3,4]. However, there is no definitive laboratory test available for the diagnosis of HSP [4].

The mammalian microbiota is highly variable at lower taxonomic levels. The four dominant phyla in the human microbiota are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [5]. Recent studies found that the gut microbiota was a crucial factor for shaping and modulating immune system responses [6,7], and an imbalance in gut microbiota composition (dysbiosis) may link to several autoimmune and immune-mediated diseases, such as food sensitization, allergic rhinitis, allergic asthma, atopic dermatitis, eczema and inflammatory bowel disease [8–11]. However, there has been no report discussing the microbiota of HSP patients.

In this study, we aim to identify differences in the intestinal

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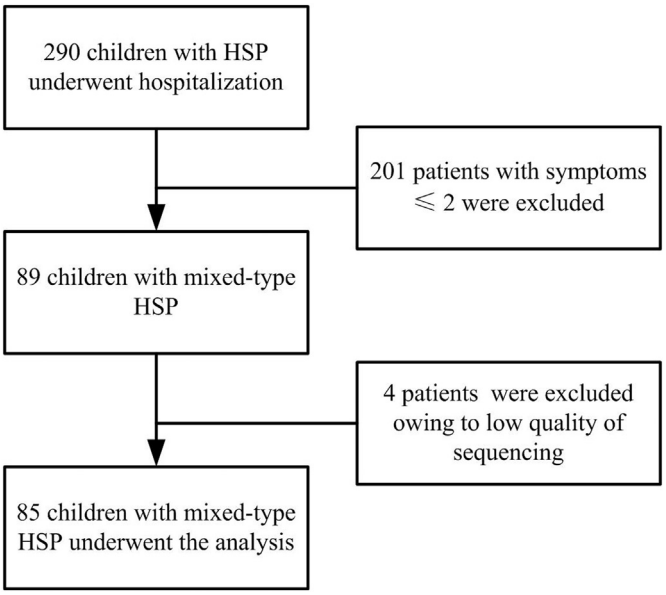

Fig. 1. The flow chart of this study.

microbiota between healthy children and children with HSP by next-generation DNA sequencing. We identify specific microbial signatures of HSP and the relationships between the microbiota and clinical indices.

2. Methods

2.1. Study population

A total of 290 children with a clinical diagnosis of HSP who were hospitalized in Qilu Children's Hospital of Shandong were screened. The inclusion criteria stated that children must be under 18 years of age; could not be suffering from an infectious disease, malnutrition, immune system defects, or congenital genetic metabolic disorders; and could not be undergoing treatment with immunoglobulins or immunomodulating agents [12]. Enrolled patients were classified as mixed-type HSP if they had > 2 clinical manifestations of the disorder. Healthy children were used as controls. The protocol of this study was approved by the local Ethical Committee of the Qilu Children's Hospital of Shandong University (Fig. 1). All volunteers received information concerning their participation in the study and gave written informed consent.

2.2. Fecal sample collection, DNA extraction, and pyrosequencing

Fecal samples for genomic DNA extraction were collected and immediately frozen by the participants in their home freezer at −20 °C (maximum 24 h) and then brought to the laboratory in a freezer pack, where they were stored at −80 °C. Genomic DNA was extracted using the CTAB assay as described by Tang et al. [13]. An equivalent of 1 mg of each sample was used for DNA quantification using a NanoDrop ND-1000 Spectrophotometer (Nucliber).

To analyze the microbial populations within the samples, amplification of the variable region V1-V2 of the 16S rRNA gene was performed. PCR was conducted using the bacterial universal primers 27F (5′ AGA GTTTGA TCM TGG CTC AG 3′) and 338R-I (5′ GCW GCC TCC CGT AGG AGT 3′) and 338R-II (5′ GCW GCCACC CGT AGG TGT 3′). Amplicons were first purified using the QIAquick PCR Purification Kit (Qiagen, Barcelona, Spain), quantified using a NanoDrop ND-1000 Spectrophotometer (Nucliber) and then pooled in equal concentrations. The pooled amplicons (2 nM) were then subjected to sequencing using Illumina MiSeq technology, following standard Illumina platform protocols.

2.3. Taxonomy quantification using 16S rRNA gene sequences

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) All reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlaps were longer than 10 bp were merged according to the overlapping sequence.

Operational taxonomic units (OTUs) were clustered using a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each representative sequence of OTUs was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU128) 16S rRNA database using a confidence threshold of 70%.

OTU abundance information was normalized using a sequence number standard corresponding to the sample with the least sequences. Subsequent analyses of alpha diversity and beta diversity were performed using these output normalized data.

Alpha diversity (Shannon) and beta diversity [weighted UniFrac, principal coordinate analysis (PCoA)] were analyzed using QIIME. Linear discriminant analysis (LDA) and effect size (LEfSe) analyses were performed with the LEfSe tool (http://huttenhower.sph.harvard.edu/galaxy). The ANOSIM (Analysis of Similarity) test within the PRIMER 6 software package (PRIMER-E Ltd., Luton, UK) was carried out to analyze significant differences in microbial communities between the HSP and control subjects.

2.4. Statistical methods

The data are presented as the mean ± SD unless otherwise stated. The normality of the distribution was determined using the Kolmogorov-Smirnoff statistical method. Chi-squared-tests were used to assess gender differences. Continuous variables were compared between independent samples and unpaired-sample *t*-tests or the Mann-Whitney *U* test. *P*-values < 0.05 were considered statistically significant. Statistical dependence between continuous variables was determined using a Spearman's rank correlation. Analyses were performed using the SPSS statistical package, version 24.0 (SPSS).

3. Results

3.1. Participants

A total of 155 participants including 85 with mixed-HSP (63 males, 22 females) and 70 healthy controls (49 males, 21 females) were recruited for this study. The HSP group and control group were matched for gender (male: 74.1% vs. 70.0%, respectively, $P = 0.569$) and age (7.4 ± 2.5 vs. 7.0 ± 2.7, respectively, $P = 0.25$).

3.2. Decrease in microbial diversity is tightly linked to the presence of HSP

After quality-filtering steps, an average of 59,508 high-quality sequences per sample was obtained. The estimate of coverage reached > 99.5% for all samples. We further applied a set of filters to remove rare microbial OTUs, those presented in < 3 samples with a number above 5, and low abundant microbial OTUs with < 50 reads across all samples. As a result, 18,916 sequences per sample and 1477 OTUs were retained for subsequent analysis.

Next, we assessed the bacterial community richness (Ace richness estimate) and diversity (Shannon index) to compare the mean values between groups. Interestingly, HSP patients displayed a significant decrease in microbial diversity (Shannon index, $P < 0.001$, Fig. 2A) and richness (Ace index, $P < 0.0001$, Fig. 2B) compared to the healthy control group.