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ARTICLE

Long-term antibiotic use during early life and risks to mental traits: an observational study and gene–environment-wide interaction study in UK Biobank cohort

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The relationships between long-term antibiotic use during early life and mental traits remain elusive now. A total of 158,444 subjects from UK Biobank were used in this study. Linear regression analyses were first conducted to assess the correlations between long-term antibiotic use during early life and mental traits. Gene–environment-wide interaction study (GEWIS) was then performed by PLINK2.0 to detect the interaction effects between long-term antibiotic use during early life and genes on the risks of mental traits. Finally, DAVID tool was used to conduct gene ontology (GO) analysis of the identified genes interacting with long-term antibiotic use during early life. We found negative associations of long-term antibiotic use during early life with remembrance (p value=1.74×10^{-6}, $b = -0.10$) and intelligence (p value=2.64×10^{-26}, $b = -0.13$), and positive associations of long-term antibiotic use during early life with anxiety (p value = 2.75×10^{-47}, $b = 0.12$) and depression (p value=2.01×10^{-195}, $b = 0.25$). GEWIS identified multiple significant genes-long-term antibiotic use during early life interaction effects, such as ANK3 (*rs773585997*, p value = 1.78×10^{-8}) for anxiety and STRN (*rs140049205*, p value = 1.88×10^{-8}) for depression. GO enrichment analysis detected six GO terms enriched in the identified genes interacting with long-term antibiotic use during early life for anxiety, such as GO:0030425~dendrite (p value = 3.41×10^{-2}) and GO:0005886~plasma membrane (p value = 3.64×10^{-3}). Our study results suggest the impact of long-term antibiotic use during early life on the development of mental traits.

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INTRODUCTION

Anxiety, depression, and alcohol dependence are common mental disorders, which contribute 26.6% of total all cause burden in Europe [1]. It has been reported that mental disorders have consistently lead to more than 14% of age-standardized years lived with disability for nearly 30 years based on the Global Burden of Diseases, Injuries, and Risk Factors Study 2017 (GBD 2017) [2]. Anxiety and depression are becoming serious global health problems worldwide, which account for 40.5% and 14.6% of disability-adjusted life years, respectively [3]. The lower intelligence is a risk factor for the whole range of mental disorders, and it is associated with illness severity [4]. Alcohol use disorder and high-volume drinking can predict of sickness absence due to mental disorders [5]. And alcohol dependence is associated with serious mental illness [5]. Smoking is a major contributor to the 10–25 years early mortality and disproportionately high morbidity burden in people with serious mental illness [6, 7].

Common mental traits and disorders are usually multi-factorial diseases, whose occurrence are impacted by both genetic and environmental factors. It has been reported that the heritability of lifetime major depression was estimated to be 38% [8]. And the estimated heritability was 26% for lifetime anxiety disorder and 31% for current anxiety symptoms [9]. Additionally, the inherited genome sequence differences account for 20% of the 50% heritability of intelligence [10]. Environmental risk factors, like smoking, stress, and early adverse childhood experiences, have been reported to be associated with depression and anxiety [11, 12]. As for alcoholism, there is evidence for both genetic and environmental factors in its pathophysiology [13]. However, the genetic mechanism of these mental traits and disorders remain largely unknown now.

Epidemiological study [14] and experimental study [15] have observed significant correlations between long-term antibiotic use during early life and mental traits. For instance, Lurie et al. [14] observed that long-term antibiotic exposure increased the risk of depression and anxiety in a nested case-control study. In another case, it was reported that a patient was diagnosed with the generalized anxiety disorder after abuse of multiple gastrointestinal antibiotics [16]. And an adolescent patient developed symptoms of delirium during treatment with cephalexin [17]. Slykerman et al. [18]. observed that children who had received antibiotics in the first 6 months of life would increase the risk of anxiety and emotional problems. Antibiotic treatment during early adolescence have a permanent impact on brain function in mice [15]. Microbiota depletion by means of chronic antibiotic

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exposure in mice has impact on anxiety and cognitive behaviors [15]. However, the biological mechanism of the long-term antibiotic use during early life on the variations of mental traits is not well understood. For instance, to the best of our knowledge, limited efforts have been paid to evaluate the potential interaction effects between long-term antibiotic use during early life and genes on the risks of mental traits.

Recently, technological and methodological breakthroughs have led to important progress in the elucidation of the genetic architecture of complex diseases, such as obesity [19] and breast cancer [20]. Considering that the amount of evidences supported a significant contribution of environmental factors to complex disease, the search for environmental factors should be reinforced accordingly [21]. Therefore, it is necessary to research the interaction effects between genes and environmental factors for complex traits and diseases. To discuss the issue of gene–environment (G × E) interactions would improve the ability to detect relevant genetic variants and draw a complete picture of etiology for complex diseases [22, 23]. The gene–environment-wide interaction study (GEWIS) takes into account the issue of G × E interactions, which can investigate the G × E interactions on a genome-wide scale [24].

In this study, we conducted an observational study to assess the associations between long-term antibiotic use during early life and mental traits in United Kingdom (UK) Biobank. We then performed the GEWIS based on the results of observational study to evaluate the potential interaction effects between genes and long-term antibiotic use during early life for mental traits. Our study results would provide novel clues for understanding the mechanism of long-term antibiotic use during early life on the development of mental traits.

MATERIALS AND METHODS

UK Biobank samples

Individual-level phenotypic and genotypic data in this study were driven from the UK Biobank health resource (http://biobank.ndph.ox.ac.uk). UK Biobank is a very large and population-based prospective study recruited from 2006 to 2010, which has over 500,000 participants aged 40–69 years. UK Biobank collects extensive phenotypic and genotypic informations of participants, including physical measures, sample assays, genome-wide genotyping and longitudinal follow-up about health-related outcomes. 158,444 individuals with long-term antibiotic use during early life data were included in this study. The phenotype of long-term antibiotic use during early life was defined as long-term or recurrent taking antibiotics as child or teenager (UK Biobank data field: 21067). UK Biobank collected the information of long-term antibiotic use during early life from 174,769 participants. The participants were asked the following question, "During childhood or as a teenager did you receive long-term or recurrent courses (three or more per year) of antibiotics (for example for tonsillitis or acne)"? The collected data for antibiotic use is categorical variable (single) and not continuous variable.

The phenotypes of anxiety and depression were defined according to the previous study [25–27]. General anxiety disorder-7 (GAD-7) [26] and Patient Health Questionnaire-9 (PHQ-9) [27] are efficient and valid self-report anxiety and depression measure for subjects in both clinical and non-clinical settings. The phenotype of remembrance was defined using the numeric memory test from UK Biobank. The longest number correctly recalled during the numeric memory test (UK Biobank data field: 4282) was analyzed. The phenotype of fluid intelligence (UK Biobank data field: 20,016) was based on a simple weighted sum of the correct answers to 13. We used the maximum number of reported past or current cigarettes (or pipes/cigars) consumed per day to define the frequency of smoking (UK Biobank data fields: 20,116, 2887, and 3456). Last, we used the sum of all alcoholic beverages per week as the frequency of drinking (UK Biobank data field: 20,117). Ethical approval of UK Biobank was granted by the National Health Service National Research Ethics Service (reference 11/NW/0382). The detailed definition of phenotypes are shown in Supplementary document 1.

UK Biobank genotyping, imputation, and quality control

Briefly, 488,377 participants of UK Biobank cohort have genotypes data [28]. Genotypes from all individuals were processed by the Affymetrix UK BiLEVE Axiom Array or the Affymetrix UK Biobank Axiom arrays (Santa Clara, CA, USA) [28]. Of note, the two arrays share 95% of marker content. The imputation was carried out in chunks of ~50,000 imputed markers with a 250 kb buffer region by IMPUTE4 (https://jmarchini.org/software/). In addition, the researchers based on the marker and sample to control data quality, including marker-based quality control and sample-based quality control. Batch effects, plate effects, departures from Hardy–Weinberg equilibrium, sex effects, array effects, and discordance across control replicates were tested by using statistical tests. The metrics of missing rate and heterozygosity computed using a set of 605,876 high-quality autosomal markers were used to identify poor quality samples. UK used an estimator implemented in the KING software and obtained the relatively independent single nucleotide polymorphism (SNP). Additionally, we removed the participants who reported inconsistencies between self-reported gender and genetic gender, who were genotyped but not imputed, and who withdraw their consents. Individuals were restricted to only "white British" based on self-reported ethnicity (UK Biobank field ID: 21000). Detailed description of array design, genotyping and quality control procedures can be found in the previous studies [28, 29].

Observational statistical analyses

All the phenotype of anxiety, depression, intelligence, remembrance, and the frequency of smoking per day and drinking per week were used as continuous variable in the current study. All the phenotypes were standardized to have mean 0 and variance 1 before further analyses. Association analyses of long-term antibiotic use during early life with anxiety, depression, intelligence, remembrance, and the frequency of smoking per day and drinking per week were tested using a linear regression model by *R* software (version 3.5.3), respectively. And the sex, age, and 10 principle components of population structure were used as covariates in the linear regression model. The significant correlation was identified at p value < 0.05 in the linear regression analyses.

Gene-environment-wide interaction analyses

We conducted GEWIS to assess the interactions between genetic factors and long-term antibiotic use during early life for mental traits, including anxiety, depression, remembrance, intelligence, and the frequency of smoking per day and drinking per week. The GEWIS was performed by PLINK2.0 [30, 31], which can test G × E interactions for quantitative and disease traits. Correspondingly, the GEWIS to detect genetic effects has more powerful tests to detect the associations between genetic variant and complex diseases [31]. Letter D is the disease outcome variable, the penetrance models of the form is represented as the following:

$$\mathrm{logit}[P(D=1|G,E)] = \beta_0 + \beta_g G + \beta_e E + \beta geGE$$

where G is genetic factors and E is the environmental factors [32]. For quality, the call rates of SNP < 0.90, Hardy–Weinberg equilibrium p values < 0.001 or minor allele frequencies (MAFs) < 0.01 were excluded in this study. Significant interaction was identified at p value < 5.0×10^{-8}. Circular Manhattan plots were generated using the "CMplot" R script (https://github.com/YinLiLin/R-CMplot).

Table 1. The associations between long-term antibiotic use during early life and mental traits.

	Samples	Female	Age, years	Regression coefficient	*p* value
Long-term antibiotic use during early life—Depression	115,129	65,339	55.92 ± 7.68	0.25	2.01×10^{-195}
Long-term antibiotic use during early life—Anxiety	115,601	65,590	55.91 ± 7.68	0.12	2.75×10^{-47}
Long-term antibiotic use during early life—Frequency of smoking	131,649	75,426	55.77 ± 7.74	0.10	7.61×10^{-40}
Long-term antibiotic use during early life—Intelligence	56,347	31,815	56.39 ± 7.82	−0.13	2.64×10^{-26}
Long-term antibiotic use during early life—Remembrance	16,597	9270	56.14 ± 7.90	−0.10	1.74×10^{-6}
Long-term antibiotic use during early life—Frequency of drinking	129,221	69,793	56.01 ± 7.71	−0.01	1.07×10^{-2}

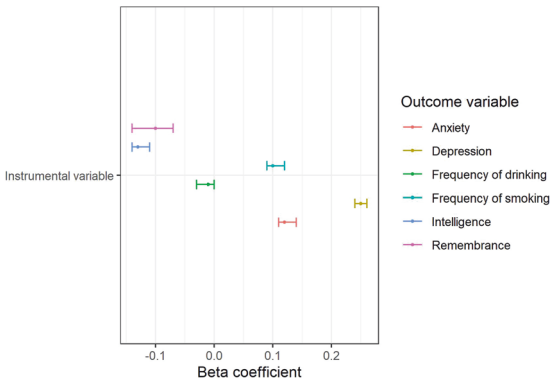

Fig. 1 The beta coefficient for long-term antibiotic use during early life and mental traits. The X-axis refers to the value of beta coefficient. The Y-axis represents the outcome variables, including anxiety, depression, frequency of drinking, frequency of smoking, intelligence and remembrance. The instrumental variable represents the long-term antibiotic use during early life. Points display the beta and 95% CIs (error bars) of beta.

Gene set enrichment analyses

To explore the functional relevance of identified genes interacting with long-term antibiotic use during early life, GO and pathway enrichment analyses of the identified target genes were performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool (https://david.ncifcrf.gov/).

RESULTS

Observational analyses results

A total of 158,444 individuals with long-term antibiotic use during early life data were included in this study. We observed positive associations between long-term antibiotic use during early life and anxiety (p value $= 2.75 \times 10^{-47}$, $b = 0.12$), depression (p value $= 2.01 \times 10^{-195}$, $b = 0.25$) and the frequency of smoking (p value $= 7.61 \times 10^{-40}$, $b = 0.10$). In addition, we found negative associations between long-term antibiotic use during early life and remembrance (p value $= 1.74 \times 10^{-6}$, $b = -0.10$), intelligence (p value $= 2.64 \times 10^{-26}$, $b = -0.13$) and the frequency of drinking (p value $= 1.07 \times 10^{-2}$, $b = -0.01$). The basic characteristics of study subjects and detailed information are presented in Table 1 and Fig. 1.

Genome-wide environmental interaction analyses results

For anxiety, we detected multiple significant genes interacting with long-term antibiotic use during early life. For instance, we detected eight SNPs located in TLX1NB, such as rs78872526 (p value $= 2.28 \times 10^{-9}$), rs79769150 (p value $= 3.02 \times 10^{-8}$), and rs79286138 (p value $= 2.65 \times 10^{-8}$). In addition, we detected eight SNPs located in FBN2, such as rs114452724 (p value $= 1.63 \times 10^{-9}$), rs148469465 (p value $= 1.66 \times 10^{-9}$), and rs28763939 (p value $= 1.08 \times 10^{-8}$). For depression, we also observed several significant genes-long-term antibiotic use during early life interactions, such as STRN (rs140049205, p value $= 1.88 \times 10^{-8}$), SLC8A1-AS1 (rs72791062, p value $= 1.45 \times 10^{-8}$), and MMD2 (rs34858681, p value $= 4.25 \times 10^{-8}$). The significant SNPs are presented in Table 2 and the circular Manhattan plots are shown in Fig. 2.

GO-enrichment analysis results

GO-enrichment analysis identified six GO terms enriched in the identified genes interacting with long-term antibiotic use during early life for anxiety, such as GO:0007528–neuromuscular junction development (p value $= 2.47 \times 10^{-2}$), GO:0005886–plasma membrane (p value $= 3.64 \times 10^{-3}$), and GO:0030425–dendrite (p value $= 3.41 \times 10^{-2}$). The detailed information is shown in Table 3.

DISCUSSION

Mental traits are the result of multiple genetic and environmental factors which may interact in complicated ways. To evaluate the roles of long-term antibiotic use during early life on the development of mental traits, we conducted an integrative analyses of observational study with GEWIS in UK biobank. We observed that long-term antibiotic use during early life was associated with mental traits. Considering that G × E interactions determine common disease risk factors and biomedically relevant complex traits [33], we performed the GEWIS to assess the potential interactions between long-term antibiotic use during early life and genetic factors for mental traits. The associations between long-term antibiotic use during early life and anxiety and depression were also replicated in GEWIS.

The population-based observational analyses found the positive associations of long-term antibiotic use during early life with anxiety and depression, which were consistent with previous published epidemiological study [14] and animal study [15]. In particular, growing experimental evidences suggested that long-term antibiotic use during early life may have significant detrimental consequences for increasing vulnerability to mental disorders [14, 16, 18, 34, 35]. The antibiotic treatment would alter the gut microbiome [36], which may influence gut–brain communication and then alter the trajectory of brain development [15]. It has been shown that the gut microbiota can be one determinant for long-term complex behavioral skills, such as social interactions, risk perception, and anxiety [34]. One experimental study revealed that low-dose penicillin in late pregnancy and early postnatal life has lasting effects on gut microbiota in mice, which would increase cytokine expression in frontal cortex, modify blood–brain barrier integrity and alter behavior. Moreover, the antibiotic exposure exhibited impaired anxiety-like and social behaviors in mice [35].

One important result of GEWIS is the disclosure of the Ankyrin 3 (ANK3), which showed correlation evidence with the anxiety [37–39]. ANK3 encodes AnkyrinG (AnkG), which was originally found at the axonal initial segment and nodes of Ranvier of neurons in the central and peripheral nervous systems [37]. ANK3 has been strongly implicated as a risk gene in many neuropsychiatric and neurodevelopmental disorders in human [37, 38].

Table 2. Identified SNPs for anxiety and depression ($p < 5.0 \times 10^{-8}$).

Anxiety						Depression		
SNP	Gene	*p* value of interaction	SNP	Gene	*p* value of interaction	SNP	Gene	*p* value of interaction
4:158853135_CTTT_C		4.57×10^{-12}	rs111658275		1.59×10^{-8}	2:37154458_CTGTGCAA_C		9.76×10^{-10}
rs77684557	FBN2	5.38×10^{-11}	rs773585997	ANK3	1.73×10^{-8}	rs601785		4.64×10^{-9}
rs34343197	LRRC20	2.32×10^{-10}	rs117643427	TLX1NB	1.74×10^{-8}	rs648904		4.67×10^{-9}
rs180851257	AC058822.1、CHIC2	3.46×10^{-10}	rs73124001		1.77×10^{-8}	rs681360		4.80×10^{-9}
rs35221250	NPFFR1	7.08×10^{-10}	rs73129945		1.80×10^{-8}	rs505166		4.90×10^{-9}
rs115350425	PDE4B	1.18×10^{-9}	rs35340924	AC116366.3、C5orf56	1.82×10^{-8}	rs504177		4.92×10^{-9}
rs114452724	FBN2	1.63×10^{-9}	rs76261103	CASC9	1.90×10^{-8}	rs680372		4.94×10^{-9}
rs148469465	FBN2	1.66×10^{-9}	rs765409009	LRRC20	2.05×10^{-8}	rs588169		4.99×10^{-9}
rs80033324	FBN2	1.89×10^{-9}	rs111770456		2.06×10^{-8}	rs552025		5.01×10^{-9}
rs3805635	FBN2	1.90×10^{-9}	rs71645571		2.12×10^{-8}	rs474620		5.06×10^{-9}
rs181056150		1.98×10^{-9}	rs76326207	AP003066.1	2.17×10^{-8}	rs622328		5.17×10^{-9}
rs72743649		2.24×10^{-9}	rs79387392	TLX1NB	2.35×10^{-8}	rs141364585		8.26×10^{-9}
rs75641268	FBN2	2.25×10^{-9}	rs66496417	TLX1NB	2.50×10^{-8}	3:175762717_ATAT_A		8.88×10^{-9}
rs78872526	TLX1NB	2.28×10^{-9}	rs191065934	TRPC6	2.62×10^{-8}	rs116393147		1.18×10^{-8}
rs554504775	FBN2	2.57×10^{-9}	rs79286138	TLX1NB	2.65×10^{-8}	rs72791062	SLC8A1-AS1	1.45×10^{-8}
rs34253529		2.75×10^{-9}	rs17313272	ZFPM2、ZFPM2-AS1	2.69×10^{-8}	rs140049205	STRN	1.88×10^{-8}
rs79931159		3.16×10^{-9}	rs116973723	TLX1NB	2.75×10^{-8}	rs114709366	AC009478.1	2.04×10^{-8}
rs77524295	NLGN1	3.89×10^{-9}	rs75127064		2.76×10^{-8}	rs1475391		2.47×10^{-8}
rs72743645		4.12×10^{-9}	rs77362007	SEC23IP	2.78×10^{-8}	5:166588008_CTG_C		2.58×10^{-8}
rs72793306		4.68×10^{-9}	rs80153007	KCNJ3	2.86×10^{-8}	rs17206544		3.79×10^{-8}
rs182739995	AL356534.1	6.07×10^{-9}	rs148569010	BRINP3	2.93×10^{-8}	rs34858681	MMD2	4.25×10^{-8}
rs111687473		6.38×10^{-9}	rs111726700		2.93×10^{-8}	rs61769782		4.41×10^{-8}
rs142073651	MSR1	6.49×10^{-9}	rs73137514		2.95×10^{-8}			
rs79432312	LINC02240、AC116362.1	6.70×10^{-9}	rs77959253	BBOX1、BBOX1-AS1	3.01×10^{-8}			
rs73136897	LINC02027	7.31×10^{-9}	rs79769150	TLX1NB	3.02×10^{-8}			
rs185260499		7.63×10^{-9}	rs117015324	AL356534.1	3.08×10^{-8}			
rs72743642		7.74×10^{-9}	rs79987105	AC116366.3、C5orf56	3.18×10^{-8}			
rs137882922	GFRA1	8.06×10^{-9}	rs2277260	SFXN3	3.28×10^{-8}			
rs72791277		8.09×10^{-9}	rs11712474	CACNA2D2	3.40×10^{-8}			
rs146123096		8.17×10^{-9}	rs149772093		3.53×10^{-8}			
rs143986132		8.34×10^{-9}	rs75959162		3.71×10^{-8}			
3:176096480_TG_T		8.82×10^{-9}	rs73129993		3.75×10^{-8}			
rs72701794		8.94×10^{-9}	rs73137517	LINC02027	4.05×10^{-8}			
rs80246073	AL356534.1	1.03×10^{-8}	rs9491621	AL356534.1	4.08×10^{-8}			
rs28763939	FBN2	1.08×10^{-8}	rs144984524	CASC9	4.27×10^{-8}			
rs181063355	AP003066.1	1.16×10^{-8}	rs76965325		4.49×10^{-8}			
rs142147122	TLX1NB	1.34×10^{-8}	rs76956944	RALYL	4.58×10^{-8}			
rs78419938		1.41×10^{-8}	rs301695	SLC9C2	4.65×10^{-8}			
rs9425731	SLC9C2、AL139142.1	1.58×10^{-8}						

SNP single nucleotide polymorphism.

In addition, it has been reported that knockout of the large isoforms of AnkG in mice would increase anxiety levels and anxiety-related behavior [37]. Similarly, researchers found that AnkG hemizygous mice exhibited elevated anxiety-like and depression-like traits, as well as cognitive impairment [40]. However, Leussis et al. [38] observed the paradoxical results. They found that ANK3 involved in the regulation of psychiatric-related behaviors and stress reactivity. And the behavioral alteration of reduced anxiety was exhibited in ANK3 knock-out mice. ANK3 has been implicated in the influence of anxiety-related personality traits [39]. BRINP3 is another anxiety-associated gene identified by GEWIS. Berkowicz et al. [41] observed that BRINP3−/− mice altered sociability and exhibited marked changes in anxiety-response on the elevated plus maze.

In addition, we also identified several candidate genes which may interact with long-term antibiotic use during early life for anxiety, such as NPFFR1, TRPC6, and GFRA1. Neuropeptide FF (NPFF) and its two cognate G protein-coupled receptors, NPFFR1 and NPFFR2, which have been reported to induce anxiety-like or depression-like behaviors [42]. In addition, NPFFR1 and NPFFR2 represent a new target system for therapeutic applications of anxiety [43]. Kim et al. [44] reported that transient receptor potential canonical (TRPC) channels involved in various patho-physiological functions, including seizure, anxiety-like behavior

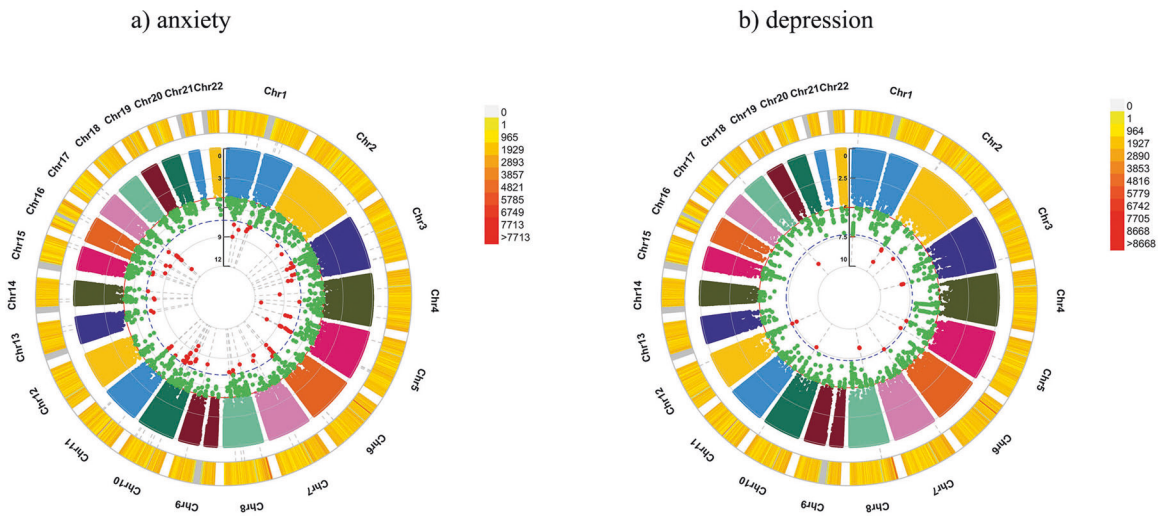

Fig. 2 Genomic regions interacting with long-term antibiotic use during early life on anxiety and depression. *From the center, the first circos depicts the $-\log_{10}$ *p*-values of each variant due to double exposure, i.e., the effect of both SNP allele and long-term antibiotic use during early life. The second circos shows chromosome density. Red plots represent the $p < 5 \times 10^{-8}$ and green plots represent $p < 1 \times 10^{-5}$. The plots were generated using the "CMplot" R script (https://github.com/YinLiLin/R-CMplot).

Table 3. List of anxiety-associated GO terms.

Category	Term ID	Term description	*p* value	Genes
BP	GO:0007528	Neuromuscular junction development	2.47×10^{-2}	ANK3, CACNA2D2
BP	GO:0045184	Establishment of protein localization	3.52×10^{-2}	ANK3, NLGN1
CC	GO:0005886	Plasma membrane	3.64×10^{-3}	CHIC2, MSR1, TRPC6, ANK3, NLGN1, GFRA1, SLC9C2, NPFFR1, CACNA2D2, KCNJ3
CC	GO:0005891	Voltage-gated calcium channel complex	2.52×10^{-2}	PDE4B, CACNA2D2
CC	GO:0030315	T-tubule	3.29×10^{-2}	ANK3, KCNJ3
CC	GO:0030425	Dendrite	3.41×10^{-2}	ANK3, NLGN1, BRINP3

GO gene ontology.

and many others. Glerup et al. [45] found that SorLA acted as sorting receptor for the GDNF/GFRA1 complex, directing it from the cell surface to endosomes. Interestingly, SorLA-deficient mice displayed marked hyperactivity and reduced anxiety.

In particular, GO analysis detected several GO terms associated with anxiety. One interesting result is dendrite (GO:0030425). Miller et al. [46] illustrated significant variability in dendritic morphology in the prefrontal cortex of healthy adult male rats, which was correlated with anxiety-like behavior. In another study, researchers found that higher levels of trait anxiety scores were associated with decreased densities of dendrites in CA3 region [47]. Of note, many neuropsychiatric disorders were characterized by dendritic and synaptic pathology [48]. Dendritic remodeling of bed nucleus of stria terminalis (BNST) neurons would facilitate anxiety after chronic stress [49]. Adamec et al. [50] found interesting research findings. First, they found that stress can cause neural expansion in basolateral amygdala dendrites related to the enhanced anxiety in extremely anxious animals. Moreover, they observed that rats with longer dendrites can predict greater anxiety [50]. In addition to the GO terms discussed above, the investigations about plasma membrane (GO:0005886) and voltage-gated calcium channel complex (GO:0005891) were also reported in the previous studies [51, 52]. For instance, Gilman et al. [51] reported that constitutive plasma membrane monoamine transporter deficiency can impact anxiety-like behaviors. SNPs in the CACNA1C, the a1C subunit of the voltage-gated L-type calcium channel Ca(v)1.2, were associated with major depression, schizophrenia, and bipolar disorder [52].

For depression, we have identified four candidate genes in which genetic effects may be modified by long-term antibiotic use during early life in GEWIS, such as striatin (STRN) and SLC8A1-AS1. Previous study reported that proteins of the STRN family are enriched in dendritic spines and are principally expressed in neurons [53]. STRN had differential expression in peripheral blood among the patients with major depressive disorder, subsyndromal symptomatic depression, and healthy controls [54]. Another significant result is SLC8A1-AS1 in the current study. Lisowski et al. [55] observed that SLC8A1 was upregulated in low analgesia mice, which was involved in calcium-signaling biochemical pathway that may impact physiological processes. Interestingly, the researchers speculated that the mechanisms of stress-induced analgesia could be a potential therapeutic target for depression-related disorders [55].

Besides confirming functional relevance of previously reported associations between antibiotic use with anxiety and depression [14, 15, 35], our study also observed negative associations between long-term antibiotic use during early life and remembrance, intelligence and the frequency of drinking, and positive

correlation between long-term antibiotic use during early life and the frequency of smoking. However, few efforts have been paid to elevate the associations between long-term antibiotic use during early life and remembrance, intelligence and the frequency of drinking and smoking. Certainly, there are three limitations of this study that should be noted. Firstly, all study subjects of this study were from the UK Biobank. Therefore, this study results should be interpreted with caution when applied to other populations due to the different genetic background. Secondly, it is helpful to discuss the identified SNP status to observed association to explore their biological mechanism implicated on the development of mental traits. However, there are no relevant studies to investigate the impact of identified SNPs on the biological mechanism of anxiety and depression. Further studies with large samples and biological studies are needed to confirm our findings and clarify the potential roles of novel genetic variants in the pathogenesis of mental traits. Finally, the phenotype of long-term antibiotic use during early life was defined as long-term or recurrent taking antibiotics as child or teenager in UK Biobank. The participants who received long-term or recurrent courses (three or more per year) of antibiotics during childhood or as a teenager were collected in UK Biobank. The collected data for antibiotic use is categorical variable and not continuous variable. And it is difficult to know the exact time of how long the sample took antibiotic. We will collect novel samples with more accurate data for antibiotic use, and investigate the associations between antibiotic use time and mental traits in our future studies.

In summary, utilizing observational study and GEWIS, we evaluated the impact of the long-term antibiotic use during early life on the development of the mental traits. We observed correlations between the long-term antibiotic use during early life and mental traits. We hope that our study results could provide novel clues for the pathogenic and precaution of mental traits.

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This research has been conducted using the UK Biobank Resource. The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

XL contributes to the design of the work, analysis of data and draft the paper; JY contributes to the part of data analysis and revises the paper; YW contributes to the part of data analysis; BLC contributes to the part of data analysis and revises the paper; PL contributes to the part of data analysis; SQC contributes to the artwork of Fig. 1; LL revises the paper; LZ revises the paper; MM revises the paper; XQ drafts the table work; CJL drafts the table work; XMC corrects the grammar issue; OPK corrects the grammar issue; YMJ approve of the version to be published; FZ contributes to the acquisition of UK biobank data and agree all aspects of the work in ensuring the work to be appropriately investigated and resolved.

ADDITIONAL INFORMATION

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