Bidirectional relationships between the gut microbiome and sexual traits

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Running title: Sexual traits and the gut microbiome

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Abstract

The human gut microbiota is known to be shaped by a variety of environmental factors (diet, drugs, geography and sanitation) and host intrinsic factors (age and sexual development). The differences in gut microbiota between sexes are minimal before adulthood and late adulthood, and marked during adulthood. For instance, consistent higher relative abundances of *Akkermansia* and *Ruminococcus* have been observed in adult women compared to men and most studies have found higher relative abundances of *Prevotella* and *Fusobacterium* (linked to a diet rich in animal proteins) in adult men compared to women. The gut microbiota taxonomy and functionality present in women is more similar to men once reached the menopause. In fact, specific taxa have been associated with the levels of different sexual hormones and their precursors in blood. The gut microbiota composition and circulating testosterone levels are also tightly linked to the extent that microbial signatures can predict its levels in blood. At the same time, the gut microbiota participates in the metabolism of sexual hormones, with some bacteria being able to metabolize gonadal steroid hormones (one example is 3β-hydroxysteroid dehydrogenase, a testosterone degrading enzyme). In summary, the relationships between the gut microbiome and sexual traits are bidirectional. In addition, other phenotypes and cultural gender-related factors could drive sex-related differences. It is important to note that other members of the microbiome (Archeae, viruses and fungi) have been largely unexplored in relation to this sexual dimorphism. More research is needed on this topic.

INTRODUCTION

The study of gut microbial communities has gained attention in recent years as a novel point of view to understand human health physiology in its interactions with internal and external agents. The human gut microbiota is known to be shaped by a variety of factors, including environmental (*e.g.* diet or drug intake), host intrinsic (*e.g*. age, sex) and microbiome intrinsic factors (*e.g*. microbe-microbe interactions) (Zeevi et al., 2015; O'Keefe et al, 2016; Vermuri et al, 2019; Zhang et al, 2021; Kåhrström et al, 2016; Sonnenburg et al, 2016; Rotschild et al, 2018; Vujkovic-Cvijin et al, 2020). Recent evidences have shown that the gut microbiota composition and function show sex-specific characteristics and change with sexual maturation (Mayneris-Perxachs et al., 2020; Zhang et al., 2021). At the same time, some observations suggest that the microbiome could even participate in sexual dimorphism through the modulation and metabolism of gonadal steroids (Mayneris-Perxachs et al., 2020; Zhang et al., 2021; Pernigoni et al., 2021; Li et al., 2022). In the last decade, the term 'microgenderome' has been proposed to refer a sexually dimorphic microbiome (Flak et al., 2013). However, this term could be deceiving, as the factors driving female-male differences in the microbiota seem to be mainly determined by biological sex rather than gender (Vemuri et al., 2019).

Some recent reviews have developed the role of the microbiome in sexually dimorphic diseases (type 1 diabetes), or in diseases exclusive of women (polycystic ovary syndrome, ovarian cancer, postmenopausal osteoporosis) (He et al., 2021). Other reviews have focused on sex-dependent changes in local gastrointestinal inflammation, systemic immunity and susceptibility to a range of inflammatory diseases (Vemuri et al., 2019), or the role of the gut microbiome in sex differences in arterial pressure (Beale et al., 2019). Some authors have even revised the findings hinting at a sexually dimorphic communication between the gut microbiome and the brain (Jašarević et al., 2016). In this mini-review, we aim to explore recent findings regarding sex differences and gut microbiota bidirectional relationships that have appeared over the past three years.

In general, the results of the studies that have evaluated sex-associated differences in the gut microbiome are inconsistent. These inconsistencies may be explained, to some extent, by the relative small number of subjects studied, poor characterized cohorts, different methodologies or limitations intrinsic to technical capabilities. All these factors are still apparent in recent studies assessing the influence of sex on the gut microbiota (**Table 1**). Controlling for all these factors remains a challenge in the microbiome field.

Sex-associated differences in the gut microbial communities have been found to be dynamic and age-dependent (**Figure 1**). In fact, age and the degree of sexual maturation are critical to understand these differences. We will stratify the findings according to these important points.

Findings before adulthood

The acquisition of microbes takes place at birth, mainly from maternal sources (Ferretti *et al*., 2018). This early microbiota rapidly evolves within the first three years of life mainly influenced by environmental factors, increasing in diversity until the microbial composition resembles an adult microbiome. During the first and second years of life, there are no differences in gonadal steroid production between sexes. In line with this, no microbiome sex-associated differences have been reported (Ferretti *et al*., 2018; Laue *et al*., 2021; Rao *et al*., 2021). However, Laue *et al* (2021) found that host sex may influence the relationship between early-microbiome and infant neurodevelopment. These authors observed that microbial diversity and composition were associated with behavioral development in a sex-specific manner. Their results also highlighted the need to develop more intervention studies to assess causality.

Few studies have analyzed sex differences in the gut microbial communities during childhood. Zhang *et al* (2021) did not find significant differences among prepubertal children (girls and boys aged 6 to 9 years). Another study found some differing taxa in prepubertal children, including a higher abundance of the Lactobacillaceae family in girls and a higher abundance of the *Saccharibacteria* class in boys (Yuan *et al*., 2020). This sexual dimorphism was more evident in postpubertal subjects where they identified *Dorea*, *Megamonas*, *Bilophila*, *Parabacteroides* and

Phascolarctobacterium as microbial signatures of pubertal status. In a Finnish cohort, bacteria belonging to Firmicutes phylum were increased while Bacteroidetes were decreased during puberty in girls (Korpela *et al*., 2021). Specifically, puberty in girls was characterized by the increase of the Ruminococcaceae and Lachnospiraceae families, and a decrease of the Bacteroidales order and the *Streptococcus* genus. A similar pattern was found during puberty in boys, which was also characterized by the significant decrease of *Lactobacillus*, *Escherichia* and *Coriobacteriaceae* genera. Overall, the gut microbiome of girls was more similar to that of adults with pubertal progression. This was not the case in boys, possibly due to the delayed development of puberty. However, a longitudinal study is needed to evaluate and confirm microbial dynamics during puberty.

Puberty is characterized by an increased production of gonadal steroid hormones that lead to the development of secondary sexual characteristics in both sexes secondary to the dimorphic secretion of sexual hormones (**Figure 1**). In women, there is an increase in estrogen and progesterone levels until they reach a steady state where progesterone and estradiol will fluctuate during female reproductive age. In men, testosterone levels increase and remain high compared to women, while estrogen levels remain low during reproductive age.

Findings during adulthood

While no sex-associated differences have been reported in childhood, sex-related changes in the gut microbiome composition have been described between women and men in the adulthood, (Mayneris-Perxachs *et al*., 2020; Zhang *et al*., 2021). Results among studies considering post-pubertal populations are more consistent. A total of ten bacteria species have been found to characterize the gut microbiome in young and middle-aged adult women and these findings have been validated in human cohorts from different geographies (**Zeevi et al, 2015;** Zhang *et al*., 2021). Similar taxa have been identified in other studies assessing sex-associated differences during adulthood (Mayneris-Perxachs *et al*., 2020; Sinha *et al*., 2019; Takagi *et al*., 2019). Overall, consistent higher abundances of *Akkermansia* and *Ruminococcus* have been observed in

adult women compared to men (**Table 1**). *Akkermansia* genus has been proposed to modulate energy metabolism and glucose tolerance, and *Akkermansia muciniphila* supplementation in obese humans have improved metabolic health and reduce inflammation (Depommier *et al*., 2019; Yoon *et al*., 2021). Despite its beneficial effect in the host, the reason for the higher abundance in women compared to men remains unknown. Finally, women have higher abundance of taxa belonging to Firmicutes phylum. A gut bacterial community dominated by Firmicutes has been associated with a putative healthy state (Costea *et al*., 2018). On the other hand, most studies have found higher abundances of *Prevotella* and *Fusobacterium* in adult men compared to women (**Table 1**). Notably, both *Prevotella* and *Fusobacterium* genera have been associated with a diet rich in animal proteins (Costea *et al*., 2018). Several *Prevotella* species have been associated with gut inflammation. In particular, *Prevotella copri* may contribute to inflammation during HIV (Armstrong *et al*., 2018; Kaur *et al*., 2018). *Fusobacterium* genus has been associated with colorectal cancer, known to be more prevalent in men (O'Keefe, 2016; Sun *et al*., 2019). Thus, not also Fusobacterium is more frequently present in men but it is also associated with the development of prostate cancer. As Fusobacterium relative abundance also increases with age (Ghosh et al, 2020; Shanahan et al, 2021), gender-specific differences seem to confluence with age-specific changes leading to differential gender-specific susceptibility to a given disease.

Findings in late adulthood

In middle- and late-aged adults, progesterone levels in women gradually decline, while estrogen levels are reduced dramatically after menopause (**Figure 1**). In men, testosterone levels progressively decline with age. Contrary to the trends observed in the gut microbiota during puberty, the abundance of Bacteroidetes increases and Firmicutes decreases during menopause (Santos-Marcos *et al*., 2018). No sex-associated differences in the gut microbiome have been reported in older individuals, coinciding with the establishment of menopause in women (Mayneris-Perxachs *et al*., 2020; Zhang *et al*., 2021). The microbiota present in women is more similar to men once reached menopause (Mayneris-Perxachs *et al*., 2020). In addition to the

absence of differences in the gut bacterial composition between post-menopausal women and men, no differences were found in the microbiome functionality between men and post-menopausal women. These results suggest that menopause status may play a role in the inconsistent results observed in other studies assessing the gut microbiome.

In the search for the mechanism responsible for the differences in the gut microbiome according to sex, the influence of gonadal steroids has been explored, mainly in animal models.

Gonadal steroids and the microbiome

Gonadal steroids affect the microbiome

The interplay between gonadal steroid hormones and the gut microbiota has been extensively studied in mice. Pernigoni *et al* (2021) found that the elimination of male sexual hormones via castration altered the gut microbial composition of mice. Another study demonstrated that mice castration reduced sex-differences in the gut microbiota (Yurkovetskiy *et al*., 2013). Similarly, ovariectomy in female mice altered their gut microbiome (Cox-York *et al*., 2015). In humans, bilateral ovariectomy also impacted the gut microbial composition and the resulted microbial dysbiosis was linked to the side effects of this intervention (Sinha *et al*., 2019). Additionally, the use of oral contraceptives also led to a different microbial composition in women (Sinha *et al*., 2019).

Specific taxa have been associated with the levels of different sex hormones and their precursors in blood: *Acinetobacter*, *Dorea*, *Megamonas* and *Ruminococcus* genera were associated with increased levels of serum testosterone (Shin *et al*., 2019). Testosterone was also positively associated with the Fibrobacteriaceae and Idiomarinaceae families from the Bacteroidetes phylum, and negatively associated with Verrucomicrobia and Akkermansiaceae (Mayneris-Perxachs *et al*., 2020). Noteworthily, the *Akkermansia* genus was enriched in the gut microbiome of adult women in most studies (**Table 1**). The gut microbiota composition and circulating testosterone levels are tightly linked to the extent that microbial signatures can predict the levels

of progesterone and testosterone in blood (Mayneris-Perxachs *et al*., 2020). Although, no taxa have been associated with estrogen levels in the latest studies (Mayneris-Perxachs *et al*., 2020; Zhang *et al*., 2021), some authors found that estradiol was positively correlated with the relative abundance of Gammaproteobacteria class and negatively correlated with Prevotellaceae family (Santos-Marcos *et al.,* 2018). Notably, *Prevotella* was enriched in the gut microbiome of adult men in most studies (**Table 1**).

The microbiome influences the level of host gonadal steroids

Not only sexual hormones seem to influence the composition and function of the gut microbiome, but also the latter participates in the metabolism of these hormones, as some bacteria are able to metabolize gonadal steroid hormones and their precursors. Interestingly, the gut microbiome of pre-menopausal women was enriched in genes participating in the steroid biosynthesis and degradation pathways, which are necessary for the metabolism of sexual hormones. Indeed, circulating levels of testosterone and progesterone were associated with bacteria capable of catabolizing steroids in pre-menopausal women (Mayneris-Perxachs *et al*., 2020). Similarly, another study found the bacterial gene *β-glucuronidase*, which encodes for an enzyme that can catabolize estrogens, was enriched in the women´s microbiome (Zhang *et al*., 2021). Li *et al* (2022) isolated *Mycobacterium neoaurum* from testosterone-deficient patients with depression. *M. neoaurum* produces 3β-hydroxysteroid dehydrogenase (3β-HSD), a testosterone degrading enzyme. These authors found that gavaging rats with 3β-HSD-producing *E. coli* reduced their serum and brain testosterone levels and caused depression-like behaviors. Although these studies support the existence of bacterial enzymes that can metabolize sex hormones, further investigation is required to identify the complete bacterial pathways responsible for sex hormones metabolism and their impact on human physiology. Pernigoni *et al* (2021) studied to what extent steroid metabolism mediated by the gut microbiota could influence human health. These authors identified a *Ruminococcus* sp. enriched in castrate-resistant prostate cancer (CRPC) patients with poor prognosis and associated with high levels of serum testosterone. This species was able to convert pregnenolone and hydroxypregnenolone into downstream androgenic steroids. Their

205 results suggested that the gut microbiota could be sustaining prostate cancer tumor growth via
206 testosterone synthesis in CRPC (Pernigoni et al., 2021).

207 ***Other important factors and final thoughts***

208 There is no conclusive interpretation of the role of sex hormones in driving these sex-dependent
209 trajectories of the gut microbiome. A study in mice suggested that the relationship between sexual
210 hormones and the microbiota is more complex than expected. These authors observed that germ
211 free mice had a defective sexual maturation (Weger *et al*., 2019). The absence of gut microbiota
212 attenuated liver sexual dimorphism and sex-specific circadian rhythm signatures and also altered
213 the sex-specific secretion of the growth hormone.

214 In addition to gonadal steroid hormones, other phenotypes and cultural gender-related factors
215 could drive sex-related differences of the gut microbiome. In one study, obesity eliminated most
216 of the differences observed in the gut microbiome composition and functionality among non-
217 obese pre-menopausal women, post-menopausal women, and men (Mayneris-Perxachs *et al*.,
218 2020). Another cultural and influential factor stated above is the diet. In a Chinese cohort, men,
219 that consumed more red meat than women, had higher abundance of *Prevotella* and
220 *Fusobacterium* genera which are associated with a diet rich in animal protein (Zhang *et al*., 2021).
221 Medication use has also been linked to sex-specific differences in gut microbiome composition
222 (Sinha *et al*., 2019). Thus, women had a higher prevalence of antibiotic resistance genes in their
223 microbiome than men. Additionally, the effects of antibiotic exposure during childhood need to
224 be further explored, as antibiotic exposure at early age in mice led to more feminized microbiome
225 in male adults (Weger *et al*., 2019). Lastly, it is worth noting that the gut microbiome contains
226 other members such as archaea, viruses and fungi that have been largely unexplored. By
227 neglecting the non-bacterial biomes of the gut, an important component of the gut microbiome
228 that could play significant roles, has been missed. Future studies need to fill up this gap.

229

Conclusions

It seems clear that the relationships between the gut microbiome and sexual traits are bidirectional, as also recently suggested (Beale et al., 2019). Altogether, age-dependent gonadal steroid hormones secretion seems to lead to the sexual dimorphism of the gut microbiome composition and functionality. The role of cultural gender-related factors, including diet and drug intake, on the microbiome sexual dimorphism should be further investigated. This research should also take into account the role of bacteria but also of other microorganisms. Finally, it is important to mention that taxonomic classification of the microbiomes usually lack resolution if the species level cannot be reached. This and intra-individual variability make it difficult to identify sex-specific differences among cohorts. However, it is increasingly recognized that the functions of the metagenome are more reproducible that the taxonomy of the microbiota at the individual level. Functional information enables us to better understand the bidirectional relationship between the gut microbiota and human body in a sex-specific manner.

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Table and Figure legends.

Table 1. Human studies focused in sex-associated differences of the gut microbiome in the past three years. In bold, sex-associated taxa found in most studies.

Figure 1. Simplified scheme of age-dependent trajectory of the gut microbiota in women and men along with sex hormone levels over life course. Representation of sex-associated taxa (left) and sexual dimorphism of the gut microbiota during adulthood (right). There are differences in the gut microbiome composition between women (dijon yellow) and men (ocean blue) during adulthood, while no sex-associated differences have been reported in childhood or the elderly. Sex-associated differences at each life stage are driven by critical hormonal shifts. Overview of testosterone (dashed line) and estradiol (dotted line) levels across the life span.

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