**Q1: What was the hypothesis being tested here? Is the hypothesis likely or unlikely. What type of evidence and prior information would alter your opinion?**

The study hypothesizes that specific gut microbial genera are genetically linked with Alzheimer’s disease (AD), and that genetic factors like the APOE genotype influence these associations. The authors suggest that certain microbes are risk elevators through increasing neuroinflammation and gut dysbiosis, while others may maintain healthier, neuroprotective situations. Such a hypothesis is practically plausible as the brain and gut are associated through the microbiota-gut-brain axis, a biochemical signalling pathway. Dysbiosis is manifested in many chronic ailments and hence it is practical to extend this to neurodegenerative diseases. Moreover, the composition of the gut microbiota is impacted by genetic background, environment and diet, hence targeting it through interventions like tailored diets, probiotics or microbiota-modifying therapies seems to be feasible strategies for AD risk mitigation. While the hypothesis is likely, it assumes a level of causality that has not yet been proven. To further solidify the hypothesis, there is a need for more direct evidence, an example of such would be studies that show specific microbial metabolites lead to amyloid aggregation or inflammatory cascades in the brain. Additionally, longitudinal studies that show distinctive microbial shifts that precede clinical symptoms of AD, could suggest that these microbial communities actively play a role in disease onset. Similarly, if interventions that are designed to change gut microbiota composition can slow the rate of or prevent the progression of AD, it would underscore the biological relevance and practicality of this approach.

**Q2: How well did the group report on and control for confounders. Can you identify confounders that they missed? How might this affect the results?**

The study accounted for confounding factors like sex, age and APOE genotype in their multivariate logistic regression models. Factors like age and the APOE genotype, specifically the ε4 are significant risk factors for AD, and confounding variables in AD research as well. By controlling for such variables, the study can decrease the likelihood that the genetic correlations observed between AD and gut microbiota were due to such confounding factors. Although such variables were accounted for, the dataset omitted information on environmental and lifestyle factors like socioeconomic status, comorbidities, medications, diet and more, all of which can heavily influence the composition of the gut microbiota, and may influence AD independently of genetics. Although the article mentions diet, detailed dietary data from participants is lacking. Moreover, the participant population was predominantly European-American, and thus race and ethnicity can confound results because of variations in genes and the microbiome.

Additionally, the interaction with other genes was not explored, and other epigenetic variables were not addressed. The lack of consideration for such factors can result in skewed estimations of the genetic associations identified. For example, if a patient with AD in the study is on a diet that changes the gut microbiota, any observed microbial affiliations may be indicative of these external influences rather than true genetic causes. Likewise, geographical and cultural differences in diet were not accounted for, which can impact the generalizability of the findings, especially with the predominantly European ancestry of the participants. Lack of consideration of such confounding factors can result in a weaker statistical power that detects the genetic associations, or mislabels, microbial genera as significant. Accounting for such factors by including a comprehensive dataset will provide a clearer picture of the interplay between gut microbiota, genetics and AD.

**Q3: What is the effect size that you anticipate for any association? How does your preconceived idea fit with their observations? What is the likelihood that an independent replication will find comparable results?**

The anticipated effect size for any association between gut microbiota and AD is small or moderate to small, especially when considering the multifactorial nature of AD. It is unlikely that a single factor, like a specific microbial genera will dominantly influence on AD. The effect size would represent the contribution of microbiota in a broader context of factors that interact with each other, for example lifestyle, genetic and environmental factors. Such statement is in alignment with the study’s findings, where the recognized associations are made evident by statistical significance but did not suggest very strong impacts. An example of this is the odds ratios (ORs) for the identified microbial general ranged between protective and risk effects in decent ranges which indicating that these genera were contributing factors, but did not play primary roes in AD pathology. My preconceived notion about the likely effect size fits in conjunction with their observations. Gut microbiota impacts neuroinflammatory and systemic inflammatory pathways which are influenced by many other variables such as age, APOE genotype and more in AD. The study adequately recognizes that the impact of the microbiota is part of a larger interplay of factors and does not claim a causal relationship. The likelihood that an independent replication would yield comparable results, in my perspective is low, however does depend on several key factors, like methodological consistency. One primary concern in replicability of a study as such is the variability in microbiota composition across populations due to diet, lifestyle and geographical location. Considering the variability in different factors, especially concerning lifestyle that can skew results, the likelihood of replication is low.

**Q4: Are the results and conclusions well-justified? What data is available? what data is not? how does that affect your conclusions?**

Although the results of this study are not definitive, they are reasonably justified, as the researchers used robust methods, which includes genome-wide association studies (GWAS), polygenic risk score analyses and meta-analyses to examine associations between AD and certain gut microbiota genera. By controlling for confounders like age, sex, and APOE genotype the associations were strengthened, and the biological likelihood of certain genera behaving as protective, or risk factors aligns with the known methods involving neuroinflammation and the interactions between the gut and the brain. However, the conclusion surrounding using genera as biomarkers or therapeutic targets is not well-justified as causality was not established. The lack of longitudinal does not allow us to determine whether microbiota changes come before AD or result from the disease. Moreover, the lack of data surrounding environment and lifestyle factors like diet, medications and comorbidities, which can heavily influence gut microbiota composition can confound observations of associations. The predominant European ancestry of the cohorts’ limits generalizability of the results to more diverse populations. These limitations attest for a need of the conclusions to be interpreted for cautiously and for future longitudinal studies for substantiation.

**Q5: What are the potential motivations of the research team?**

Potential motivations of the research team can involve wanting to contribute to a deeper understanding of AD and exploring different therapeutic interventions. Another motivation is to discover new biomarkers or targets for prevention and treatment. Upon analyzing the article there seems to be no potential conflicts of interest, however the study focuses on correlation rather than causality which can bring much attention around interventions tailored to the microbiome.

**Q6: Provide a summary tweet/skeet of 300 characters or less that summarizes this paper**

There is a connection between gut microbiota and AD! Study finds 10 genera that are associated with AD risk/protection, with APOE genotype playing a primary role. The gut-brain axis can serve as a potential therapeutic target, however more research is needed.

**Reference:**

Cammann, D., Lu, Y., Cummings, M.J. *et al.* Genetic correlations between Alzheimer’s disease and gut microbiome genera. *Sci Rep* **13**, 5258 (2023). https://doi.org/10.1038/s41598-023-31730-5