Research Article

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Causal association between ceramide levels and central precocious puberty: a mendelian randomization study

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Abstract

Objectives: Ceramides have been implicated in metabolic disorders, but their role in central precocious puberty (CPP) is unclear. This study aimed to assess the causal relationship between ceramide species and CPP using Mendelian randomization (MR).

Methods: Genome-wide association study (GWAS) data were used to construct a ceramide database. MR analyses, including inverse variance weighting (IVW) and Wald Ratio methods, were performed to evaluate causal associations. Sensitivity analyses tested robustness. Gene Ontology (GO) and KEGG enrichment analyses were conducted to explore biological pathways and regulatory genes.

Results: The MR predicted that 17 ceramide species were associated with CPP. Cer(d17:1/20:0), Cer(d17:1/22:0), Cer(d17:1/24:0), and Cer(d18:1/14:0, d16:1/16:0) were linked to increased CPP risk, while total ceramide levels and 12 other subtypes showed protective associations. Enrichment analyses indicated involvement of sphingolipid metabolism and related signaling pathways, with SPTLC1, SPTLC3, and CERS4 framed as plausible pathways.

Conclusions: Our analysis suggests a potential causal relationship between specific ceramide species and CPP. We need more experimental research on specific pathological and physiological mechanisms in the future.

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Keywords: ceramide; central precocious puberty; mendelian randomization; blood metabolites; sphingolipid

Introduction

Sphingolipids, found in all mammalian cells, are unique lipids containing amino alcohols in their structure. This family includes ceramide (Cer), monohexosyl ceramide, sphingomyelin, and sphingosine [1]. Ceramide has been identified as a key lipotoxic molecule implicated in the pathogenesis of various metabolic disorders [2–4]. Elevated ceramide levels, for instance, impair insulin signaling and promote lipid buildup, leading to insulin resistance and atherosclerosis [5, 6]. Notably, animal studies reveal that early-onset obesity raises hypothalamic ceramide levels, advancing puberty via ovarian sympathetic circuits, a process reversible by inhibiting ceramide synthase SPTLC1. It suggests that targeting the ceramide pathway may provide a treatment strategy for precocious puberty [7]. Another observational clinical study using non-targeted ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for lipid analysis also identified Cer(18:0/15:0), Cer(18:1/16:0), and Cer(18:1/26:0) as potential biomarkers for distinguishing central precocious puberty (CPP) in girls (AUC=0.964) [8]. However, the results of such observational studies are susceptible to unmeasured confounding factors and may not truly reflect the relationship between ceramides and CPP.

Central precocious puberty is a common pediatric endocrine disorder characterized by the early activation of the hypothalamic-pituitary-gonadal axis (HPGA), leading to secondary sexual development before age eight in girls and before age nine in boys. CPP not only promotes the rapid progress of sexual development and affects adult height and mental and behavioral health, but is also accompanied by an increased risk of diseases such as obesity in adulthood, type 2 diabetes, cardiovascular disease, and breast cancer [9]. With a global trend toward earlier puberty, CPP incidence has notably increased in many countries [10, 11]. Understanding the pathogenesis of CPP is thus vital to public health efforts.

While risk factors, such as mutations in the MKRN3 gene, obesity, and exposure to endocrine disruptors, have been linked to CPP, its underlying mechanisms and biomarkers remain largely unexplored [12–14]. In particular, research on ceramide metabolism in CPP is still sparse. Current clinical reports linking ceramide and precocious puberty in children are based on cross-sectional data, which cannot confirm causal relationships [8]. The recent advancements in metabolomics technology have yielded innovative and efficient analytical methods that facilitate the investigation of intricate biological processes associated with metabolic alterations underlying various diseases [15].

Understanding the regulation of ceramides in sphingolipid metabolism is essential for uncovering the mechanisms of CPP and developing early diagnostic and preventive strategies. Research has shown that ceramides, lipid metabolites that accumulate with aging and overnutrition, play a crucial role in obesity-related dysfunction of thermogenic fat cells [16]. Specifically, C16:0 and C18:0 ceramides act as key lipotoxic agents in adipose tissue, skeletal muscle, and liver, disrupting insulin sensitivity, beta-cell function, vascular reactivity, and mitochondrial metabolism [3]. Inhibiting ceramide biosynthesis or promoting its breakdown in rodent models has demonstrated improvements in various metabolic diseases, including diabetes, cardiomyopathy, insulin resistance, atherosclerosis, and fatty liver disease [17]. Early studies have used metabolomic methods to explore the relationship between specific metabolites and disease. Combined with the close relationship between nutrition and energy metabolism and adolescent development, ceramide has a potential association with CPP, but the causal relationship between the two is still unclear [8]. Traditional randomized controlled trials (RCTs) rely on statistical models to quantify relationships between measured variables. However, for studies involving complex, multiomics data, RCTs face practical constraints. Mendelian Randomization (MR) studies offer a robust alternative by using genetic variants, single nucleotide polymorphisms (SNPs), to proxy for exposures, allowing causal inferences between exposure and outcome [18]. This technique leverages the random assortment of alleles at conception to mitigate confounding factors and reduce the risk of reverse causality [19]. The high heritability of ceramide further supports the utility of MR in these analyses [20].

Given the sparse research on the relationship between ceramide in the blood and CPP, further investigation is essential. This study establishes a novel blood ceramide database using genome-wide association study (GWAS) data for MR analysis, aiming to clarify the causal link between blood ceramide levels and CPP. This approach provides novel insights and potential strategies for CPP.

Materials and methods

Study design and MR assumptions

The process of this study was conducted strictly in accordance with the statement (STROBE-MR), which used Mendelian randomization to enhance observational epidemiological findings [21]. Three key assumptions must be met in MR studies (Figure 1A): (i) the genetic variants (instruments) used must be strongly associated with the exposure; (ii) these variants should be independent of any confounding factors affecting the exposure-outcome relationship; and (iii) the genetic variants should influence the outcome solely through the exposure. The flow chart (Figure 1B) illustrates the overall study design. All methods were performed in accordance with the relevant guidelines and regulations.

Data source

The four parts of the blood ceramide database established in this study were all from the IEU OpenGWAS project database (**https://gwas.mrcieu.ac.uk/**), derived from Cadby [22]、McGurk [23]、Chen [24]、Ottensmann [20], et al., and integrated lipidomics and genomics analysis. Each study obtained ethics committee approval and participant informed consent. The first part included 4,492 participants of European ancestry, analyzed approximately 13.68 million SNPs, including 88 ceramide classes, and was validated by 1,565 individuals of European ancestry. The second part included lipidomic GWAS association results for 999 participants of European ancestry, including 12 ceramide classes, seven of which overlapped with the first part. Considering the timing and number of data updates, the final results of this part of the data were based on the metabolites selected in the first part. The third part included 8,096 participants of European ancestry and analyzed approximately 15.4 million SNPs, including four ceramide classes. The last part included 7,147 participants of European ancestry and analyzed approximately 1.28 million SNPs, including four ceramide classes. For the outcome, CPP data were sourced from the FinnGen Consortium (R11), encompassing 202 cases and 434,894 controls of European ancestry (https://www.finngen.fi/en/). Table 1 presents summary information on the data sources for exposure and outcomes, while detailed information for each

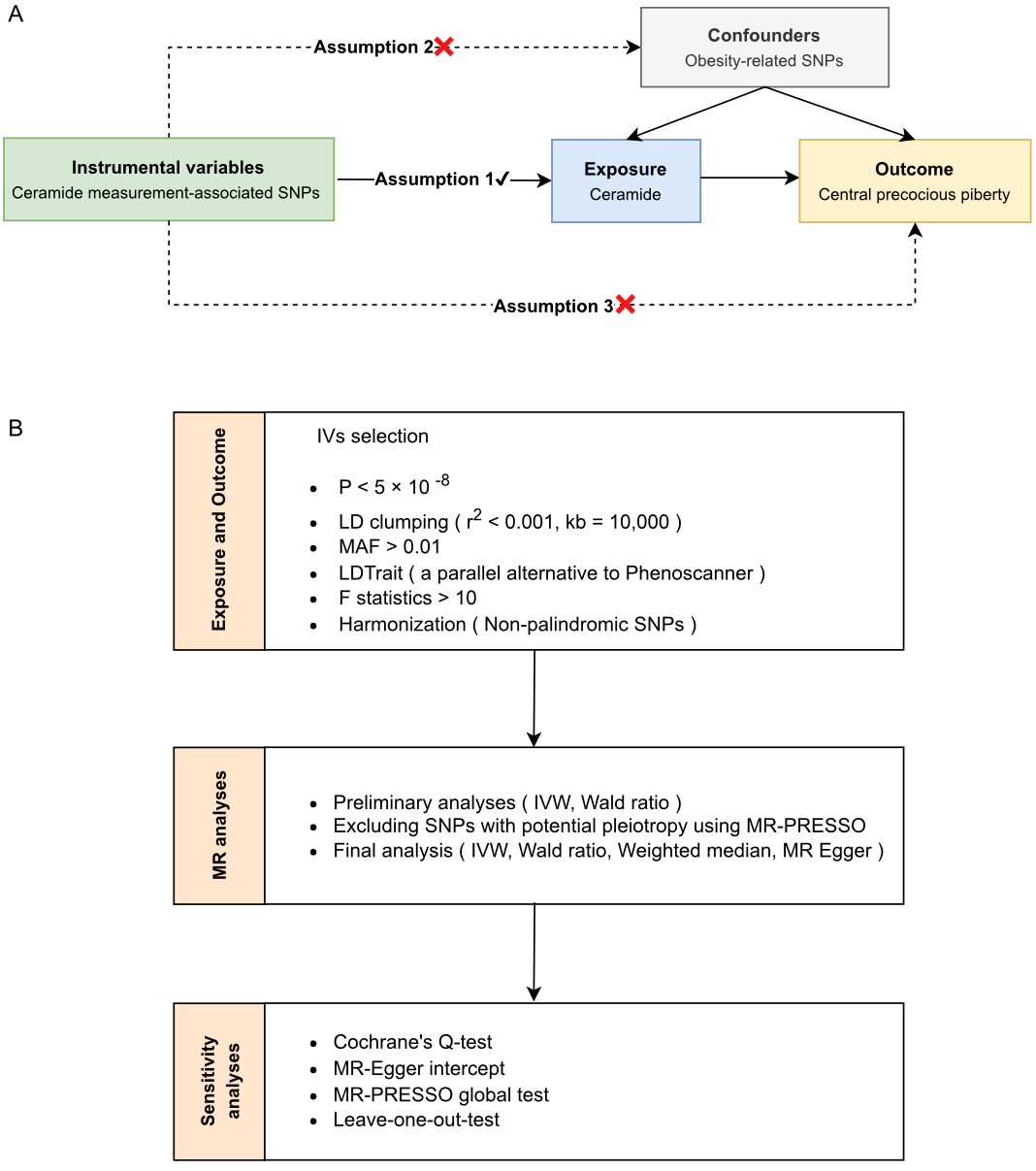

Figure 1: Flowchart of the study. (A) Diagram of the MR assumptions of ceramide and central precocious puberty association. (B) Flowchart of the MR study.

Table 1: Detailed information of GWAS summary statistics data.

Trait	Population	Sample size, n	Journal/Consortium	PubMed ID	First author	Pub.date
Ceramide	European	4,492	Nat commun	35,668,104	Cadby G	2022-6-6
	European	999	Hum mol genet	33,437,986	McGurk KA	2021-1-12
	European	8,091	Nat genet	36,635,386	Chen Y	2023-1-12
	European	7,174	Nat commun	37,907,536	Ottensmann L	2023-10-31
Central precocious puberty	European	435, 096	The FinnGen consortium (R11)	–	–	2024-6-24

ceramide is presented in Supplementary Table S1. There is no overlap between exposure and outcome samples.

Instrument selection

To ensure the accuracy and validity of MR assessment, Stringent criteria were implemented for the selection of SNPs as instrumental variables (IV): (i) SNPs were required to have genome-wide significance ($p<5\times10^{-8}$) for association with ceramide levels. (ii) Only unique, independent SNPs were selected, minimizing linkage disequilibrium (LD) bias with thresholds of $r^2<0.001$ and a clumping distance of 10,000 kb. (iii) minor allele frequency (MAF) > 0.01. (iv) SNPs that may be associated with confounding factors were excluded using the LDtrait online tool (obesity: rs72999033, rs150268548, rs8100204). (v) The assessment using F-statistics ($F=R^2\times(n-2)/(1-R^2)$) aims to identify and eliminate weak instrumental variables, setting a minimum F statistical threshold of 10 for SNP inclusion [25]. (vi) Harmonization is performed to align alleles of SNPs associated with exposure and outcome, explicitly discarding palindromic SNPs with intermediate allele frequencies or incompatible alleles.

MR analysis and statistical analysis

In the absence of IV pleiotropy, the inverse variance-weighted method (IVW) is considered to be the most reliable MR estimation technique [26]. In our study, we used IVW (IV≥2) and wald ratio (IV=1) with random effects as the primary methods for MR analysis, adjusting the significance threshold to $p<0.05$ to initially explore the association between ceramide and CPP. In addition, MR-Egger [27] and weighted median (WM) [28] were used as complementary methods to verify the robustness of the results. MR-Egger regression provided estimates of MR adjusted for horizontal pleiotropy [27]. When more than half of the information came from valid instrumental variables, the WM method generated estimates consistent with actual effects [28]. The IVW method served as the main criterion for causal inference, contingent on consistent results across the three approaches.

Sensitivity analyses were performed to evaluate pleiotropy, heterogeneity, and robustness. First, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) [29] and MR-Egger intercept tests [27] assessed horizontal pleiotropy, with $p<0.05$ indicating its presence. Second, Cochrane Q tests evaluated heterogeneity between IVW and MR-Egger estimates ($p<0.05$ indicating heterogeneity) [30]. Leave-one-out (LOO) analysis is employed to identify pleiotropic outlier SNPs that significantly impact the causal effect. Finally, funnel plots were also used to assess SNP heterogeneity visually.

All statistical tests were performed with two-tailed analysis, and a p-value of less than 0.05 in each test indicated a significant causal relationship. 99 statistical tests were conducted, and the p-values for each ceramide type were adjusted for multiple comparisons to control for the false discovery rate (FDR). The corrected p-value, denoted as p_adj, was calculated using the Benjamini-Hochberg method, with a significance threshold set at 0.05 divided by 99 (5.56×10^{-3}). A p-value < 0.05, but above the Bonferroni-corrected threshold, was considered as suggestive evidence for a potential causal association. Analyses were conducted using R version 4.4.1.

Statistical power analysis

We computed the power in our main MR analyses for each exposure using the online sample size and power calculator for Mendelian randomization with a binary outcome (https://sb452.shinyapps.io/power) based on the sample size of the outcome and an alpha level of 0.05.

Metabolic pathway analysis

To determine the potential functional impact of association variants identified in the MR analysis, we used the Variant Effect Predictor (VEP) annotation tool (**https://asia.ensembl.org/Tools/VEP**) to obtain the genes corresponding to the SNPs used in the forward MR analysis. Gene enrichment analyses were performed using the "clusterProfiler" R package to identify biological functions and pathways, including KEGG and GO, with a significance level of $p<0.05$.

Ethics statement

Since every piece of data used in this study was taken from publically accessible databases, no further ethical approval was needed. Each group that makes data available to the public got participants' informed consent and the go-ahead from the appropriate ethical committees before conducting their research.

Results

After screening for instrumental variables, we selected 99 classes from the established pool of ceramides in blood for

MR analysis. All SNPs demonstrated F-statistics exceeding 10, indicating strong instrumental variables.

The causal impact of ceramide levels on CPP

Through MR analysis using the IVW and Wald ratio methods, we identified significant associations between CPP and levels of 16 ceramide classes and the total ceramide, as shown in Figure 2. The information corresponding to valid SNPs is detailed in Table 2. Among these, the levels of Ceramide (d19:1/20:0), Ceramide (d19:1/24:0), Ceramide (d19:1/24:1), Ceramide (d19:1/18:0), Ceramide (d19:1/22:0), Ceramide (d18:1/24:1), Ceramide (d20:1/24:0), Total Ceramide, Ceramide [n(23)S(20)], Ceramide [n(24)DS(19)], Ceramide [n(24)DS(20)], Ceramide [n(25)S(20)], and Glycosyl-N-tricosanoyl-sphingadienine (d18:2/23:0) each showed a significant decrease in the risk of central precocious puberty (CPP) by 37.3 % (OR=0.627, 95 % CI 0.426–0.923, p=0.018), 31.9 % (OR=0.681, 95 % CI 0.487–0.951, p=0.024), 30.7 % (OR=0.693, 95 % CI 0.501–0.959, p=0.027), 41.7 % (OR=0.583, 95 % CI 0.361–0.942, p=0.027), 29.2 % (OR=0.708, 95 % CI 0.518–0.968, p=0.031), 49.5 % (OR=0.505, 95 % CI 0.259–0.984, p=0.045), 36.2 % (OR=0.638, 95 % CI 0.410–0.992, p=0.046), 45.0 % (OR=0.550, 95 % CI 0.309–0.980, p=0.042), 51.3 % (OR=0.487, 95 % CI 0.254–0.932, p=0.030), 40.5 % (OR=0.595, 95 % CI 0.372–0.950, p=0.030), 53 % (OR=0.470, 95 % CI 0.238–0.929, p=0.030), 54.1 % (OR=0.459, 95 % CI 0.228–0.927, p=0.030), and 56.4 % (OR=0.436, 95 % CI 0.191–0.992, p=0.048) for each standard deviation (SD) increase. In contrast, the levels of Ceramide (d17:1/20:0), Ceramide (d17:1/22:0), Ceramide (d17:1/24:0), and Ceramide (d18:1/14:0, d16:1/16:0) were associated with an increase in the risk of CPP by 161.8 % (OR=2.618, 95 % CI 1.168–5.867, p=0.019), 176.9 % (OR=2.769, 95 % CI 1.158–6.619, p=0.022), 168.7 % (OR=2.687, 95 % CI 1.153–6.261, p=0.022), and 237.7 % (OR=3.377, 95 % CI 1.100–10.366, p=0.033) for each SD increase. The other two methods of MR analysis showed similar directional trends, but not all results were significant (Table S2). When applying the Benjamini-Hochberg method to adjust for multiple comparisons between ceramide classes, none of the results reached the FDR-corrected significance threshold ($p\_adj<5.56 \times 10^{-3}$). However, it is important to note that the findings in this exploratory study present suggestive evidence, with p_adj values ranging from 5.56×10^{-3} to 0.05.

Evaluation of heterogeneity and horizontal pleiotropy, and power

Supplementary Table S2 presents the MR-PRESSO, MR-Egger intercept, and Cochrane Q test results, confirming no evidence of horizontal pleiotropy across all analyses (p>0.05). Cochrane Q tests further indicated no heterogeneity among the selected SNPs (p>0.05). Although funnel plots lacked significant symmetry due to the limited number of IVs (Supplementary Figure S1), the overall results support the robustness of the causal relationship. As shown in Supplementary Table S3, the total ceramide levels and 16 subclasses appeared underpowered due to the scarcity of CPP cases and the strict criteria for selecting IVs.

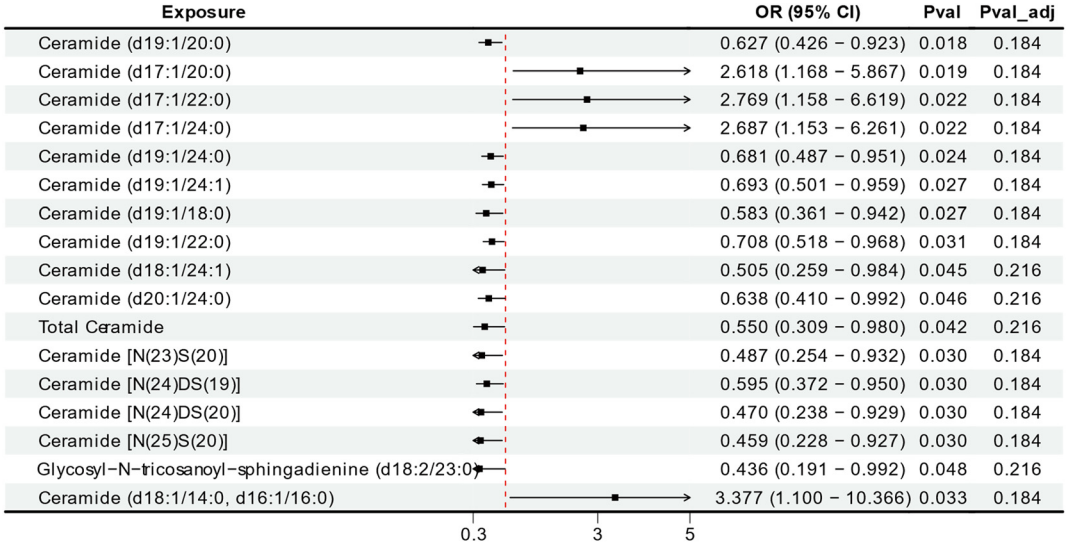

Figure 2: MR analysis results of the effects of ceramide levels in blood on CPP in the database.

Table 2: Summary of instrumental variables used for all significant causal association evidence in the database.

SNP	Chr	Pos	EA	OA	Beta	Se	p-Value	Palindromic	mr_keep	EAF	F	PMID
Total ceramide levels												35,668,104
rs168622	20	12,966,089	G	T	−0.33	0.022	7.34193E-51	FALSE	TRUE	0.610	221	
rs7412	19	45,412,079	T	C	−0.290	0.040	4.16772E-13	FALSE	TRUE	0.090	61	
Ceramide (d17:1/20:0) levels												35,668,104
rs17101394	14	64,232,386	A	G	0.250	0.030	7.85975E-17	FALSE	TRUE	0.160	74	
rs7248003	19	8,301,048	T	C	0.150	0.025	1.97318E-09	FALSE	TRUE	0.710	41	
rs7412	19	45,412,079	T	C	−0.250	0.040	4.10453E-10	FALSE	TRUE	0.090	45	
Ceramide (d17:1/22:0) levels												35,668,104
rs17101394	14	64,232,386	A	G	0.330	0.029	5.30173E-30	FALSE	TRUE	0.160	128	
Ceramide (d17:1/24:0) levels												35,668,104
rs17101394	14	64,232,386	A	G	0.340	0.029	9.58721E-32	FALSE	TRUE	0.160	135	
Ceramide (d18:1/24:1) levels												35,668,104
rs2076711	22	47,064,837	C	A	−0.160	0.025	1.55377E-10	FALSE	TRUE	0.280	46	
rs680379	20	12,969,400	G	A	−0.250	0.022	6.34475E-30	FALSE	TRUE	0.600	131	
rs7412	19	45,412,079	T	C	−0.230	0.040	8.92434E-09	FALSE	TRUE	0.090	39	
Ceramide (d19:1/20:0) levels												35,668,104
rs6520049	22	46,996,309	T	C	−0.140	0.025	2.14E-08	FALSE	TRUE	0.270	34	
rs680379	20	12,969,400	G	A	−0.520	0.021	2.31E-135	FALSE	TRUE	0.600	670	
Ceramide (d19:1/18:0) levels												35,668,104
rs168622	20	12,966,089	G	A	−0.430	0.021	3.51065E-93	FALSE	TRUE	0.610	360	
Ceramide (d19:1/22:0) levels												35,668,104
rs116940708	9	94,738,467	G	A	−0.460	0.074	5.09286E-10	FALSE	TRUE	0.027	49	
rs2423713	20	12,881,846	A	G	0.150	0.026	7.96342E-09	FALSE	TRUE	0.770	35	
rs364585	20	12,962,718	G	A	−0.620	0.020	5.3905E-211	FALSE	TRUE	0.610	671	
rs6138379	20	24,789,626	A	G	0.130	0.023	1.58431E-08	FALSE	TRUE	0.330	33	
Ceramide (d19:1/24:0) levels												35,668,104
rs2423713	20	12,881,846	A	G	0.150	0.026	7.96342E-09	FALSE	TRUE	0.770	35	
rs364585	20	12,962,718	G	A	−0.590	0.021	1.12E-173	FALSE	TRUE	0.610	620	
rs75431233	9	94,781,894	A	G	−0.440	0.074	2.74865E-09	FALSE	TRUE	0.027	45	
Ceramide (d19:1/24:1) levels												35,668,104
rs116940708	9	94,738,467	G	A	−0.460	0.075	8.60566E-10	FALSE	TRUE	0.027	49	
rs364585	20	12,962,718	G	A	−0.620	0.020	5.3905E-211	FALSE	TRUE	0.610	671	
Ceramide (d20:1/24:0) levels												35,668,104
rs168622	20	12,966,089	G	T	−0.440	0.021	1.78481E-97	FALSE	TRUE	0.610	375	
rs8119743	20	13,120,092	A	G	0.210	0.033	1.97032E-10	FALSE	TRUE	0.120	41	
Ceramide [n(23)S(20)] levels												33,437,986
rs1321940	20	12,959,885	A	G	0.317	0.048	4.62E-11	FALSE	TRUE	0.366	576	
Ceramide [n(24)DS(19)] levels												33,437,986
rs1321940	20	12,959,885	A	G	0.440	0.047	3.14E-21	FALSE	TRUE	0.366	575	
Ceramide [n(24)DS(20)] levels												33,437,986
rs680379	20	12,969,400	A	G	0.302	0.047	1.48E-10	FALSE	TRUE	0.368	581	
Ceramide [n(25)S(20)] levels												33,437,986
rs680379	20	12,969,400	A	G	0.294	0.048	9.18E-10	FALSE	TRUE	0.368	580	
Glycosyl-N-tricosanoyl-sphingadienine (d18:2/23:0) levels												36,635,386
rs10762405	10	70,808,679	G	A	0.108	0.0175	7.49074E-10	FALSE	TRUE	0.306	36	
rs2381400	9	35,776,422	A	G	0.103	0.0178	6.39868E-09	FALSE	TRUE	0.291	32	
rs9790720	4	47,552,471	A	T	0.226	0.0189	4.93315E-33	TRUE	TRUE	0.237	136	
Ceramide (d18:1/14:0, d16:1/16:0) levels												36,635,386
rs7160525	14	63,765,502	A	G	0.258	0.0217	9.17091E-33	FALSE	TRUE	0.169	139	

SNP, single nucleotide polymorphisms; Chr, chromosome; Pos, position; EA, effect allele; OA, other allele; SE, standard error; EAF, effect allele frequency; F, F-statistic.

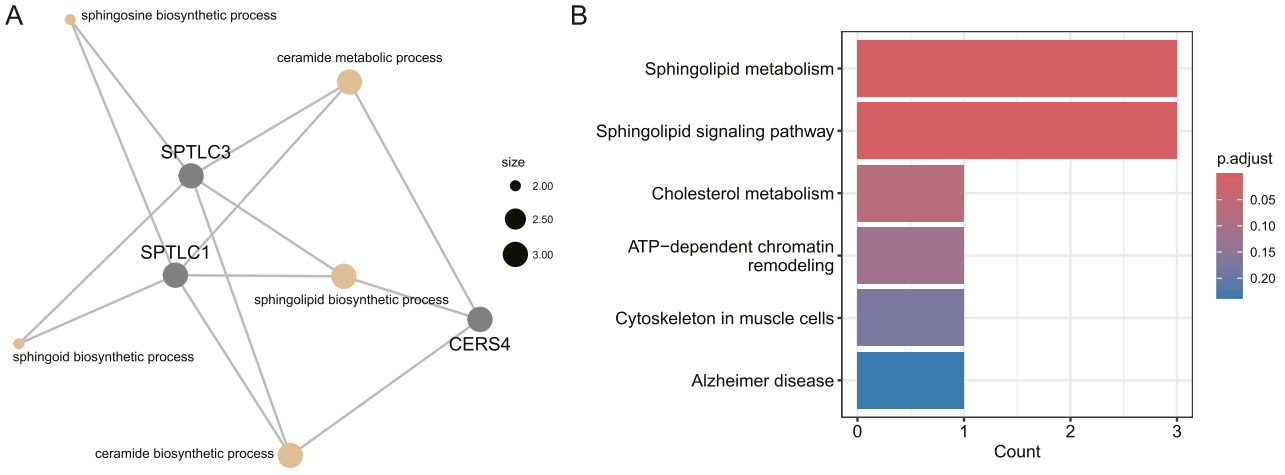

Figure 3: Enrichment analysis. (A) GO analysis network graph. The graph shows the relationships between enriched GO terms, where node size represents the number of genes and color indicates significance. (B) KEGG analysis. Bar length corresponds to the number of genes involved in each pathway, and color denotes adjusted significance levels.

Metabolic pathway analysis

In the forward MR analysis, we employed the VEP annotation tool to identify gene symbols linked to the SNPs. GO analysis highlighted pathways such as ceramide biosynthesis, sphingolipid biosynthesis, and ceramide metabolism, with key genes like SPTLC1, SPTLC3, and CERS4 playing crucial roles in these processes (Figure 3A). KEGG pathway analysis identified five enriched functional pathways (Figure 3B), with strong associations in sphingolipid metabolism and signaling pathways, underscoring their potential importance in CPP.

Discussion

In this study, we utilized GWAS data to investigate associations between total ceramide levels and 16 subclasses from an established pool of blood ceramides with CPP. Among the identified types, total ceramide levels and 12 specific subclasses demonstrated a protective effect associated with reduced risk of CPP. These include Ceramide (d19:1/20:0), Ceramide (d19:1/24:0), Ceramide (d19:1/24:1), Ceramide (d19:1/18:0), Ceramide (d19:1/22:0), Ceramide (d18:1/24:1), Ceramide (d20:1/24:0), Total Ceramide, Ceramide [n(23)S(20)], Ceramide [n(24)DS(19)], Ceramide [n(24)DS(20)], Ceramide [n(25)S(20)], and Glycosyl-N-tricosanoylsphingadienine (d18:2/23:0). In contrast, four subclasses were found to increase the risk of CPP, namely Ceramide (d17:1/20:0), Ceramide (d17:1/22:0), Ceramide (d17:1/24:0), and Ceramide (d18:1/14:0, d16:1/16:0). Various sensitivity analysis methods further validated these findings.

Ceramides are a large family of bioactive lipid signaling molecules with prominent roles in metabolic regulation and cellular processes such as growth, differentiation, and apoptosis. Emerging evidence suggests that ceramides are key mediators in various metabolic disorders and function as crucial modulators of leptin and ghrelin, two hormones central to metabolism and pubertal onset [31, 32]. Increased hypothalamic ceramide levels interfere with leptin's anorectic effects while leptin itself inhibits ceramide synthesis, establishing a complex feedback loop [33]. Conversely, ghrelin promotes ceramide production, and blocking ceramide synthesis within the hypothalamus disrupts ghrelin's appetite-stimulating actions [34]. This interplay aligns with the leptin-mTOR-Kisspeptin axis, as research by Kim et al. reveals that C16-ceramide negatively regulates mTOR, while sphingosine-1-phosphate (S1p), a ceramide metabolite, enhances mTOR signaling [35]. Additionally, hypothalamic ceramide plays a critical role in regulating the HPG axis at reproductive levels – extending beyond its metabolic function related to central estradiol (E2), which induces negative energy balance by mitigating ceramide-induced lipotoxicity and endoplasmic reticulum stress [36]. Heras et al. identified a novel non-classical pathway involving ceramide-controlled ovarian sympathetic innervation within the paraventricular nucleus of the hypothalamus as a new mediator contributing to the obesity-induced acceleration of puberty in female rats [7]. Moreover, a growing body of literature has identified that

sphingolipids, including ceramides, sphingosine, S1p, sphingomyelin, and gangliosides, also play indispensable roles in steroid hormone biosynthesis, impacting both gene expression and signaling cascades involved in steroidogenesis [37].

In addition, studies have shown that the S1p/S1PR1/ceramide axis, in particular, has been implicated in hunger regulation within the hypothalamus, promoting lipolysis and energy expenditure while reducing lipogenesis, resulting in an anti-obesity effect that may have implications for metabolic and reproductive health [38]. Although previous lipidomic studies have found that phosphocholine(16:1(9Z)/16:1(9Z)) may be a potential biomarker for CPP, confounding factors such as BMI, diet and other life characteristics have not been controlled [39]. Recent findings indicate that certain ceramides, such as Cer18:0/15:0, Cer18:1/16:0, and Cer18:1/26:0, may distinguish CPP girls from controls, though observational limitations persist [8]. Furthermore, a longitudinal lipidomic study in children revealed persistent alterations in plasma ceramide levels associated with maternal obesity, suggesting their potential as early predictors of metabolic risk in offspring [40]. Another longitudinal metabolomic study found that the expression of PE [19:1(9Z)0:0] was elevated in female infants with CPP but decreased after treatment, indicating the possible utility of lipid metabolites in evaluating therapeutic efficacy [41]. Beyond blood-based findings, intestinal microbial metabolomics have also provided insights. Huang et al. reported that nitric oxide synthesis and Streptococcus abundance were closely related to CPP based on 16 S rRNA sequencing and non-targeted metabolomics [42]. In contrast, another fecal metabolomics study in girls with CPP and corresponding animal models identified thymine and inosine as differential metabolites compared with prepubertal controls [43]. Moreover, metabolomic analysis of mouse hypothalamic tissue revealed that (S)-abscisic acid, methylmalonic acid, and two-oxo-4-methylthiobutyric acid were significantly altered in the CPP group [44]. Overall, metabolomic studies in pediatric endocrinology remain limited, while some findings suggest an association between ceramides and CPP, the causative role of ceramides is still ambiguous. Future research is critical to elucidate the underlying mechanisms, particularly in non-obese settings, to clarify ceramide's broader role in pubertal timing and identify potential therapeutic interventions.

A primary strength of this study is the demonstration of a suggestive causal link between the two. We have found a suggestive causal relationship between overall blood levels and specific subclasses of ceramide and CPP, though several of classes differs from trends observed in metabolic diseases like obesity. This discrepancy may be partially due to the distinct subcellular sites where ceramides are synthesized and their specific, poorly understood transport mechanisms. Sphingolipid levels within cells are precisely regulated by enzymes that localize to distinct subcellular compartments, thus controlling ceramide homeostasis in specific microenvironments [45]. Further, the biological impact of ceramides varies among subclasses. While certain ceramides are implicated in metabolic diseases, others may have protective roles. For instance, C24:1 ceramide levels, which are reduced in liver, heart, and plasma in type 1 diabetes and high-fat diet models, show beneficial effects when restored, improving glucose tolerance, insulin sensitivity, and fatty acid oxidation [46]. Similarly, elevated liver C18:1 ceramide levels, as induced by alkaline ceramidase (Acer3) inhibition, mitigate oxidative stress and reduce nonalcoholic steatohepatitis severity [47]. A longitudinal bi-racial cohort study also found that the mean levels of plasma monounsaturated ceramide and sphingolipid in prediabetic progressors were significantly reduced after adjusting for various factors such as age, gender, race/ethnicity, and BMI [48]. This finding is consistent with Floegel et al.'s prospective study on the association between serum metabolites and type 2 diabetes, which highlighted the protective effect of baseline levels of C16:1 sphingomyelin against diabetes [49]. Elevated levels of saturated sphingolipids and lower monounsaturated forms are linked to higher prediabetes risk, supporting recommendations to replace saturated fats with unsaturated fats to improve metabolic health [50, 51]. Collectively, these observations underscore the significance of distinguishing ceramide subclasses, backbones, and metabolites, alongside their subcellular distribution, in metabolic disease contexts.

In addition, GO and KEGG enrichment analyses identified three plausible genes and two potentially significant metabolic pathways associated with CPP. The identified genes include SPTLC1, SPTLC3, and CERS4, while the pathways are involved in sphingolipid metabolism and sphingolipid signaling transduction. The serine palmitoyltransferase genes SPTLC1 and SPTLC3 are pivotal in sphingolipid synthesis, and plasma ceramide levels are modulated by their enzymatic activity. In animal studies, increased SPTLC1 expression and elevated ceramide levels in the hypothalamic paraventricular nucleus are linked to early puberty in obese female rats [52, 53]. At the same time, CERS4 plays an important role in regulating sphingolipid types and metabolic balance. According to Kim et al., inhibition of CERS4 expression improves liver metabolic characteristics in mice [54]. In addition, CERS4

can produce C20 and C22 ceramides, which have protective effects in the development of heart failure [55]. Further research is needed to fully understand the roles of SPTLC3 and CerS4 in the occurrence of CPP. Overall, disruptions in bioactive sphingolipid signaling likely serve as both root causes and linking factors between metabolic diseases and their pathological consequences. Interventions targeting key enzymes or metabolic pathways, as described above, hold promise for future breakthroughs in diagnostics, therapeutics, and fundamental understanding of CPP.

The research exhibits several key advantages and distinctive characteristics, as outlined below. Primarily, we leveraged genetic instruments as proxies for blood ceramide levels and applied rigorous methodologies to mitigate potential violations of MR assumptions, thereby reducing bias and minimizing confounding factors. This study also significantly reduced potential biases caused by population stratification by limiting the data to participants of European ancestry. Sensitivity analyses reinforced the reliability of our results across different conditions and assumptions. Additionally, by constructing an extensive ceramide dataset, we enabled more comprehensive comparative analyses, bolstering the robustness of our findings. These specific ceramides with established suggestive causal links may serve as clues to promising therapeutic targets. This study also highlights several metabolic pathways potentially implicated in CPP development, suggesting new avenues for early identification and prevention of CPP in clinical settings.

Despite these strengths, there are several limitations to our study. First, the genetic data were derived solely from blood samples. Although we attempted to source cerebrospinal fluid metabolite data via public GWAS databases, we could not obtain suitable genetic instruments. Second, the GWAS data for both exposure and outcomes were sourced from European populations, which may limit the generalizability of our conclusions across diverse ethnic groups. Additionally, the incidence of idiopathic CPP in women is 5–10 times that of men, making it essential to distinguish sex-specific mechanisms in CPP etiology [56]. However, we were unable to obtain sufficient information to conduct a subgroup analysis to explore potential sex differences in ceramide levels. Furthermore, the MR analysis had limited statistical power, likely due to the case–control imbalance in the FinnGen Consortium's CPP dataset. To address this limitation, we performed multiple sensitivity analyses, including MR-PRESSO, MR-Egger regression, and the Cochrane Q test, all of which supported the robustness of the results. Nevertheless, these findings should be interpreted with caution and validated in future studies with larger sample sizes and more cases. Finally, while this study demonstrates a suggestive association between various ceramide species and CPP, the underlying mechanism for this association remains unclear. Further research, including functional or experimental validation, is necessary to elucidate their precise roles in CPP pathogenesis.

Conclusions

This two-sample Mendelian randomization study supports a suggestive causal relationship between ceramide and CPP. The overall and specific subtype levels of ceramide in plasma have great potential as objective indicators for evaluating potential protective or risk factors for the development of CPP in children. Our findings provide valuable evidence of ceramide's impact on CPP, offering insights into new diagnostic and preventive approaches. Moreover, interventions targeting ceramide levels or correcting their imbalances hold promise for CPP management, though further studies are necessary to validate these findings and explore therapeutic applications.

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Supplementary Mate-rials, further inquiries can be directed to the corresponding author.

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