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Impact of sleep problems on the cardiometabolic risks: an integrated epidemiological and metabolomics study

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Abstract

Background We investigated the association between sleep problems and cardiometabolic risks and the potential linking effect of metabolites and metabolic pathways based on multi-layered research, including observational, mendelian randomization (MR), and metabolomics analysis.

Methods A cross-sectional analysis of the 2015–2018 National Health and Nutrition Examination Survey (NHANES) dataset was conducted to identify the association between sleep problems and cardiometabolic risks. A subsequent MR study based on genetic data was performed to explore the causal correlation of significant associations in the NHANES study. The underlying alteration of metabolism was explored by constructing zebrafish models and wide-targeted metabolomics analysis.

Results The cross-sectional analysis of the NHANES database revealed a significant association of snoring with obesity [OR = 2.65, 95% confidence intervals (CI): 1.87, 3.74]; sleep apnea with hypertension (OR = 1.68, 95% CI: 1.14, 2.48) and obesity (OR = 1.44, 95% CI: 1.05, 1.96); trouble sleeping with hypertension (OR = 1.84, 95% CI: 1.18, 2.86), obesity (OR = 1.56, 95% CI: 1.07, 2.26), and type 2 diabetes (T2DM) (OR = 1.52, 95% CI: 1.02, 2.25). MR analysis verified the causal relationship between genetically proxied sleep apnea or snoring and obesity. The decreased activity levels and altered expression levels of six circadian genes (*bmal1b*, *cry1aa*, *cry1ab*, *clock1a*, *per1b*, *per2*) were identified in the zebrafish of the sleep disorder group. Multiple metabolites related to disturbed glucose metabolism (e.g., 20-HETE), lipid metabolism (e.g., inosine), and vascular-related metabolites (e.g., riboflavin) were finally identified, indicating the latent effect of metabolism.

Conclusions This study identified the chain of sleep-circadian rhythm-metabolism-cardiometabolic risks. These findings can promote improved prevention implementation and therapeutic strategies.

Keywords Sleep disorder, Cardiometabolic risks, Circadian rhythm, Clock genes, Targeted metabolomics

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Introduction

According to the Global Burden of Disease (GBD), cardiovascular disease (CVD) is the leading cause of death and disability worldwide in 2019 [1]. Since the late 1940s, a large number of scientific research results have suggested some risk factors of CVD [2], mainly including behavioral risk factors, metabolic risk factors (hyperlipidemia, hypertension, diabetes, and obesity), grip strength, and so on [3]. With the rise of unhealthy lifestyles, the prevalence rate of obesity, metabolic disorders, and type 2 diabetes (T2DM) has significantly increased. 70% of CVDs and all-cause deaths can be attributed to a series of common changeable risk factors, of which 41.2% are metabolic risk factors [4].

CVD is associated with genetic factors and lifestyle, among which lifestyle is a key link in the treatment of obesity, T2DM, and hyperlipidemia. In addition to reasonable physical exercise and a healthy diet, healthy sleep behavior is considered an essential lifestyle for preventing and treating CVDs [5, 6]. The most common type is insomnia, with approximately 5–15% of the population in the United States experiencing varying degrees of insomnia [7]. In addition, sleep problems include sleep apnea syndrome, snoring, and daytime sleepiness. Snoring is very common in the population, and the prevalence is about 20–40% [8]. Sleep apnea syndrome is another sleep problem, defined as an average of at least five apnea or insufficient breathing per hour of sleep [5].

Multiple studies support the association between sleep problems and cardiometabolic risk factors. Insufficient sleep can lead to hormonal disorders in the body and cause damage to various systems throughout the body. Some studies have shown that the duration and quality of sleep are related to the increased incidence of CVD, obesity, insulin resistance, and T2DM [5, 9]. FadaeiR et al. found that patients with sleep apnea symptoms have impaired HDL function [10], and successful management of sleep apnea symptoms positively impacts systolic blood pressure in hypertensive patients [11].

Most of the research is cross-sectional and belongs to clinical research, making it challenging to identify causal relationships and elucidate the specific mechanisms underlying the correlation between sleep problems and metabolic risk factors of CVD.

As a diurnal animal, zebrafish have the same sleep pattern as humans. At least 70% of the zebrafish genome sequence is homologous to the human genome, and the regulation mode of the biological clock of is similar, zebrafish is an ideal model for exploring the sleep and biological clock [12]. Therefore, this study combined the National Health and Nutrition Examination Survey (NHANES) with Mendelian Randomization (MR) to verify our hypothesis that there were potential causal relationships between sleep problems and cardiometabolic risks. Construction of zebrafish models with sleep disorders and metabolomics analysis were conducted to identify the underlying mechanism of the connection between sleep problems and cardiometabolic risk factors from the circadian rhythm and metabolism perspective. In the meantime, it is hoped that regulating sleep problems can be a more cost-effective way to control the development of cardiometabolic risks.

Methods

Study design

As shown in Fig. 1A, this study first explored the relationship between sleep problems and cardiometabolic risks through the NHANES database. MR analysis was conducted to explore the causal relationship between sleep problems and cardiometabolic risks based on the significant results of cross-sectional NHANES studies. To further explore the underlying mechanism bridging the association of sleep problems and cardiometabolic risks, zebrafish models of sleep disorders were constructed and sent for metabolomics analysis to discover altered metabolic pathways.

NHANES analysis

NHANES is an epidemiological survey project that utilizes multistage, complex, and probabilistic sampling methods conducted by the National Health Survey Center (NCHS). It aims to assess the health and nutritional status of adults and children in the United States.

The data used in this study was from NHANES 2015–2018. As presented in Fig. 1B, the participants aged < 20 years or pregnant or with cancer or malignancy were excluded.

Participants with missing data on exposures and outcomes were subsequently excluded, as were participants < 18 years old at the time of their first diagnosis of diabetes.

NHANES has obtained permission from the Institutional Review Committee of the Centers for Disease Control and Prevention (CDC), and all participants have signed informed consent forms before the investigation.

Definition of exposures, outcomes, and covariables

The five sleep behaviors (sleep time, snoring, sleep apnea symptoms, excessive daytime sleep, and trouble sleeping) obtained from self-report in the questionnaire were defined as the exposure variables. The outcomes were defined as cardiometabolic risk factors, which included hypertension, obesity, hyperlipidemia, and T2DM.

Covariates included age, marital status, sex (biological sex, male/female), education level, race, triglyceride (TG), physical activity category, total calories taken, alcohol intake, family income-to-poverty ratio (PIR), sedentary time, and tobacco exposure.

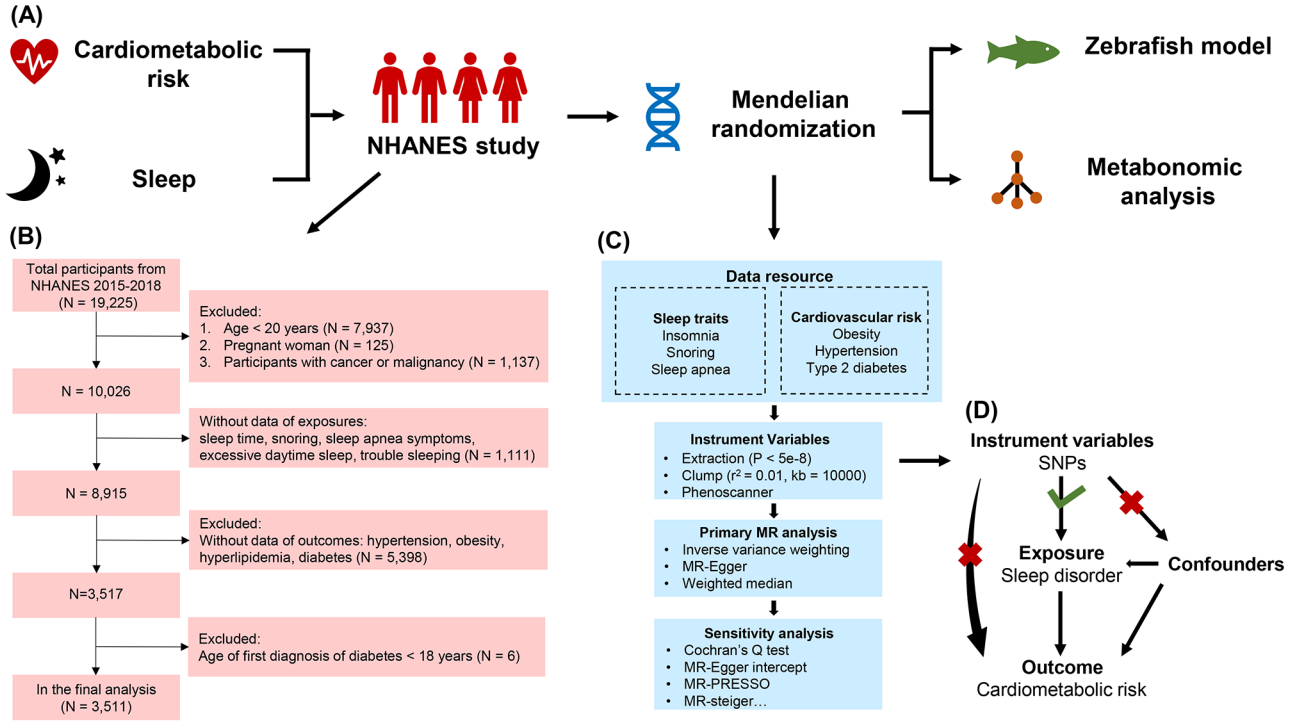

Fig. 1 Research scheme and flowchart of data process. (**A**) The schematic illustration comprised the NHANES study, Mendelian randomization analysis, zebrafish model construction, and metabolomics analysis. (**B**) The flowchart of the participants from NHANES 2015–2018. (**C**) Data resource and study design of our MR analysis. (**D**) Three assumptions in the MR analysis

Statistical analysis

All analyses used weighted sampling weights, stratification, and primary sampling units to make the results more nationally representative. The standard for selecting covariates was to change the effect value by more than 10%. The missing variables of the covariate were divided into a separate group and named "Missing". Data were described as means and SDs for normally distributed continuous variables and as medians and interquartile ranges for nonnormally distributed continuous variables. The chi-square test was used for categorical variables (%). Three weighted logistic regression models (model 1: adjusted no variables; model 2: adjusted age, race, sex; model 3: adjusted sex, race, age, education level, marital status, TG, physical activity category, total calories taken, alcohol intake, PIR, sedentary time, and tobacco exposure) were employed to calculate the regression odds ratio (OR) and 95% confidence intervals (CIs) between five sleep problems and cardiometabolic risk factors.

All data analyses were conducted using software R (version 4.2.1, R core team) and EmpowerStats (version 4.0, EmpowerStats core team). $P<0.05$ (two-sided) was considered significant.

MR analysis

As shown in Fig. 1C, a bi-directional MR study was conducted, following the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline for MR (STROBE-MR), to explore the potential causal relationship between three sleep problems (insomnia, snoring, sleep apnea) and three cardiovascular risks (obesity, hypertension, T2DM), which has been preliminarily identified in the NHANES study. Single-nucleotide polymorphism (SNP) was extracted as instrument variables (IVs) from previous summary-level GWAS data on sleep apnea [13], snoring [14], and insomnia [14]. While GWAS data on snoring and insomnia was generated from the UK biobank, GWAS data on sleep apnea was from a meta-analysis across five independent cohorts from the United Kingdom (UKB), Canada (Canadian Longitudinal Study of Aging, CLSA), the United States (Partners Healthcare Biobank), Australia (Australian Genetics of Depression Study, AGDS), and Finland (FinnGen) [13]. GWAS data on insomnia, snoring, and sleep apnea included the following covariates, namely, age, sex, and genetic principal components. GWAS summary statistics of outcomes were acquired from the research of the FinnGen consortium [15]. The eligibility criteria, methods of assessment, diagnostic criteria for diseases, ethics committee approval, and participants' consent can be found in the original articles. We checked the sample description of each GWAS study to ensure that the GWAS data used for exposure was independent of those for the outcomes. For snoring and insomnia, all participants were

from the UK biobank, indicating there wasn't a sample overlap between snoring-cardiometabolic risks or insomnia-cardiometabolic risks. For sleep apnea, 66,216 participants were from the FinnGen consortium, leading to a sample overlap proportion of 12.65% (66216/523366). For IVs to be valid, selected IVs should conform to three assumptions in Fig. 1D: (1) SNPs were robustly associated with sleep problems; (2) SNPs were not related to confounders; (3) SNPs did not affect the cardiometabolic risks except through the potential effects of the sleep problems. After harmonization, a bi-directional MR was performed to calculate the MR estimate of each sleep problem-cardiovascular risk pair by multiple methods, including Inverse weighted median (IVW), Weighted median (WME), and MR-Egger. Comprehensive sensitivity analyses such as heterogeneity, pleiotropy, and directionality were conducted to exclude potential bias.

Instrumental variables selection

SNPs closely ($P<5\times10^{-8}$) associated with sleep problems were extracted as candidate IVs. After SNPs were extracted at a significance level of $P<5\times10^{-8}$, they were clumped ($r^2<0.01$, kb > 10000, population = EUR) to guarantee the independence of each SNP. SNPs that directly related to outcomes were identified by Phenoscanner and were removed to reduce pleiotropy bias. Palindromic SNPs were subsequently excluded, and the F statistic of each IV was calculated. Sample sizes, number of controls and cases, population, and consortium of the GWAS were listed in Table 1.

Mendelian randomization study

After the harmonization, a two-sample MR analysis by the Two-Sample MR R package (version 0.5.6) was performed. The IVW method was used to calculate combined association across the Wald ratios for all IVs. Although the IVW method possessed strong statistical power, this method required all included instruments to be valid. Therefore, we also included results of MR-Egger, WME, and weighted mode providing unbiased reference even in the presence of invalid IVs [16]. Results of IVW with multiplicative random effect were referred to in the presence of heterogeneity. The mean F-statistics of each exposure generated from the calculation of the F statistic of each SNP was applied to exclude potential weak-instrument bias. Moreover, the significance *P* value was set as 0.0167 (0.05/3) based on bonferroni correction to exclude multiple-test bias.

The MR-PRESSO test by MR-PRESSO R package (1.0) was conducted to exclude horizontal pleiotropic outliers for the pleiotropy correction. The MR-Egger intercept result was considered an additional reference of pleiotropy. MR-PRESSO results after excluding outliers were referred to in the presence of pleiotropy. The Q tests of IVW and MR-Egger were performed to identify potential heterogeneity. In addition, the directionality between the exposure and outcome was validated by the results of the MR-Steiger analysis. Leave-one-analysis recalculated the MR estimate after excluding SNPs individually to identify whether a single SNP drove the bias of MR results. All data analyses were conducted using software R (version 4.2.1, R core team). The procedures of reverse MR analysis were the same.

Experimental animals-zebrafish

Zebrafish husbandry and construction of sleep disorder model

Adult wild-type AB zebrafish were cultured in a circulating water system at a temperature of 28.5±1℃. They were fed three times daily and given a 14:10 h light-dark (LD) cycle. Pair male and female zebrafish on the night before and lay eggs within 1 h of turning on the light the following day to obtain embryos. The obtained embryos were placed in a light-controlled (14:10 h LD) incubator at 28.5℃, and cultured with Holt Buffer containing 0.5 mg/L methylene blue.

Embryos that were well developed at 1-day postfertilization (dpf) were selected and divided into two groups. The control group was given a 14:10 h LD cycle lasting three days. The experimental group was given a 24 h light-light (LL) environment for three days to construct a sleep disorder model.

Analysis of behavior after building the sleep-disorder model

After the construction of the model, 144 (repeat three times with 48 larvae each group) larvae in each group were randomly selected for motor behavior detection. The movement of zebrafish was tracked using the

Table 1 Characteristics of included GWAS summary-level data of sleep, cardiovascular risks

Variable	Consortium of study	Sample size	Population	Journal/GWAS ID	Year	PMID
Sleep						
Insomnia	CNCR CTG lab	386,533	European	Nature Genetics	2018	30,804,565
Snoring	CNCR CTG lab	359,916	European	Nature Genetics	2018	30,804,565
Sleep apnea	NA	362,638	European	Sleep	2022	36,525,587
Obesity	FinnGen	218,735	European	finn-b-E4_OBESITY	2021	/
Type 2 diabetes	FinnGen	215,654	European	finn-b-E4_DM2	2021	/
Hypertension	FinnGen	218,754	European	finn-b-I9_HYPTENS	2021	/

zebrafish behavior analysis system, and two environments of 14:10 h LD and 24 h dark-dark (DD) were set, respectively.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Samples (repeat three times with 30 larvae each group) every 4 h for a total of 24 h were collected, and the total RNA content was determined by NanoDrop (NanoDrop, DE, USA). The purity and quality of RNA were determined by 260/280nm ratio and agarose gel electrophoresis. The first-strand cDNA was synthesized by the Prime-Script RT Reagent Kit. Furthermore, relevant instruments (StepOnePlus™ Real-Time PCR System, ABI) were used for RT–qPCRs. Internal reference group *β-actin*, with the $2^{-\Delta\Delta CT}$ method, was applied to calculate the relative changes of each gene (*bmal1b*, *cry1aa*, *cry1ab*, *clock1a*, *per1b*, *per2*). The primers for each gene are shown in Supplementary Table S1.

Widely targeted metabolomics analysis

The 150 larvae (repeat three times with 50 larvae each group) from the control group (normal light-dark cycle, 14:10 h LD) and experimental group (continuous light cycle for 3 days, 24 h LL) were collected at 9:00 and 21:00, respectively, for targeted metabolomics analysis. Use R software to perform principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) dimensionality reduction analysis on the samples. Metabolites with P value < 0.05 and VIP value > 1 were selected (considered to have significant statistical differences).

Statistical analysis

SPSS 26.0 and GraphPad Prism 8.4 were used for statistical analysis. If the P value of the variance homogeneity test is greater than 0.05, the T-test is used. If the P value of the result of the homogeneity test of variance is less than 0.05, the T_2 test is used. $P < 0.05$ was deemed statistically significant.

Results

NHANES results

Characteristics of study objects

The study objects' socio-demographic characteristics and health indicators stratified by sex were summarized in Supplementary Table S2. Of 3,511 objects, 48.87% were males. Age, sleep time, TG, race, marital status, hyperlipidemia, physical activity category, snoring, sleep apnea symptoms, trouble sleeping, excessive daytime sleep, total calorie intake, alcohol intake, and tobacco exposure all differed significantly across sex ($P < 0.05$).

Association of five sleep problems with cardiometabolic risk factors

We constructed three models (model 1: no covariables were adjusted, model 2: sex, age race were adjusted, model 3: all the covariables were adjusted) to explore the associations between five sleep problems and cardiometabolic risk factors (Supplementary Tables S3-S4 and Table 2).

As shown in model 1 (Supplementary Table S3), snoring, sleep apnea symptoms and insomnia were significantly positively correlated with all cardiometabolic risk factors (hypertension, hyperlipidemia, obesity, and T2DM). There was a significant positive correlation between excessive daytime sleep and obesity.

In model 2 (Supplementary Table S4), there were significant positive correlations between insomnia and hypertension, obesity, and T2DM. Snoring and sleep apnea symptoms were significantly positively correlated with hypertension, hyperlipidemia, and obesity. Excessive daytime sleep was positively correlated with hypertension and obesity ($P < 0.05$).

As shown in model 3 (Table 2), there were insignificant correlations with all cardiometabolic risk factors regarding sleep time and excessive daytime sleep. In terms of snoring, it was positively and significantly associated with obesity (OR = 2.65, 95% CI: 1.87, 3.74). In terms of sleep apnea symptoms, it was positively and significantly associated with hypertension (OR = 1.68, 95% CI: 1.14, 2.48) and obesity (OR = 1.44, 95% CI: 1.05, 1.96). In terms of trouble sleeping, it was positively and significantly associated with hypertension (OR = 1.84, 95% CI: 1.18,

Table 2 Association of five sleep problems with cardiometabolic risk factors after adjusted all the covariables

Exposures	OR (95% CI)			
	Hypertension	Hyperlipidemia	Obesity	Type 2 diabetes
Sleep time, hours	0.99 (0.87, 1.12)	1.02 (0.89, 1.15)	0.96 (0.86, 1.06)	1.03 (0.92, 1.15)
Snoring	1.56 (0.95, 2.56)	1.28 (0.89, 1.84)	2.65 (1.87, 3.74) ***	1.30 (0.88, 1.91)
Sleep apnea symptoms	1.68 (1.14, 2.48) *	1.29 (0.75, 2.21)	1.44 (1.05, 1.96) *	1.36 (0.75, 2.49)
Trouble sleeping	1.84 (1.18, 2.86) *	1.05 (0.67, 1.65)	1.56 (1.07, 2.26) *	1.52 (1.02, 2.25) *
Excessive daytime sleep	1.36 (0.92, 2.01)	0.97 (0.66, 1.44)	1.21 (0.86, 1.70)	1.27 (0.83, 1.95)

Note: Age, race, sex, triglyceride, sedentary time, physical activity category, total calories take, alcohol intake, family income-to-poverty ratio (PIR), tobacco exposure, education level and marital status were adjusted, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

2.86), obesity (OR=1.56, 95% CI: 1.07, 2.26), and T2DM (OR=1.52, 95% CI: 1.02, 2.25).

MR results

Based on the significant results of the NHANES study, three sleep problems (insomnia, sleep apnea, snoring) and three cardiovascular risk outcomes (hypertension, obesity, T2DM) were included for MR analysis. After clumping and harmonization, the MR analysis was conducted between each exposure and outcome to explore whether the potential causal relationship existed. IVs that were finally included for MR analysis were exhibited in Supplementary Table S5. F statistics were larger than 10, excluding weak instrument bias.

After multiple-testing correction, as shown in Fig. 2A, the causal correlation of snoring-obesity (P_{IVW} = 0.002496) was identified, and sensitivity analyses including the leave-one-out analysis in Fig. 2B indicated the robustness of the result. Figure 2C and D also exhibited the robust correlation of sleep apnea-obesity (P_{IVW} = 0.000547). While the OR of snoring with obesity was 1.38 (95% CI: 1.12, 1.70), the OR of obesity per 1-standard deviation increase in genetically predicted sleep apnea was 3.37 (95% CI: 1.69, 6.72). Heterogeneity test (Q test) and pleiotropy test (MR-Egger and MR-PRESSO) did not identify heterogeneity or pleiotropy that may violate the validity of the assumptions. As for the reverse MR result, although *P* values of hypertension-sleep apnea, obesity-sleep apnea, obesity-insomnia, and obesity-snoring were lower than 0.05, these associations did not pass multiple-testing corrections. MR-PRESSO results also indicated that associations of obesity-cardiometabolic risks were probably influenced by pleiotropy. Thus, these results were not considered as significant. All MR results of three sleep problems on three cardiovascular risk outcomes were summarized in Supplementary Table S6, and all sensitivity analysis results were exhibited in Supplementary Table S7.

Behavioral experiment of zebrafish

Sleep disorder model of zebrafish was constructed and recorded as LL group (experimental group: given 24 h LL light environment for 3 days) and LD group (control group: given 14:10 h LD environment for 3 days). The behavior observation and activity evaluation of larvae was conducted under the condition of continuous

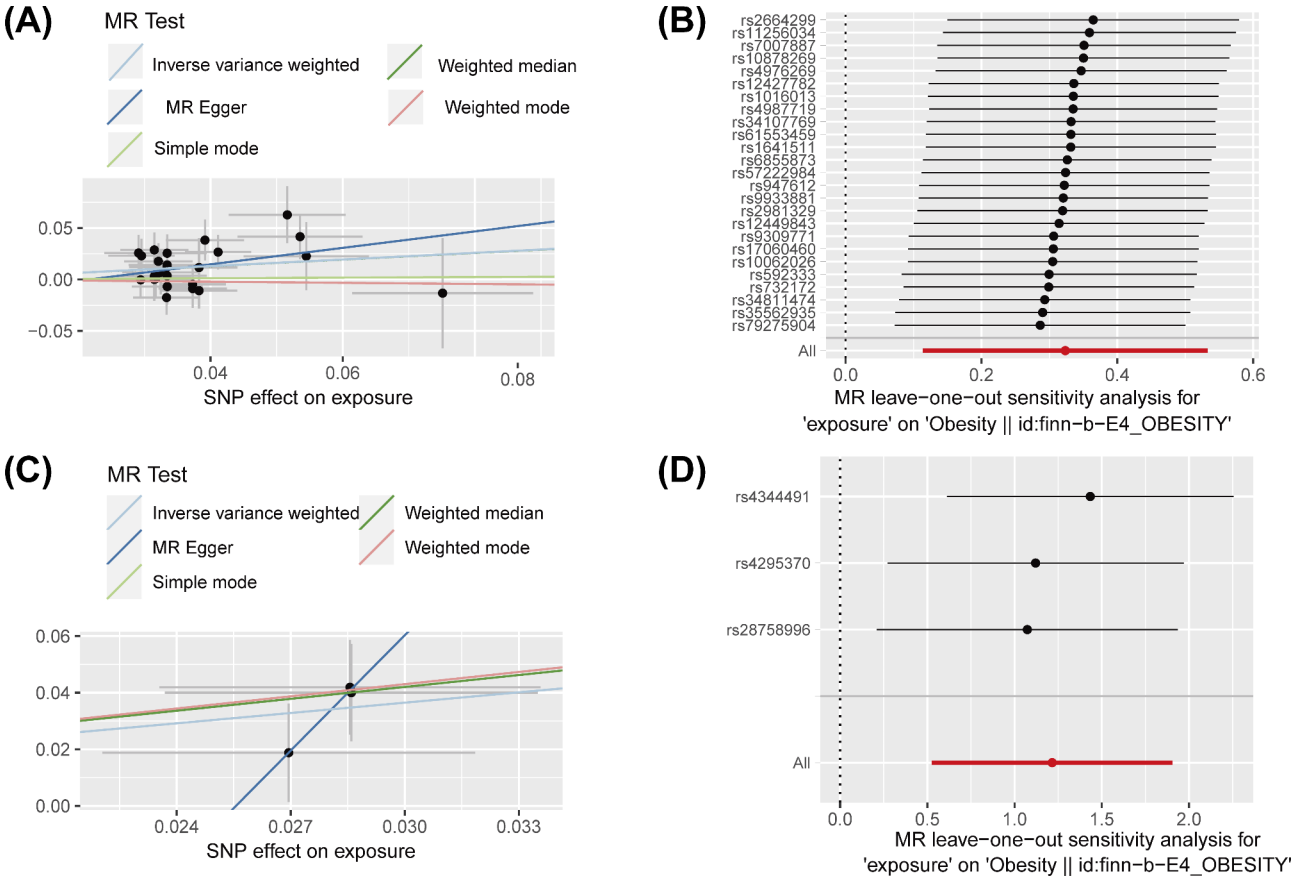

Fig. 2 Significant Mendelian randomization results. (**A**) Scatter plot and (**B**) leave-one-out analysis of snoring on obesity. (**C**) Scatter plot and (**D**) leave-one-out analysis of sleep apnea on obesity

darkness (DD) or regular light-darkness shift (LD) in the next two days (5 dpf-6 dpf).

As shown in Fig. 3A, the activity level of the LL group decreased in the LD observed condition compared with that of the LD group. Based on the statistical analysis in Fig. 3B, there was an apparent decrease in activity in the first dark state. However, no significant statistical difference was observed in other periods. Besides, Fig. 3C exhibited the decreased activity level of the LL group, a more extended period, a decrease in amplitude, and a shift of peak active time in the DD observed condition. The activity in the first expected daytime state and the second expected daytime state decreased significantly. However, there was no noticeable decrease in the activity amount in the expected dark state through the quantitative analysis of the activity in the DD state in Fig. 3D.

Alteration of circadian gene expression level

After construction of the sleep disorder model, samples of zebrafish were collected every 4 h the next day for the quantitative analysis of circadian gene expression level. Based on the RT-PCR results in Fig. 4A-F, compared with the regular circadian rhythm group (LD), *bmalb*, *clockl1a*, and *per2* expression levels decreased in the continuous light group (LL). In contrast, the expression levels of *cry1aa*, *cry1ab*, and *per1b* increased in the LL group.

Metabolomics signatures of sleep disorder

Zebrafish control or sleep disorder samples were collected in the 9 (LL_9 vs. LD_9) or 21 o'clock group (LL_21 vs. LD_21) and sent for widely targeted metabolomics analysis. Secondary metabolites were chosen for subsequent analysis because they were more specific and informative of metabolic pathways than primary metabolites. Figure 5A exhibits the amount of differentially expressed metabolites (up or down-regulation) in each group, and the cluster heatmap of the 9 and 21 o'clock group in Supplementary Figure S1-S2 showed distinct expression patterns of metabolites. As the circadian clock influences the expression level of genes, only 36 metabolites (Supplementary Table S8) in Fig. 5B whose expression levels were altered significantly at 9 and 21 o'clock were thought to be associated with sleep disorders. Scatter plots (X: mass-to-charge ratio, Y: *P* value) of secondary metabolites of the 9 o'clock group (LL_9 vs. LD_9) in Fig. 5C and the 21 o'clock group (LL_21 vs. LD_21) in Fig. 5D displayed the differentially expressed metabolites

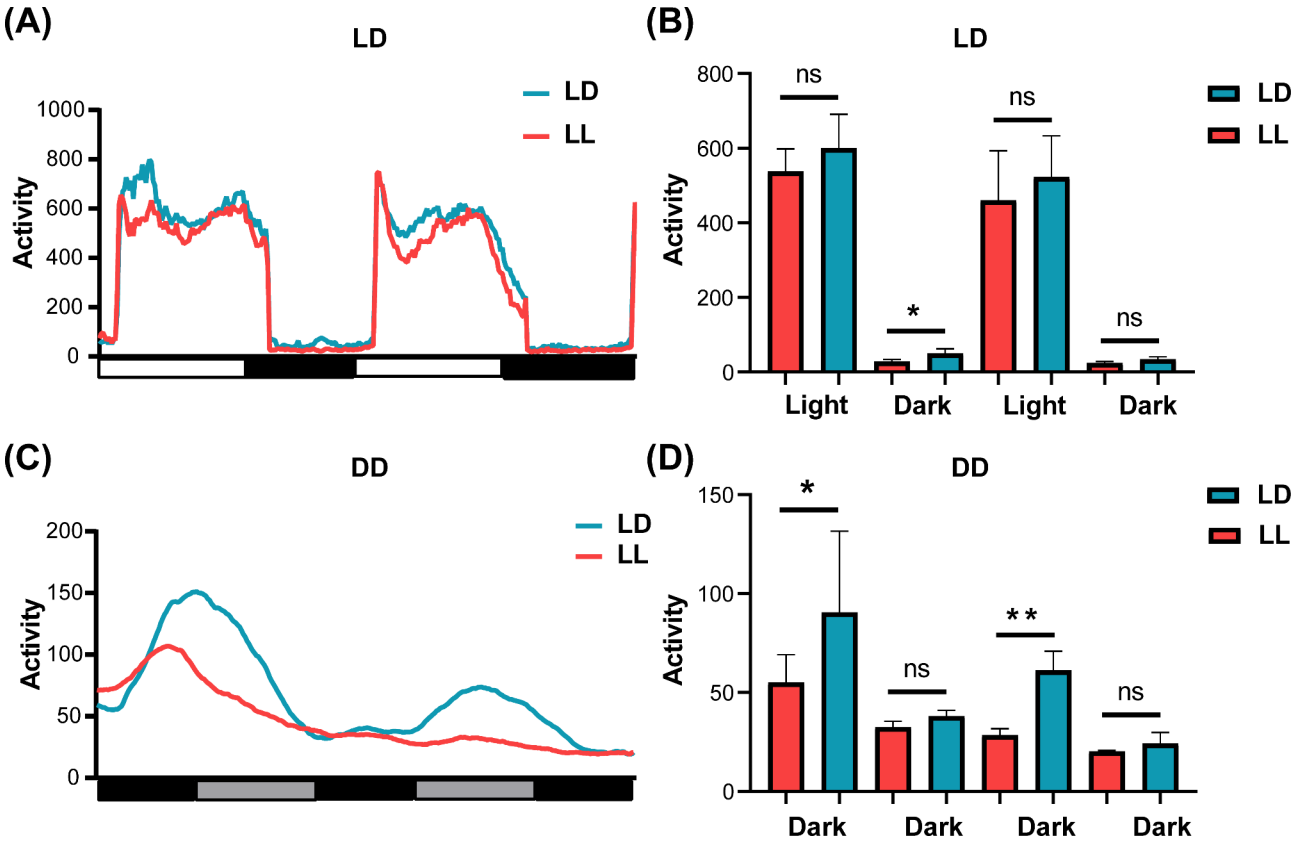

Fig. 3 Activity evaluation in the zebrafish model. (**A, C**) Curve graph of activity level alteration accompanied with the time of LD and LL group under the condition of (**A**) LD or (**C**) DD. (**B, D**) Comparison of mean activity levels of LD and LL groups in different periods (Light or Dark) under the condition of (**B**) LD or (**D**) DD

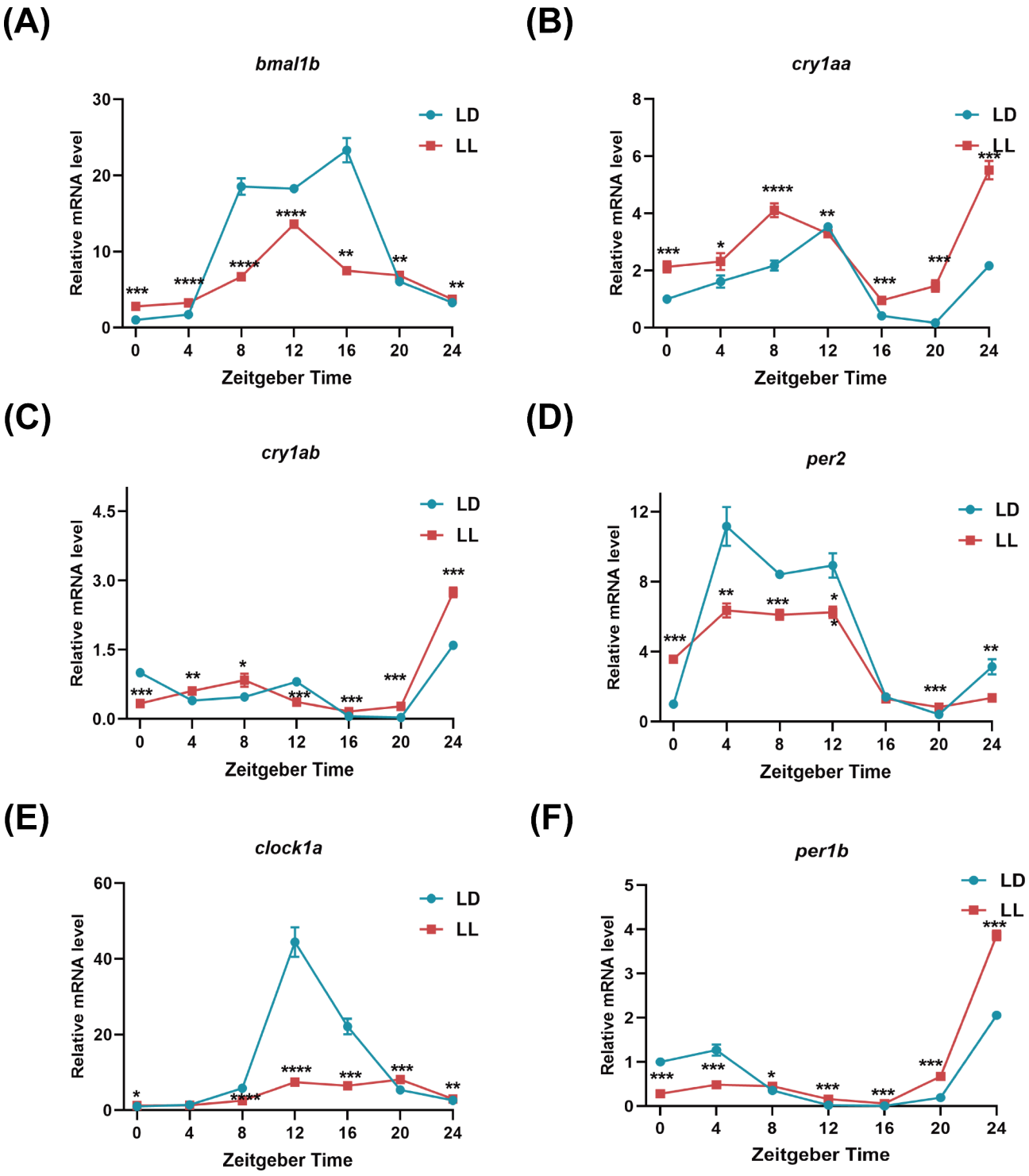

Fig. 4 Expression level of circadian rhythm genes in the zebrafish model. (**A-F**) Expression levels of (**A**) *bmal1b*, (**B**) *cry1aa*, (**C**) *cry1ab*, (**D**) *per2*, (**E**) *clock 1a*, (**F**) *per1b* in 24 h of LD and LL groups

after sleep disorder. Besides, multiple substances were known to be related to glucose or lipid metabolism and vascular endothelial function. Thus, the enrichment analysis was conducted to explore the underlying metabolism pathways. Based on the amounts of up or down-regulated metabolites after the normal sleep rhythm was interrupted by continuous light, differential abundance score figures in Fig. 6A-B were plotted. The formula calculated the DA score: DA score = (count of up-regulated metabolites-count of down-regulated metabolites)/ (total count

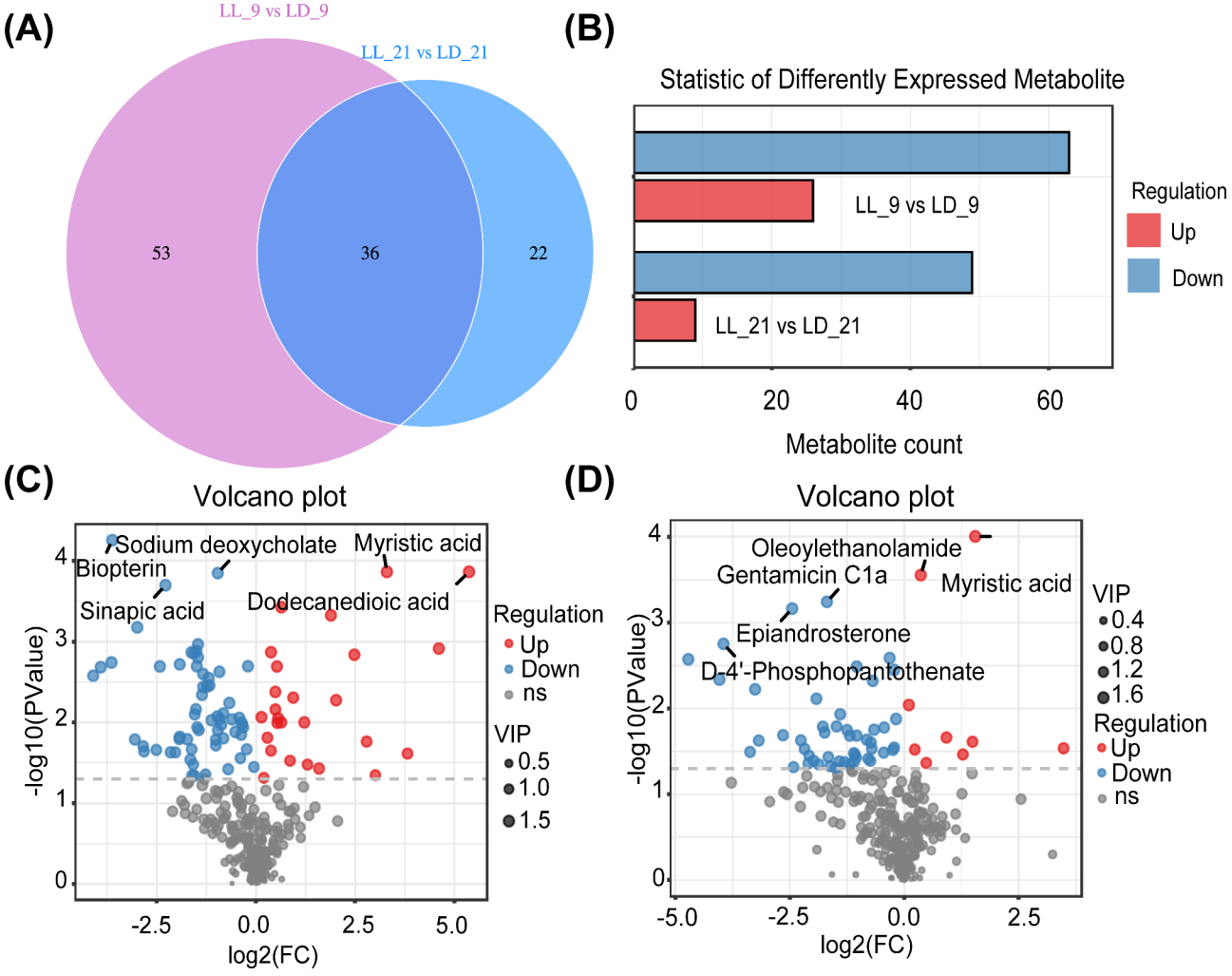

Fig. 5 Disturbed metabolism after sleep disorder based on the metabolomics results. (**A, B**) (**A**) Statistic and (**B**) Venn diagram of differentially expressed metabolites in 9 (LL_9 vs. LD_9) and 21 (LL_21 vs. LD_21) o'clock group. (**C, D**) Scatter plots of mass-to-charge ratio and *P*-value of differentially expressed metabolites in (**C**) 9 and (**D**) 21 o'clock group

of differentially expressed metabolites in that pathway) [17]. While arachidonic acid metabolism was activated, the activity of most metabolic pathways, such as glycolysis/gluconeogenesis, citrate cycle, and vascular smooth muscle contraction, were down-regulated.

Discussion

This is the first study that explores the correlation between sleep problems and cardiometabolic risks by combining NHANES, MR, and the metabolomics analysis of zebrafish models with sleep disorders.

NHANES, a cross-sectional study, preliminarily revealed the significant association of three types of sleep problems (snoring, sleep apnea symptoms, and trouble sleeping) with three types of cardiometabolic risk factors (hypertension, obesity, and T2DM). Subsequent MR analyses verified two causal relationships: genetically proxied snoring with obesity, and sleep apnea and obesity, which further indicated the close correlation of sleep problems and cardiometabolic risks. Multiple metabolites that expressed differentially after the normal circadian rhythm was interrupted (e.g., myristic acid, inosine, biliverdin) were related to numerous metabolic pathways such as inhibited citrate cycle and enhanced arachidonic acid metabolism.

The circadian rhythm, also known as the biological clock, is an endogenous timing system controlled by various genes that repeats approximately every 24 h, which is closely related to sleep [18, 19]. Several circadian genes are essential in human physiology, including *CLOCK*, *PER1*, *PER2*, *CRY1*, *and BMAL1* [19]. Sleep can affect the expression level of circadian genes, which was verified by our results. Disturbances in the biological clock also affect metabolism. The possible mechanism is that the *CLOCK* gene can regulate metabolic rhythm through rhythmic acetylating metabolic enzymes, thereby

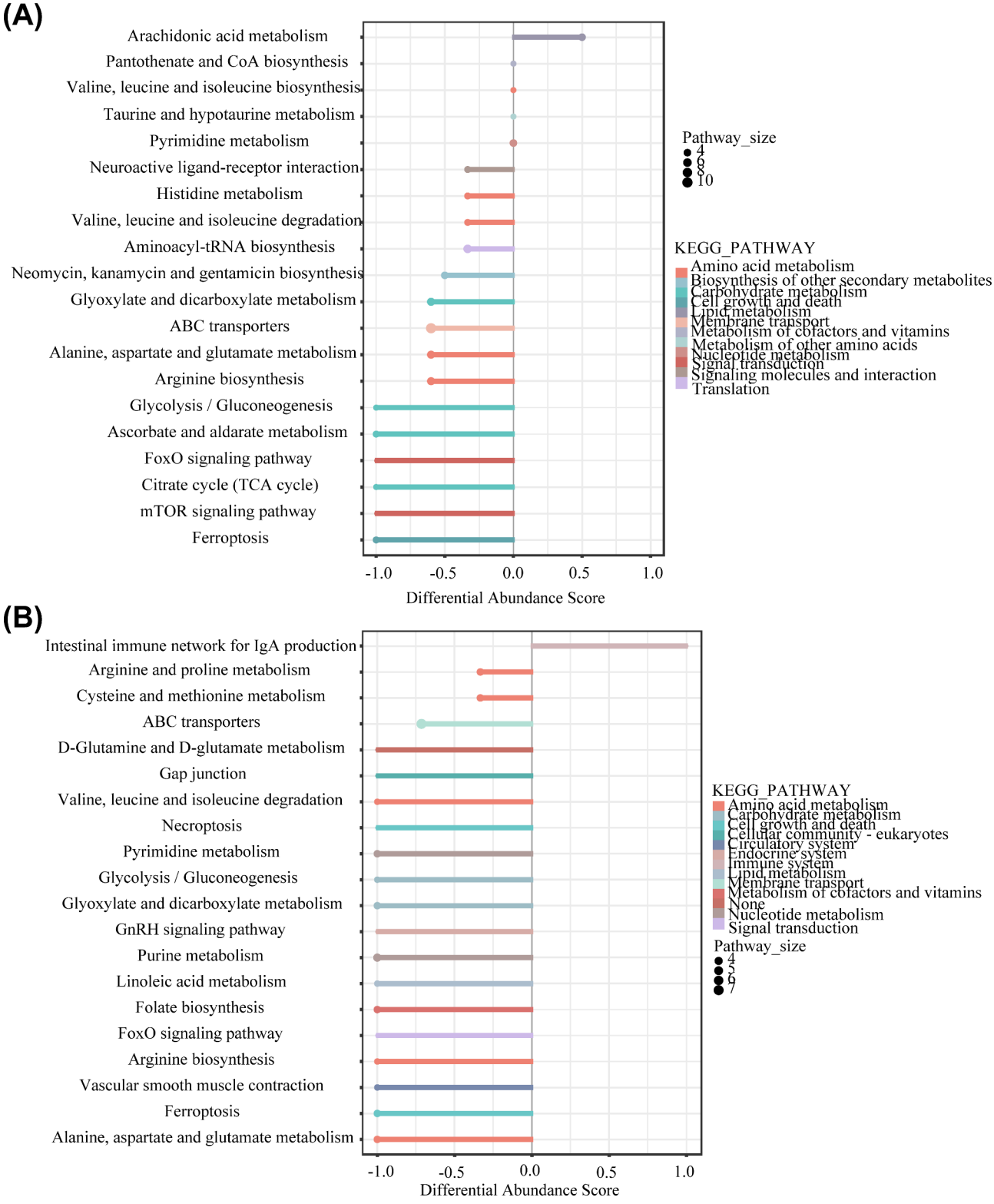

Fig. 6 Differential enrichment score map of metabolic pathways. (**A, B**) Differential abundance (DA) score map of (**A**) 9 and (**B**) 21 o'clock group. The ordinate is the metabolic pathway, and the size of the point at the top of the column indicates the number of differential metabolites enriched in this pathway

affecting the production of metabolites such as arginine and urea [20]. *CLOCK* and *PER2* genes regulate glutamate uptake levels [21]. In addition to metabolites, the expression of *BMAL1*, *CLOCK*, *CRY1*, and *PER2* genes in adipose tissue is associated with human metabolic syndrome [22]. *BMAL1* is vital in adipose tissue development, contributing to adipogenesis and differentiation [23]. In the meantime, genetic variations in the *BAML1* gene have been linked to T2DM and high blood pressure [24]. Therefore, the dysrhythmia of metabolites in zebrafish may be related to abnormal *clock*, *bmal1*, *per*, *cry* gene expression levels in the biological clock. Moreover, circadian disorders can interfere with human and animal behavior [25]. Seasonal light hours can modulate the peripheral clocks and eating behavior with consequential interference on energy metabolism [26]. And results of our research showed that zebrafish's activity levels in the model group (LL) were reduced compared to that of the control group (LD). The behavior alteration can exert an additional effect on human metabolism and cardiometabolic risks. Therefore, the sleep-circadian rhythm-metabolism-cardiometabolic risks link was proposed, which was discussed in detail below from the perspective of metabolism.

We found that snoring and sleep apnea were associated with obesity in both NHANES and MR analyses. Among 36 metabolites with significant changes identified in our research, inosine, myristic, and dodecanoic acid were associated with the development of obesity. In terms of lipid metabolism, while white fat stores excessive energy, brown and beige fat tissue is thermogenic, which can produce heat by energy consumption. Intriguingly, the expression level of inosine, a novel activator of brown adipose tissue (BAT) and energy homeostasis, decreased under continuous light exposure [27]. Inosine can stimulate the metabolism of BAT and activate energy expenditure (EE), which is a promising target to tackle the obesity pandemic [28, 29]. We postulated that the interference of sleep disorder on inosine release and function was a probable explanation of sleep-related obesity. Given the correlation results of sleep disorders (e.g., snoring, sleep apnea) and cardiometabolic risks (e.g., obesity), inosine and BAT were potential mediators bridging this connection. Besides, the correlation of fatty acid metabolism with sleep and cardiometabolic risks was also worthy of attention. Levels of saturated fatty acid (SFA) including dodecanoic acid and myristic acid were up-regulated in the group of sleep disorder (LL_9 and LL_21) compared with that of control (LD_9 and LD_21). In addition, the saturated fat-rich diet was believed to be more obesogenic than the lower SFA diet [30]. The association of increased SFA levels and obesity is an important therapeutic perspective for further improvements in obesity and related metabolic disorders [31].

In addition, we found that insomnia was associated with hypertension, T2DM, obesity, and sleep apnea is closely related to hypertension in the NHANES study. In the MR analysis, the limited GWAS data on sleep problems and cardiac metabolic risks reduced the possibility of verifying the significant correlation in the NHANES analysis. Given that, other significant results in NHANES analysis need to be further explored. In the meantime, we have found some metabolites associated with the development of hypertension and T2DM in 36 significant metabolites in our study.

Riboflavin, ruscogenin, and biopterin are associated with the development of hypertension. Riboflavin, a cofactor of methylenetetrahydrofolate reductase (MTHFR), which was previously reported that low or lack of riboflavin biomarkers in the human body can exacerbate the genetic risk of hypertension [32]. Ruscogenin, as a natural anti-inflammatory and antithrombotic agent, can weaken the pulmonary hypertension induced by monocrotaline in rats [33]. Biopterin is an essential cofactor in NO production, and its decrease in level is related to the pathogenesis of hypertension by increasing oxidative stress [34]. These metabolites are related to riboflavin metabolism and folate biosynthesis. Concerning glucose metabolism, 20-HETE is a lipid-based factor released from platelets, which can interfere with normal glucose and insulin homeostasis and promote insulin secretion from pancreatic β cells [35]. L-arabinose is a simple sugar not easily absorbed by the intestine and can improve insulin resistance and the intestinal environment [36, 37]. At the same time, it can act on intestinal flora to play an anti-obesity role [37]. It is related to the metabolic pathway of ascorbate and aldarate metabolism. L-glutamic acid is closely related to insulin secretion [38] and participates in glyoxylate and dicarboxylate metabolism, which plays a crucial role in the glycolysis of mitochondrial energy metabolism. Ethyl icosapentate (EPA) improves insulin resistance in non-insulin-dependent diabetes mellitus [39]. Metabolomic analysis showed reduced metabolite expression levels of 20-HETE, L-arabinose, and EPA in the sleep disorder groups (LL_9 and LL_21) compared to the control groups (LD_9 and LD_21). These results suggest that sleep disorders may be closely related to the occurrence of diabetes and hypertension.

Limitation

There are some limitations. First, sleep behaviors in the NHANES study were defined by the self-report of questionnaire, which was not reliable as other objective approach such as polysomnography monitoring. Secondly, limited GWAS data on sleep problems and cardiometabolic risks restricted the replication of MR results. As all GWAS datasets used in this research were from the

European ancestry, the generalizability to other populations in addition to the European ancestry was uncertain. Thirdly, we currently only explore the relationship between sleep disorders and cardiovascular metabolic risk factors by constructing the zebrafish sleep disorder model, which needs to be verified at other animal models (e.g., intermittent hypoxia model). The deeper mechanism needs to be explored. Finally, future prospective cohort studies in humans are needed to confirm this.

Conclusion

Our research first proposed and confirmed the correlation between sleep problems (snoring and sleep apnea) and cardiometabolic risks (obesity) based on cross-sectional observation of NHANES and genetic association study of MR analysis. Subsequent metabolomics analysis on zebrafish models of sleep disorders suggested that altered metabolites-related pathways, including lipid and glucose metabolism and vascular function, were potential mediators linking sleep problems and cardiometabolic risks. The chain of sleep-circadian rhythm-metabolism-cardiometabolic risks was proposed and ascertained. Sleep management was crucial for controlling cardiometabolic risks, and therapeutic targets related to circadian rhythm deserved attention for the therapy of cardiometabolic diseases. These findings would benefit further clinical research on patients with cardiometabolic diseases or sleep problems.

Supplementary Information

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Supplementary Fig. S1. Cluster heat map of differential metabolites at 9 a.m. Note: The column represents the sample, the row represents the metabolite, the cluster tree on the left is the differential metabolite cluster tree, and the top is the sample cluster tree. The gradient color represents the size of the quantitative value—the redder the color, the higher the expression, and the bluer the expression

Supplementary Fig. S2. Cluster heat map of differential metabolites at 9 p.m. Note: The column represents the sample, the row represents the metabolite, the cluster tree on the left is the differential metabolite cluster tree, and the top is the sample cluster tree. The gradient color represents the size of the quantitative value—the redder the color, the higher the expression, and the bluer the expression

Supplementary Material 3

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Author contributions

Mingcong Chen: Conceptualization, Methodology, Software, Writing - Original Draft. Yuzhen Ouyang: Methodology, Software, Validation, Resources, Writing - Original Draft. Yang Yang: Visualization, Supervision. Zihao Liu.: Investigation, Data Curation. Mingyi Zhao: Writing - Review & Editing, Supervision, Funding acquisition. All authors contributed to the article and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

NHANES has obtained permission from the Institutional Review Committee of the Centers for Disease Control and Prevention (CDC), and all participants have signed informed consent forms before the investigation. All the zebrafish experiments in this study strictly adhere to the ethical guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washing-ton, D.C.) and comply with the welfare standards for experimental animals stipulated in the Chinese national standard GB/T 35,892–2018.

Competing interests

The authors declare no competing interests.

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