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News & Views

Gut microbiota have blood types as human

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ABO blood group system was firstly recognized by Landsteiner in 1900 [1]. Since then, the antigens of the ABO system (A, B and H determinants, respectively) have been shown to encompass complex carbohydrate structures [2]. A and B antigen were synthesized by the sequential action of glycosyltransferases, with A and B glycosyltransferases catalyzing the addition of N-acetylgalactosamine and D-galactose to precursor H antigen, respectively. Group O individuals lack such transferase enzymes and consequently continue to express the basic H structure only [3,4]. It is determined that approximate 2 million ABH glycan antigen sites are presented on each red blood cell [5]. Additionally, the ABH antigens are widely expressed in other human cells and tissues, including the sensory neurons, epithelium, the vascular endothelium and platelets [6].

Human beings harbor extremely dense and diverse microbiota in gastrointestinal tract, comprising 0.15 kg of microbial biomass harboring hundreds of bacterial and archaeal species [7–9]. Gut microbiota as an ignored organ has been verified to adapt to host genetics, ie., *APOA1*, Mediterranean Fever, *FUT2* and *NOD2* in human evolutionary history [10–14]. For example, individuals with functional *FUT2* alleles colonized more taxa of bifidobacterial, ie., *B. angulatum*, *B. catenulatum* and *Bifidobacterium* spp., compared with individuals with non-functional *FUT2* alleles [14]. Nevertheless, to date, little is known regarding how ABO blood group as a genetic marker affects enterotypes of gut microbiota after longitudinal human evolution. Previous studies have tried to verify the relationship between them. The analysis of gut microbiota in Finland cohort manifested that microbiota from the individuals harboring the B antigen (sector B and AB) differed from the non-B antigen groups (A and O) and also showed higher diversity of the *Eubacterium rectale-Clostridium coccoides* (EREC) and *Clostridium leptum* (CLEPT) – groups in comparison with other blood groups [15]. However, another research based on 1503 individuals cohort suggested that microbiota were not associated with ABO blood group [16]. Such contradictory consequences confused us with the relationship between blood group types and gut microbiota. Herein, we desired to illustrate the relationship between blood group and gut microbiota from three aspects, blood types and composition of gut microbiota, blood types and blood group activity of gut microbiota, blood types and composition of gut microbiota with surface blood group antigens.

Initially, we investigated whether composition of gut microbiota varied with blood types in Chinese cohort. A cohort of 149 healthy volunteers, consisting of 41 A individuals, 47 B individuals, 49 O individuals and 12 AB individuals, participated in this study (Table S1 online). The results of 16S rRNA gene sequencing suggested there was no correlation between blood types and composition of gut microbiota on the basis of α and β diversity (Fig. S1 online). This result was consistent with previous estimates [16].

Next, we sought to explore the relationship between blood types and blood group activity of gut microbiota. Blood group activity of gut microbiota was assessed by staining methods (enzyme-linked immunosorbent assay and flow cytometry) using A and B blood group antibodies in individuals, respectively. Fluorescence value derived from ELISA (enzyme-linked immunosorbent assay) correlated with intensity of blood group antigens. The results of ELISA from 149 subjects suggested gut microbiota from A blood individuals enriched more A blood antigen than B blood antigen in the surface (412.7 ± 44.89 vs 216.2 ± 38.61, $P = 0.0014$, $t = 3.320$, df = 80, $n = 41$), whereas gut microbiota from B blood individuals enriched more B blood antigen than A blood antigen in the surface (440.9 ± 60.12 vs 289.6 ± 23.94, $P = 0.0216$, $t = 2.337$, df = 92, $n = 47$) (Fig. 1a; Table S2 online). Additionally, gut microbiota from O and AB blood individuals presented equivalent A and B blood group antigens in the surface (O: 258.8 ± 29.96 vs 239.2 ± 35.54 (A vs B), $P = 0.6748 > 0.05$, $t = 0.4208$, df = 96, $n = 49$; AB: 123.7 ± 75.04 vs 86.8 ± 66.84 (A vs B), $P = 0.7167$, $t = 0.3676$, df = 22, $n = 12$) (Fig. 1a; Table S2 online). Additionally, flow cytometry was further applied to assessed the blood group reactivity of gut microbiota. *E. coli* O86:H2 whose O-antigen was similar with blood group B antigen [17], as positive control, was chosen to confirm the feasibility of flow cytometry. The results suggested that *E. coli* O86:H2 almost exclusively enriched B antigen, whereas it had no A antigen (Fig. 1b). Then the blood group activity of gut microbiota from A, B, O and AB blood individuals respectively was analyzed by flow cytometry, and the typical sample result of each blood-type individual was presented. Gut

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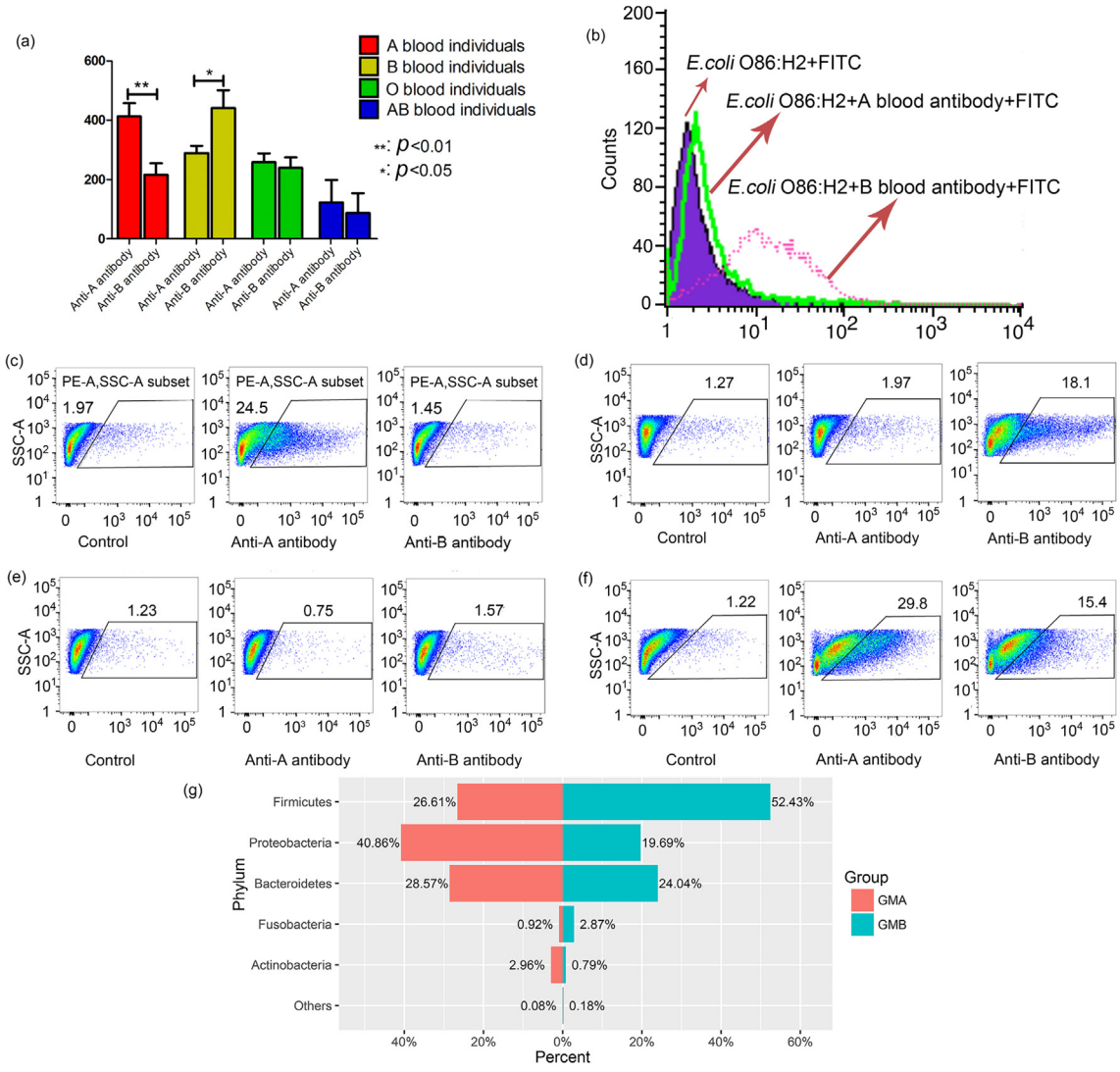

Fig. 1. The amount of A or B blood group antigen in gut microbial surface from four blood-type individuals (a). The detection of blood group antigens of *E.coli* O86:H2 (b), A-individual gut microbiota (c), B-individual gut microbiota (d), O-individual gut microbiota (e) and AB-individual gut microbiota (f) by flow cytometry. The composition of gut microbiota with A blood group (GMA) antigen and B blood group antigen (GMB) at phylum level (g).

microbiota from A and B blood individuals enriched self-blood group antigen (Fig. 1c, d). Additionally, gut microbiota from O blood individual presented no A and B blood group antigens (Fig. 1e), while gut microbiota from AB blood individual presented A and B blood group antigens simultaneously (Fig. 1f). However, divergence emerged between the data of ELISA and flow cytometry about blood group activity of O-individual gut microbiota, which profound works need to illustrate. Together, these data indicated that gut microbiota of human beings enriched more self-blood group antigens, declaring gut microbiota as an organ of human body was identical to other organs expressing self-blood group antigens.

Ultimately, these observations prompted us to identify gut microbiota with A or B blood group antigen in the surface. Therefore, we purified and characterized gut microbiota expressing blood group antigens by the combination of immunomagnetic beads and 16S rRNA sequencing (Fig. S2 online). *E. coli* O86:H2 as positive control was incubated respectively with immunomagnetic beads binding A or B blood antibody, confirming the specificity of immunomagnetic beads. Notably, the results of transmission electron microscope suggested that immunomagnetic beads with B blood antibody exclusively bound to *E. coli* O86:H2 (Fig. S3b online), whereas immunomagnetic beads with A blood antibody were unable to bind to it (Fig. S3a online). Then the credible immunomagnetic beads with blood group antibody were utilized to separate gut microbiota with A blood antigen (GMA) from 10 A blood individuals and gut microbiota with B blood group antigen (GMB) from 10 B blood individuals, respectively (Table S3 online). After separation, analysis of 16S rRNA gene sequencing suggested gut microbiota with A or B blood group antigen showed a parallel but typically distinct pattern of microbial taxa. The predominant subsets of GMA resembled that of GMB, *Firmicute* (26.61% vs 52.43%, GMA vs GMB), *Proteobacteria* (40.86% vs 19.69%, GMA vs GMB), *Bacteroidetes* (28.57, vs 24.04%, GMA vs GMB) (Fig. 1g). However, analysis of β diversity suggested that a few subsets of GMA was dramatically different from that of GMB (Fig. S4 online), in which eight phyla were different significantly between GMA and GMB, including *AD3*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes*, *OD1*, *Planctomycetes*, *Proteobacteria* ($P < 0.05$) (Table S4). *Proteobacteria* (40.86%) and *Firmicute* (52.43%) were the most abundant taxa in GMA and GMB, respectively (Fig. 1g). Additionally, *AD3*, *Chlamydiae*, *Chloroflexi*, *Crenarchaeota*, *Elusimicrobia*, *GAL15*, *OD1*, *Planctomycetes*, *Spirochaetes* and *WPS-2* were unique

to GMA, whereas those were not found in GMB (Table S4 online). Notably, gut microbiota with self-blood antigen was different significantly between A and B blood individuals.

In short, owing to long-term coevolution, blood types as a genetic factor significantly affected the gut microbiota in Chinese cohort. Gut microbiota as an organ is mostly enriched with the same blood group antigen as the host's blood type. These microbial blood group antigens may not have the exact same structures as the human ABO blood group antigen, but they have the same immunological properties. Gut microbiota with self-blood antigen exhibits different pattern of classification, which will help to understand disease susceptibility among different blood-type populations. Furthermore, previous works focused on the relationship between host genetics and composition of gut microbiota, whereas our work highlights the interplay between host genetics and glycan phenotype of gut microbiota. Finally, we hope that our work enables new applications in the field of personalized nutrition and medicine.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

All raw data of 16S rRNA amplicon reads from 149 individuals and 10 A or 10 B blood individuals considered are publicly available on NCBI SRA datasets (SRA accession: SRP155477, SRP155336). Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2018.09.013.

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