ORIGINAL ARTICLE

Causal Effect Between Gut Microbiota, Gut Bacterial Pathway, and Chronic Spontaneous Urticaria: A Large-Scale Bidirectional Mendelian Randomization Analysis

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Abstract

Background: To analyze causality between gut microbiota and chronic spontaneous urticaria (CSU) and to investigate the mediating effect of metabolic pathways.
Methods: We extracted genome-wide association study summary statistics for 211 microbiota taxa from the MiBioGen consortium (N=18 340), 205 microbiota metabolic pathways from the Dutch Microbiome Project (N=7738), and CSU from the FinnGen genomics initiative (N=450). Bidirectional Mendelian randomization (MR) was performed to detect genetic causality between gut microbiota, gut bacterial pathways, and CSU. Sensitivity analyses were performed to validate the robustness of the results. Mediation MR investigated mediators in the association between gut microbiota and CSU.
Results: MR analysis suggested that the family Peptococcaceae and its child taxon, the genus *Peptococcus*, were risk factors for CSU. In addition, the genera *Collinsella, Lachnospiraceae UCG004, Ruminococcaceae UCG004*, and *Sellimonas* were also risk factors for CSU, whereas *Family XIII UCG001*, *Lachnospiraceae UCG010*, and *Methanobrevibacter* had protective effects on CSU. As for metabolic pathways, NONMEVIPP-PWY, PWY-5022, and PWY-7221 were positively associated with CSU, although others, such as KDO-NAGLIPASYN-PWY, PWY-6353, and PWY-7400 presented a suggestive association with CSU. Moreover, PWY-7400 was a mediator in causality between the family Peptococcaceae and CSU. These results were based on nominal significance ($P<.05$). None of the Bonferroni-corrected P values were <.05.
Conclusions: Our study confirmed a causal association between gut microbiota and CSU, with the metabolic pathway being a potential mediator. Our findings provide new insights for further mechanistic and clinical studies in CSU.

Key words: Chronic spontaneous urticaria. Mendelian randomization. Mediation effect. Gut microbiota. Metabolic pathway.

Resumen

Antecedentes: Determinar la relación causal entre la microbiota intestinal (MI) y la urticaria crónica espontánea (UCE) e investigar el efecto mediador de las vías metabólicas.
Métodos: Se calcularon los estadísticos de síntesis de GWAS de 211 taxones de la microbiota de MiBioGen (N = 18.340), de 205 vías metabólicas de la microbiota del Estudio Holandés de Microbioma (N = 7738) y de UCE de la iniciativa genómica FinnGen (N = 450). Se realizó una aleatorización mendeliana bidireccional (AM) para detectar la relación de causalidad genética entre la microbiota intestinal, las vías bacterianas intestinales y la UCE. Se realizaron análisis de sensibilidad para validar la solidez de los resultados. La AM de mediación investigó los mediadores en la asociación entre la microbiota intestinal y la UCE.
Resultados: El análisis de AM sugirió que la familia Peptococcaceae y su taxón el género *Peptococcus* eran factores de riesgo de UCE. Además, los géneros *Collinsella*, *Lachnospiraceae UCG004*, *Ruminococcaceae UCG004* y *Sellimonas* también eran factores de riesgo de UCE, mientras que los géneros Family XIII UCG001, *Lachnospiraceae UCG010* y *Methanobrevibacter* tenían efectos protectores sobre la UCE. En cuanto a las vías metabólicas, NONMEVIPP-PWY, PWY-5022 y PWY-7221 se hallaban positivamente relacionadas con la UCE, pero el resto, como KDO-NAGLIPASYN-PWY, PWY-6353 y PWY-7400 presentaban una asociación sugestiva con la UCE. Además, PWY-7400 fue un mediador en la causalidad entre la familia Peptococcaceae y la UCE. Estos resultados se basaron en la significación nominal ($p < 0,05$) y ninguna de las p corregidas por Bonferroni fue inferior a 0,05.
Conclusiones: Este estudio confirmó una asociación causal entre MI y UCE, siendo la vía metabólica el posible mediador, lo que proporciona nuevos elementos para futuros estudios mecanísticos y clínicos sobre UCE.

Palabras clave: Urticaria crónica espontánea. Aleatorización mendeliana. Efecto de mediación. Microbiota intestinal. Vía metabólica.

Summary box

- **What do we know about this topic?**
  Chronic spontaneous urticaria (CSU) is a common disease that is associated with autoimmunity. Altered gut bacteria and related metabolites have been observed in CSU patients.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  Gut microbes were protective factors or risk factors for CSU. Gut bacteria causally affected CSU through metabolic pathways. These findings offer fresh avenues for CSU treatment and for further research into the mechanism of CSU.

Introduction

Chronic spontaneous urticaria (CSU) is a debilitating inflammatory disease characterized by recurrent itchy wheals, angioedema, or both for a period exceeding 6 weeks [1]. The diagnosis of CSU requires exclusion of other types of chronic urticaria caused by physical triggers or specific allergens [2]. Two main autoimmune mechanisms underlie the pathogenesis of CSU. Type I CSU is associated with IgE antibodies against autoantigens and type IIb CSU is mediated by autoantibodies [3]. However, the etiology and pathogenesis of CSU are sophisticated and remain unclear. The gut microbiota comprises the microorganisms residing in the intestinal tract, which play a key role in metabolism and in host immune and inflammatory pathways [4]. Cumulative evidence shows significant differences in the diversity, composition, and metabolites of the gut microbiota between patients with immune and/or allergic disease, especially CSU, and healthy controls [5-8]. Studies have revealed potential associations between unsaturated fatty acids and butanoate metabolism pathways related to gut microbiota and CSU [9-11]. Altered gut bacteria in CSU patients may reduce concentrations of short-chain fatty acids and promote skin inflammation driven by mast cells [11]. These studies elucidated the potential microbial pathogenesis and the relevant metabolic pathways underlying the pathogenesis of CSU. However, given the lack of evidence from randomized controlled trials, the causal relationship between gut microbiota and CSU remains unknown [12].

Two-sample Mendelian randomization (MR) analysis is an effective alternative [13] for exploring the causal association between gut microbiota and CSU. MR analysis is based on Mendel's law, which involves the random distribution of parental alleles during gametogenesis and mimics the randomization process of randomized controlled trials (RCTs). MR integrates genetic variants as instrumental variables (IVs) to identify the common genetic background and potential causal relationship between human phenotypes and genetic variants [14,15]. Genome-wide association studies (GWASs) have tested millions of genetic variants, mainly single-nucleotide polymorphisms (SNPs), to identify genotype-phenotype associations, thus providing an approach for investigating the genetic basis of complex disease [16]. Mediation analysis is used to decompose the effects of an exposure on an outcome, which act directly, and those that act via mediating variables. These analyses could estimate the causal effects between 3 types of variables: exposures, mediators, and an outcome.

Given the lack of studies on the causal relationship between gut microbiota and CSU mediated by metabolic pathways, the present study was based on a comprehensive 2-sample MR analysis and mediation MR analysis.

Material and Methods

Study Design

Firstly, summary statistics of gut microbiota, gut bacterial pathway abundance, and CSU were extracted from the respective consortiums. Secondly, we utilized a bidirectional 2-sample MR to assess the causal association between gut microbiota, metabolic pathways, and chronic idiopathic urticaria. Finally, mediation MR analysis was performed to investigate the interaction between the gut microbiota, metabolic pathways, and CSU. A summary of the study design is illustrated in Figure 1. Figure 1B shows the basic principles of MR analysis. The study conformed to the STROBE-MR guidelines [17], and several methods were adopted to obey the 3 fundamental assumptions of MR (Table S1).

Data Sources and Selection of Instrumental Variables

Summary statistics for gut microbiota abundance in 18 340 individuals (85% European ancestry) from 24 cohorts were extracted from the MiBioGen consortium (Table S2) [18]. A total of 211 taxa (9 phyla, 16 classes, 20 orders, 35 families, and 131 genera) determined using 16S ribosomal RNA gene sequencing were included. Summary statistics for microbial metabolic pathways were extracted from the Dutch Microbiome Project for 7738 participants of European ancestry, and 205 bacterial metabolic pathways determined by shotgun metagenomic sequencing were also included [19]. Summary statistics for CSU were from the FinnGen genomics initiative R8 (https://r8.finngen.fi/) [20]. The FinnGen study is a large-scale genomics initiative that has analyzed over 500 000 Finnish biobank samples and correlated genetic variation with health data to understand disease mechanisms and predisposition. The project is a collaboration between research organizations and biobanks in Finland and international industry partners.

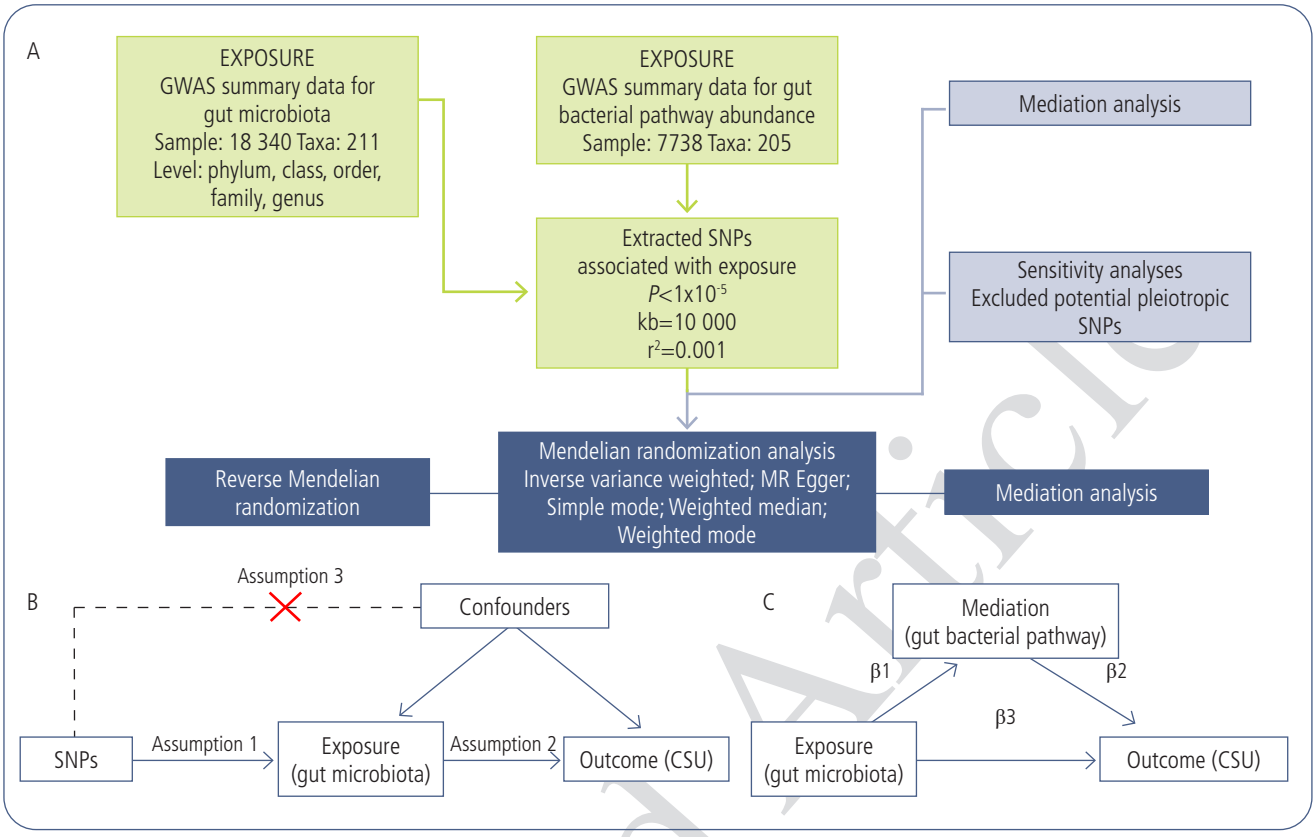


Figure 1. Overall study design. A, Study design and flowchart. B, Basic principles of Mendelian randomization. Assumption 1: the genetic variants selected as instrumental variables are robustly associated with exposure; Assumption 2: the instrumental variables have no association with the outcome except through the exposure; Assumption 3: the IVs are not associated with confounders. C, Basic principles of mediation Mendelian randomization. GWAS indicates genome-wide association study; SNP, single-nucleotide polymorphism; CSU, chronic spontaneous urticaria.

The gut microbiota and gut bacterial pathway were selected as exposure variables and CSU as an outcome variable for this MR study. SNPs significantly associated with gut microbiota in bidirectional MR were selected as IVs based on strict inclusion and exclusion criteria. SNPs with genome-wide significance $P<1\times10^{-5}$ were included for each microbiota taxon and pathway. The linkage disequilibrium threshold for clumping was set to $r^2<0.001$, and the clumping window size was set to 10 000 kb using the 1000 Genomes European reference panel [21]. The strength of the SNPs selected was assessed based on an equation, which was used to calculate the F statistics for each bacterial taxon, as follows:

$$F=\frac{R^2\,(n-1-k)}{(1-R^2)k}$$

R^2 is the portion of exposure variance explained by the IVs, n is the sample size, and k is the number of IVs. An F statistic ≥10 indicates no strong evidence of a weak instrument. IVs with F statistics <10 were considered weak IVs and were excluded.

Genetic Causality of Microbiota and Chronic Spontaneous Urticaria

We obtained the MR estimates for the causal effect using inverse variance weighting (IVW) as the principal analysis. Additional methods such as the weighted median method, simple mode, and weighted mode were applied to validate the results. We also used the MR-Egger method to assess horizontal pleiotropy. The estimate was provided as effect size (β) with the 95%CI. The heterogeneity of effects was assessed using the Cochran Q test, with $P<.05$ indicating significant heterogeneity. Given the lower accuracy and statistical power of MR-Egger regression, MR pleiotropy residual sum and outlier (MR-PRESSO) was performed to detect any outliers reflecting likely pleiotropic biases and correct horizontal pleiotropy. In addition, several sensitivity analyses were performed to validate the results from the IVW method. We used corrected *P* values (Bonferroni approach) according to different taxonomic ranks of bacteria, as follows: genus, 0.05/131 (3.8×10^{-4}); family, 0.05/35 (1.4×10^{-3}); order, 0.05/20 (2.5×10^{-3}); class, 0.05/16 (3.1×10^{-3}); and phylum, 0.05/9 (5.5×10^{-3}).

Mediation Analysis of Microbiota and Metabolic Pathways in Chronic Spontaneous Urticaria

To investigate whether the effect of the gut microbiota on CSU was mediated by the gut bacterial pathway, mediation analysis was conducted using MR. Firstly, we conducted an MR of the causal effect of the metabolic pathway on CSU based on a Bonferroni-corrected *P* value ($0.05/205=2.44\times10^{-4}$).

Secondly, we performed an MR analysis of the causal effect of 9 microbiota (significantly affecting CSU) on the metabolic pathway (β1). Finally, we performed MR to determine the mediation effect of the bacterial metabolic pathway on CSU. The MR estimated the effect of the gut bacterial metabolic pathway on CSU after adjusting for bacteria (β2) and the effect of bacteria on CSU after adjusting for bacteria. To calculate the indirect mediation effect of bacteria on disease outcomes, we used the product of coefficients method as our primary approach. This shows the causal effect of bacteria on outcomes via the gut bacterial pathway (β1×β2). Therefore, the proportion of the total effect mediated by the gut bacterial pathway was estimated by dividing the indirect effect by the total effect (Figure 1C).

All statistical analyses were performed using TwoSampleMR 0.5.10, ieugwasr 0.2.2, and MendelianRandomization 0.9.0 in R, version 4.3.1. All estimations were expressed as ORs per SD increment of the corresponding exposure. A *P* value less than the Bonferroni-corrected threshold for MR results can be considered significant. *P*<.05 was considered nominal significance.

Results

Characteristics of SNPs

The characteristics of the selected SNPs for each gut microbiota are presented in Table S3. After a series of quality control steps, 2238 SNPs were identified as IVs for 211 taxa in the MiBioGen consortium and 1727 SNPs were included for 205 metabolism pathways in the Dutch Microbiome Project. Additionally, we used 6 SNPs as IVs for CSU in FinnGen. Details for the selected IVs are shown in Tables S3-S5.

Supplementary Figure 1 shows the impact of changes on the abundance of gut bacterial taxa at 5 levels (phylum, class, order, family, genus) and the effect of metabolic pathway abundance on CSU risk.

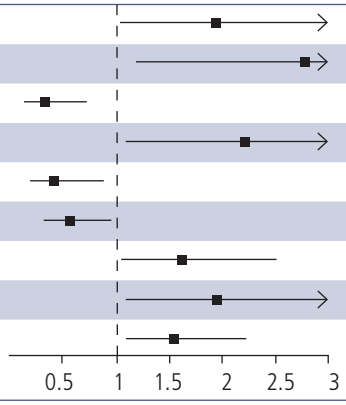
Causal Associations Between Gut Microbiota and CSU

In the MR analysis, the IVW test was used as the principal analysis for causal associations to obtain unbiased estimates. Tables 1 and S6 detail the causal effect of gut microbiota on CSU. IVW revealed a positive association between CSU and the family Peptococcaceae (OR, 1.94; 95%CI, 1.03-3.63; *P*=.0388) and the genus *Peptococcus* (OR, 1.61; 95%CI, 1.04-2.49; *P*=.0328). A further 4 bacterial genera were also found to be risk factors for CSU, namely, *Collinsella* (OR, 2.77; 95%CI, 1.18-6.50; *P*=.0189), *Lachnospiraceae UCG004* (OR, 2.21; 95%CI, 1.09-4.47; *P*=.0279), *Ruminococcaceae UCG004* (OR, 1.93; 95%CI, 1.08-3.45; *P*=.0255), and *Sellimonas* (OR, 1.54; 95%CI, 1.08-2.20; *P*=.0184). Interestingly, we found that gut microbiota was also a protective factor for CSU: the IVW analysis revealed a negative association between CSU and the genera *Family XIII UCG001* (OR, 0.33; 95%CI, 0.15-0.71; *P*=.0044), *Lachnospiraceae UCG010* (OR, 0.42; 95%CI, 0.20-0.87; *P*=.0197), and *Methanobrevibacter* (OR, 0.56; 95%CI, 0.33-0.95; *P*=.0320).

A reverse MR analysis was performed to explore whether CSU had a causal impact on gut bacteria. Seven taxa were causally influenced by CSU according to IVW (Table 2, S7). Specifically, CSU showed a suggestive association with the family Peptostreptococcaceae (OR, 1.05; 95%CI, 1.00-1.09; *P*=.0426) and the genera *Eubacterium xylanophilum* group (OR, 1.05; 95%CI, 1.00-1.10; *P*=.0408) and *Intestinibacter* (OR, 1.06; 95%CI, 1.01-1.11; *P*=.0215). In contrast, CSU had a negative effect on the genera *Eubacterium brachy* group (OR, 0.94; 95%CI, 0.90-0.98; *P*=.0028), *Candidatus Soleaferrea* (OR, 0.93; 95%CI, 0.87-1.00; *P*=.0474), *Christensenellaceae R7* group (OR, 0.95; 95%CI, 0.91-0.99; *P*=.0116), and *Lachnospiraceae FCS020* group (OR, 0.95; 95%CI, 0.90-0.99; *P*=.0242). Detailed information can be seen in Tables S6 and S7. None of these *P* values underwent Bonferroni correction, although they remained <.05, that is, nominally significant.

Table 1. Causal Effects of Gut Microbiota on Chronic Spontaneous Urticaria Estimated Using Mendelian Randomization Analysis Based on Inverse Variance Weighting.

Levels	Gut microbiota	nSNPs	IVW-P	OR (95%CI)
Family	Peptococcaceae	9	0.0388	1.94 (1.03-3.63)
Genus	*Collinsella*	9	0.0189	2.77 (1.18-6.50)
Genus	*Family XIII UCG001*	8	0.0044	0.33 (0.15-0.71)
Genus	*Lachnospiraceae UCG004*	12	0.0279	2.21 (1.09-4.47)
Genus	*Lachnospiraceae UCG010*	10	0.0197	0.42 (0.20-0.87)
Genus	*Methanobrevibacter*	6	0.0320	0.56 (0.33-0.95)
Genus	*Peptococcus*	11	0.0328	1.61 (1.04-2.49)
Genus	*Ruminococcaceae UCG004*	11	0.0255	1.93 (1.08-3.45)
Genus	*Sellimonas*	9	0.0184	1.54 (1.08-2.20)



Abbreviations: nSNPs, number of single-nucleotide polymorphisms; IVW-P, inverse variance–weighted *P* value.

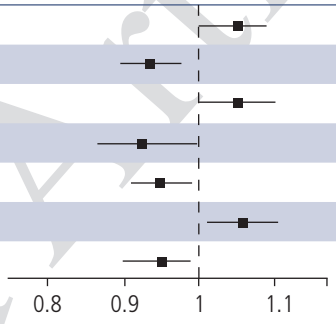
Causal Associations Between Gut Bacterial Pathways and CSU

According to the results of the IVW methods of MR analysis, a total of 8 gut bacterial metabolism pathways were involved in the metabolism of nucleic acids and amino acids, and suggestively significant causal associations with CSU were observed for other vital biological activities. In particular, a protective effect against CSU was observed for the following: NONMEVIPP-PWY, methylerythritol phosphate pathway I (OR, 0.46; 95%CI, 0.24-0.89; *P*=.0201); PWY-5022, 4-aminobutanoate degradation V (OR, 0.34; 95%CI, 0.16-0.76; *P*=.0080); and PWY-7221, guanosine ribonucleotides de novo biosynthesis (OR, 0.56; 95%CI, 0.36-0.87, *P*=.0106). On the other hand, the risk of CSU increased for other pathways, as follows: KDO-NAGLIPASYN-PWY, superpathway of $(Kdo)_2$-lipid A biosynthesis (OR, 1.51; 95%CI, 1.11-2.05; *P*=.0079); PWY_HEME-BIOSYNTHESIS-II, heme biosynthesis I (aerobic) (OR, 1.67; 95%CI, 1.04-2.68; *P*=.0340); PWY-6353, purine nucleotides degradation II (aerobic) (OR, 1.79; 95%CI, 1.10-2.91; *P*=.0192); PWY-7211, superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (OR, 1.90; 95%CI, 1.09-3.31; *P*=.0233); and PWY-7400, L-arginine biosynthesis IV (archaebacteria) (OR, 2.09; 95%CI, 1.18-3.70; *P*=.0117) (Table 3).

Three pathways are affected by CSU. The disease negatively regulates ASPASN-PWY (superpathway of L-aspartate and L-asparagine biosynthesis) (OR, 0.93; 95%CI, 0.88-0.99; *P*=.0311) and GALACT-GLUCUROCAT-PWY (superpathway

Table 2. Causal Effects of Chronic Spontaneous Urticaria on Gut Microbiota Estimated Using Mendelian Randomization Analysis Based on Inverse Variance Weighting.

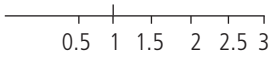
Levels	Gut microbiota	nSNPs	IVW-P	OR (95%CI)
Family	Peptostreptococcaceae	3	0.0426	1.94 (1.03-3.63)
Genus	*Eubacterium hallii group*	3	0.0028	2.77 (1.18-6.50)
Genus	*Eubacterium xylanophilum group*	3	0.0408	0.33 (0.15-0.71)
Genus	*Candidatus Soleaferrea*	3	0.0474	2.21 (1.09-4.47)
Genus	*Christensenellaceae R7 group*	3	0.0116	0.42 (0.20-0.87)
Genus	*Intestinibacter*	3	0.0215	0.56 (0.33-0.95)
Genus	*Lachnospiraceae FCS020 group*	3	0.0242	1.61 (1.04-2.49)



Abbreviations: nSNPs, number of single-nucleotide polymorphisms; IVW-P, inverse variance–weighted *P* value.

Table 3. Causal Effects of Gut Bacterial Pathway on Chronic Spontaneous Urticaria Estimated Using Mendelian Randomization Analysis.

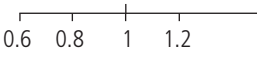
Gut bacterial pathway	nSNPs	IVW-P	OR (95%CI)
KDO-NAGLIPASYN-PWY: superpathway of (Kdo)2-lipid A biosynthesis	7	0.0079	1.51 (1.11-2.05)
NONMEVIPP-PWY: methylerythritol phosphate pathway I	7	0.0201	0.46 (0.24-0.89)
PWY_HEME-BIOSYNTHESIS-II: heme biosynthesis I (aerobic)	10	0.0340	1.67 (1.04-2.68)
PWY-5022: 4-aminobutanoate degradation V	4	0.0080	0.34 (0.16-0.76)
PWY-6353: purine nucleotides degradation II (aerobic)	11	0.0192	1.79 (1.10-2.91)
PWY-7211: superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis	9	0.0233	1.90 (1.09-3.31)
PWY-7221: guanosine ribonucleotides de novo biosynthesis	13	0.0106	0.56 (0.36-0.87)
PWY-7400: L-arginine biosynthesis IV (archaebacteria)	9	0.0117	2.09 (1.18-3.70)



Abbreviations: nSNPs, number of single-nucleotide polymorphisms; IVW-P, inverse variance–weighted *P* value.

Table 4. Causal Effects of Chronic Spontaneous Urticaria on Gut Bacterial Pathway Estimated Using MR Analysis Based on Inverse Variance Weighting.

Gut bacterial pathway	nSNPs	IVW-P	OR (95%CI)
ASPASN-PWY: superpathway of L-aspartate and L-asparagine biosynthesis	3	0.0311	0.93 (0.88-0.99)
GALACT-GLUCUROCAT-PWY: superpathway of hexuronide and hexuronate degradation	3	0.0208	0.93 (0.87-0.99)
GALACTARDEG-PWY: D-galactarate degradation I	3	0.0368	1.13 (1.01-1.27)



Abbreviations: nSNPs, number of single-nucleotide polymorphisms; IVW-P, inverse variance–weighted *P* value.

of hexuronide and hexuronate degradation) (OR, 0.93; 95%CI, 0.87-0.99; *P*=.0208). Conversely, CSU exerts a positive effect on GALACTARDEG-PWY (D-galactarate degradation I) (OR, 1.13; 95%CI, 1.01-1.27; *P*=.0368, Table 4). Detailed information can be seen in Tables S8 and S9. Tables S10 and S11 demonstrate the results of the sensitivity analyses.

Mediation Analysis of Microbiota, the Gut Bacterial Pathway, and CSU

Mediation analysis showed the indirect effect of the family Peptococcaceae on CSU via PWY-7400 and the indirect effect of the genus *Lachnospiraceae UCG010* on CSU via PWY-7221. However, these mediation effects were not statistically significant. Importantly, KDO-NAGLIPASYN-PWY mediated in a causal relationship between the genus *Lachnospiraceae UCG004* and CSU. In addition, we found a masking effect of KDO-NAGLIPASYN-PWY on the relationship between the genus *Methanobrevibacter* and CSU (Figure 2, Tables S12-13).

Discussion

In this study, we performed bidirectional 2-sample MR analysis and mediation MR analysis to investigate the causal effects of GM on CSU and further explore the mediation effect of metabolic pathways. We found a positive association between CSU and the family Peptococcaceae and the genera *Peptococcus*, *Collinsella*, *Lachnospiraceae UCG004*, *Ruminococcaceae UCG004*, and *Sellimonas*. A negative association was found for the genera *Family XIII UCG001*, *Lachnospiraceae UCG010*, and *Methanobrevibacter*. Regarding metabolic pathways, while NONMEVIPP-PWY, PWY-5022, and PWY-7221 were protective factors for CSU, KDO-NAGLIPASYN-PWY, PWY_HEME-BIOSYNTHESIS-II, PWY-6353, PWY-7211, and PWY-7400 increased the risk of CSU. Mediation analysis revealed 4 pathways associated with the gut microbiota, metabolic pathways, and CSU, although the only statistically significant difference was recorded for the mediating effect of KDO-NAGLIPASYN-PWY on causality between *Lachnospiraceae UCG004* and CSU.

Research has led CSU to be considered not only an allergic disease, but also an autoimmune disease with an abnormal inflammatory response. Links have been reported between gut microbiota and skin diseases, such as atopic dermatitis [22], acne [23], systemic lupus erythematosus [24], and multiple sclerosis [7]. Our study provided ample evidence of the gut–skin axis in CSU. We found that the family Peptococcaceae had a bidirectional relationship with CSU and that the genus *Peptococcus* could increase the risk of CSU. Furthermore, the genera *Peptococcus* and *Peptostreptococcus* were dominant in the affected skin area of acute urticaria patients [25]. Factors that promote inflammation or reduce anti-inflammatory mechanisms contribute to CSU. Researchers exploring the clinical application of anti-inflammatory molecular biological agents in CSU found that dupilumab was effective for targeting the IL-4/IL-13 signaling pathway [26,27]. Tezepelumab is useful for treating asthma by targeting thymic stromal lymphopoietin [28], which is elevated in CSU patients [29]. *Peptococcus* was identified as a proinflammatory taxon, and the abundance of *Peptococcus* was shown to be associated with intestinal inflammation and gut barrier function [30,31], thus implying the potential of *Peptococcus* to regulate inflammation in clinical therapy, such as dietary therapy or traditional Chinese medicine [32,33]. Moreover, the present study provides new evidence of how the family Peptococcaceae participates in regulation of inflammation. We identified the indirect effect of the family Peptococcaceae on CSU via the pathway PWY- 7400 (L-arginine biosynthesis IV) (archaebacteria). L-arginine is a versatile amino acid and a central intestinal metabolite in humans and microbes that participates in regulating cell division and growth [34]. It has been proved that disrupted L-arginine metabolism is associated with immune-mediated or infectious and inflammatory diseases [35]. Biosynthesis of L-arginine is positively correlated with the severity of psoriasis [36]. In CSU patients, plasma nitric oxide synthesized from L-arginine was significantly increased [37]. Rat mast cells were shown to synthesize nitric oxide–like factor from L-arginine [38] and were induced to release histamine by poly-L-arginine [39].

Our study revealed a positive association between CSU and the genera *Ruminococcaceae UCG004* and *Collinsella*.

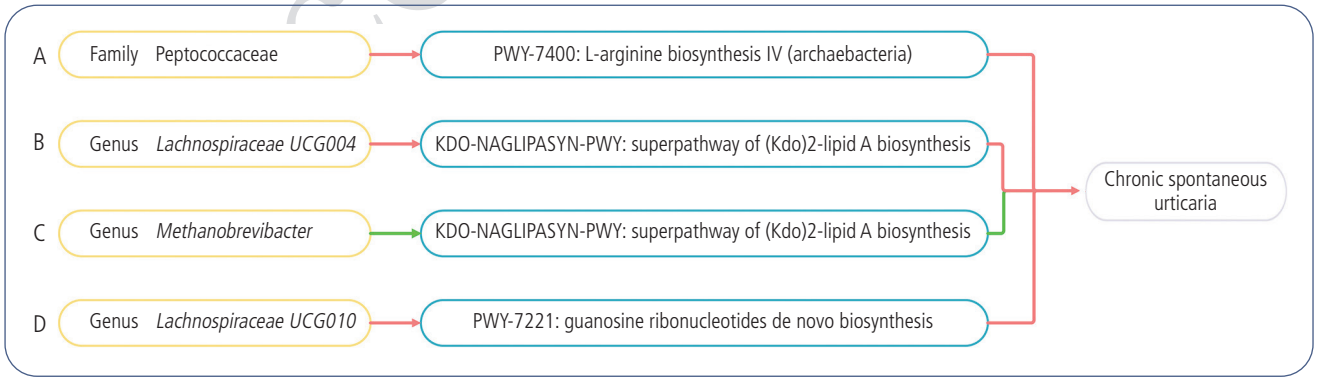


Figure 2. Mediation effects of metabolic pathway on the impact of gut microbiota on chronic spontaneous urticaria. A, The mediation effect of PWY-7400 on the causal relationship between the family Peptococcaceae and CSU. B, The mediation effect of KDO-NAGLIPASYN-PWY in the causal relationship between genus *Lachnospiraceae UCG004* and CSU. C, *Methanobrevibacter* and KDO-NAGLIPASYN-PWY had reverse effects on CSU. D, The mediation effect of PWY-7221 on the causal relationship between genus *Lachnospiraceae UCG010* and CSU. Red arrow, the same direction of the effects of gut bacteria and metabolic pathways on CSU; green arrow, the opposite direction of the effects of gut bacteria and metabolic pathways on CSU. CSU indicates chronic spontaneous urticaria.

Previous studies reported that *Collinsella* and *Ruminococcus* played a vital role in allergic disease, with the abundance of both genera increasing significantly in food allergy patients [40]. The abundance of Ruminococcaceae was significantly reduced in CSU patients [9], and increased *Ruminococcaceae UCG011* may reduce the risk of urticaria [41]. The *Ruminococcaceae* species are also associated with other chronic allergic diseases such as eczema, hives, and rhinitis [42]. *Ruminococcus albus* lysates induced the anti-inflammatory cytokine in a dose-dependent manner in asthma [43], and orally administered *Ruminococcus* significantly reduced the skin inflammation of atopic dermatitis in mice [44]. Based on genetics, MR analysis predicted that *Collinsella* increased the risk of childhood-onset asthma [45]. These findings confirmed the influence of *Collinsella* and *Ruminococcus* in allergy and regulation of inflammation, suggesting that regulation of these intestinal flora may be an appropriate therapeutic strategy in inflammatory disease.

Ćesić et al [46] discovered that the abundance of Lachnospiraceae was decreased in CSU patients. Liu et al [47] found that Lachnospiraceae and its subordinate taxa were also a signature for predicting the efficacy of antihistamines in patients with CSU. In addition, Lachnospiraceae participates in the metabolism of tryptophan, an essential amino acid, as well as in gut immune regulation. Lachnospiraceae was the target of *Bifidobacterium longum CCFM1029*, which upregulates tryptophan metabolism and increases fecal and serum I3C, thus activating the immune response to alleviate atopic dermatitis. The supplementation of Lachnospiraceae increased cecal butyrate content and alleviated visceral hypersensitivity [48]. In this study, however, *Lachnospiraceae UCG004* and *Lachnospiraceae UCG010* showed the opposite effect in CSU. This might be explained by further exploration of gut bacterial pathway mediators. Our findings confirm the indirect effect of *Lachnospiraceae UCG010* on CSU via PWY-7221 and the mediation effect of KDO-NAGLIPASYN-PWY in the causal relationship between *Lachnospiraceae UCG004* and CSU. PWY-7221 has been shown to be involved in nucleotide production, and nucleotides from colostrum and mature milk are vital to enhance intestinal integrity and immunological functions in newborns [49]. (Kdo)2-lipid A is an essential component of lipopolysaccharide [50] and could be recognized by Toll-like receptor 4 in immune cells, including mast cells, and thus trigger downstream proinflammatory cytokine production and antigen presentation during infection [51-54]. Therefore, the imbalance of gut flora may contribute to the increase in (Kdo)2-lipid A, which could in turn trigger the host immune response, leading to immune dysregulation. Similarly, KDO-NAGLIPASYN-PWY was recently found to be enriched in patients with AD [55]. Combining the above findings and those of the present study, treatment targeting metabolic pathways could be a feasible option in CSU.

We also identified a masking effect of KDO-NAGLIPASYN-PWY on the association between the genus *Methanobrevibacter* and CSU. Methanogens, including *Methanobrevibacter smithii*, *Methanosphaera stadtmanae*, and *Methanobrevibacter oralis* microbiota, as ancient domains of single-celled organisms, are major archaea in the digestive system [48,56]. The relationship between methanogens and autoimmune disease has been reported, with authors finding that *M smithii* was less frequent, but *M stadtmanae* significantly more frequent, in inflammatory bowel disease patients than in healthy individuals [57,58]. *M stadtmanae* was shown to be increased in patients with inflammatory bowel disease and to induce an anti–*M stadtmanae* IgG response [58], indicating the participation of *M stadtmanae* in inflammatory disease. Another study found that individuals under 21 years old with multiple sclerosis exhibited a greater abundance of *Methanobrevibacter* [59]. Combining data from the above studies and our findings, the potential mechanism of *Methanobrevibacter* in CSU could be assumed through inflammatory regulation.

Our study found that *Sellimonas* was a risk factor for CSU. *Sellimonas* is a part of the gut microbiota fraction, although it remains uncultivated because of its oxygen-sensitive nature [60], thus contributing to the lack of studies. Therefore, MR analysis is a strong tool for exploring the function of *Sellimonas* in disease [61,62]. The relationship between *Sellimonas* and neurobiological features has been reported. *Sellimonas* is considered an important predictor of depressive symptoms [62]. Previous studies demonstrated that patients with CSU have increased levels of emotional distress with underlying anxiety and depression [5]. Vice versa, increased levels of stress may perhaps predispose individuals to CSU [63]. Interestingly, the present work found *Sellimonas* and *Lachnospiraceae* to be positively associated with CSU. Both genera have been proven to be imbalanced under pressure [48,62], indicating that gut microbiota affects CSU by regulating stress state.

Our study complements and adds to the scarce data on the relationship between metabolic pathways and CSU. A key finding was that gut microbiota affects CSU via metabolic pathways, thus emphasizing the influence of the immune response, especially in terms of inflammatory and anti-inflammatory networks and mental illness.

Strengths and Limitations

This study has several strengths. It is the first bidirectional MR study to be combined with mediation analysis to identify the metabolic pathway as a mediator in facilitating the impact of the gut microbiota on CSU. In addition, our genetic analysis of the gut microbiota, metabolic pathways, and CSU was based on large-scale GWAS data, thus largely mitigating confounding factors such as environment and lifestyle. Consequently, the results were relatively reliable. Finally, the causal relationship identified may provide candidate bacteria and metabolic pathways for subsequent studies on clinical diagnosis and treatment of CSU and promote further research on pathogenesis.

Our study is also subject to a series of limitations. First, given the scarcity of studies on CSU, GWAS based on the gut microbiota is still in its infancy in terms of sample size; therefore, bacterial taxa were only analyzed at the family and genus level but not at a more specialized level such as species or strain. Second, the genomic data used in this investigation were extracted from European cohorts, and it remains to be verified whether the results are representative of other

populations. Third, while MR was instrumental in inferring causality, clinical implications necessitate more direct and further experiments for verification.

Conclusion

Our findings confirmed potential causal associations between gut microbiota and CSU, in which metabolic pathways are the mediators. A pathogenic effect was observed for the family Peptococcaceae, the genus *Peptococcus*, and other genera of gut microbiota, including *Collinsella*, *Lachnospiraceae UCG004*, *Peptococcus*, *Ruminococcaceae UCG004*, and *Sellimonas*. A protective effect was observed for Family XIII U CG001, *Lachnospiraceae UCG010*, and *Methanobrevibacter*. As for metabolic pathways, NONMEVIPP-PWY, PWY-5022, and PWY-7221 were protective factors for CSU, whereas KDO-NAGLIPASYN-PWY, PWY_HEME-BIOSYNTHESIS-II, PWY-6353, PWY-7211, and PWY-7400 were associated with a higher risk of CSU. Mediation analysis indicated that *Lachnospiraceae UCG004* had a causal influence on CSU through KDO-NAGLIPASYN-PWY. This study provides new targeted gut microbiota and metabolic pathways for further mechanistic and clinical studies on CSU.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Statement

Raw data were obtained from the MiBioGen, Dutch Microbiome Project (DMP), and FinnGen databases. The original contributions presented in the study are included in the article/supplementary material. More data supporting the findings of this study are available from the corresponding authors, Jiao Q and Jiang J, on request.

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