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A SIMPLIFIED MODEL HIGHLIGHTING THE NECESSITY TO REEVALUATE THE DESIGN OF CLINICAL TRIALS INVESTIGATING MICROBIOTA: HOW TO ERADICATE "FRENCH KISS BIAS"?

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Abstract – *Objective:* The aim of the study was to address the possibility that microbial exchange between individuals in close contact may introduce a hidden bias due to differing microbiota phenotypes.
Materials and Methods: A random sample of 296 spouses experiencing insomnia and 296 spouses experiencing hypersomnia was selected from a total of 1,740 couples initially enrolled in previously conducted trials. Overall, 296 otherwise healthy spouses of individuals with insomnia were compared with 296 healthy spouses of individuals with hypersomnia in terms of microbiota phenotypes. Microbiota phenotypes were extracted from the existing literature and further categorized into different phenotypes. The microbiota phenotypes in otherwise healthy spouses were evaluated, and differences between groups were assessed using Chi-square tests.
Results: There were significant differences (p-value < 0.01) in the occurrence of microbiota phenotypes between the two groups of otherwise healthy spouses of individuals with (i) insomnia and (ii) individuals with hypersomnia in terms of microbiota phenotypes.
Conclusions: We recommend that microbiota research incorporates the baseline characteristics of peers, spouses, and immediate family members. This short communication holds substantial therapeutic and pharmacological significance for various types of clinical trials, as well as for the fields of family medicine and personalized medicine.

Keywords: Social network dynamic, Microbiota phenotype, Bacterial transmission, Disease communication, Bias, Methodology.

INTRODUCTION

Several years ago, a novel hypothesis was introduced that sought to establish a link between the human intestinal microbiome and non-communicable diseases[1]. This concept was subsequently expanded upon by Finlay et al[2]. The hypothesis suggests that certain non-communicable diseases, which may initially appear to be unrelated to microbial influences, could indeed possess a microbial component that might be transmissible via the microbiota[2]. This proposition is increasingly gaining traction and has already inspired a number of compelling theories[1-5].

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While it is acknowledged that our microbial identity is unique[6], it is also characterized by a high degree of dynamism and responsiveness to various perturbations, including alterations in dietary habits, antibiotic administration, and dysbiosis changes that may precede the onset of symptomatic disease[7]. Although this assertion is valid to some extent, it is crucial to understand that humans do not exist in isolation; rather, we participate in intricate social interactions that involve diverse physical contact with multiple individuals, each harboring their own distinct microbiome. The degree to which these social interactions influence the individual genetic composition of the microbiome and its potential implications within and across communities remains an area of uncertainty.

A recent and intriguing study published in Nature suggests oral and gut microbiota may be transmitted through person-to-person contact[8]. The authors introduced a metric referred to as "shared strains and species," demonstrating that the presence of five or more shared strains or fifty or more shared species (edges) among individuals (nodes) implies that overlooking transmission could constitute a methodological oversight. This groundbreaking research has opened a new avenue of inquiry into the transfer of microbial strains among individuals in close proximity. However, there remains a paucity of rigorously designed studies to substantiate this hypothesis.

To address this research gap, we tested several hypotheses derived from the existing literature. Due to ethical considerations, we conducted non-invasive, prospective, and observational studies involving non-clinical participants. Recently, our team reported findings that were both unexpected and remarkable, surpassing even the expectations of the most optimistic and conservative researchers[9-11].

Since the previous year, research has demonstrated that the transmission of microbiota between individuals can significantly impact various medical and psychological conditions. These include alterations in sleep patterns[10], the incidence of dry eye disease[9], as well as levels of depression and anxiety among couples who have been married and cohabiting for an average duration of two years and two months[11].

A critical aspect of the methodology employed in the studies conducted by Rastmanesh et al[9-11] was the enrollment of newly married couples to effectively manage the transmission of microbiota between individuals. This deliberate selection and recruitment of participants facilitated an investigation into the impact of continuous and short- to mid-term transmission of oral, gut, and ocular bacteria on various outcomes of interest in a real-world context. Specifically, these outcomes included sleep patterns (experiment A), the frequency and severity of dry eye (experiment B), and levels of anxiety and depression (experiment C).

The findings were significant. Within a period of three to six months following marriage, spouses exhibiting normal sleep patterns, psychometric parameters, and healthy tear film and ocular surface conditions demonstrated a marked tendency to adopt characteristics associated with insomniac or hypersomniac phenotypes (experiment A)[10], as well as phenotypes related to depression and anxiety (experiment B)[11], and dry eye disease (experiment C)[9]. These alterations were concomitant with changes in gut, oral, and ocular microbiota. Notably, the microbiota composition in healthy spouses underwent significant modifications, aligning more closely with that of their partners. The mediation analyses for experiments A, B, and C accounted for 18%, 24%, and 35% of the variability in the data, respectively. Such statistics are difficult to overlook.

There is, however, a significant concern. If non-communicable diseases, such as dry eye, anxiety, depression, and sleep disorders, can be transmitted through microbial means, then there is a high likelihood that microbial exchange between individuals in close contact (such as couples and partners) may introduce a hidden bias induced by differing microbiota phenotypes, which could confound the results. This study aims to address this possibility.

PATIENTS AND METHODS

Data were collected from our patient database at two private sleep clinics located in Tehran, Iran. In summary, individuals who had been legally married for at least six months and were cohabiting were invited to participate in this study along with their partners.

For this study, we randomly selected 296 spouses experiencing insomnia and 296 spouses experiencing hypersomnia from a total of 1,740 couples initially enrolled in trials previously conducted[9-11]. Overall, 296 healthy spouses of individuals with insomnia were compared with 296 healthy spouses of individuals with hypersomnia in terms of microbiota phenotypes.

Microbiota phenotypes were extracted from the existing literature and further categorized into insulin-resistant[12], obesity or lean[13], aging[14], inflammatory[15], diet-induced obesity[16], diabetic[13,17], pro-colitogenic[18], and normal microbiota phenotypes[13].

The microbiota phenotypes in healthy spouses were evaluated, and differences between groups were assessed using Chi-square tests.

RESULTS

Table 1 presents the microbiota phenotypes of two groups: the spouses of individuals with insomnia and hypersomniac individuals. The Chi-square test results showed significant differences (p-value < 0.01) in the occurrence of microbiota phenotypes between the two groups.

DISCUSSION

We demonstrated that the randomization produced two equal groups of spouses with insomnia and hypersomnia concerning microbiota phenotypes. However, this protocol did not ensure the formation of two groups of otherwise healthy, corresponding spouses.

While our study was designed as a prospective and observational investigation, the results offer initial insights that may inform future research in diagnostics, prognostics, therapeutics, and pharmaceuticals. This implies that the associations identified may be causal, highlighting a substantial likelihood that microbiota transfer among individuals in extended close contact could introduce confounding variables in various statistical analyses. These analyses may include between-group comparisons, studies on side effects, efficacy assessments, translational research, and other related investigations.

It is well established that careful and systematic randomization is crucial in minimizing the potential for selection and allocation bias[19,20]. To assess whether the randomization of participants experiencing sleep disturbances yields comparable groups, we conducted a real data simulation study utilizing a stringent randomization methodology. As expected, the randomization process resulted in two group participants – those with insomnia and those with hypersom-

TABLE 1. THE PREVALENCE OF VARIOUS MICROBIOTA PHENOTYPES IN THE TWO GROUPS.

	Spouses of individuals with insomnia (n=296)	Spouses of individuals with hypersomnia (n=296)	*p*-value
Insulin-resistant	140, 47.9	43, 14.52	
Obesity	270, 91.21	267, 90.20	
Diet-induced obesity	260, 87.83	226, 76.35	
Aging	45, 15.20	12, 4.05	< 0.01
Diabetic	65, 21.95	31, 10.47	
Pro-colitogenic	10, 3.38	3, 1.01	
Normal	37.16	32, 10.81	

The results of the Chi-square test indicated significant differences (p-value < 0.01) in the prevalence of microbiota phenotypes between the two groups.
*Since there is a significant overlap between the phenotypes of insulin resistance and diabetes, as well as between obesity and diet-induced obesity, we combined the data across various scenarios. In all scenarios, the results were significant, with the p-value falling below 0.01.

nia – exhibiting comparable baseline characteristics, with no statistically significant differences identified. However, when we applied the same statistical analyses to the spouses of these randomly selected participants, we discovered that the baseline characteristics of the spouses in the two groups were significantly different. Notably, spouses classified within the healthy and normal control group (hereafter referred to as control spouses) exhibited differences in several aspects, including pet ownership, interactions with other animals, hydration status, and the incidence of both communicable and non-communicable diseases. Additionally, various other factors directly or indirectly related to our primary and secondary outcomes of interest were also identified.

This finding holds significant importance and can be elucidated through several factors pertaining to control spouses. In this brief discussion, we will focus on the concept of social closeness, which is defined as the average distance from one node to all others[21]. Several parameters warrant consideration:

- Microbial identities are uniquely distinctive[6].
- The human microbiome is capable of being transmitted between individuals[1-5, 9-11].
- Certain individuals who own pets exhibit distinct bacterial profiles[22].
- Significant variations in social closeness and dynamism exist among individuals[23,24].

This comment aims to integrate the aforementioned premises and offer a more precise representation. Figure 1 illustrates a model that elucidates how the interplay between the simplicity or complexity of social networks and the diverse microbiota phenotypes of randomly selected individuals can result in notable variations in the baseline characteristics of peers, spouses, and analogous groups, ultimately complicating the interpretation of any findings.

Numerous confounding variables may pose challenges to effective control through randomization, particularly individual habits. For instance, the presence of pets in a household has been shown to facilitate significant bacterial exchange between humans and animals, including dogs and livestock[25,26]. Furthermore, there is evidence of microbiota exchange occurring in the penile and genital regions between sexual partners[27,28]. These factors can potentially influence the integrity of randomization protocols, which will be briefly discussed in the following sections.

The author has previously noted that, from a methodological perspective, randomization protocols are designed to ensure the equitable distribution of potential confounding variables across treatment groups, however, in the context of microbiota studies, uncertainties may emerge due to several factors, including the inherent characteristics of the gut and oral ecosystems, the transmissibility of the microbiome, the dynamic nature of microbial populations, and the varying temporal patterns of interactions and interventions, such as supplementation and medication effects[29-32]. I have referred to these uncertainties as “French Kiss Bias”[29]. Additionally, altered or differential social dynamics may further complicate these interactions [21]. Consequently, it is questionable whether randomization protocols alone can be considered a reliable methodological approach in studies aimed at investigating the potential for disease transmission among close individuals and family members.

One possible statistical approach may involve the implementation of cross-over designs, which are frequently regarded as standard methodologies across numerous scientific disciplines. Nevertheless, in the context under discussion, we contend that cross-over designs are likewise unsuitable. This claim is substantiated by several important considerations:

- Seasonal fluctuations have a significant impact on the profile and composition of microbiota[33];
- Challenges exist in ascertaining the duration necessary for the establishment of bacterial communities[34-36];
- The interaction among circadian rhythms, the body’s microbiota, and dietary habits is complex and may be further influenced by social dynamics[37-39]. These parameters can also undermine crossover designs.

These arguments have significant implications for pharmacological and toxicological research in various ways. For instance, drug-microbiota interactions modulate drug pharmacokinetics and bioavailability[40-42].

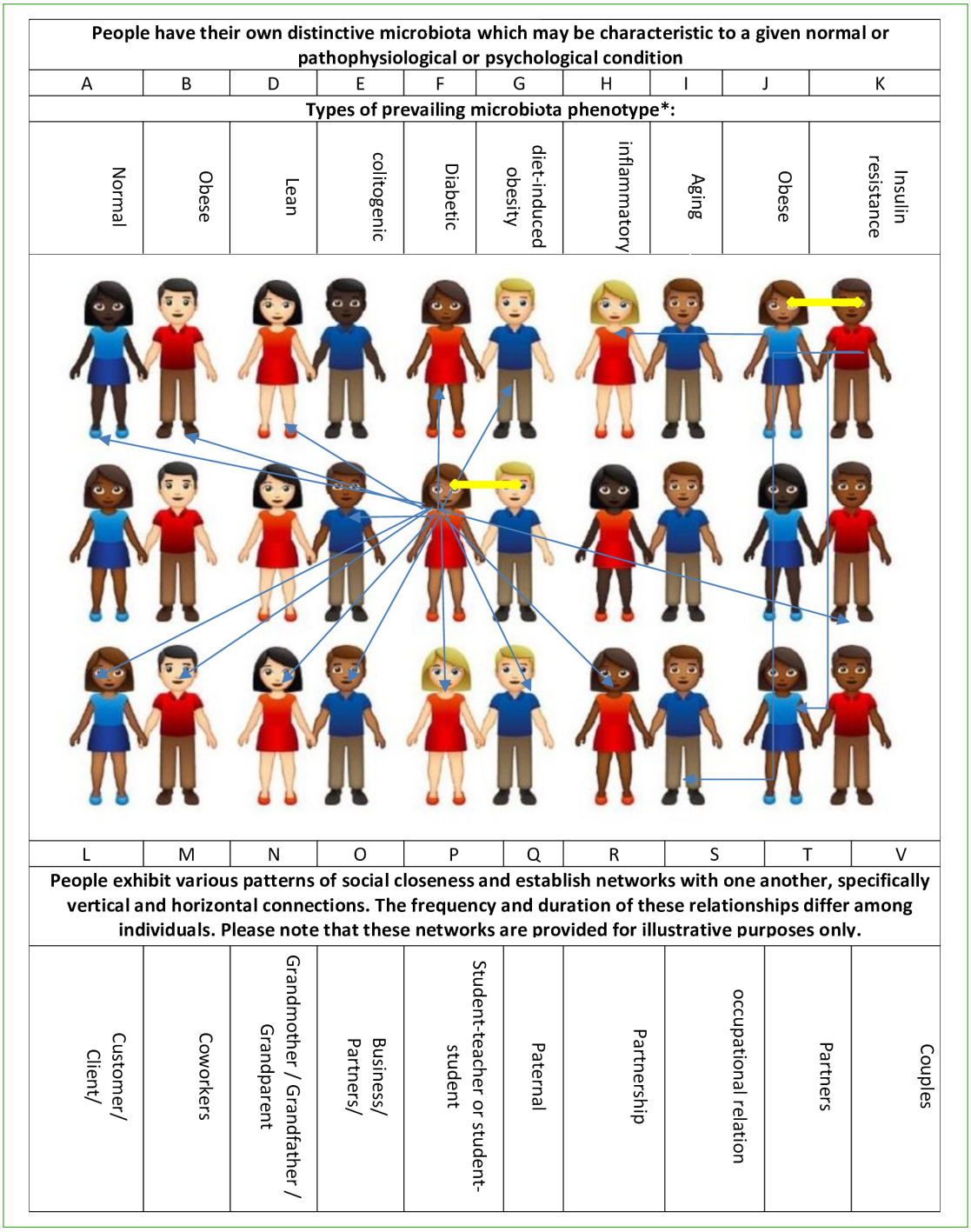

Figure 1. *These phenotypes are simply examples. Persons J and K are a relatively isolated couple with a limited social network, each exhibiting an obese and insulin-resistant microbiota phenotype. Person K is in direct contact with Persons S and T, who also display obese and aging microbiota phenotypes. In contrast, there is another individual situated in the center of the network who possesses a highly dynamic social network, interacting with individuals of various microbiota phenotypes. When a set of couples is randomly selected, there will undoubtedly be (i) couples with relatively limited social networks and (ii) couples whose social networks are highly dynamic. At this stage, proper randomization with a moderate-to-high sample size can ensure that the baseline characteristics of the two groups are comparable, with no significant differences. However, due to person-to-person bacterial transmission and the diversity of microbiota phenotypes, there is a high likelihood that the baseline characteristics of their corresponding spouses become non-comparable. Our model, for the first time, demonstrates how bacterial transmission can confound microbiota research and lead to potential biases, residuals, or partialities, depending on the study design, the outcomes of interest, and possibly the duration of the study. These factors are all influenced by the interaction between baseline microbiota phenotypes, the nature and duration of contact between individuals, and numerous other variables. The design of clinical trials involving the body microbiota requires careful reconsideration.

CONCLUSIONS

It is recommended that microbiota research incorporates the baseline characteristics of peers, spouses, and immediate family members. The findings from the studies examined by our team[9-11] are not limited to clinical trials; instead, they have broader implications for various medical conditions, nutritional status, and diseases that are either influenced by or can be altered through microbiota. Nonetheless, this brief study has only been able to explore a limited number of statistical implications associated with a select body of research. This discussion undoubtedly holds substantial therapeutic and pharmacological significance for various types of clinical trials, as well as for the fields of family medicine and personalized medicine.

One day, it may be possible to realize the dream of next-generation statistical analyses that simultaneously incorporate and account for all the confounding effects imposed by the baseline characteristics of peers, spouses, and immediate family members.

Conflict of Interest

The author declares no conflict of interest.

Informed Consent and Ethics Approval

All participants were guaranteed that their personal information would remain confidential, and that the data collected would solely be used for research purposes, with their identities protected throughout the study and beyond. This research was conducted at two private sleep clinics, and all participants willingly provided their informed consent. Written consent was secured from each participant. Ethical approval was granted by the Human Research Ethics Committee (IT. 24001670, date: January 08, 2024), and the principles outlined in the Declaration of Helsinki were followed.

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Authors' Contributions

RR wrote the manuscript. RR critically reviewed the manuscript. RR approved the final version of the manuscript.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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No AI tools were used in the writing of this manuscript. The author utilized https://wordvice.ai/ for grammar check.

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