A bidirectional Mendelian randomisation study of the association between gut microbiota and malignant non-melanoma skin cancer

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Article

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A bidirectional Mendelian randomisation study of the association between gut microbiota and malignant non-melanoma skin cancer

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Abstract

Gut microbiota are suggested to be associated with a variety of cancers.However, the correlation between gut microbiota and the development of malignant non-melanoma skin cancer (MNMSC) remains unknown. Thus, this study employed a bidirectional Mendelian randomisation (MR) method to investigate the potentially causal association

between gut microbiota and MNMSC. Here the detailed genetic data on the gut microbiota available from the MiBioGen consortium and the Dutch Microbiota Project were analysed, as well as the MNMSC data from the UK Biobank.Several sophisticated statistical methods involving inverse variance weighting, weighted median estimator, MR Egger, and simple and weighted mode-based estimator models were utilised to assess the potential association between gut microbiota and MNMSC. Reverse MR and sensitivity analyses were also performed. Eight gut microbiota were identified as potentially causally related to MNMSC. Genera *Holdemanella* (odds ratio [OR]=0.99, 95% confidence interval [CI]: 0.99–1.00, 4.5×10^{-3}), *Ruminococcaceae* UCG014 (OR=0.99, 95% CI: 0.99–1.00, $P=1.3\times10^{-2}$), and *Sutterella* (OR=0.99, 95% CI: 0.99–1.00, $P=4.2\times10^{-2}$), *Bifidobacterium adolescentis* (OR=0.99, 95% CI: 0.99–1.00, $P=8.8\times10^{-3}$), *Bacteroides dorei* (OR=1.00, 95% CI: 0.99–1.00, $P=9.9\times10^{-3}$), and the family *Veillonellaceae* (OR=0.99, 95% CI: 0.99–1.00,$P=3.8\times10^{-4}$) were negatively correlated with MNMSC. *Clostridia* (OR=1.01, 95% CI: 1.00–1.02, $P=2.1\times10^{-3}$) and *Clostridiales* (OR=1.01, 95% CI: 1.00–1.02, $P=2.1\times10^{-3}$) were positively associated with MNMSC. The inverse association between MNMSC and *Veillonellaceae* was more pronounced following Bonferroni correction. No causal relationship was determined between MNMSC and any of these eight gut microbiota by reverse MR analysis. This study suggests that specific gut microbiota have potential causal effects on MNMSC. This study indicates that certain gut microbiota may have a causal effect on MNMSC. Thus, gut microbiota modulation may be a potential means to prevent MNMSC.

1 Introduction

Malignant non-melanoma skin cancer (MNMSC), which is the most common form of malignancy among the Caucasian population (Cantisani et al., 2019), develops from epidermal cell types other than melanocytes (Rong et al., 2015). According to the American Cancer Society, the estimated annual incidence of MNMSC in the United States is over one million cases, which is approximately equal to that of all other human malignancies combined (Ridky, 2007). Risk factors associated with MNMSC include light-colored ethnicity, eye color, red and blonde hair color, sunburn, and exposure to ionizing radiation (Connolly et al., 2017). Intensive research on the gut–skin axis has highlighted the need to further explore the correlation between gut dysbiosis and skin cancer (De Pessemier et al., 2021).

A growing body of evidence suggests a strong relationship between gut microbiota and skin diseases, including psoriasis and acne (Cao et al., 2023; Long et al., 2023; Zang et al., 2023), with gut microbiota exerting immune and metabolic effects on human skin through the action of commensal

bacteria and their metabolites (Polkowska-Pruszyńska et al., 2020; Šuler Baglama and Trčko, 2022). The integrity of the intestinal barrier and the action of mucus produced by intestinal epithelial cells, immune cells, secretory IgA, and antimicrobial peptides prevent the entry of intestinal bacteria into the bloodstream, thereby preserving skin homeostasis (Macpherson et al., 2009). In particular, secretory IgA controls the inflammatory response of gut microbes by spatially segregating host tissue from the microbes (Roland et al., 2020). Nevertheless, the correlation between dysbiosis of gut microbiota and skin cancer remains unclear (Lou et al., 2024).

To fill that research void, Mendelian randomisation (MR) was used by this study, which is a powerful statistical method in epidemiological studies. The core principle of MR is its ability to use genetic variation as a tool to assess causal relationships between risk factors and particular diseases (Li et al., 2022; Liu et al., 2022). MR employs similar principles to those of randomized controlled trials to establish unbiased causal relationships between exposures and outcomes. In this study, we use a bidirectional MR design to randomly assign genetic variants associated with gut microbiota as instrumental variables (IVs), the causal relationship between gut microbiota and MNMSC was then investigated.

2 Materials and methods

2.1 Study design

The process for designing this bidirectional MR analysis is as follows (refer to Figure 1). We used single nucleotide polymorphisms (SNPs) as IVs to evaluate causality between the variables exposure (gut microbiota) and outcome (MNMSC) by means of MR analysis. To ensure reliable results, two-sample bidirectional MR must fulfill three main assumptions (Emdin et al., 2017; Bowden and Holmes, 2019). First, the IVs should correlate with the gut microbiota. Second, the IV must be independent of any potential confounders that may affect the correlation. Lastly, IV may affect MNMSC risk only through gut microbiota (Figure 2A). Then, MNMSC was used as the exposure variable and gut microbiota was used as the outcome variable, and reverse MR analyses were performed to test for reverse causality effects (Figure 2B).

2.2 Data sources and instrument selection

This genome-wide association study (GWAS) of MNMSC included 23,694 cases and 372,016 controls from the European population. The study data were obtained from the UK Biobank and are publicly available from the IEU

OpenGWAS project. Genetic data on the human gut microbiota were acquired from the MiBioGen consortium, which consists of 24 population-based cohorts with a total of 18,340 participants (Kurilshikov et al., 2021). The dataset comprises altogether 211 gut microbial groups, which represent 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. However, 15 microbial taxa were excluded, leaving 196 for MR analysis. The Dutch Microbiota Project (DMP) provides a summary of species-level statistics on the gut microbiota, covering 105 species from 7738 participants of European origin (Lopera-Maya et al., 2022).

To gain more holistic results, the IV with genome-wide locus significance was selected ($P<1\times10^{-5}$). Simultaneously, The PLINK clumping method was used to exclude SNPs in linkage disequilibrium ($r^2 < 0.001$, kb=10,000). Then, SNPs with F-statistics [formula: $R^2/K\times(N-K-1)/(1-R^2)]<10$] were also removed (Palmer et al., 2012; Davies et al., 2018) (Supplementary Table 1). As our study was grounded in existing public available databases, no additional ethical approval or informed consent was required.

2.3 Statistical analysis

The inverse variance weighting (IVW) method is the primary analytical method for assessing the potential causal relationship among each phenotype and MNMSC risk. IVW combines the ratio estimates for each SNP to estimate the causal effectiveness of the exposure on the outcome, thus converting the MR estimates into a weighted regression of the SNP outcome effect on the SNP exposure effect (Choi et al., 2019). To ensure the accuracy of the results, further robustness validation was conducted via MR-Egger regression and simple mode, weighted median, and weighted mode estimator models. The MR-Egger method detects causality from the slope coefficient of the Egger regression and detects small study bias (Bowden et al., 2015). The simple model represents a non-weighted representation of the empirical density function used to estimate causality (Hemani et al., 2018). Even if up to half of the information is derived from invalid IVs, the weighted median method will still result in unbiased estimates (Bowden et al., 2016). When the largest number of causal effect estimates of similar individual instruments are derived from valid instruments, the weighted mode method exhibits consistent performance, even when the IVs are invalid (Hartwig et al., 2017). The results of these supplementary tests were consistent with those estimated using the IVW method (Levin et al., 2020).

2.4 Heterogeneity and pleiotropy test

To quantify potential heterogeneity and test for horizontal multidimensionality, we calculated Cochran's Q statistic and the MR-Egger

intercept test, respectively, where results with P>0.05 were excluded. To identify and eliminate potential outliers that could independently influence the causality of the observation, leave-one-out analyses were performed. For major MR assessments, the MR-PRESSO test was employed to detect outliers and adjust for heterogeneity. If any heterogeneity is identified in IVs, the outliers are discarded and the MR analysis is repeated (Bowden et al., 2016). The analyses were conducted using "Two-Sample MR" and "MR-PRESSO" packages in R (version 4.3.1).

3 Results

3.1 Overview

The analysis involved a totally 3467 eligible IVs from 301 gut microbiota that passed a sequence of IV selection steps, 77 SNPs associated with three genera (*Holdemanella, Ruminococcaceae* UCG014, and *Sutterella*), and five species groups (*Bifidobacterium adolescentis, Bacteroides dorei, Veillonellaceae, Clostridia, and Clostridiales*) (Supplementary Table 1). We observed no evidence of heterogeneity between pairs of samples (Cochran's Q statistic, P>0.05) or multidirectionality at the IV level (MR-PRESSO global, P>0.05; MR-Egger intercept, P>0.05).

IVW analysis showed that *Holdemanella* (ratio [OR]=0.99, 95% confidence interval [CI]: 0.99–1.00, $P=4.5\times10^{-3}$), *Ruminococcaceae* UCG014 (OR=0.99, 95% CI: 0.99–1.00, $P=1.3\times10^{-2}$), *Sutterella* (OR=0.99, 95% CI: 0.99–1.00, $P=4.2\times10^{-2}$), *B. adolescentis* (OR=0.99, 95% CI: 0.99–1.00, $P=8.8\times10^{-3}$), *B. dorei* (OR=1.00, 95% CI: 0.99–1.00, $P=9.9\times10^{-3}$), and the family *Veillonellaceae* (OR=0.99, 95% CI: 0.99–1.00, $P=3.8\times10^{-4}$) were negatively correlated with MNMSC, whereas *Clostridia* (OR=1.01, 95% CI: 1.00–1.02, $P=2.1\times10^{-3}$) and *Clostridiales* (OR=1.01, 95% CI: 1.00–1.02, $P=2.1\times10^{-3}$) were positively associated with an increased risk of MNMSC (Table 1). The IVW results of all eight gut microbiota indicated the same causal effect as the corresponding MR-Egger, weighted median, simple mode, and weighted mode results (i.e., the straight lines obtained from each method in the scatter plot had the same monotonicity, as shown in Figure 3), indicating that the results were stable and reliable. A forest plot of the causal effect of SNPs on MNMSC for each category of gut microbiota is shown in Figure 4.

3.2 Bonferroni correction and sensitivity analysis

Bonferroni's method was used to set significance thresholds for multiple testing separately at each taxonomic level according to the count of bacteria under each taxonomic level to further ensure the accuracy of the causal relationships (Table 1). Nominal potential positive causal effects were

assumed when p-values reached nominal significance (P<0.05). Following Bonferroni correction, only *Veillonellaceae* remained strongly negatively correlated with MNMSC (OR=0.99, 95% CI: 0.99–1.00, $P=3.8\times10^{-5}$). To prevent overly biased effects, we employed a range of measures to verify the sensitivity of the MR analyses and to examine the potential pleiotropic effects of IV on each phenotype. The MR-PRESSO method was used to detect outliers. However, no outliers with pleiotropic effects that could be causally related to MNMSC were detected in the IVs of eight types of gut microbiota (Table 2). The results of leave-one-out analysis also showed us that removing SNPs did not fundamentally affect the outcome (Supplementary Figure. 1). Cochran's Q-test showed no heterogeneity, with P>0.05 for all eight taxa (Table 2). Similarly, the funnel plot is relatively symmetrical, demonstrating the absence of heterogeneity (Supplementary Figure 2). There is no sign of horizontal pleiotropy in the intercepts of the MR-Egger regressions in all eight taxa.

3.3 Reverse MR analysis

A total of 63 eligible SNPs related to MNMSC were selected as IVs ($P<1\times10^{-5}$) (Supplementary Table 2). After subjecting MNMSC to reverse MR, none of the eight types of microbiota showed bidirectional causality with MNMSC. Supplementary table 3 provides detailed results.

4 Discussion

To our knowledge, this is the first MR study to explore the causal effect of gut microbiota on the risk of MNMSC using large-scale genetic data. Our bidirectional MR analysis based on the largest GWAS dataset not only showed a causal effect of *Veillonellaceae* on a reduced risk of MNMSC, but also identified seven additional bacterial taxa that may be causally involved in MNMSC. This suggests an important role for gut microbiota in the pathogenesis of MNMSC and its potential as a complementary therapeutic approach for skin malignancies.

The genera *Holdemanella* and *Ruminococcaceae* UCG014 exhibited a negative causal relationship with MNMSC. Immune checkpoint blockade therapy is highly effective for enhancing the anticancer effects of treatment against aggressive cancers (Hodi et al., 2010). One of the most compelling target protein pairs in immune checkpoint blockade therapy is programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) (Davar and Zarour, 2022; Huang et al., 2023). Many tumors display increased PD-1 expression, which facilitates their ability to evade immune responses (Boussiotis, 2016). A combination of CTLA-4 and PD-1 blockade has been successful in the treatment of advanced melanomas, including

brain metastases (Khayyati Kohnehshahri et al., 2023). This is consistent with the findings of Huang et al. (Huang et al., 2023), who observed a negative correlation between PD-1 and the genus *Holdemanella*, as well as a negative correlation between PD-L1 and the genus *Ruminococcaceae* UCG014. Therefore, the genera *Holdemanella* and *Ruminococcaceae* UCG014 may counteract MNMSC through this mechanism. The genus *Sutterella* also exhibited a negative causal relationship with MNMSC. *Sutterella* is a widely used commensal bacterium in the human digestive tract (Wexler, 2005); however, it does not appear to cause intestinal dysbiosis. Instead, its presence (especially in the duodenum) and its ability to adhere to intestinal epithelial cells suggests that *Sutterella* and intestinal epithelial cells are mutualistic organisms that help to keep the immune system alert (Hiippala et al., 2016). This may explain its relationship with MNMSC.

Bifidobacterium adolescentis (*B. a*) is a true symbiont that is not a promoter of systemic or intestinal inflammation when in a healthy state. (Roberts et al., 2020). *B. a* can reduce the inflammatory response of the host to intestinal injury and pathogen invasion, and has potent immunomodulatory properties in disease states (Guo et al., 2019). According to previous research, *B. a* can protect rats from infection by *Yersinia* spp. and toxigenic *Escherichia coli* and antagonize the growth of harmful bacteria (Resta-Lenert and Barrett, 2003; Frick et al., 2007). Additionally, *B.a* can improve the lifespan of various species (Chen et al., 2021). Further, *B.a,* identified by 16S ribosomal RNA sequencing, has been linked to antitumor effects and improved efficacy of melanoma immunotherapy (Sivan et al., 2015). *B. a* also carries the endothelial repressor gene, which serves as a selective inhibitor of angiogenesis and hypoxic tumor growth (Li et al., 2003). These studies are consistent with our results and suggest the potential mechanism underlying the relationship between *B. a* and MNMSC. Interestingly, *B. a* inhibits colon tumorigenesis by inducing CD143+ cancer-associated fibroblasts (with high expression of GAS1) through the Wnt/β-catenin pathway (Chen et al., 2023a). High GAS1 expression protects against tumor formation and metastasis in colon and gastric cancers (Segovia and Zarco, 2014). However, Sternfeld et al. showed that a small number of locally advanced basal cell carcinomas had high levels of GAS1 before treatment, suggesting that these tumors may be more aggressive and thus capable of spreading, even with high levels of GAS1, making them refractory to systemic treatments (Sternfeld et al., 2020). Therefore, further investigation is needed to better understand the mechanism of action of *B. a* and its potential relevance to MNMSC.

The increase in *Veillonellaceae* microbiota may produce carbohydrates and short-chain fatty acids (SCFAs) (Chen et al., 2023b), which are by-products of the bacterial fermentation of dietary fiber in the colon (Maslowski and

Mackay, 2011). In humans, SCFAs regulate immune homeostasis in the gut, affecting Tregs, effector T cells, and γδ T cells (Matsuoka and Kanai, 2015), which is important for the gut–skin axis. Two independent studies conducted on patients with cancer undergoing anti-PD-1 therapy found that a favorable therapeutic response was associated with higher levels of SCFAs (Botticelli et al., 2020; Nomura et al., 2020). Moreover, the alkali-producing bacteria *Veillingerella* spp. ATCC 17745, a strictly anaerobic Gram-negative *Chlorella*, requires putrescine or cadaverine for growth (Arpaia et al., 2013). Polyamines, which are necessary for cell proliferation, are essential in many cancers, especially those driven by oncogenes, and may be sensitive to disturbances in polyamine metabolism (Casero et al., 2018). This provides a promising avenue for further research into the effects of gut microbiota on MNMSC.

The mechanisms underlying the effect of *Bacteroides. dorei* in MNMSC may involve the ability of *Bacteroides. dorei* to enhance earlier interferon expression, modulate the balance of pro- and anti-inflammatory cytokines, and restore the composition of the gut microbiota (Song et al., 2021).

As for the class *Clostridia* and order *Clostridiales*, *Clostridioides difficile* includes a wide range of organisms, and various mechanisms have been identified to explain its role in tumorigenesis, including increased Wnt/β-catenin signaling, reactive oxygen species generation, and an oncogenic mucosal immune response characterized by IL17 production from multiple sources and an increased presence of myeloid cells (Drewes et al., 2022).

In summary, this is the first known study to use MR analysis to investigate the causal relationship between 301 gut microbiota species and MNMSC. The results of this study are more reliable than those of traditional observational studies that are vulnerable to confounding factors and reverse causality. Our findings shed light on microbiota exhibiting a defined causal relationship with MNMSC, providing a novel and valuable strategy for the prevention and treatment of MNMSC mediated by gut microbiota. The robustness of the IVs is confirmed using gut microbiota-associated SNPs from the largest GWAS meta-analysis to date. The large sample size coupled with the use of diversity sensitivity analyses, reverse MR, and Bonferroni correction further confirm the validity of our results. Although the P-values of the causal effects estimated by the IVW method for the seven types of microbiota other than *Veillonellaceae* fall short of those following Bonferroni correction, the results still suggest a reliable causal effect of these microbiota on MNMSC. Our study has the following main strengths. On the one hand, the Bonferroni correction is stringent. On the other hand, the directionality of the WM, MR-Egger regression, simple mode, weighted

median, and weighted mode models is consistent with that of the IVW results. What's more, the results estimated by the IVW method have high statistical power (Verbanck et al., 2018; Luo et al., 2023). Fourth, this study is exploratory and inherently aimed to find as many potential positive results as possible; therefore, we included the other seven gut microbiota in the exposure, which is conducive to an in-depth analysis of the potential factors underlying the effect of gut microbiota on MNMSC.

However, the MR method used in this study only identified a causal relationship between gut microbiota and MNMSC, and no additional specific experiments were performed to corroborate the intricate and specific mechanisms underlying the effect of microbiota on MNMSC. Furthermore, the small differences in some microbial taxa explained by genetic IVs may have hampered the estimation of correlations owing to limited statistical power. Additionally, all data are derived from European populations, with limited representation of non-European populations; this unavoidable bias due to demographic origin restricts the applicability of our results to populations from other geographic regions. Consequently, more research should be conducted in future to better understand the mechanisms behind the effect of gut microbiota on MNMSC. Such efforts will contribute greatly to the etiology, prevention, and treatment of MNMSC, as well as the development of new preventive and therapeutic strategies.

5 Conclusion

To conclude, our study suggests that gut microbiota may be involved in causing MNMSC, targeting gut microbiota to identify new ways to prevent and treat MNMSC. It is necessary to conduct further longitudinal and laboratory studies to explore the mechanisms and pathways by which the gut microbiota influences MNMSC.

6 Data Availability Statement

The sources of data on gut microbiota publicly available datasets, which can be found at https://mibiogen.gcc.rug.nl/ and https://dutchmicrobiomeproject.molgeniscloud.org/;The sources of data on malignant non-melanoma skin cancer(ieu-b-4959), which can be found at https://gwas.mrcieu.ac.uk/datasets/ieu-b-4959/

7 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8 Author Contributions

XXQH:Conceptualization, Writing - original draft, Data curation, Formal Analysis, Visualization; XGL: Writing - original draft;XMG: Writing - review & editing; XYZ: Writing - review & editing; PSC: Writing - review & editing; RRX: Writing - review & editing; WTY: Writing - review & editing; YYH: Writing - review & editing; HYL: Writing - review & editing; FZ: Writing - review & editing, Supervision, Validation, Project administration;CYW: Writing - review & editing, Supervision, Validation, Project administration.

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11 References

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504(7480)**,** 451-455. doi: 10.1038/nature12726.

Botticelli, A., Vernocchi, P., Marini, F., Quagliariello, A., Cerbelli, B., Reddel, S., et al. (2020). Gut metabolomics profiling of non-small cell lung cancer (NSCLC) patients under immunotherapy treatment. *J Transl Med* 18(1)**,** 49. doi: 10.1186/s12967-020-02231-0.

Boussiotis, V.A. (2016). Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. *N Engl J Med* 375(18)**,** 1767-1778. doi: 10.1056/NEJMra1514296.

Bowden, J., Davey Smith, G., and Burgess, S. (2015). Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 44(2)**,** 512-525. doi: 10.1093/ije/dyv080.

Bowden, J., Davey Smith, G., Haycock, P.C., and Burgess, S. (2016). Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 40(4), 304-314. doi: 10.1002/gepi.21965.

Bowden, J., and Holmes, M.V. (2019). Meta-analysis and Mendelian randomization: A review. *Res Synth Methods* 10(4), 486-496. doi: 10.1002/jrsm.1346.

Cantisani, C., Kiss, N., Naqeshbandi, A.F., Tosti, G., Tofani, S., Cartoni, C., et al. (2019). Nonmelanoma skin cancer associated with Hydroxyurea treatment: Overview of the literature and our own experience. *Dermatol Ther* 32(5), e13043. doi: 10.1111/dth.13043.

Cao, Q., Guo, J., Chang, S., Huang, Z., and Luo, Q. (2023). Gut microbiota and acne: A Mendelian randomization study. *Skin Res Technol* 29(9), e13473. doi: 10.1111/srt.13473.

Casero, R.A., Jr., Murray Stewart, T., and Pegg, A.E. (2018). Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nat Rev Cancer* 18(11), 681-695. doi: 10.1038/s41568-018-0050-3.

Chen, S., Chen, L., Qi, Y., Xu, J., Ge, Q., Fan, Y., et al. (2021). Bifidobacterium adolescentis regulates catalase activity and host metabolism and improves healthspan and lifespan in multiple species. *Nat Aging* 1(11), 991-1001. doi: 10.1038/s43587-021-00129-0.

Chen, S., Fan, L., Lin, Y., Qi, Y., Xu, C., Ge, Q., et al. (2023a). Bifidobacterium adolescentis orchestrates CD143(+) cancer-associated fibroblasts to suppress colorectal tumorigenesis by Wnt signaling-regulated GAS1. *Cancer Commun (Lond)* 43(9), 1027-1047. doi: 10.1002/cac2.12469.

Chen, Z., Shi, W., Chen, K., Lu, C., Li, X., and Li, Q. (2023b). Elucidating the causal association between gut microbiota and intrahepatic cholangiocarcinoma through Mendelian randomization analysis. *Front Microbiol* 14, 1288525. doi: 10.3389/fmicb.2023.1288525.

Choi, K.W., Chen, C.Y., Stein, M.B., Klimentidis, Y.C., Wang, M.J., Koenen, K.C., et al. (2019). Assessment of Bidirectional Relationships Between Physical Activity and Depression Among Adults: A 2-Sample Mendelian Randomization Study. *JAMA Psychiatry* 76(4), 399-408. doi: 10.1001/jamapsychiatry.2018.4175.

Connolly, K.L., Nehal, K.S., and Disa, J.J. (2017). Evidence-Based Medicine: Cutaneous Facial Malignancies: Nonmelanoma Skin Cancer. *Plast Reconstr Surg* 139(1), 181e-190e. doi: 10.1097/prs.0000000000002853.

Davar, D., and Zarour, H.M. (2022). Facts and Hopes for Gut Microbiota Interventions in Cancer Immunotherapy. *Clin Cancer Res* 28(20), 4370-4384. doi: 10.1158/1078-0432.Ccr-21-1129.

Davies, N.M., Holmes, M.V., and Davey Smith, G. (2018). Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *Bmj* 362, k601. doi: 10.1136/bmj.k601.

De Pessemier, B., Grine, L., Debaere, M., Maes, A., Paetzold, B., and Callewaert, C. (2021). Gut-Skin Axis: Current Knowledge of the Interrelationship between Microbial Dysbiosis and Skin Conditions. *Microorganisms* 9(2). doi: 10.3390/microorganisms9020353.

Drewes, J.L., Chen, J., Markham, N.O., Knippel, R.J., Domingue, J.C., Tam, A.J., et al. (2022). Human Colon Cancer-Derived Clostridioides difficile Strains Drive Colonic Tumorigenesis in Mice. *Cancer Discov* 12(8), 1873-1885. doi: 10.1158/2159-8290.Cd-21-1273.

Emdin, C.A., Khera, A.V., and Kathiresan, S. (2017). Mendelian Randomization. *Jama* 318(19), 1925-1926. doi: 10.1001/jama.2017.17219.

Frick, J.S., Fink, K., Kahl, F., Niemiec, M.J., Quitadamo, M., Schenk, K., et al. (2007). Identification of commensal bacterial strains that modulate Yersinia enterocolitica and dextran sodium sulfate-induced inflammatory responses: implications for the development of probiotics. *Infect Immun* 75(7), 3490-3497. doi: 10.1128/iai.00119-07.

Guo, Y., Xie, J.P., Deng, K., Li, X., Yuan, Y., Xuan, Q., et al. (2019). Prophylactic Effects of Bifidobacterium adolescentis on Anxiety and Depression-Like Phenotypes After Chronic Stress: A Role of the Gut Microbiota-Inflammation Axis. *Front Behav Neurosci* 13, 126. doi: 10.3389/fnbeh.2019.00126.

Hartwig, F.P., Davey Smith, G., and Bowden, J. (2017). Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol* 46(6), 1985-1998. doi: 10.1093/ije/dyx102.

Hemani, G., Zheng, J., Elsworth, B., Wade, K.H., Haberland, V., Baird, D., et al. (2018). The MR-Base platform supports systematic causal inference across the human phenome. *Elife* 7. doi: 10.7554/eLife.34408.

Hiippala, K., Kainulainen, V., Kalliomäki, M., Arkkila, P., and Satokari, R. (2016). Mucosal Prevalence and Interactions with the Epithelium Indicate Commensalism of Sutterella spp. *Front Microbiol* 7, 1706. doi: 10.3389/fmicb.2016.01706.

Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., et al. (2010). Improved survival with ipilimumab in patients with

metastatic melanoma. *N Engl J Med* 363(8), 711-723. doi: 10.1056/NEJMoa1003466.

Huang, Y.F., Zhang, W.M., Wei, Z.S., Huang, H., Mo, Q.Y., Shi, D.L., et al. (2023). Causal relationships between gut microbiota and programmed cell death protein 1/programmed cell death-ligand 1: A bidirectional Mendelian randomization study. *Front Immunol* 14, 1136169. doi: 10.3389/fimmu.2023.1136169.

Khayyati Kohnehshahri, M., Sarkesh, A., Mohamed Khosroshahi, L., HajiEsmailPoor, Z., Aghebati-Maleki, A., Yousefi, M., et al. (2023). Current status of skin cancers with a focus on immunology and immunotherapy. *Cancer Cell Int* 23(1), 174. doi: 10.1186/s12935-023-03012-7.

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet* 53(2), 156-165. doi: 10.1038/s41588-020-00763-1.

Levin, M.G., Judy, R., Gill, D., Vujkovic, M., Verma, S.S., Bradford, Y., et al. (2020). Genetics of height and risk of atrial fibrillation: A Mendelian randomization study. *PLoS Med* 17(10), e1003288. doi: 10.1371/journal.pmed.1003288.

Li, P., Wang, H., Guo, L., Gou, X., Chen, G., Lin, D., et al. (2022). Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study. *BMC Med* 20(1), 443. doi: 10.1186/s12916-022-02657-x.

Li, X., Fu, G.F., Fan, Y.R., Liu, W.H., Liu, X.J., Wang, J.J., et al. (2003). Bifidobacterium adolescentis as a delivery system of endostatin for cancer gene therapy: selective inhibitor of angiogenesis and hypoxic tumor growth. *Cancer Gene Ther* 10(2), 105-111. doi: 10.1038/sj.cgt.7700530.

Liu, X., Tong, X., Zou, Y., Lin, X., Zhao, H., Tian, L., et al. (2022). Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. *Nat Genet* 54(1), 52-61. doi: 10.1038/s41588-021-00968-y.

Long, J., Gu, J., Yang, J., Chen, P., Dai, Y., Lin, Y., et al. (2023). Exploring the Association between Gut Microbiota and Inflammatory Skin Diseases: A Two-Sample Mendelian Randomization Analysis. *Microorganisms* 11(10). doi: 10.3390/microorganisms11102586.

Lopera-Maya, E.A., Kurilshikov, A., van der Graaf, A., Hu, S., Andreu-Sánchez, S., Chen, L., et al. (2022). Effect of host genetics on the gut

microbiome in 7,738 participants of the Dutch Microbiome Project. *Nat Genet* 54(2), 143-151. doi: 10.1038/s41588-021-00992-y.

Lou, J., Cui, S., Li, J., Jin, G., Fan, Y., and Huang, N. (2024). Causal relationship between the gut microbiome and basal cell carcinoma, melanoma skin cancer, ease of skin tanning: evidence from three two-sample mendelian randomisation studies. *Front Immunol* 15, 1279680. doi: 10.3389/fimmu.2024.1279680.

Luo, M., Cai, J., Luo, S., Hong, X., Xu, L., Lin, H., et al. (2023). Causal effects of gut microbiota on the risk of chronic kidney disease: a Mendelian randomization study. *Front Cell Infect Microbiol* 13, 1142140. doi: 10.3389/fcimb.2023.1142140.

Macpherson, A.J., Slack, E., Geuking, M.B., and McCoy, K.D. (2009). The mucosal firewalls against commensal intestinal microbes. *Semin Immunopathol* 31(2), 145-149. doi: 10.1007/s00281-009-0174-3.

Maslowski, K.M., and Mackay, C.R. (2011). Diet, gut microbiota and immune responses. *Nat Immunol* 12(1), 5-9. doi: 10.1038/ni0111-5.

Matsuoka, K., and Kanai, T. (2015). The gut microbiota and inflammatory bowel disease. *Semin Immunopathol* 37(1), 47-55. doi: 10.1007/s00281-014-0454-4.

Nomura, M., Nagatomo, R., Doi, K., Shimizu, J., Baba, K., Saito, T., et al. (2020). Association of Short-Chain Fatty Acids in the Gut Microbiome With Clinical Response to Treatment With Nivolumab or Pembrolizumab in Patients With Solid Cancer Tumors. *JAMA Netw Open* 3(4), e202895. doi: 10.1001/jamanetworkopen.2020.2895.

Palmer, T.M., Lawlor, D.A., Harbord, R.M., Sheehan, N.A., Tobias, J.H., Timpson, N.J., et al. (2012). Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res* 21(3), 223-242. doi: 10.1177/0962280210394459.

Polkowska-Pruszyńska, B., Gerkowicz, A., and Krasowska, D. (2020). The gut microbiome alterations in allergic and inflammatory skin diseases - an update. *J Eur Acad Dermatol Venereol* 34(3), 455-464. doi: 10.1111/jdv.15951.

Resta-Lenert, S., and Barrett, K.E. (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut* 52(7), 988-997. doi: 10.1136/gut.52.7.988.

Ridky, T.W. (2007). Nonmelanoma skin cancer. *J Am Acad Dermatol* 57(3), 484-501. doi: 10.1016/j.jaad.2007.01.033.

Roberts, J.L., Liu, G., Darby, T.M., Fernandes, L.M., Diaz-Hernandez, M.E., Jones, R.M., et al. (2020). Bifidobacterium adolescentis supplementation attenuates fracture-induced systemic sequelae. *Biomed Pharmacother* 132**,** 110831. doi: 10.1016/j.biopha.2020.110831.

Roland, M.M., Mohammed, A.D., and Kubinak, J.L. (2020). How MHCII signaling promotes benign host-microbiota interactions. *PLoS Pathog* 16(6)**,** e1008558. doi: 10.1371/journal.ppat.1008558.

Rong, Y., Zuo, L., Shang, L., and Bazan, J.G. (2015). Radiotherapy treatment for nonmelanoma skin cancer. *Expert Rev Anticancer Ther* 15(7)**,** 765-776. doi: 10.1586/14737140.2015.1042865.

Segovia, J., and Zarco, N. (2014). Gas1 is a pleiotropic regulator of cellular functions: from embryonic development to molecular actions in cancer gene therapy. *Mini Rev Med Chem* 14(14)**,** 1139-1147. doi: 10.2174/1389557514666141127142301.

Sivan, A., Corrales, L., Hubert, N., Williams, J.B., Aquino-Michaels, K., Earley, Z.M., et al. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 350(6264)**,** 1084-1089. doi: 10.1126/science.aac4255.

Song, L., Huang, Y., Liu, G., Li, X., Xiao, Y., Liu, C., et al. (2021). A Novel Immunobiotics Bacteroides dorei Ameliorates Influenza Virus Infection in Mice. *Front Immunol* 12**,** 828887. doi: 10.3389/fimmu.2021.828887.

Sternfeld, A., Rosenwasser-Weiss, S., Ben-Yehuda, G., Shefer, H.K., Friedman-Gohas, M., Yassur, I., et al. (2020). Gene-Related Response of Basal Cell Carcinoma to Biologic Treatment with Vismodegib. *Sci Rep* 10(1)**,** 1244. doi: 10.1038/s41598-020-58117-0.

Šuler Baglama, Š., and Trčko, K. (2022). Skin and gut microbiota dysbiosis in autoimmune and inflammatory skin diseases. *Acta Dermatovenerol Alp Pannonica Adriat* 31(3)**,** 105-109.

Verbanck, M., Chen, C.Y., Neale, B., and Do, R. (2018). Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* 50(5)**,** 693-698. doi: 10.1038/s41588-018-0099-7.

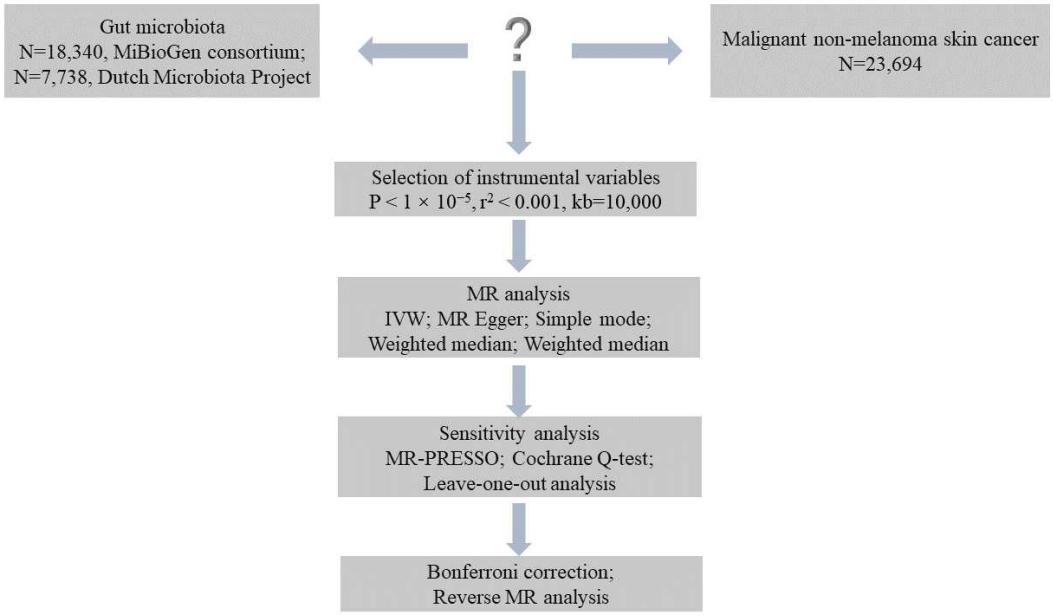
Wexler, H. (2005). "Genus VIII. Sutterella, p 682–683. Brenner DJ, Krieg NR, Staley JT (ed), Bergey’s manual of systematic bacteriology, vol 2, part C. The Proteobacteria". Springer-Verlag, New York, NY).

Zang, C., Liu, J., Mao, M., Zhu, W., Chen, W., and Wei, B. (2023). Causal Associations Between Gut Microbiota and Psoriasis: A Mendelian

Randomization Study. *Dermatol Ther (Heidelb)* 13(10)**,** 2331-2343. doi: 10.1007/s13555-023-01007-w.

12 Figure Legends

Figure 1.



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587 Figure 1. Flowchart of a two-sample MR analysis of the gut microbiota and
588 MNMSC.

589 MR:Mendelian randomization

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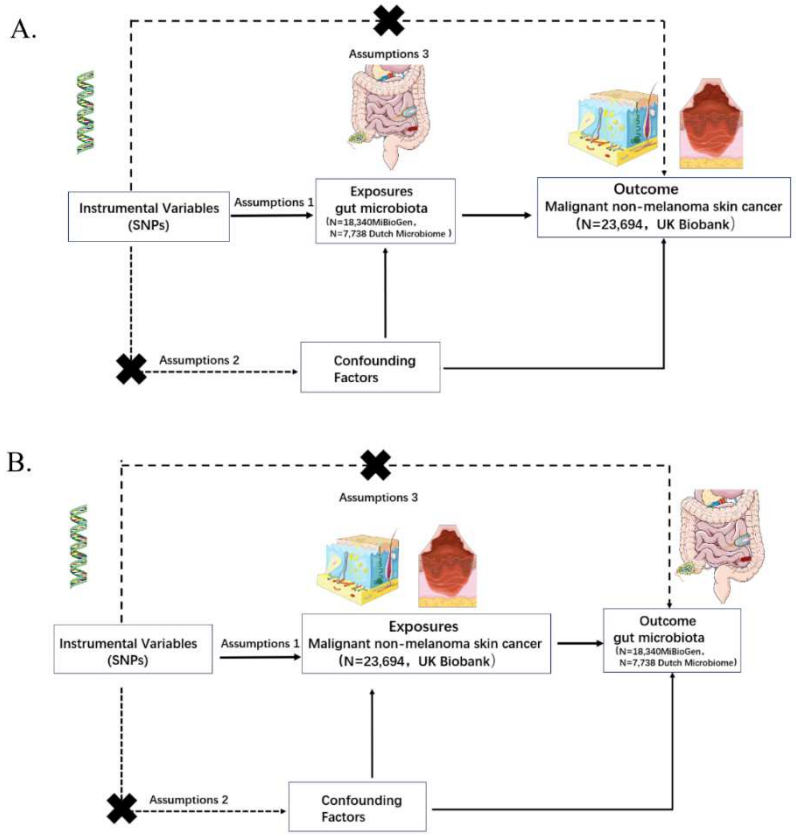
601 **Figure 2.**



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603 The principle of bidirectional MR analysis. A: MR framework employed to
604 investigate the causal relationship between the gut microbiome and
605 MNMSC. B: MR framework employed to investigate the causal relationship
606 between the MNMSC and gut microbiome. MR: Mendelian randomization
607 study; MNMSC: malignant non-melanoma skin cancer; IV: instrumental
608 variables; SNP: single nucleotide polymorphisms.

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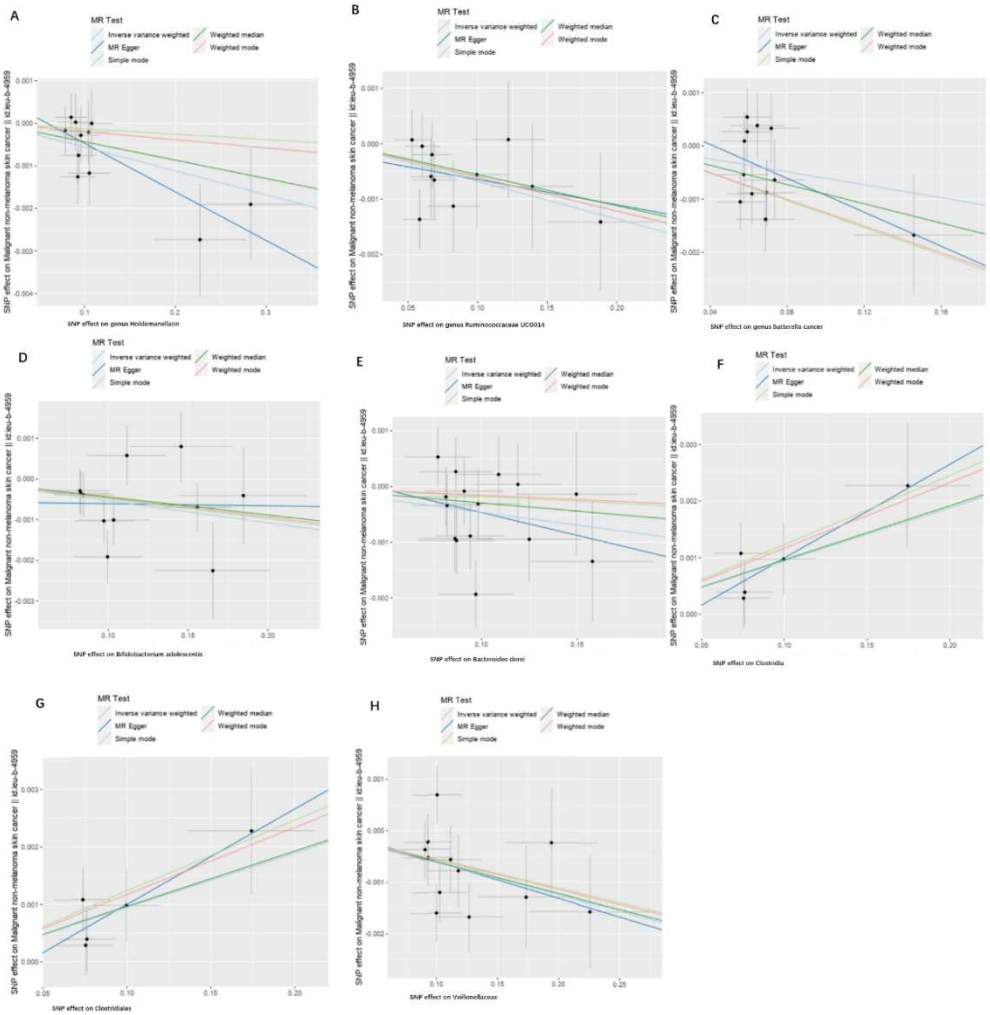
Figure 3.



Scatter plot of genetic correlations of gut microbiome and MNMSC using different MR methods. A: genus Holdemanella; B: genus Ruminococcaceae UCG014; C: genus Sutterella; D: bifidobacterium adolescentis; E: bacteroides dorei; F: Veillonellaceae; G: Clostridia; H: Clostridiales. MNMSC: malignant non-melanoma skin cancer; SNP: single nucleotide polymorphisms; GWAS: gut microbiome genome-wide association study; MR: Mendelian randomization.

Figure 4.

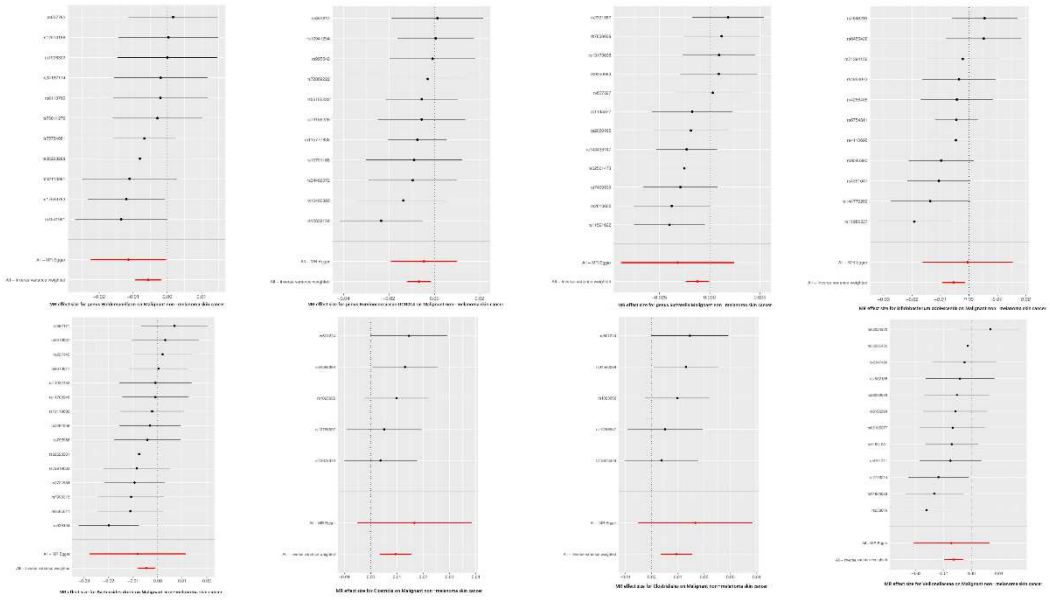


Figure 4. Forest plot of the causal effects of different gut microbiome associated SNPs on MNMSC. The red and black dot/bar indicate the causal estimate of gut microbiome level on risk of patients with MNMSC. A: genus Holdemanella; B: genus Ruminococcaceae UCG014; C: Genus Sutterella; D: bifidobacterium adolescentis; E: bacteroides dorei; F: Veillonellaceae; G: Clostridia; H: Clostridiales. MNMSC: malignant non-melanoma skin cancer; SNP: single nucleotide polymorphisms.

13 Tables

Table 1 Causal effects of the gut microbiome on MNMSC

Bacterial taxa(exposure)	MR method	Number of SNP	OR	Beta	95%CI	P value
Genus Holdemanella	IVW	11	0.99	-0.006	0.99 - 1.00	0.004*
	MR Egger	11	0.99	-0.011	0.98 - 1.00	0.073
	Weighted median	11	1.00	-0.004	0.99 - 1.00	0.096
	Weighted Mode	11	1.00	-0.002	0.99 - 1.01	0.670
	Simple mode	11	1.00	-0.001	0.99 - 1.01	0.770
Genus Ruminococcaceae UCG014	IVW	11	0.99	-0.007	0.99 - 1.00	0.013*
	MR Egger	11	1.00	-0.005	0.98 - 1.01	0.556
	Weighted median	11	0.99	-0.006	0.99 - 1.00	0.118
	Weighted Mode	11	0.99	-0.006	0.98 - 1.00	0.271
	Simple mode	11	0.99	-0.006	0.98 - 1.01	0.314
Genus Sutterella	IVW	12	0.99	-0.006	0.99 - 1.00	0.042*
	MR Egger	12	0.98	-0.016	0.96 - 1.01	0.294
	Weighted median	12	0.99	-0.009	0.98 - 1.00	0.025
	Weighted Mode	12	0.99	-0.013	0.97 - 1.00	0.135
	Simple mode	12	0.99	-0.013	0.97 - 1.00	0.127
Bifidobacterium_adolescentis	IVW	11	0.99	-0.005	0.99 - 1.00	0.009*
	MR Egger	11	1.00	-0.001	0.98 - 1.02	0.950
	Weighted median	11	1.00	-0.004	0.99 - 1.00	0.069
	Weighted Mode	11	1.00	-0.005	0.99 - 1.00	0.191
	Simple mode	11	1.00	-0.005	0.99 - 1.00	0.286
Bacteroides_dorei	IVW	15	1.00	-0.005	0.99 - 1.00	0.010*
	MR Egger	15	0.99	-0.008	0.97 - 1.01	0.429
	Weighted median	15	1.00	-0.003	0.99 - 1.00	0.221
	Weighted Mode	15	1.00	-0.002	0.99 - 1.01	0.735
	Simple mode	15	1.00	-0.002	0.99 - 1.01	0.690

Veillonellaceae	IVW	12	0.99	-0.006	0.99 - 1.00	0.0004*
	MR Egger	12	0.99	-0.007	0.98 - 1.01	0.333
	Weighted median	12	0.99	-0.006	0.99 – 1.00	0.007
	Weighted Mode	12	0.99	-0.006	0.99 – 1.00	0.161
	Simple mode	12	0.99	-0.006	0.99 – 1.00	0.197
Clostridia	IVW	5	1.01	0.009	1.00 – 1.02	0.002*
	MR Egger	5	1.02	0.017	0.99 – 1.04	0.232
	Weighted median	5	1.01	0.010	1.00 – 1.02	0.019
	Weighted Mode	5	1.01	0.012	1.00 – 1.02	0.091
	Simple mode	5	1.01	0.012	1.00 – 1.02	0.089
Clostridiales	IVW	5	1.01	0.009	1.00 - 1.02	0.002*
	MR Egger	5	1.02	0.017	0.99 – 1.04	0.232
	Weighted median	5	1.01	0.010	1.00 - 1.02	0.022
	Weighted Mode	5	1.01	0.012	1.00 - 1.02	0.103
	Simple mode	5	1.01	0.012	1.00 – 1.02	0.086

* Bonferroni correction:[5.6×10-3 (0.05/9) for phylum, 3.1×10-3 (0.05/16) for class, 2.5×10-3 (0.05/20) for order, 1.6×10-3(0.05/32) for family, 4.2×10-4 (0.05/119) for genus, and 5.0×10-4 (0.05/101) for species].

MNMSC: malignant non-melanoma skin cancer; IVW:inverse-variance weighting; MR: Mendelian randomization; OR: odds ratio; CI: confidence interval; SNP: single nucleotide polymorphism.

Table 2 The heterogeneity and pleiotropy test outcome of gut microbiome on MNMSC

	Heterogeneity			MR-PRESSO		pleiotropy	
Gut microbiota	Method	Q	Q P value	MR-PRESSO Outlier-corrected	MR-PRESSO results Global Test P value	Egger intercept	P value
genus Holdemanella	IVW	6.41	0.780	NA	0.614	0.001	0.298
	MR Egger	5.19	0.818	-	-	-	-
genus Ruminococcaceae UCG014	IVW	5.75	0.774	NA	0.846	0	0.756
	MR Egger	5.65	0.836	-	-	-	-
genus Sutterella	IVW	13.7	0.219	NA	0.271	0.001	0.499
	MR egger	13.1	0.249	-	-	-	-
Bifidobacterium adolescentis	IVW	13.4	0.202	NA	0.254	-0.001	0.549
	MR egger	12.8	0.170	-	-	-	-
Bacteroides dorei	IVW	15.3	0.358	NA	0.349	0.001	0.723
	MR egger	15.1	0.298	-	-	-	-
Clostridia	IVW	1.79	0.774	NA	0.810	-0.001	0.549
	MR egger	1.34	0.721	-	-	-	-
Clostridiales	IVW	1.79	0.774	NA	0.798	-0.001	0.549
	MR egger	1.34	0.720	-	-	-	-
	IVW	12.9	0.299	NA	0.272	0	0.9

Veillonellaceae	MR egger	12.9	0.230	-	-	-	-

IVW: inverse variance weighting; NA: not available.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1MRleaveoneoutsensitivityanalysis.tif
- SupplementaryFigure2MRfunnelplot.tiff
- SupplementaryTable1TheinstrumentalvariablesfordifferentGutmicrobiotausedintheMRanalysis..xlsx
- SupplementaryTable2TheinstrumentalvariablesforMNMSCusedintheMRanalysis..xlsx
- SupplementaryTable3theoutcomeofreverseMRanalysis.docx