

Blood group-gut microbiome–health axis gains further support from landmark multi-omics study in swines

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Research into gut microbiome is one of the most productive areas of life sciences in the last decade, revealing great potential for both fundamental and translational researches. The effects of microbiota on host immunity and metabolism in healthy humans as well as in disease have been widely reported; however, how hosts regulate the composition of the gut microbiota, which requires large populations to mitigate interference from confounding environmental factors (Falony et al., 2016), has been comparatively slow. In humans, a series of genome-wide association studies (GWASs) have revealed that multiple host genes may have a significant role in these regulations (Bonder et al., 2016; Wang et al., 2016) since 2016. Among these, *LCT* that encodes lactase enzyme to hydrolyze lactose, has been shown to determine host digestibility of dairy products and regulates the abundance of *Bifidobacteria* which metabolize lactose (Blekhman et al., 2015). Another gene vitamin D receptor (*VDR*) forming heterodimerization with the retinoid X receptor (*RXR*), and participating in the sensing of microbial and dietary metabolites, most importantly bile acids, was shown to be able to affect the abundance of bile acid-producing bacteria and then the composition of the microbiome (Wang et al., 2016). Another set of genes, namely ABO-related genes have been associated with the abundance of the gut microbiome in several more recent genome-wide association studies (Rühlemann et al., 2021). And a recent study by Huang group in

Nature provides more compelling evidence for ABO blood types' effect in shaping microbiome as well as feedback on the hosts, using a large population of swines and multi-omics approach (Yang et al., 2022).

Indeed, ABO and other blood group-related genes have been reported to be functionally important in host-pathogenic microbial interactions. A range of genes that determine blood group simultaneously determine the structure of oligosaccharide chain and type of glycosyl groups of glycoproteins on the mucosal surfaces of the intestinal, respiratory and reproductive tracts, as well as the oral cavity, serving as the key molecules in the process of pathogen attachment and invasion of the host cells (Ewald and Sumner, 2016). The original host of one of the most common infectious agents, influenza viruses, is waterfowl, but some influenza viruses have adapted to several mammalian species, including humans because the viruses' key protein haemagglutinin (HA) can bind to the specific sialic acid on cell surface glycoproteins. Furthermore, numerous influenza subtypes (H1, H2, H3) that were previously only able to attach to the 2,3-linked sialylated glycan receptor in avians, can now bind to the 2,6-linked sialylated glycan receptor in humans due to the mutations in the receptor-binding region, allowing for host jumps (Shi et al., 2014). The ABO blood group is also a genetically important factor associated with disease severity and fatality in the SARS-CoV-2 pandemic, but the exact mechanism is not yet elucidated (Ellinghaus et al., 2020). In

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contrast, one of the blood type-related genes *FUT2* has been repeatedly implicated in the research of host gene interactions with better understanding of the mechanisms (Knights et al., 2014). This gene has been identified in GWAS of inflammatory bowel disease (IBD), an autoimmune disease including Crohn's disease and ulcerative colitis, more prevalent in specific European populations, and regulated by an interaction of genetic and environmental factors (particularly gut microbial factors). *FUT2* encodes fucosyltransferase 2 and add terminal fucose residues to mucosal glycans on intestinal epithelial cell, thus regulates both the expression of blood group antigens and the physiological characteristics of the gastrointestinal tract, and its genotype can be classified as secretors or non-secretors based on the presence or absence of terminal fucose residues on mucosal glycans. Although the *FUT2* non-secretors were reported to have a high risk for IBD in Caucasians and significantly affect the composition of the gut microbiome (Rausch et al., 2011), the phenotype was not selected against in long-term evolution; one essential explanation is that the secretory phenotype in turn enhances host sensitivity to Norwalk and respiratory viruses, hence heterozygotes are most favorable in long-term selection (Lindsmith et al., 2003).

In the *Nature* study, Huang et al. established a large mosaic population of swines with strictly uniform rearing standards, thus reducing the interference of complex environmental factors. Comprehensive 16S analyses of different growth periods (25, 120, and 240 d) and different sample types (ileum, cecum, feces) were conducted, and it was discovered that the variations in microbial compositions across sampling types were higher than the differences across generations (F6, F7), highlighting the limitation of previously used, single sampling type in humans. Subsequently, significant negative correlations between genetic similarity and microbiota dissimilarity supported an effect of genetics on microbiota composition. They used F6 and F7 jointly to evaluate the heritability (h^2) of the abundances of individual taxa and, respectively, identified 1,050 and 955 in F6 and F7, of which 55 were considered to be stably heritable. Some of the identified taxa/OTUs, such as *Christensenella*, were consistent with the results in the human studies. To further investigate the genetic structure of the gut microbiome, they conducted a genome-wide association analysis (GWAS) of the abundance and presence/absence on 8,490 bacterial taxa, and to identify SNPs with a consistent effect, a cross sample-type, meta-analysis was run in F6 and F7. Ultimately, six most significant SNPs and their strong associations with three OTUs (OTU476, OTU327, and p-75-a5) in the *Erysipelotrichaceae* were identified. All of these lead SNPs mapped to the ABO blood group gene (N-acetyl-galactosaminyl transferase gene) and had a high degree of linkage disequilibrium (LD) with a 2.3 kb deletion containing exon 8, resulting from a 3.5 million-year-old ABO blood type

gene mutation. The transcript of this gene shows that the mutation results in a deletion of 62% of the amino acids in the protein encoded by the gene, which in turn results in losing glycosyltransferase activity and ultimately a reduction in the concentration of GalNAc (N-acetyl-galactosamine) in the gut.

To further verify that the change in the abundance of miQTL-responsive bacteria was caused by a decrease in the concentration of GalNAc in gut, a series of validation experiments were carried out. GalNAc concentrations in the cecum contents of different blood group individuals were measured using targeted metabolomics, from which a positive correlation between GalNAc concentrations and the abundance of some *Erysipelotrichaceae* species was found. The genomes of two OTU476-like strains cultured from this population were compared to 3111 metagenomic assembled genomes, and a more complete GalNAc transport and metabolic pathway was found in *Erysipelotrichaceae* species influenced by ABO blood type. Further *in vitro* culture and gavage experiments in mice have demonstrated that the concentration of GalNAc in gut significantly affects the abundance of *Erysipelotrichaceae* that can import and catabolize GalNAc. Thus, this landmark work provides a detailed and systematic association analysis of bacterial abundances, more importantly in various intestinal locations, eventually revealing the interaction between microbiota and host genes. Experimentally, the study provided the functional link and mechanism in which the ABO blood type gene interacts with bacteria species with particular functional adaptation. This study is the first to show how host genetics impacts gut microbiome in pigs, both an important commercial animal for agriculture and model organism of increasing medical importance.

To summarize, the findings in this study echo previous discoveries of the interactions between genes, such as ABO blood groups, and symbiotic microbiome in humans, providing new evidence in non-primate mammals. At the same time, the Huang group focuses on research in pig genetic breeding and has interpreted biological processes such as microbial-host interactions, fat deposition (Zhang et al., 2021), and chromosomal inactivation (Zhu et al., 2022) from a genetic perspective, which provides an important framework and data for subsequent research in both genetic breeding and medical research (Lunney et al., 2021). In particular, considering that while AB antigen is expressed in the most commonly used mouse model, there is no blood group corresponding to humans, hindering many functional validations and the emerging blood group- microbiome-disease axis. In this aspect, pigs are more similar to humans in terms of the physiological structure and the composition of the gut microbiome than the mouse model, and future studies in pigs can certainly provide a higher reference value. More attention should be paid to investigate the relationship be-

tween health, disease, and the microbiome in future studies, and systematically explore the mechanisms of their interactions through more functional analysis.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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