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Studying the direct and maternal effect of ABO genotype on the intestinal microbiota of the pig

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Introduction

The composition of the intestinal microbiome is correlated with physiological and pathological parameters such as cardiovascular diseases, body mass index and fasting glucose levels in humans (Rothschild *et al.,* 2018; Li *et al.,* 2024), and, additionally, with production traits such as feed conversion ratio, carcass composition and methane production in livestock (O'hara *et al.,* 2020). These correlations may in part reflect a direct effect of the microbiome on the host, which may open possibilities to affect the host by modulating its microbiome. Pedigree studies suggest that microbiome composition is in part inherited in humans and animals (Goodrich *et al.,* 2014; Bonder *et al.,* 2016; Hughes *et al.,* 2020; Yang *et al.,* 2022), suggesting that part of the heritability of host traits may be mediated by microbiome composition. Genome Wide Association Studies (GWAS) in humans have revealed a small number of reproducible association signals including with variants underpinning intestinal lactase persistence, and the ABO blood group (Sanna *et al.*, 2022; Zhernakova *et al.*, 2024). In pigs, an association between the orthologous AO blood group and the abundance of members of the genus p75.a.5 was also reported and shown to be due to the effect of AO blood group on the abundance of GalNAc in the gut (Yang *et al.*, 2022). Bacteria sensitive to AO blood group were shown to use GalNAc as carbon source by means of a non-inducible GalNac operon (Yang *et al.*, 2022).
We herein reproduce the effect of AO genotype on p.75.a5 abundance in a Belgian pig population. We also discuss ongoing experiments to study the effect of AO genotype on the milk glycome of sows and the effect of glycome composition of the milk on the intestinal microbiome of the piglets.

Materials & Methods

Populations. The first study was conducted using 250 (Landrace x Large White) x Piétrain cross-bred pigs. Fecal samples were collected manually from the rectum at ~180-days, and DNA extracted from tail samples. The second study used ~1,000 (Landrace x Large White) x Piétrain pigs from 91 (Landrace x Large White) sows and six Piétrain boars. Fecal samples were collected from the piglets at 26 days, 8 and 22 weeks, and DNA extracted from hair samples (piglets and sows) or sperm (boars).

Genotyping. DNA was extracted using standard procedures. All animals were genotyped using the GeneSeek Genomic Profiler (GGP) Porcine HD (70 K) array, and with a custom-made PCR assay targeting the 2.3Kb deletion in the N-acetyl-galactosaminyl-transferase gene for AO blood group typing (Yang *et al.*, 2022). SNPs were filtered based on call rate and Hardy-Weinberg disequilibrium. Samples were filtered using genotyping rate. We conducted parentage control and cosegregation of ABO and linked SNPs for additional QC.

Microbiota profiling. The V3-V4 region of the 16S-rRNA gene was amplified using standard primers (Yang *et al.*, 2022), and the PCR product sequenced on a MiSeq instrument (Illumina).

The resulting reads were analyzed using FastQC, MultiQC, Trimmomatic, QIIME2 and Dada2, phyloseq and the Greengenes 2 database. Raw abundances were CLR (Centered Log-ratio) transformed with or without rarefaction.

Milk glycome composition. We performed a near-full glycome analysis (MS/MS-based N-glycan profiling and xCGE-LIF glycoprofiling) of milk samples collected from the 91 sows.

Statistical analyses. The effect of AO genotype on the abundance of p.75.a5 was tested using a linear model including sex and dosage of the A allele as fixed effects, and kinship and degree of pen-sharing as random effects. While being highly significant when performing one test (as we did), the p-value of 2.3×10^{-7} would not have been significant if we had not known about the Yang *et al.* (2022) results, and performed a GWAS. In eQTL analyses, its customary to correct transcript levels using expression principal components, with the aim to account for hidden confounders. We applied the same approach to our analyses using microbial principal components (PCs). We then conducted a GWAS at the genus level (426 genera) using the same model, including 22 PCs and performed a GWAS for the 22 PCs. We only used ~70K SNPs in these analyses, and did not perform genotype imputation as we didn't have whole genome sequence data matching our population. We nevertheless performed a haplotype-based analysis using HiddenPhase (Druet & Georges, 2010) and Glascow (Zhang *et al.,* 2012). All analyses were conducted in R.

Results

Microbiota composition. For population 1, we produced an average of 47,301 reads per sample clustering in 15,481 amplicon sequence variants (ASV), of which 10,492 were observed in more than one sample and kept for further analysis. These were assigned to 25 phyla, 32 classes, 73 orders, 132 families, 426 genera and 768 species. Alpha (mean: 5.5) and beta (mean 0.8) diversities were in line with previous reports (Yang *et al.*, 2022).

Effect of AO genotype on p.75.a5 abundance. We obtained a highly significant effect in the expected direction (A increasing abundance): 2.3×10^{-7}, hence confirming the effect reported by Yang *et al.* (2022). The effect of the A allele was dominant as expected.

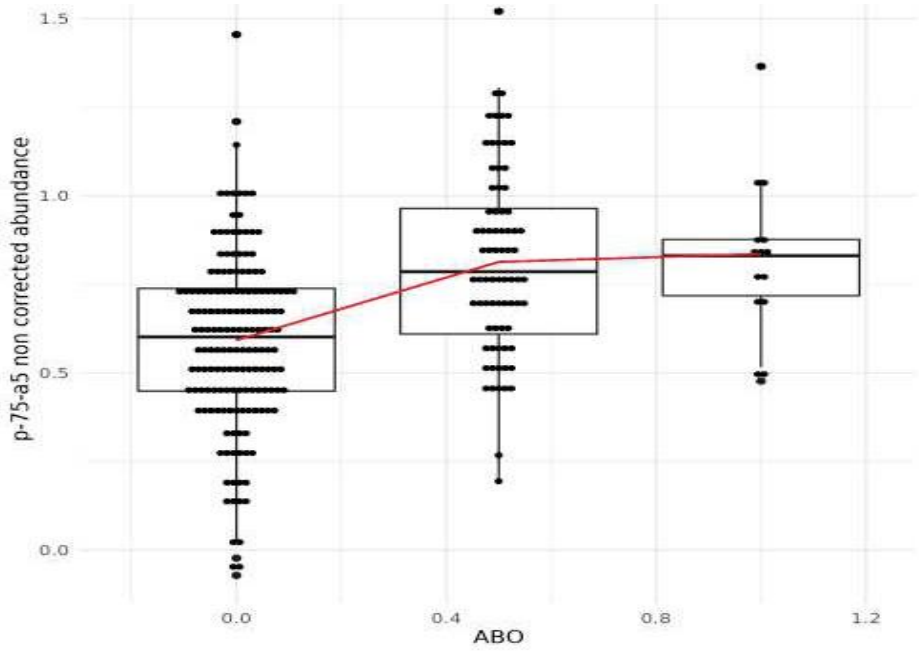

Figure 1: Effect of A allele dosage on p.75.a5 abundance. Boxplots for uncorrected abundances.

Increasing statistical power with microbial principal components. The p-value improved to 2.2 x 10^{-11} when adding the first 22 PCs in the model, hence exceeding the experiment-wide significance level assuming GWAS for 1000 taxa. Of note, the effects of PCs and AO genotype had to be estimated simultaneously to reach this level of significance, i.e., precorrecting the abundances for PC and working on residuals did not yield as good results. Also, correcting for kinship using the LOCO (Leave One Chromosome Out) approach was better than we using SNPs covering the entire genome. There was no correlation between any of the PCs and known covariates.

No convincing evidence for additional GWAS signals. No additional significant associations were identified in genus-level GWAS analyses beyond the AO–p.75.a5 relationship. Similarly, GWAS performed on the 22 PCs did not reveal locus-specific effects. The haplotype-based analysis did not reveal new signals either.

Studying the glycome of sow milk. We are in the process of analysing the effect of the sows AO genotype on the glycome composition of her milk and the microbiota composition of her offspring. We are also looking at relationships between sow's milk glycome and offspring's microbiota composition irrespective of AO genotype. Latest results will be presented.

Discussion

We herein confirm the effect of AO genotype on the abundance of p.75.a5 previously reported by Yang *et al.* (2022) in an independent, Belgian pig population, hence confirming its veracity. The AO locus was shown to segregate in this Belgian population as it was in all eight founder populations of the Chinese mosaic population used by Yang *et al.* (2022), in agreement with the fact that the AO locus is under balancing selection in pigs. Of note, the p.75.a5 genus is, to a large extend, livestock specific. The effect of the ABO locus on microbiota composition in humans is affecting other taxa. We didn't find any evidence of other associations with intestinal microbiota composition in agreement with Yang et al. (2022). We provide strong evidence that the power of GWAS for microbiota composition can be substantially increased by adding microbiota principal components in the model to correct for unidentified hidden confounders, that may include biological as well as technical effects.

Conclusions

We confirm the effect of AO genotype on the abundance of p.75.a5 in an independent commercial pig population and show that the power of microbial QTL detection can be increased by adding microbial principal components in the models. Understanding such host–microbiome interactions could support the development of novel selection strategies or microbiome-targeted interventions aimed at improving productivity and sustainability in pig production.

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