

Article

Evaluating the Genetic Effects of Gut Microbiota on the Development of Neuroticism and General Happiness: A Polygenic Score Analysis and Interaction Study Using UK Biobank Data

Yumeng Jia [†], Shiqiang Cheng [†], Li Liu , Bolun Cheng, Chujun Liang, Jing Ye, Xiaomeng Chu, Yao Yao, Yan Wen, Om Prakash Kafle and Feng Zhang ^{*}

Key Laboratory of Trace Elements and Endemic Diseases of National Health and Family Planning Commission, School of Public Health, Health Science Center, Xi'an Jiaotong University, Xi'an 710061, China

* Correspondence: fzhxjtu@xjtu.edu.cn

† These authors contributed equally to this work.

Abstract: Limited efforts have been invested in exploring the interaction effects between genetic factors and gut microbiota on neuroticism and general happiness. The polygenic risk scores (PRS) of gut microbiota were calculated from individual-level genotype data of the UK Biobank cohort. Linear regression models were then used to assess the associations between individual PRS of gut microbiota and mental traits and interaction analysis was performed by PLINK2.0. KOBAS-i was used to conduct gene ontology (GO) enrichment analysis of the identified genes. We observed suggestive significant associations between neuroticism and PRS for the genus *Bifidobacterium* (rank-normal transformation, RNT) ($\beta = -1.10$, $P = 4.16 \times 10^{-3}$) and the genus *Desulfovibrio* (RNT) ($\beta = 0.54$, $P = 7.46 \times 10^{-3}$). PRS for the genus *Bifidobacterium* (hurdle binary, HB) ($\beta = 1.99$, $P = 5.24 \times 10^{-3}$) and the genus *Clostridium* (RNT) ($\beta = 1.26$, $P = 9.27 \times 10^{-3}$) were found to be suggestive positively associated with general happiness. Interaction analysis identified several significant genes that interacted with gut microbiota, such as *RORA* (rs575949009, $\beta = -45.00$, $P = 1.82 \times 10^{-9}$) for neuroticism and *ASTN2* (rs36005728, $\beta = 19.15$, $P = 3.37 \times 10^{-8}$) for general happiness. Our study results support the genetic effects of gut microbiota on the development of neuroticism and general happiness.

Keywords: gut microbiota; polygenic risk scores; neuroticism; general happiness



Citation: Jia, Y.; Cheng, S.; Liu, L.; Cheng, B.; Liang, C.; Ye, J.; Chu, X.; Yao, Y.; Wen, Y.; Kafle, O.P.; et al. Evaluating the Genetic Effects of Gut Microbiota on the Development of Neuroticism and General Happiness: A Polygenic Score Analysis and Interaction Study Using UK Biobank Data. *Genes* **2023**, *14*, 156. <https://doi.org/10.3390/genes14010156>

Academic Editors: Xingguang Luo and Silvia Turrioni

Received: 15 November 2022

Revised: 3 January 2023

Accepted: 4 January 2023

Published: 6 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Mental disorders are highly prevalent, placing a heavy burden on global public health [1]. The estimated prevalence of mental disorders was 0.97 billion globally in 2017 [2]. According to a recent report, mental disorders have consistently accounted for more than 14% of age-standardized years lived with disability for nearly three decades [2]. Neuroticism and positive emotion are common personality traits that are often of concern [3,4]. Mental traits are the product of the interplay between genetic and environmental influences. The estimated heritability was 40~60% for neuroticism and 37~64% for positive emotion [5,6]. With regards to environmental influences, adversities during development, such as “emotional neglect and sexual abuse”, were found to be positively associated with these two common mental traits [5,7,8]. However, the pathogenesis of mental traits remains elusive even now.

Gut microbiota co-develops with the host from birth and changes throughout growth depending on different physiological, pathological, dietary patterns, and environmental conditions [9]. Increasing evidence has indicated that the gut microbiota is closely related to host health and plays an important role in the etiology of a variety of complex human disorders, including mental illness [10–12]. Cryan et al. suggested that the gut microbiota is a pivotal part of the signaling along the microbiota–gut–brain axis, a bi-directional

communication network encompassing the gut microbiota and the nervous system, as well as neuroendocrine and neuroimmune pathways [13–15]. However, limited efforts have been invested in exploring the impact of the interaction effects between genetic factors and gut microbiota on mental traits.

Genome-wide association studies (GWAS) have successfully revealed single nucleotide polymorphisms that are associated with mental traits. Recently, using fecal 16S ribosomal RNA gene sequences and host genotype data from the Flemish Gut Flora Project (n = 2223) and two German cohorts (n = 1667), Hughes et al. [16] identified several genetic associations involving multiple microbial traits, which were described as continuous (relative abundance, α -diversity), binary (presence/absence), multinomial (enterotypes), and multivariate (β -diversity) traits. However, the results obtained by GWAS are usually derived from individual causal loci and the effect sizes are relatively weak [17]. To overcome this difficulty, polygenic risk scores (PRS) has been used to predict the risk of human disease, which is a sum of trait-associated alleles across many genetic loci, typically weighted by effect sizes estimated from an independent large-scale discovery GWAS [18]. More importantly, since there are no available gut microbiota data for mental traits in UK Biobank, utilizing microbiota-based PRS can be used to explore the relationship between emotional traits and gut microbiota. By using microbiota-based PRS, previous studies have explored the effects of gut microbiota interactions with C-reactive protein or brain aging on psychiatric disorders such as depression and anxiety [19,20]. Until now, precise association loci and interactions effects between gut microbiota on the development of neuroticism and general happiness remain unidentified despite the role of the gut microbiota on the etiology of mental disorders having already been demonstrated.

In this study, we first calculated the PRS of gut microbiota in the UK Biobank cohort in relation to emotional traits. Briefly, linear regression analyses were first performed to detect the associations between individual PRS values of gut microbiota and the phenotypic data of neuroticism and general happiness in the UK Biobank cohort. Finally, interaction analysis was conducted to explore candidate gene–gut microbiota interactions on the development of mental traits. The results of this study may expand our genetic understanding of the effect of the gut microbiota on these two mental traits.

2. Materials and Methods

2.1. UK Biobank Dataset

The UK Biobank study is a large prospective cohort study including health data, hospital records, and genetic data from 502,656 participants aged 40–69 in 2006 and 2010 [21]. UK Biobank received electronically signed consent from the study participants, and ethical approval was obtained from the Northwest Multi-Centre Research Ethics Committee (reference 11/NW/0382). We used the imputed genotype dataset made available by the UK Biobank in its July 2017 release. We restricted participants to “white British” individuals based on self-reported ethnicity. Subjects who had a self-reported sex inconsistent with the genetic sex, who were genotyped but not imputed, or who withdraw their consents were removed. After removing the participants without the calculated gut microbiota-related PRS, 306,161 participants for *G_Bifidobacterium_RNT* and neuroticism, 153,483 participants for *G_Desulfovibrio_RNT* and neuroticism, 89,206 participants for *G_Bifidobacterium_HB* and general happiness, and 115,013 participants for *G_Clostridium_sensu_stricto_RNT* and general happiness were included for association analysis. All participants agreed to allow the use of their anonymous data to conduct any health-related studies and to reconnect for further sub-studies.

Genotyping, quality control, and imputation were performed by the UK Biobank. DNA samples of all participants in the UK Biobank were genotyped using either the Affymetrix UK BiLEVE (807,411 markers) or Affymetrix UK Biobank Axiom (825,927 markers) array [22]. Single-nucleotide polymorphisms (SNPs) were imputed by IMPUTE2 against the reference panel of the Haplotype Reference Consortium, 1000 Genomes, and UK10K projects. Full details regarding these data are available elsewhere [23]. This research has

been conducted using the UK Biobank Resource under Application Number 46478. The authors thank all UK Biobank participants and researchers who contributed or collected data.

2.2. Phenotypes Definition

Neuroticism score is derived based on 12 domains of neurotic behavior as reported from UK Biobank data fields 1920, 1930, 1940, 1950, 1960, 1970, 1980, 1990, 2000, 2010, 2020, and 2030 from the touchscreen questionnaire at baseline. Participants were assessed for the 12 domains of neurotic behaviors via the touchscreen questionnaire. Neuroticism score summarizes the number of “Yes” answers across the 12 questions into a single integer score for each participant. The detailed questionnaire is shown in Supplementary File S1. General happiness was collected from the response to the UK Biobank online “Thoughts and Feelings” mental health questionnaire: “In general how happy are you?” by choosing “Do not know (−121)”, “Extremely happy (1)”, “Very happy (2)”, “Moderately happy (3)”, “Moderately unhappy (4)”, “Very unhappy (5)”, “Extremely unhappy (6)”, and “Prefer not to answer (−818)”. The subjects whose answers are “Do not know (−121)” and “Prefer not to answer (−818)” were excluded from this study. The detailed definition of phenotypes is shown in Supplementary File S1.

2.3. GWAS Summaries of Gut Microbiota

The gut microbiota-associated SNPs were derived from a recently published GWAS [16]. Briefly, using fecal 16S ribosomal RNA gene sequences and host genotype data from the Flemish Gut Flora Project ($n = 2223$) and two German cohorts ($n = 1667$), Hughes et al. [16] identified several genetic associations involving multiple microbial traits. Sequencing was carried out on the Illumina HiSeq platform at the VIB Nucleomics core laboratory (Leuven, Belgium), with 500 cycles (sequencing kit HiSeq-Rapid SBS v.2). After merging paired sequences and the removal of chimeras, compositional matrices for each taxonomical level were carried out using the Ribosomal Database Project (RDP) training set ‘rdp_train_set_16’. Microbial taxa were described as relative abundance profiles using the rank normal transformed (RNT) model, while those with zero-inflated abundance distributions were described using a hurdle binary (HB) model. For association analysis, any taxa that met the following two criteria were included: (1) comprise $\geq 5\%$ of the reads for at least one individual; and (2) have $\geq 15\%$ of individuals with non-zero data were included. In total, 114 taxa across all phylogenetic levels met these criteria. We selected microbial traits associated with SNPs with a significant threshold of $P < 5 \times 10^{-6}$ to calculate the PRSs. A detailed description of sample characteristics, array design, quality control, and statistical analysis can be found in the previous study [16].

2.4. PRS Analysis

Using the genotype data of the UK Biobank cohort, PRS analysis was performed by using the PLINK’s “-score” command [24]. Briefly, let PRS_g denote the PRS value of each microbial trait for the g th subjects, where i ($i = 1, 2, 3, \dots, l$) and g ($g = 1, 2, 3, \dots, k$) denote the number of genetic markers and sample size, respectively. β_i is the effect parameter of the risk allele of the i th significant SNP related to each microbial trait obtained from the previously published study. SNP_{ig} is the dosage (0 to 2) of the risk allele of the i th SNP for the g th subject. The PRS values were standardized to have a mean of 0 and a variance of 1 before further analyses. Using computed PRSs as the instrumental variables of gut microbiota, a linear regression model was finally used to detect the potential associations between gut microbiota and target traits. In this study, the significant association thresholds should be $P < 2.19 \times 10^{-4}$ [$0.05/(114 \times 2)$] after strict Bonferroni correction. The suggestive significance threshold was set as $P < 0.05$. All statistical analyses were performed using R3.5.3 (<https://www.r-project.org/> (accessed on 5 February 2021)). Additionally, the sex, age, and 10 principal components of the population structure were used as covariates in the regression model.

2.5. Statistical Analysis

Based on the result of the regression model, interaction analysis was then conducted to explore the impact of the interaction between genetic factors and gut microbiota traits related to PRS on mental traits in the UK Biobank cohort. The outcome variables, including neuroticism and general happiness, were adjusted by sex, age, and 10 principal components of population structure. The instrumental variables are the PRS of gut microbiota traits. Based on the previous study, the genetic additive (ADD) model of PLINK2.0 was used in this study [25]. SNPs with a call rate <0.95 , Hardy–Weinberg equilibrium testing of $P < 0.001$, and minor allele frequencies (MAFs) < 0.01 were excluded for quality control [25]. A significant threshold was set at $P = 5.0 \times 10^{-8}$. Circular Manhattan plots were generated using the “CMplot” R script (<https://github.com/YinLiLin/R-CMplot> (accessed on 3 April 2021)).

2.6. Gene Set Enrichment Analyses

To explore the functional relevance of identified genes interacting with individual PRS of gut microbiota for neuroticism and general happiness, gene ontology (GO) enrichment analyses of the identified target genes were performed by using KOBAS-i (short for KOBAS intelligent version) (<http://kobas.cbi.pku.edu.cn/> (accessed on 29 December 2022) [26].

3. Result

3.1. Basic Characteristics of Study Samples

The general characteristics of the subjects are presented in Table 1. For the association analysis of neuroticism and G_*Bifidobacterium*_RNT, 306,161 participants were selected; 161,977 of them were female, with mean age (SD) was 56.81 (7.93) years. For the association analysis of neuroticism and G_*Desulfovibrio*_RNT, 153,483 participants were selected; 81,310 of them were female, with mean age (SD) was 56.80 (7.93) years. For the association analysis of general happiness and G_*Bifidobacterium*_HB, 89,206 participants were selected; 49,806 of them were female, with mean age (SD) was 56.27 (7.62) years. For the association analysis of general happiness and G_*Clostridium_sensu_stricto*_RNT, 115,013 participants were selected; 64,229 of them were female, with mean age (SD) was 56.28 (7.61) years.

We observed suggestive significant associations between neuroticism and the PRS of the genus *Bifidobacterium* (RNT) (beta = -1.10 , $P = 4.16 \times 10^{-3}$) and genus *Desulfovibrio* (RNT) (beta = 0.54 , $P = 7.46 \times 10^{-3}$). The PRS of the genus *Bifidobacterium* (HB) (beta = 1.99 , $P = 5.24 \times 10^{-3}$) and the genus *Clostridium* (RNT) (beta = 1.26 , $P = 9.27 \times 10^{-3}$) were found to be positively suggestive associated with general happiness.

We further compared the above association analysis results, and we found that the genus *Bifidobacterium* was shared by both participants with neuroticism and general happiness, genus *Bifidobacterium* (beta_{neuroticism} = -1.10 , $P_{neuroticism} = 4.16 \times 10^{-3}$; beta_{general happiness} = 1.99 , $P_{general happiness} = 5.24 \times 10^{-3}$)

Table 1. The associations between gut microbiota traits and mental traits.

Mental Traits	Gut Microbiota Traits	Sample Size	Sex (Female)	Age \pm SD	Beta	SE	R ²	P
Neuroticism	G_ <i>Bifidobacterium</i> _RNT	306,161	161,977	56.81 \pm 7.93	-1.10	0.38	4.16×10^{-3}	4.16×10^{-3}
	G_ <i>Desulfovibrio</i> _RNT	153,483	81,310	56.80 \pm 7.93	0.54	0.20	7.46×10^{-3}	7.46×10^{-3}
General happiness	G_ <i>Bifidobacterium</i> _HB	89,206	49,806	56.27 \pm 7.62	1.99	0.71	5.23×10^{-3}	5.24×10^{-3}
	G_ <i>Clostridium_sensu_stricto</i> _RNT	115,013	64,229	56.28 \pm 7.61	1.26	0.49	9.27×10^{-3}	9.27×10^{-3}

Note: G, genus; RNT, rank-normal transformation; HB, hurdle binary; SD, standard deviation; SE, standard error.

3.2. Interaction Analysis of Gut Microbiota with Mental Traits

For neuroticism, the interaction analysis identified 17 significant SNPs interacted with the genus *Bifidobacterium* (RNT) at $P < 5.0 \times 10^{-8}$, such as *RORA* (rs575949009, beta = -45.00 , $P = 1.82 \times 10^{-9}$) and *NDUFS1* (rs148934517, beta = -15.60 , $P = 3.71 \times 10^{-8}$), and 249 significant SNPs interacted with the genus *Desulfovibrio* (RNT) were identified at $P < 5.0 \times 10^{-8}$, such as *KCNQ3* (rs58613338, beta = -14.79 , $P = 2.62 \times 10^{-8}$) (Table 2,

Figure 1). The detailed significant interaction results ($P < 5.0 \times 10^{-8}$) for neuroticism are summarized in Table S1.

For general happiness, the interaction analysis found 17 significant SNPs interacted with the genus *Bifidobacterium* (HB) at $P < 5.0 \times 10^{-8}$, such as *DCTN4* (rs252157, beta = 41.95, $P = 9.23 \times 10^{-10}$) and *MYOZ3* (rs194134, beta = 43.12, $P = 4.74 \times 10^{-10}$), and 130 significant SNPs interacted with the genus *Clostridium* (RNT) were identified, such as *ASTN2* (rs36005728, beta = 19.15, $P = 3.37 \times 10^{-8}$) (Table 3, Figure 2). The detailed significant interaction results ($P < 5.0 \times 10^{-8}$) for general happiness are summarized in Table S2.

3.3. GO Enrichment Analysis Results

GO enrichment analysis identified five GO terms enriched in the significant genes interacting with the genus *Bifidobacterium* (RNT), such as GO:0005515–protein binding ($P = 8.12 \times 10^{-3}$), and 15 GO terms enriched in the significant genes interacting with the genus *Desulfovibrio* (RNT) for neuroticism, such as GO:0005925–focal adhesion ($P = 1.56 \times 10^{-2}$), GO:0045202–synapse ($P = 1.57 \times 10^{-2}$). Fourteen GO terms enriched in the significant genes interacting with the genus *Clostridium* (RNT) for general happiness were identified, such as GO:0045666–positive regulation of neuron differentiation ($P = 2.46 \times 10^{-4}$). The detailed enrichment analysis results are summarized in Tables S3 and S4.

Table 2. Summary of interaction analysis between SNP and gut microbiota for neuroticism.

	Chromosome	Gene	Beta	P
G_ <i>Bifidobacterium</i> _RNT	5	<i>GRK6</i>	−19.43	1.10×10^{-10}
	2	<i>NDUFS1</i>	−15.60	3.71×10^{-8}
	5	<i>NSD1</i>	−6.28	7.91×10^{-10}
	15	<i>RORA</i>	−45.00	1.82×10^{-9}
G_ <i>Desulfovibrio</i> _RNT	15	<i>ARNT2</i>	−6.40	7.16×10^{-10}
	14	<i>CCDC85C</i>	−12.94	7.27×10^{-10}
	4	<i>CCNG2</i>	−9.75	2.73×10^{-8}
	19	<i>CERS4</i>	−7.55	1.21×10^{-10}
	13	<i>FLT3</i>	−4.94	8.55×10^{-10}
	8	<i>KCNQ3</i>	−14.79	2.62×10^{-8}
	4	<i>LAMTOR3</i>	−13.25	2.09×10^{-50}
	4	<i>MANBA</i>	−9.25	1.18×10^{-11}
	8	<i>MSR1</i>	5.15	1.47×10^{-15}
	4	<i>NR3C2</i>	−13.59	1.49×10^{-8}
	12	<i>PPFIBP1</i>	−3.43	3.27×10^{-13}
	1	<i>PRSS38</i>	−6.85	2.76×10^{-9}
	2	<i>RAMP1</i>	−7.73	6.59×10^{-11}
	12	<i>RIMBP2</i>	−5.15	4.32×10^{-11}
4	<i>SLC9B1</i>	−10.70	3.01×10^{-12}	
7	<i>TPST1</i>	−15.90	5.34×10^{-10}	
4	<i>UBE2D3</i>	−9.78	3.03×10^{-12}	
19	<i>ZNF317</i>	−12.96	1.01×10^{-12}	

Note: SNP, single-nucleotide polymorphism; G, genus; RNT, rank-normal transformation.

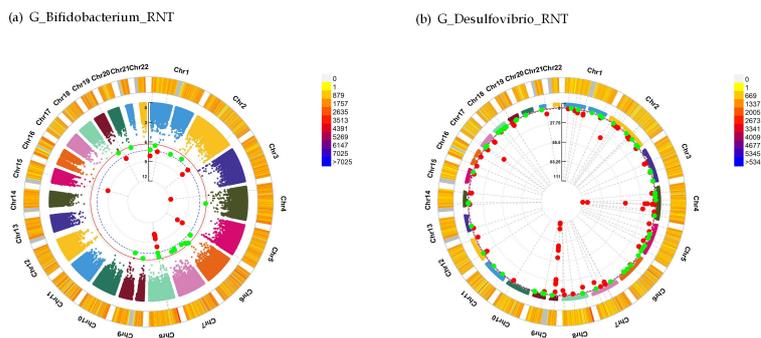


Figure 1. Interaction analysis between SNP and gut microbiota for neuroticism. Chromosomal regions interacting with *G_Bifidobacterium_RNT* (a) and *G_Desulfovibrio_RNT* (b) for neuroticism. From the center, the first circo depicts the $-\log_{10} P$ of each variant owing to double exposure, i.e., the effect of both single nucleotide polymorphism allele and gut microbiota. The second circo shows chromosome density. Red plots indicate the P -value threshold for genome-wide significance ($P < 5 \times 10^{-8}$), while the green plots indicate the P -value threshold for suggestive significance ($P < 5 \times 10^{-7}$). The plots were generated using the “CMplot” R script (<https://github.com/YinLiLin/R-CMplot> (accessed on 3 April 2021)). SNP, single-nucleotide polymorphism; G, genus; RNT, rank-normal transformation.

Table 3. Summary of interaction analysis between SNP and gut microbiota for general happiness.

	Chromosome	Gene	Beta	P
<i>G_Bifidobacterium_HB</i>	5	<i>DCTN4</i>	41.95	9.23×10^{-10}
	12	<i>MAP1LC3B2</i>	-15.80	6.11×10^{-9}
	5	<i>MYOZ3</i>	43.12	4.74×10^{-10}
	16	<i>PRSS54</i>	-11.58	2.38×10^{-8}
	1	<i>RGS21</i>	14.30	1.67×10^{-8}
<i>G_Clostridium_sensu_stricto_RNT</i>	9	<i>ASTN2</i>	19.15	3.37×10^{-8}
	1	<i>ATF6</i>	8.28	2.54×10^{-8}
	6	<i>BMP6</i>	7.55	1.98×10^{-9}
	1	<i>CAMTA1</i>	9.60	8.80×10^{-10}
	3	<i>CCDC14</i>	13.67	1.72×10^{-9}
	3	<i>ECT2</i>	14.69	2.17×10^{-18}
	2	<i>FMNL2</i>	7.78	1.66×10^{-19}
	1	<i>LAMB3</i>	10.86	4.61×10^{-10}
	3	<i>MYLK</i>	13.61	3.95×10^{-9}
17	<i>RHBDL3</i>	8.91	3.35×10^{-16}	
9	<i>SVEP1</i>	12.88	5.36×10^{-19}	

Note: SNP, single-nucleotide polymorphism; G, genus; RNT, rank-normal transformation; HB, hurdle binary.

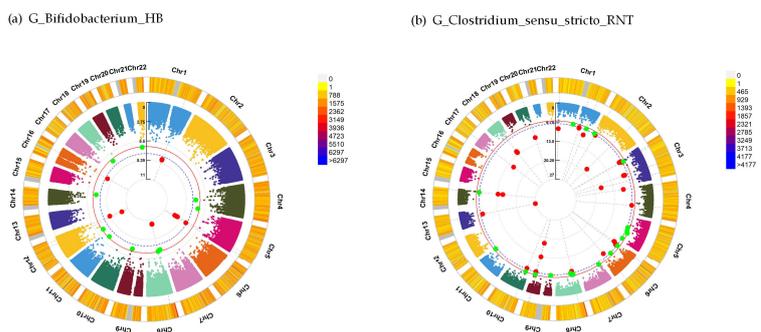


Figure 2. Interaction analysis between SNP and gut microbiota for general happiness. Chromosomal regions interacting with *G_Bifidobacterium_HB* (a) and *G_Clostridium_sensu_stricto_RNT* (b) for general happiness. From the center, the first circo depicts the $-\log_{10} P$ of each variant owing to double exposure, i.e., the effect of both single nucleotide polymorphism allele and gut microbiota. The second circo shows chromosome density. Red plots indicate the P -value threshold for genome-wide significance ($P < 5 \times 10^{-8}$), while the green plots indicate the P -value threshold for suggestive significance ($P < 5 \times 10^{-7}$). The plots were generated using the “CMplot” R script (<https://github.com/YinLiLin/R-CMplot> (accessed on 3 April 2021)). SNP, single-nucleotide polymorphism; G, genus; HB, hurdle binary; RNT, rank-normal transformation.

4. Discussion

In this study, we conducted PRS and interaction analyses to explore the relationship between gut microbiota and two common mental traits, neuroticism and general happiness. We found significant associations between gut microbiota and the risk of neuroticism and general happiness. In addition, we identified multiple genes which interacted with the gut microbiota influencing neuroticism and general happiness.

It has been demonstrated by previous studies that the gut microbiota is associated with certain behaviors and psychiatric disorders, which are consistent with our findings. For example, according to an observational and cross-sectional study in 672 adults, high neuroticism was correlated with a high abundance of *Gammaproteobacteria* and *Proteobacteria*, respectively, when age, sex, BMI, and nutrient intake were controlled as covariates. The results of the beta diversity analysis indicate a lower diversity and closer similarity within paired samples in the neuroticism group [27]. Michels et al. suggested that adjusted and unadjusted taxonomic differences in the gut microbiota were the most pronounced for happiness, which was associated with 24 operational taxonomic units (=11.8% of bacterial counts) in 93 Belgian adolescents [28]. Another research from healthy Korean adults revealed that positive emotion and gut microbiome diversity (Shannon Index) were associated with substantial relationships in the *Prevotella*-dominating group [29].

We observed a suggestive significant negative correlation between the PRS of the genus *Bifidobacterium* and neuroticism and a positive correlation between the genus *Desulfovibrio* and neuroticism. Although evidence on the genus *Bifidobacterium* and neuroticism is limited, anxiety and depression, typical characteristics among populations with high neuroticism [30], have been shown to be strongly associated with *Bifidobacterium*. Animal experiments have observed that long-term exposure to a 150 mT static magnetic field improved the abundance of *Bifidobacterium* and *Clostridium* and further improved anxiety in C57BL/6J mice [31]. A recent clinical study also confirmed the potential of *Bifidobacterium* in the treatment of major depression [32]. They found that oral administration of freeze-dried *Bifidobacterium* breve CCFM1025 significantly reduced the serum serotonin turnover in patients compared to the placebo [32]. The genus *Desulfovibrio* is anaerobic and Gram-negative, also known as a sulfate-reducing bacterium. Recently, our group identified that *Desulfovibrio* was significantly related to attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder (BD), schizophrenia (SCZ), and major depressive disorder (MDD) [33]. As a potentially harmful bacterium, *Desulfovibrio* was found to be enriched in diseased individuals in inflammatory disease models [34,35]. The abundance of *Desulfovibrio* was negatively correlated with short-chain fatty acids (SCFAs), the production of microbial metabolites [34]. SCFAs, such as acetate, butyrate, and propionate, are important immunomodulatory and anti-inflammatory molecules in the gut and have shown promising therapeutic effects on symptoms of depression and anxiety in animal studies [36]. Despite limited research on *Desulfovibrio* and neuroticism, further multi-omics studies integrating metagenomics, transcriptomics and metabolomics may provide the basis for revealing the mechanisms underlying this interaction.

We also observed suggestive significant positive correlations between the PRS of the genus *Bifidobacterium* and the genus *Clostridium* with general happiness. The genus *Clostridium* is Gram-positive, and most of them are non-pathogenic bacteria. A recent comparative study identified a significantly lower relative abundance of *Clostridium* in patients with schizophrenia than in healthy controls [37]. Emerging evidence supported the beneficial role of *Clostridium* in the anti-inflammatory activity, immune protection, and remodeling of the gut microbiome [38,39]. For example, oral administration of Ataining (containing *Clostridium* butyricum, CGMCC0313.1) to gastrectomized patients resulted in reduced early postoperative inflammation, enhanced immune ability, restored intestinal microbiota eubiosis, and increased intestinal SCFAs [38]. Similarly, *Clostridium* butyricum SLZX19-05 treatment of weaned piglets inhibited inflammation levels, remodeled the ileal microbiome and increased propionate production [39]. Since inflammation and immune system activation play an important role in brain function and the development of mental

health [40], the above evidence also supports to some extent the relationship between *Clostridium* and general happiness. Collectively, genus *Bifidobacterium* and *Clostridium* are promising candidate psychological gut microbiota that may improve emotional health. These mechanisms may be related to the production of neuroactive substances, as well as anti-inflammatory effects and immune system activation. These findings support the future clinical use of psychobiotics to improve emotional health.

Notably, we found that the PRS of the genus *Bifidobacterium* was suggestive negatively correlated with neuroticism but positively correlated with general happiness. Mechanistically, as a psychobiotic, *Bifidobacterium* has been reported to secrete γ -aminobutyric acid (GABA, the “happy” chemical) [41]. Moreover, supplementation of *Bifidobacterium* could increase tryptophan, a precursor of serotonin (another “happy” chemical) [42]. Neuroticism, a health-related personality factor that includes negative emotions such as anxiety, moodiness, and depression [43], is opposed to the positive trait of general happiness. Taken together, the positive correlation between the genus *Bifidobacterium* and general happiness and the negative correlation between *Bifidobacterium* and neuroticism can be speculated.

The interaction effects between genetic factors and gut microbiota traits for mental traits remain largely unknown now. The key element of this study is that we conducted an interaction analysis of mental traits and identified multiple loci and candidate genes for the modulation of genetic response to the gut microbiota, which may provide novel insights to help disentangle its underlying etiological mechanisms.

Interaction analysis identified several candidate genes interacting with gut microbiota for neuroticism, such as *RORA*, *KCNQ3*, and *NDUFS1*. *RORA* is a member of the nuclear hormone receptor superfamily. Genetic association studies have identified that *RORA* is a suggestive gene linked more specifically to the depression facet of neuroticism [44]. In the mouse nervous system, *RORA* is localized in the cerebellum, thalamus, cerebral cortex, suprachiasmatic nucleus, and other structures [45]. As recently demonstrated by multiple studies of animal models, GWA, and linkage studies converging on variants in the *RORA* gene, it may be linked to bipolar disorder and trait depression [46,47]. *RORA* proteins play an important role in the maintenance of circadian rhythms [48]. Clinically, the disruption of circadian rhythms may contribute to anxiety disorders [49]. In addition, *RORA* proteins are involved in protecting neurons and glial cells from oxidative stress-induced apoptosis [50], which is a potential mechanism implicated in the pathophysiology of depression and anxiety disorders. Direct evidence for *RORA* and neuroticism, although limited, suggests these physiological roles of *RORA* protein may support the biological plausibility of its association with neuroticism. Sands et al. revealed that specific gain-of-function variants in *KCNQ3* cause neurodevelopmental disability, autism, and ASD [51]. In addition, Kaminsky et al. suggested that epigenetic alterations in the *KCNQ3* gene may be important in the aetiopathogenesis of bipolar disorder [52]. As a voltage-gated potassium channel, *KCNQ* is an important regulator of cell membrane excitability [53]. Preclinical studies suggest that the *KCNQ* channel is a potential target for the treatment of depression and anhedonia [54]. Specifically, *KCNQ2* and *KCNQ3* form homologous or heterodimers (such as *KCNQ2/3* channels), which constitute M-shaped channels regulating nerve excitability [55]. Up-regulation of the *KCNQ3* channel restored the hyperactivity of dopamine neurons and reversed depressive behavior in susceptible mice [56]. A genetic variant in the *NDUFS1* gene was reported to be associated with schizophrenia and negative symptoms in Han Chinese subjects [57]. According to a previous study, neuroticism may share common genetic variants with most mental disorders, such as BD and MDD [58]. *NDUFS1* knockdown in neurons reduced complex I affiliation into supercomplexes, resulting in decreased oxygen consumption and increased mitochondrial reactive oxygen species [59]. Further functional studies are warranted to validate the role of those genes in neuroticism.

ASTN2 encodes a large vertebrate-specific transmembrane protein that is expressed primarily in developing and adult brains. We observed that it interacted with the genus *Clostridium* to influence general happiness. In recent studies on *ASTN2* and general happiness, although very limited, copy number variants of *ASTN2*, both deletions and dupli-

cations, have been identified in patients with neurodevelopmental disorders, including ASD, SCZ, ADHD, BD, intellectual disability, and global developmental delay [60–63]. The hippocampus is thought to be involved in emotional and cognitive regulation [64]. A dataset test of 724 twins and siblings found a non-linear correlation between subjective well-being (SWB) and hippocampal volume, characterized by lower SWB in subjects with relatively smaller hippocampal volume compared to those with medium and high hippocampal volume [65]. A previous GWAS study identified nine SNPs at 9p33 in *ASTN2* that were significantly associated with hippocampal volume [66]. Another GWAS study also identified one novel independent locus that was significantly associated with hippocampal volume lying within the *ASTN2* gene [67]. Emotional or stressful events, on the other hand, are associated with a large release of corticosterone, which rapidly enhances synaptic plasticity [68]. *ASTN2* can play a role in emotional events by regulating synaptic strength through endocytic transport and the degradation of surface proteins [69]. Conclusively, the above studies suggest that *ASTN2* may influence general happiness by altering the hippocampal volume or regulating synaptic strength, which supports our findings.

GO enrichment analysis identified multiple GO terms associated with neuroticism and general happiness. One notable finding is focal adhesion (GO:0005925). It plays an important role in cell migration during brain development. Previous studies suggest that focal adhesion and extracellular matrix receptor interaction pathways are significantly associated with certain facets of neuroticism (i.e., angry hostility and depression) [70]. It has also been reported that focal adhesion is involved in abnormal neurodevelopment in SCZ patients compared with healthy control subjects [71]. Synapse (GO:0045202) is another notable GO term that we found. A recent finding has raised the importance of glypicans in the development and functions of synapses, and the dysfunctions of glypicans may lead to the malfunction of synapses and abnormal neurodevelopmental disorders, such as neuroticism and SCZ [72].

It is important to emphasize that our study has certain limitations. First, like GWAS, some significant SNPs found by the interaction analysis are located in the non-coding region, which still poses a challenge for us to better illustrate our results. Second, all subjects in this study are of European ancestry. Therefore, one should be careful when applying our study results to other ethnic groups. Next, because the use of antibiotics may disrupt the composition of the microbiota [73], future studies to explore the impact of antibiotic use on mental traits by affecting gut microbiota are warranted. Further experiments are needed to confirm our findings and reveal the potential molecular mechanisms underlying the associations observed in this study. Finally, the gut microbiota-related SNP sets were derived from previous GWAS. The accuracy of our findings may be influenced by the power of the previous study. Further replication studies with other genetic background individuals and experimental studies are required to verify the results of this study.

5. Conclusions

In conclusion, we observed correlations between gut microbial traits and neuroticism and general happiness in the UK Biobank cohort. Interaction analysis identified multiple candidate genes which may serve as the underlying genetic mechanisms of the observed association. Our study findings could provide novel insights into the impacts of gut microbial traits on the two common mental traits.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/genes14010156/s1>, Supplementary File S1: Supplement to the neuroticism score questions; Table S1: Interactions between individual SNPs and gut microbiota for neuroticism with $P < 5 \times 10^{-8}$; Table S2: Interactions between individual SNPs and gut microbiota for general happiness with $P < 5 \times 10^{-8}$; Table S3: List of neuroticism-associated GO terms; Table S4: List of general happiness-associated GO terms.

Author Contributions: Y.J., S.C. and F.Z. conceived and designed the study, and wrote the manuscript; Y.J., S.C. and F.Z. collected the data and carried out the statistical analyses; L.L., Y.W., B.C., C.L., X.C.,

J.Y., Y.Y. and O.P.K. made preparations for the manuscript at first. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Scientific Foundation of China: 82103959, 81922059.

Institutional Review Board Statement: The original studies providing data sources for this study were all conducted with appropriate institutional review board approval (Northwest Multi-Center Research Ethics Committee, reference 11/NW/0382).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Antunes, A.; Frasilho, D.; Azeredo-Lopes, S.; Neto, D.; Silva, M.; Cardoso, G.; Caldas-de-Almeida, J.M. Disability and common mental disorders: Results from the World Mental Health Survey Initiative Portugal. *Eur. Psychiatry* **2018**, *49*, 56–61. [[CrossRef](#)] [[PubMed](#)]
- James, S.L.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A.; et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2018**, *392*, 1789–1858. [[CrossRef](#)] [[PubMed](#)]
- Ormel, J.; Bastiaansen, A.; Riese, H.; Bos, E.H.; Servaas, M.; Ellenbogen, M.; Rosmalen, J.G.M.; Aleman, A. The biological and psychological basis of neuroticism: Current status and future directions. *Neurosci. Biobehav. Rev.* **2013**, *37*, 59–72. [[CrossRef](#)] [[PubMed](#)]
- Etkin, A. The genetics of happiness. *Sci. Transl. Med.* **2016**, *8*, 359ec159. [[CrossRef](#)]
- Ormel, J.; Jeronimus, B.F.; Kotov, R.; Riese, H.; Bos, E.H.; Hankin, B.; Rosmalen, J.G.M.; Oldehinkel, A.J. Neuroticism and common mental disorders: Meaning and utility of a complex relationship. *Clin. Psychol. Rev.* **2013**, *33*, 686–697. [[CrossRef](#)] [[PubMed](#)]
- Gigantesco, A.; Stazi, M.A.; Alessandri, G.; Medda, E.; Tarolla, E.; Fagnani, C. Psychological well-being (PWB): A natural life outlook? An Italian twin study on heritability of PWB in young adults. *Psychol. Med.* **2011**, *41*, 2637–2649. [[CrossRef](#)]
- Hudson, A.; Wekerle, C.; Goldstein, A.L.; Ellenbogen, S.; Waechter, R.; Thompson, K.; Stewart, S.H. Gender differences in emotion-mediated pathways from childhood sexual abuse to problem drinking in adolescents in the child welfare system. *J. Child Adolesc. Trauma* **2017**, *10*, 19–28. [[CrossRef](#)]
- Rosenthal, M.Z.; Cheavens, J.S.; Lejuez, C.W.; Lynch, T.R. Thought suppression mediates the relationship between negative affect and borderline personality disorder symptoms. *Behav. Res. Ther.* **2005**, *43*, 1173–1185. [[CrossRef](#)]
- Nicholson, J.K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.R.; Jia, W.; Pettersson, S. Host-Gut Microbiota Metabolic Interactions. *Science* **2012**, *336*, 1262–1267. [[CrossRef](#)]
- Clemente, J.C.; Ursell, L.K.; Parfrey, L.W.; Knight, R. The impact of the gut microbiota on human health: An integrative view. *Cell* **2012**, *148*, 1258–1270. [[CrossRef](#)]
- Bosch, J.A.; Nieuwdorp, M.; Zwinderman, A.H.; Deschasaux, M.; Radjabzadeh, D.; Kraaij, R.; Davids, M.; de Rooij, S.R.; Lok, A. The gut microbiota and depressive symptoms across ethnic groups. *Nat. Commun.* **2022**, *13*, 7129. [[CrossRef](#)] [[PubMed](#)]
- Chen, Y.-H.; Zhou, C.-H.; Yu, H.; Wu, W.-J.; Wang, Y.-W.; Liu, L.; Hu, G.-T.; Li, B.-J.; Peng, Z.-W.; Wang, H.-N. Gut microbial signatures and differences in bipolar disorder and schizophrenia of emerging adulthood. *CNS Neurosci. Ther.* **2022**, *00*, 1–13. [[CrossRef](#)] [[PubMed](#)]
- Cryan, J.F.; Dinan, T.G. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* **2012**, *13*, 701–712. [[CrossRef](#)] [[PubMed](#)]
- Mayer, E.A.; Knight, R.; Mazmanian, S.K.; Cryan, J.F.; Tillisch, K. Gut Microbes and the Brain: Paradigm Shift in Neuroscience. *J. Neurosci.* **2014**, *34*, 15490–15496. [[CrossRef](#)] [[PubMed](#)]
- Sampson, T.R.; Mazmanian, S.K. Control of Brain Development, Function, and Behavior by the Microbiome. *Cell Host Microbe* **2015**, *17*, 565–576. [[CrossRef](#)]
- Hughes, D.A.; Bacigalupe, R.; Wang, J.; Rühlemann, M.C.; Tito, R.Y.; Falony, G.; Joossens, M.; Vieira-Silva, S.; Henckaerts, L.; Rymenans, L.; et al. Genome-wide associations of human gut microbiome variation and implications for causal inference analyses. *Nat. Microbiol.* **2020**, *5*, 1079–1087. [[CrossRef](#)]
- Visscher, P.M.; Brown, M.A.; McCarthy, M.I.; Yang, J. Five years of GWAS discovery. *Am. J. Hum. Genet.* **2012**, *90*, 7–24. [[CrossRef](#)]
- Burgess, S.; Thompson, S.G. Use of allele scores as instrumental variables for Mendelian randomization. *Int. J. Epidemiol.* **2013**, *42*, 1134–1144. [[CrossRef](#)]
- Chen, Y.; Meng, P.; Cheng, S.; Jia, Y.; Wen, Y.; Yang, X.; Yao, Y.; Pan, C.; Li, C.E.; Zhang, H.; et al. Assessing the effect of interaction between C-reactive protein and gut microbiome on the risks of anxiety and depression. *Mol. Brain* **2021**, *14*, 133. [[CrossRef](#)]

20. Zhang, H.; Liu, L.; Cheng, S.; Jia, Y.; Wen, Y.; Yang, X.; Meng, P.; Li, C.e.; Pan, C.; Chen, Y.; et al. Assessing the joint effects of brain aging and gut microbiota on the risks of psychiatric disorders. *Brain Imaging Behav.* **2022**, *16*, 1504–1515. [[CrossRef](#)]
21. Sudlow, C.; Gallacher, J.; Allen, N.E.; Beral, V.; Burton, P.R.; Danesh, J.; Downey, P.; Elliott, P.; Green, J.; Landray, M.J. UK biobank: An open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* **2015**, *12*, e1001779. [[CrossRef](#)]
22. Bycroft, C.; Freeman, C.; Petkova, D.; Elliott, L.T.; Sharp, K.; Motyer, A.; Vukcevic, D.; Delaneau, O.; O'Connell, J.; Cortes, A. The UK Biobank resource with deep phenotyping and genomic data. *Nature* **2018**, *562*, 203–209. [[CrossRef](#)] [[PubMed](#)]
23. Canelaxandri, O.; Rawlik, K.; Tenesa, A. An atlas of genetic associations in UK Biobank. *Nat. Genet.* **2018**, *50*, 1593–1599. [[CrossRef](#)]
24. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.; Bender, D.; Maller, J.; Sklar, P.; de Bakker, P.I.; Daly, M.J.; et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **2007**, *81*, 559–575. [[CrossRef](#)]
25. Chang, C.C.; Chow, C.C.; Tellier, L.C.A.M.; Vattikuti, S.; Purcell, S.; Lee, J.J. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience* **2015**, *4*, 7. [[CrossRef](#)] [[PubMed](#)]
26. Bu, D.; Luo, H.; Huo, P.; Wang, Z.; Zhang, S.; He, Z.; Wu, Y.; Zhao, L.; Liu, J.; Guo, J.; et al. KOBAS-i: Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res.* **2021**, *49*, W317–W325. [[CrossRef](#)] [[PubMed](#)]
27. Kim, H.N.; Yun, Y.; Ryu, S.; Chang, Y.; Kwon, M.J.; Cho, J.; Shin, H.; Kim, H.L. Correlation between gut microbiota and personality in adults: A cross-sectional study. *Brain Behav. Immun.* **2018**, *69*, 374–385. [[CrossRef](#)] [[PubMed](#)]
28. Michels, N.; Van de Wiele, T.; Fouhy, F.; O'Mahony, S.; Clarke, G.; Keane, J. Gut microbiome patterns depending on children's psychosocial stress: Reports versus biomarkers. *Brain Behav. Immun.* **2019**, *80*, 751–762. [[CrossRef](#)]
29. Lee, S.-H.; Yoon, S.-H.; Jung, Y.; Kim, N.; Min, U.; Chun, J.; Choi, I. Emotional well-being and gut microbiome profiles by enterotype. *Sci. Rep.* **2020**, *10*, 20736. [[CrossRef](#)]
30. Park, E.; Yun, K.E.; Kim, M.-H.; Kim, J.; Chang, Y.; Ryu, S.; Kim, H.-L.; Kim, H.-N.; Jung, S.-C. Correlation between Gut Microbiota and Six Facets of Neuroticism in Korean Adults. *J. Pers. Med.* **2021**, *11*, 1246. [[CrossRef](#)]
31. Yang, X.; Yu, B.; Song, C.; Feng, C.; Zhang, J.; Wang, X.; Cheng, G.; Yang, R.; Wang, W.; Zhu, Y. The Effect of Long-Term Moderate Static Magnetic Field Exposure on Adult Female Mice. *Biology* **2022**, *11*, 1585. [[CrossRef](#)] [[PubMed](#)]
32. Tian, P.; Chen, Y.; Zhu, H.; Wang, L.; Qian, X.; Zou, R.; Zhao, J.; Zhang, H.; Qian, L.; Wang, Q.; et al. Bifidobacterium breve CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: A randomized clinical trial. *Brain Behav. Immun.* **2022**, *100*, 233–241. [[CrossRef](#)] [[PubMed](#)]
33. Cheng, S.; Han, B.; Ding, M.; Wen, Y.; Ma, M.; Zhang, L.; Qi, X.; Cheng, B.; Li, P.; Kafle, O.P.; et al. Identifying psychiatric disorder-associated gut microbiota using microbiota-related gene set enrichment analysis. *Brief. Bioinform.* **2020**, *21*, 1016–1022. [[CrossRef](#)]
34. Zhao, X.; Jiang, L.; Fang, X.; Guo, Z.; Wang, X.; Shi, B.; Meng, Q. Host-microbiota interaction-mediated resistance to inflammatory bowel disease in pigs. *Microbiome* **2022**, *10*, 115. [[CrossRef](#)] [[PubMed](#)]
35. Yin, Y.; Cai, J.; Zhou, L.; Xing, L.; Zhang, W. Dietary oxidized beef protein alters gut microbiota and induces colonic inflammatory damage in C57BL/6 mice. *Front. Nutr.* **2022**, *9*, 980204. [[CrossRef](#)] [[PubMed](#)]
36. van de Wouw, M.; Boehme, M.; Lyte, J.M.; Wiley, N.; Strain, C.; O'Sullivan, O.; Clarke, G.; Stanton, C.; Dinan, T.G.; Cryan, J.F. Short-chain fatty acids: Microbial metabolites that alleviate stress-induced brain-gut axis alterations. *J. Physiol.* **2018**, *596*, 4923–4944. [[CrossRef](#)]
37. Yan, F.; Xia, L.; Xu, L.; Deng, L.; Jin, G. A comparative study to determine the association of gut microbiome with schizophrenia in Zhejiang, China. *BMC Psychiatry* **2022**, *22*, 731. [[CrossRef](#)] [[PubMed](#)]
38. Cao, W.; Zheng, C.; Xu, X.; Jin, R.; Huang, F.; Shi, M.; He, Z.; Luo, Y.; Liu, L.; Liu, Z.; et al. Clostridium butyricum potentially improves inflammation and immunity through alteration of the microbiota and metabolism of gastric cancer patients after gastrectomy. *Front. Immunol.* **2022**, *13*, 1076245. [[CrossRef](#)]
39. Wu, J.; Wang, J.; Lin, Z.; Liu, C.; Zhang, Y.; Zhang, S.; Zhou, M.; Zhao, J.; Liu, H.; Ma, X. Clostridium butyricum alleviates weaned stress of piglets by improving intestinal immune function and gut microbiota. *Food Chem.* **2023**, *405*, 135014. [[CrossRef](#)]
40. Peirce, J.M.; Alviña, K. The role of inflammation and the gut microbiome in depression and anxiety. *J. Neurosci. Res.* **2019**, *97*, 1223–1241. [[CrossRef](#)]
41. Kong, Q.; Chen, Q.; Mao, X.; Wang, G.; Zhao, J.; Zhang, H.; Chen, W. Bifidobacterium longum CCFM1077 Ameliorated Neurotransmitter Disorder and Neuroinflammation Closely Linked to Regulation in the Kynurenine Pathway of Autistic-like Rats. *Nutrients* **2022**, *14*, 1615. [[CrossRef](#)] [[PubMed](#)]
42. Accettulli, A.; Corbo, M.R.; Sinigaglia, M.; Speranza, B.; Campaniello, D.; Racioppo, A.; Altieri, C.; Bevilacqua, A. Psycho-Microbiology, a New Frontier for Probiotics: An Exploratory Overview. *Microorganisms* **2022**, *10*, 2141. [[CrossRef](#)] [[PubMed](#)]
43. Friedman, H.S. Neuroticism and health as individuals age. *Pers. Disord.* **2019**, *10*, 25–32. [[CrossRef](#)] [[PubMed](#)]
44. Terracciano, A.; Tanaka, T.; Sutin, A.R.; Sanna, S.; Deiana, B.; Lai, S.; Uda, M.; Schlessinger, D.; Abecasis, G.R.; Ferrucci, L.; et al. Genome-wide association scan of trait depression. *Biol. Psychiatry* **2010**, *68*, 811–817. [[CrossRef](#)] [[PubMed](#)]
45. Ino, H. Immunohistochemical Characterization of the Orphan Nuclear Receptor ROR α in the Mouse Nervous System. *J. Histochem. Cytochem.* **2004**, *52*, 311–323. [[CrossRef](#)]

46. Le-Niculescu, H.; Patel, S.D.; Bhat, M.; Kuczynski, R.; Faraone, S.V.; Tsuang, M.T.; McMahon, F.J.; Schork, N.J.; Nurnberger Jr, J.I.; Niculescu Iii, A.B. Convergent functional genomics of genome-wide association data for bipolar disorder: Comprehensive identification of candidate genes, pathways and mechanisms. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **2009**, *150B*, 155–181. [[CrossRef](#)]
47. Soria, V.; Martínez-Amorós, È.; Escaramís, G.; Valero, J.; Pérez-Egea, R.; García, C.; Gutiérrez-Zotes, A.; Puigdemont, D.; Bayés, M.; Crespo, J.M.; et al. Differential Association of Circadian Genes with Mood Disorders: CRY1 and NPAS2 are Associated with Unipolar Major Depression and CLOCK and VIP with Bipolar Disorder. *Neuropsychopharmacology* **2010**, *35*, 1279–1289. [[CrossRef](#)]
48. Sato, T.K.; Panda, S.; Miraglia, L.J.; Reyes, T.M.; Rudic, R.D.; McNamara, P.; Naik, K.A.; FitzGerald, G.A.; Kay, S.A.; Hogenesch, J.B. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* **2004**, *43*, 527–537. [[CrossRef](#)]
49. Coles, M.E.; Schubert, J.R.; Nota, J.A. Sleep, Circadian Rhythms, and Anxious Traits. *Curr. Psychiatry Rep.* **2015**, *17*, 73. [[CrossRef](#)]
50. Boukhtouche, F.; Vodjdani, G.; Jarvis, C.I.; Bakouche, J.; Staels, B.; Mallet, J.; Mariani, J.; Lemaigre-Dubreuil, Y.; Brugg, B. Human retinoic acid receptor-related orphan receptor alpha1 overexpression protects neurones against oxidative stress-induced apoptosis. *J. Neurochem.* **2006**, *96*, 1778–1789. [[CrossRef](#)]
51. Sands, T.T.; Miceli, F.; Lesca, G.; Beck, A.E.; Sadleir, L.G.; Arrington, D.K.; Schönewolf-Greulich, B.; Moutton, S.; Lauritano, A.; Nappi, P.; et al. Autism and developmental disability caused by KCNQ3 gain-of-function variants. *Ann. Neurol.* **2019**, *86*, 181–192. [[CrossRef](#)] [[PubMed](#)]
52. Kaminsky, Z.; Jones, I.; Verma, R.; Saleh, L.; Trivedi, H.; Guintivano, J.; Akman, R.; Zandi, P.; Lee, R.S.; Potash, J.B. DNA methylation and expression of KCNQ3 in bipolar disorder. *Bipolar Disord.* **2015**, *17*, 150–159. [[CrossRef](#)] [[PubMed](#)]
53. Xiong, Q.; Gao, Z.; Wang, W.; Li, M. Activation of Kv7 (KCNQ) voltage-gated potassium channels by synthetic compounds. *Trends Pharmacol. Sci.* **2008**, *29*, 99–107. [[CrossRef](#)]
54. Wulff, H.; Castle, N.A.; Pardo, L.A. Voltage-gated potassium channels as therapeutic targets. *Nat. Rev. Drug Discov.* **2009**, *8*, 982–1001. [[CrossRef](#)] [[PubMed](#)]
55. Wang, H.S.; Pan, Z.; Shi, W.; Brown, B.S.; Wymore, R.S.; Cohen, I.S.; Dixon, J.E.; McKinnon, D. KCNQ2 and KCNQ3 potassium channel subunits: Molecular correlates of the M-channel. *Science* **1998**, *282*, 1890–1893. [[CrossRef](#)] [[PubMed](#)]
56. Costi, S.; Morris, L.S.; Kirkwood, K.A.; Hoch, M.; Corniquel, M.; Vo-Le, B.; Iqbal, T.; Chadha, N.; Pizzagalli, D.A.; Whitton, A.; et al. Impact of the KCNQ2/3 Channel Opener Ezogabine on Reward Circuit Activity and Clinical Symptoms in Depression: Results From a Randomized Controlled Trial. *Am. J. Psychiatry* **2021**, *178*, 437–446. [[CrossRef](#)]
57. Zhu, Y.; Wang, Z.; Ni, J.; Zhang, Y.; Chen, M.; Cai, J.; Li, X.; Zhang, W.; Zhang, C. Genetic variant in NDUF51 gene is associated with schizophrenia and negative symptoms in Han Chinese. *J. Hum. Genet.* **2015**, *60*, 11–16. [[CrossRef](#)]
58. de Moor, M.H.M.; Berg, S.M.V.D.; Verweij, K.J.H.; Krueger, R.F.; Luciano, M.; Vasquez, A.A.; Matteson, L.K.; Derringer, J.; Esko, T.; Amin, N.; et al. Meta-analysis of Genome-wide Association Studies for Neuroticism, and the Polygenic Association With Major Depressive Disorder. *JAMA Psychiatry* **2015**, *72*, 642–650. [[CrossRef](#)]
59. Lopez-Fabuel, I.; Le Douce, J.; Logan, A.; James, A.M.; Bonvento, G.; Murphy, M.P.; Almeida, A.; Bolaños, J.P. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 13063–13068. [[CrossRef](#)]
60. Glessner, J.T.; Wang, K.; Cai, G.; Korvatska, O.; Kim, C.E.; Wood, S.; Zhang, H.; Estes, A.; Brune, C.W.; Bradfield, J.P. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature* **2009**, *459*, 569–572. [[CrossRef](#)]
61. Bernardini, L.; Alesi, V.; Loddo, S.; Novelli, A.; Bottillo, I.; Battaglia, A.; Digilio, M.C.; Zampino, G.; Ertel, A.; Fortina, P. High-resolution SNP arrays in mental retardation diagnostics: How much do we gain? *Eur. J. Hum. Genet.* **2010**, *18*, 178–185. [[CrossRef](#)] [[PubMed](#)]
62. Lesch, K.P.; Timmesfeld, N.; Renner, T.J.; Halperin, R.; Röser, C.; Nguyen, T.T.; Craig, D.W.; Romanos, J.; Heine, M.; Meyer, J.; et al. Molecular genetics of adult ADHD: Converging evidence from genome-wide association and extended pedigree linkage studies. *J. Neural Transm.* **2008**, *115*, 1573–1585. [[CrossRef](#)] [[PubMed](#)]
63. Vrijenhoek, T.; Buizerboskamp, J.E.; Der Stelt, I.V.; Strengman, E.; Sabatti, C.; Van Kessel, A.G.; Brunner, H.G.; Ophoff, R.A.; Veltman, J.A. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. *Am. J. Hum. Genet.* **2008**, *83*, 504–510. [[CrossRef](#)]
64. Desmedt, A.; Marighetto, A.; Richter-Levin, G.; Calandrea, L. Adaptive emotional memory: The key hippocampal-amygdalar interaction. *Stress* **2015**, *18*, 297–308. [[CrossRef](#)] [[PubMed](#)]
65. Van't Ent, D.; den Braber, A.; Baselmans, B.M.L.; Brouwer, R.M.; Dolan, C.V.; Hulshoff Pol, H.E.; de Geus, E.J.C.; Bartels, M. Associations between subjective well-being and subcortical brain volumes. *Sci. Rep.* **2017**, *7*, 6957. [[CrossRef](#)]
66. Bis, J.C.; DeCarli, C.; Smith, A.V.; van der Lijn, F.; Crivello, F.; Fornage, M.; Dobbins, S.; Shulman, J.M.; Schmidt, H.; Srikanth, V.; et al. Common variants at 12q14 and 12q24 are associated with hippocampal volume. *Nat. Genet.* **2012**, *44*, 545–551. [[CrossRef](#)]
67. Hibar, D.P.; Adams, H.H.H.; Jahanshad, N.; Chauhan, G.; Stein, J.L.; Hofer, E.; Renteria, M.E.; Bis, J.C.; Arias-Vasquez, A.; Ikram, M.K.; et al. Novel genetic loci associated with hippocampal volume. *Nat. Commun.* **2017**, *8*, 13624. [[CrossRef](#)]
68. Wiegert, O.; Joëls, M.; Krugers, H.J. Corticosteroid hormones, synaptic strength and emotional memories: Corticosteroid modulation of memory—A cellular and molecular perspective. *Prog. Brain Res.* **2008**, *167*, 269–271.

69. Behesti, H.; Fore, T.R.; Wu, P.; Horn, Z.; Leppert, M.; Hull, C.; Hatten, M.E. ASTN2 modulates synaptic strength by trafficking and degradation of surface proteins. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E9717–E9726. [[CrossRef](#)]
70. Kim, S.E.; Kim, H.-N.; Yun, Y.-J.; Heo, S.G.; Cho, J.; Kwon, M.-J.; Chang, Y.; Ryu, S.; Shin, H.; Shin, C.; et al. Meta-analysis of genome-wide SNP- and pathway-based associations for facets of neuroticism. *J. Hum. Genet.* **2017**, *62*, 903–909. [[CrossRef](#)]
71. Fan, Y.; Abrahamsen, G.; Mills, R.; Calderón, C.C.; Tee, J.Y.; Leyton, L.; Murrell, W.; Cooper-White, J.; McGrath, J.J.; Mackay-Sim, A. Focal adhesion dynamics are altered in schizophrenia. *Biol. Psychiatry* **2013**, *74*, 418–426. [[CrossRef](#)] [[PubMed](#)]
72. Kamimura, K.; Maeda, N. Glypicans and Heparan Sulfate in Synaptic Development, Neural Plasticity, and Neurological Disorders. *Front. Neural Circuits* **2021**, *15*, 595596. [[CrossRef](#)] [[PubMed](#)]
73. Maier, L.; Goemans, C.V.; Wirbel, J.; Kuhn, M.; Eberl, C.; Pruteanu, M.; Müller, P.; Garcia-Santamarina, S.; Cacace, E.; Zhang, B.; et al. Unravelling the collateral damage of antibiotics on gut bacteria. *Nature* **2021**, *599*, 120–124. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.