Systematic Mendelian randomization study of the effect of gut microbiome and plasma metabolome on severe COVID-19

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Abstract

Background

COVID-19 could develop severe respiratory symptoms in certain infected patients, especially in the patients with immune disorders. Gut microbiome and plasma metabolome act important immunological modulators in the human body and could contribute to the immune responses impacting the progression of COVID-19.

Methods

Based on two-sample Mendelian randomization framework, the causal effects of 131 microbiota in genus or species level and 452 plasma metabolites on severe COVID-19 are estimated. Single nucleotide polymorphisms (SNPs) strongly associated with the abundance of intestinal bacteria in gut and the concentration of metabolites in plasma have been utilized as the instrument variables to infer whether they are causal factors of severe COVID-19. In addition, mediation analysis is conducted to find the potential link between the microbiota and metabolite which identified by polygenic Mendelian randomization analysis, while colocalization analysis has been performed to validate the causal relationships which identified by *cis*-Mendelian randomization analysis.

Results

Mendelian randomization support 13 microbiota and 53 metabolites, which are significantly causal association with severe COVID-19. Mediation analysis find 11 mediated relations, such as myo-inositol, 2-stearoylglycerophosphocholine and alpha-glutamyltyrosine, which appeared to mediate the association of *Howardella* and *Ruminiclostridium 6* with severe COVID-19 respectively, while *Butyrivibrio* and *Ruminococcus gnavus* appeared to mediate the association of myo-inositol and N-acetylalanine respectively. *Ruminococcus torques* abundance was colocalized with severe COVID-19 (PP.H4 = 0.77) and the colon expression of permeability related protein RASIP1 (PP.H4 = 0.95).

Conclusions

Our study results highlight the causal relationships of gut microbiome and plasma metabolome for severe COVID-19, which have the promise to be served as clinical biomarkers for risk stratification and prognostication, and novel basis to unravel the pathophysiological mechanisms of severe COVID-19.

Introduction

COVID-19 as a global pandemic continues to spread rapidly across the world causing serious concerns. The individuals with COVID-19 infection could develop fevers, coughing, difficulty in breathing, pneumonia and even death. The prognosis of COVID-19 could be greatly improved with the prevention from the development of severe symptoms. The severity of symptoms varied among the patients which may be attributed to immunity, a combination of basic diseases, ACE2 expression and genetic factors (1, 2, 3, 4, 5). Nevertheless, the mechanism needs to be further studied.

The gastrointestinal tract is the largest immunological organ in human body and plays an essential role in immunity regulation (6, 7). The microbiome as an important immune regulator in the gastrointestinal system controls host immunity by preserving intestinal mucosa and producing immune regulatory metabolites (such as short chain fatty acids) (8). The gut microbiota has been suggested to be closely associated with COVID-19 infection status and severity (9). In the COVID-19 patients, the abundance of *Faecalibacterium prausnitzii*, *Clostridium butyricum* is decreased, while the abundance of *Enterobacter* and *Enterococcus* is increased (10). In addition, the abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewai* in the feces is positively associated with the severity in hospitalized COVID-19 patients, while the abundance of *Faecalibacterium prausnitzii* is negatively correlated (11). However, these conventional correlation studies of intestinal bacteria and COVID-19 infection or severity are not able to identify a causative relationship, and thus cannot guide the development of clinical treatment regimens of COVID-19.

The severity of COVID-19 has been reported to be associated with disturbance of various metabolic pathways which are directly or indirectly associated with the systemic inflammatory response observed in patients with severe COVID-19 (12, 13, 14, 15). And the metabolites could accurately predict the course of COVID-19, such as tryptophan, kynurenine and 3-hydroxykynurenine, the metabolites of kynurenine pathway(16). In a recent study on immune metabolism, proinflammatory cytokines and chemokines were found to be closely related to metabolites originating in TCA cycle, amino acid metabolism, purine and pyrimidine metabolism and primary bile acid metabolism in severe COVID-19 patients (17). Therefore, searching for serum metabolites that cause severe COVID-19 could help doctors to prevent the onset of servious illness earlier.

Identifying the causal factors conferred to the severity of COVID-19 and further intervening could allow to possibly prevent the onsets of severe COVID-19. Causal analysis is a prominent statistical method which allows the analysis of causal relationship between exposure factors and outcomes. Mendelian randomization (MR) studies can provide strong evidence for the causal inference between exposure factors and outcomes. Based on the principle of random allocation of alleles in gamete formation, MR divides the population into exposure group and control group by using genetic loci

strongly related to exposure factors, and compares the difference in outcome between the two groups. And this could achieve an effect similar to that of RCT, so as to realize causal inference. Here, we applied Mendelian randomization, one of the most prevalent methods of causal analysis in clinical practice allowing the inference of causality from GWAS summary data (18) to examine the relationship between the gut microbiome, plasma metabolome and severe COVID-19. We find 13 microbiota and 53 metabolites act as the causing factors associating with the severity of COVID-19. Moreover, 11 mediated relations among them have been identified. This is the first study to determine the causal relationship between intestinal microbiome, plasma metabolome and the development of severe COVID-19.

Method And Materials

GWAS datasets and IV selection

The GWAS results data of gut microbiome abundance are downloaded from MiBioGen consortium (https://mibiogen.gcc.rug.nl/). They investigate the relationship between whole genome SNPs and the abundance of 211 intestinal taxa in 18473 participants from 24 cohorts in European and American countries (19). Among these taxa, 131 genus and species are selected for the subsequently analysis. The genotyping information is obtained using whole genome genotyping microarrays, and the abundance of each taxa is determined using 16S rDNA sequencing. The GWAS summary statistics of plasma metabolome are obtained from IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/). A total of 452 plasma metabolites are investigated in 7824 European subjects(20). COVID-19 research cohorts are obtained from the COVID-19 Host Genetics Initiative Program (21). The severe respiratory disease of COVID-19 dataset contains 5101 COVID-19 patients with very severe respiratory and 1383241 population controls, with a total of 9739225 SNPs genotyped. The GWAS summary statistics of severe COVID-19 (GCST011075) is obtained from GWAS Catalog (https://www.ebi.ac.uk/gwas/). Due to different p values as cutoff values, different number of IV will be screened out. In order to get a more comprehensive information, four p value levels ($p < 1\times10^{-5}$, $p < 1\times10^{-6}$, $p < 1\times10^{-7}$and $p < 1\times10^{-8}$) are set for of intestinal bacteria and plasma metabolites instrument variable (IV) screening.

Mendelian randomization (MR) and mediation analysis

At first, univariate MR analysis is performed to identify the causal relationship between intestinal bacteria and severe COVID-19 or plasma metabolite and severe COVID-19. According to the number of IV, different methods are utilized. If the number is more than 1, Inverse variance weighted (IVW) method is employed, otherwise the Wald ratio model is used. In addition, Cochran Q test and MR-Egger's intercept are used to investigate the pleiotropy and heterogeneity of the selected IVs. Only the intestinal bacteria and plasma metabolites with MR $p < 0.05$ and without pleiotropy and heterogeneity (het $Q > 0.05$, pleio $p > 0.05$) are included in follow-up analysis. Mediation analysis is an analytical method to test whether a variable is a mediating variable and to what extent it plays a mediating role. Through mediation Mendelian randomization analysis, we can construct a pathway from exposure factors to mediating factors to outcomes, helping to elucidate the potential mechanism of exposure factors affecting COVID-19 outcomes. For bacteria and metabolites that causally associated with severe COVID-19, bi-directional mediation analysis are conducted to find the possible link among bacteria and metabolites. The 'total' effect of exposure (includes both 'direct' effect and any 'indirect' effect via mediator) on severe COVID-19 is calculated by univariate MR analysis, and the 'indirect' effects is obtained from two-step MR(22, 23). The proportion of mediation effect of the mediator is calculated by the following formula: $\rho_M = \frac{\beta_{EM}\times\beta_{MO}}{\beta_{EO}}$. Where ρ_M is the proportion of mediation effect of the mediator M, β_{EM} is the MR casual effect of exposure E on mediator M, β_{MO} is the MR casual effect of mediator M on outcome O, and β_{EO} is the 'total' effect of exposure E on outcome O. In addition, for similar exposures, multivariate MR is also performed to identify the key exposures. All of above analysis are conducted by using R package TwoSampleMR (24).

Database analysis

MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) is utilized to conduct the enrichment analysis of metabolites. For the most significant exposure in the cis-MR, further colocalization analysis and eQTL analysis are conducted. GTEX database (https://gtexportal.org) is used to perform eQTL analysis, and the genes whose expression can be regulated by the IV of candidate intestinal bacteria in colon tissue are screened (25). The colocalization analysis among candidate exposure, severe COVID-19 and the expression of the genes screened by eQTL analysis is also conducted by using R package coloc (26). Web software LocusZoom (http://locuszoom.sph.umich.edu/) is used for regional association plotting, and the flanking size is set as 50kb (27).

Results

MR analysis results of gut microbiome

Using univariate MR analysis, we identify 13 significant taxa with causal relationship with severe COVID-19, including *Alloprevotella*, *Anaerotruncus, Bifidobacterium*, *Butyrivibrio*, *Gordonibacter, Howardella, Oxalobacter, Rikenellaceae RC9, Ruminiclostridium 6, Ruminococcus gnavus, Ruminococcus torques, Tyzzerella3 and unknowngenus.id.1000001215* (Table 1). Among them, *Anaerotruncus, Butyrivibrio, Gordonibacter, Oxalobacter, Rikenellaceae RC9, Ruminiclostridium 6, Tyzzerella3 and unknowngenus.id.1000001215* show protective causal effect on severe COVID-19, while *Alloprevotella*, *Bifidobacterium*, *Howardella*, *Ruminococcus torques* and *Ruminococcus gnavus* have positive causal effect. *Bifidobacterium* and *Oxalobacter* show the same causal effects on COVID-19 at two p value levels.

Table 1
Gut bacteria significantly causally associated with severe COVID-19

Microbiota	Method	nSNP	OR	Up95%CI	Low95%CI	P_{mr}	Q value	P_{pleio}	level
Alloprevotella	IVW	4	1.627	2.323	1.140	0.007	0.151	0.489	e5
Anaerotruncus	Wald ratio	1	0.213	0.595	0.076	0.003	NA	NA	e6
Bifidobacterium	IVW	2	2.092	3.808	1.149	0.016	#N/A	NA	e7
Bifidobacterium	Wald ratio	1	2.376	4.663	1.211	0.012	NA	NA	e8
Butyrivibrio	IVW	14	0.830	1.000	0.690	0.050	0.052	0.132	e5
Gordonibacter	Wald ratio	1	0.578	0.998	0.335	0.049	NA	NA	e6
Howardella	IVW	7	1.264	1.583	1.009	0.042	0.401	0.160	e5
Oxalobacter	IVW	11	0.842	1.000	0.709	0.050	0.543	0.590	e5
Oxalobacter	IVW	4	0.752	0.980	0.578	0.035	0.722	0.377	e6
Rikenellaceae RC9 gutgroup	Wald ratio	1	0.627	0.935	0.421	0.022	NA	NA	e6
Ruminiclostridium 6	IVW	14	0.708	0.921	0.544	0.010	0.331	0.500	e5
Ruminococcus gnavus group	IVW	2	1.703	2.849	1.018	0.043	0.538	NA	e6
Ruminococcus torques group	Wald ratio	1	6.660	19.937	2.225	0.001	NA	NA	e7,e8
Tyzzerella3	Wald ratio	1	0.452	0.802	0.255	0.007	NA	NA	e8
unknowngenus.id.1000001215	IVW	5	0.720	0.966	0.536	0.029	0.346	0.336	e5

MR analysis results of plasma metabolome

A total of 53 plasma metabolites have been found significant causal association with severe COVID-19 (Table 2). Among them, the plasma concentration of 12-hydroxyeicosatetraenoate, 2-linoleoylglycerophosphocholine, 4-androsten-3beta, 17beta-diol disulfate 1, alpha-glutamyltyrosine, Cis-4-decenoyl carnitine, leucylalanine (X-14304), octanoylcarnitine and urate show positive causal effect on severe COVID-19 at more than 2 p value levels with the same direction. Enrichment analysis of the 53 identified metabolites indicate that they are significantly enriched in pathways of ascorbate and aldarate metabolism, beta oxidation of very long chain fatty acids and oxidation of branched chain fatty acids (Fig. 2). 5 identified metabolites are related to carnitine metabolism, include 2-tetradecenoyl carnitine, carnitine, cis-4-decenoyl carnitine, decanoylcarnitine and octanoylcarnitine. After multivariate MR, carnitine (multivariate MR $p = 0.001$) and 2-tetradecenoyl carnitine (multivariate MR $p = 0.075$) can be retained in the multivariate MR model ($p < 0.1$) (Fig. 3A). In addition, 4 identified metabolites, aspartylphenylalanine, leucylalanine (X-14189), leucylalanine (X-14304) and N-acetylalanine are correlated to lalanine, and only N-acetylalanine (multivariate MR $p = 0.066$) is retained after multivariate MR (Fig. 3B).

Table 2
Metabolites significantly causally associated with severe COVID-19

Metabolite	Method	nSNP	OR	Up95%CI	Low95%CI	P_{mr}	Q value	P_{pleio}	level
10-nonadecenoate (19:1n9)	IVW	6	0.242	0.878	0.067	0.031	0.707	0.359	e5
1-docosahexaenoylglycerophosphocholine*	IVW	3	0.133	0.978	0.018	0.047	0.678	0.603	e5
2-hydroxybutyrate (AHB)	IVW	3	0.083	0.829	0.008	0.034	0.888	0.865	e6
2-linoleoylglycerophosphocholine*	IVW	2	27.594	501.151	1.519	0.025	0.299	NA	e6
2-linoleoylglycerophosphocholine*	Wald ratio	1	132.581	7774.598	2.261	0.019	NA	NA	e7, e8
2-stearoylglycerophosphocholine*	IVW	10	2.881	7.493	1.108	0.030	0.387	0.954	e5
4-androsten-3beta,17beta-diol disulfate 1*	IVW	4	0.581	0.919	0.367	0.020	0.352	0.366	e6
4-androsten-3beta,17beta-diol disulfate 1*	IVW	2	0.513	0.825	0.318	0.006	0.597	NA	e7, e8
4-hydroxyhippurate	Wald ratio	1	0.214	0.891	0.052	0.034	NA	NA	e6
acetylcarnitine	IVW	3	0.140	0.858	0.023	0.034	0.628	0.891	e6
acetylcarnitine	IVW	2	0.100	0.700	0.014	0.020	0.817	NA	e7, e8
alpha-hydroxyisovalerate	IVW	8	0.261	0.664	0.102	0.005	0.838	0.886	e5
aspartylphenylalanine	Wald ratio	1	3.230	10.216	1.021	0.046	NA	NA	e7, e8
carnitine	IVW	9	0.121	0.869	0.017	0.036	0.670	0.545	e8
cis-4-decenoyl carnitine	IVW	8	2.461	5.038	1.202	0.014	0.952	0.838	e5
cis-4-decenoyl carnitine	IVW	4	2.585	5.708	1.171	0.019	0.564	0.985	e6
cis-4-decenoyl carnitine	IVW	2	2.900	6.883	1.221	0.016	0.768	NA	e7, e8
citrulline	IVW	4	0.026	0.607	0.001	0.023	0.572	0.323	e7
decanoylcarnitine	IVW	3	2.721	5.795	1.278	0.009	0.309	0.737	e8
dehydroisoandrosterone sulfate (DHEA-S)	IVW	2	0.237	0.792	0.071	0.019	0.271	NA	e7, e8
gamma-glutamylisoleucine*	Wald ratio	1	0.004	0.865	0.000	0.044	NA	NA	e6
gamma-glutamylthreonine*	IVW	6	4.644	17.604	1.225	0.024	0.456	0.905	e5
hexanoylcarnitine	IVW	2	2.404	5.285	1.094	0.029	0.383	NA	e6,e7
hexanoylcarnitine	Wald ratio	1	2.636	5.951	1.168	0.020	NA	NA	e8
HWESASXX*	IVW	4	3.133	9.583	1.025	0.045	0.946	0.884	e5
laurate (12:0)	IVW	9	0.094	0.942	0.009	0.044	0.358	0.712	e6
myo-inositol	IVW	26	5.617	16.964	1.860	0.002	0.676	0.939	e5
myo-inositol	IVW	5	24.491	281.774	2.129	0.010	0.917	0.421	e6
myo-inositol	IVW	2	75.109	2217.927	2.544	0.012	0.863	NA	e7
N-acetylalanine	IVW	4	97.063	4643.843	2.029	0.020	0.732	0.745	e6
octanoylcarnitine	IVW	12	1.901	3.304	1.094	0.023	0.728	0.385	e5
octanoylcarnitine	IVW	6	2.130	3.857	1.177	0.013	0.712	0.888	e6
octanoylcarnitine	IVW	3	2.343	4.384	1.253	0.008	0.663	0.613	e7
octanoylcarnitine	IVW	2	2.386	4.549	1.251	0.008	0.380	NA	e8
pseudouridine	IVW	5	0.005	0.178	0.000	0.003	0.428	0.874	e6

Metabolite	Method	nSNP	OR	Up95%CI	Low95%CI	P_{mr}	Q value	P_{pleio}	level
scyllo-inositol	IVW	7	0.440	0.988	0.196	0.047	0.851	0.776	e5
taurodeoxycholate	Wald ratio	1	0.435	0.891	0.213	0.023	NA	NA	e6
urate	IVW	3	265.964	27719.168	2.552	0.019	0.454	0.428	e6
urate	Wald ratio	1	13946.581	47843971.004	4.065	0.022	NA	NA	e7
X-04494	IVW	2	24.449	540.287	1.106	0.043	0.277	NA	e6
X-10346	Wald ratio	1	1.928	3.257	1.141	0.014	NA	NA	e6
X-11438	IVW	21	1.972	3.819	1.018	0.044	0.164	0.914	e5
X-11485	Wald ratio	1	4.450	14.469	1.369	0.013	NA	NA	e6
X-11497	IVW	8	7.303	38.614	1.381	0.019	0.686	0.637	e5
X-11792	IVW	13	1.363	1.799	1.032	0.029	0.509	0.728	e5
X-11792	IVW	5	1.562	2.270	1.075	0.019	0.367	0.558	e6
X-11858	Wald ratio	1	2.717	5.907	1.250	0.012	NA	NA	e8
X-12013	IVW	3	1.478	2.064	1.058	0.022	0.890	0.939	e6
X-12013	Wald ratio	1	1.478	2.101	1.040	0.029	NA	NA	e7
X-12188	IVW	7	1.205	1.434	1.012	0.036	0.809	0.684	e6
X-12244	IVW	14	3.536	12.408	1.008	0.049	0.284	0.048	e5
X-12441	IVW	8	0.563	0.847	0.374	0.006	0.502	0.484	e5
X-12441	IVW	2	0.393	0.750	0.206	0.005	0.926	NA	e6
X-12442	Wald ratio	1	3.566	12.224	1.040	0.043	NA	NA	e6,e7,e8
X-12850	Wald ratio	1	0.308	0.985	0.096	0.047	NA	NA	e8
X-13477	IVW	5	6.383	37.654	1.082	0.041	0.347	0.887	e5
X-13553	IVW	8	0.231	0.976	0.055	0.046	0.036	0.075	e5
X-14056	IVW	6	0.244	0.702	0.085	0.009	0.665	0.532	e5
X-14086	IVW	11	2.321	4.422	1.218	0.010	0.639	0.271	e5
X-14086	IVW	3	3.110	9.115	1.061	0.039	0.498	0.984	e6
X-14086	Wald ratio	1	4.736	21.819	1.028	0.046	NA	NA	e7, e8
X-14189	Wald ratio	1	2.505	6.172	1.016	0.046	NA	NA	e6,e7,e8
X-14205	IVW	12	1.941	3.367	1.119	0.018	0.816	0.932	e5
X-14205	Wald ratio	1	3.389	11.242	1.022	0.046	NA	NA	e6,e7,e8
X-14208	Wald ratio	1	3.905	11.649	1.309	0.015	NA	NA	e8
X-14304	Wald ratio	1	2.741	7.380	1.018	0.046	NA	NA	e8
X-18601	IVW	2	0.220	0.696	0.069	0.010	0.823	NA	e8

Bi-directional Mediation analysis results

To explore the potential mechanism of intestinal microbiome and plasma metabolome on severe COVID-19, bi-directional mediation analysis between intestinal bacteria and plasma metabolites are conducted. This analysis is restricted to the bacteria and metabolites that significantly causal associated with severe COVID-19 in polygenic MR analysis. A total of 11 mediation relationships are identified, and 6 of them are composed by known bacteria and metabolites (Fig. 4). The indirect effect of *Howardella* via myo-inositol is 13.7% (Fig. 4A). The proportion of mediation effect of *Ruminiclostridium 6* on severe COVID-19 via 2-stearoylglycerophosphocholine, 2-tetradecenoyl carnitine, alpha-glutamyltyrosine and X-11497 are 18.0%, 14.5%, 14.5% and 16.7% respectively (Fig. 4B-E). *Butyrivibrio* mediated 12% effect of myo-inositol on severe COVID-19 (Fig. 4F). *Ruminococcus gnavus* mediated more than one third effect (36.8%) of N-acetylalanine (Fig. 4G). Due to the effects of *Ruminiclostridium 6* on severe COVID-19 is mediated by 4 plasma metabolites, multivariate MR is also performed to find the key metabolites, and alpha-glutamyltyrosine (multivariate MR p = 0.027) and 2-stearoylglycerophosphocholine (multivariate MR p = 0.051) are retained (Table 3). After adjusted 2-stearoylglycerophosphocholine, alpha-glutamyltyrosine or both, the effect of *Ruminiclostridium 6* on severe COVID-19 decreased, and the MR p value become insignificant (Table 4).

Table 3
Multivariate Mendel randomization analysis of *Ruminiclostridium 6* related metabolites

Exposure	Univariate				Multivariate			
	Beta	SE	OR (95%CI)	P	Beta	SE	OR (95%CI)	P
X-11497	1.896	0.869	6.66 (1.213 ~ 36.570)	0.029	0.492	0.806	1.635 (0.337 ~ 7.939)	0.542
2-stearoylglycerophosphocholine	1.058	0.488	2.881 (1.107 ~ 7.497)	0.030	0.982	0.503	2.671(0.996 ~ 7.155)	0.051
2-tetradecenoyl carnitine	0.656	0.303	1.927 (1.064 ~ 3.490)	0.030	0.371	0.318	1.450 (0.777 ~ 2.703)	0.243
Alpha-glutamyltyrosine	0.683	0.283	1.98 (1.137 ~ 3.448)	0.016	0.607	0.274	1.834 (1.072 ~ 3.139)	0.027

Table 4
Univariate and multivariate Mendel randomization analysis of *Ruminiclostridium 6*

Status	Beta	SE	OR (95%CI)	P
Univariate	-0.346	0.135	0.708 (0.544 ~ 0.921)	0.010
Adjusted by 2-stearoylglycerophosphocholine	-0.277	0.194	0.758 (0.519 ~ 1.109)	0.154
Adjusted by alpha-glutamyltyrosine	-0.320	0.164	0.726 (0.526 ~ 1.002)	0.051
Adjusted by both	-0.241	0.167	0.786 (0.567 ~ 1.089)	0.148

Eqtl And Colocalization Analysis

After applied multiple test correction based on Bonferroni correction method for cis-MR results, only *Ruminococcus torques* has a trend of positive causal relationship with the severe COVID-19 (Bonferroni adjusted p = 0.092, raw p = 7.0×10^{-4} [OR = 6.66, 95%CI:2.23–19.94]). Using GTEX database, we perform the eQTL analysis and identify rs35866622 (the IV of *Ruminococcus torques*) as the eQTL of RASIP1, NTN5, MAMSTR, SEC1P, IZUMO1, FAM83E, SPHK2 and FUT2. Colocalization analysis reveals that the abundance of *Ruminococcus torques* is highly colocalized with severe COVID-19 (PP.H4 = 0.77, Fig. 5). Moreover, the abundance of *Ruminococcus torques* is also found to be significantly colocalized with the mRNA expressions of RASIP1, NTN5, MAMSTR, and SEC1P (PP.H4 > 0.92, Fig. 5), which indicates that they might be affected by the same cause. Further analysis reveals that only RASIP1 expression has a higher colocalization probability with severe COVID-19. The PP.H4 value in transverse colon and sigmoid colon are 0.73 and 0.75, respectively (Fig. 5). These findings indicates that RASIP1 expression in colon may influence the risk of respiratory severity in COVID-19 patients.

Discussion

In this study, the causal relationships of gut microbiome and plasma metabolome for the severity of COVID-19 are investigated using Mendelian randomization analysis. We identify 13 microbiota (*Butyrivibrio*, *Howardella*, *Oxalobacter*, *Ruminiclostridium 6*, *Ruminococcus torques*, etc.) and 53 metabolites (2-stearoylglycerophosphocholine, alpha-glutamyltyrosine, carnitine, myo-inositol, etc.) to be putative causal for severe COVID-19. Pathway analysis of the 53 identified metabolites suggests that they are significantly enriched in pathways of ascorbate and aldarate metabolism, beta oxidation of very long chain fatty acids and oxidation of branched chain fatty acids. Mediation analysis among the identified exposures find that the associations of *Howardella, Ruminiclostridium 6*, myo-inositol and N-acetylalanine with severe COVID-19 are likely to be mediated by one or more of exposure. After multiple testing correction of cis-MR results, only *Ruminococcus torques* has a trend of positive causal relationship with the severe COVID-19. The increased abundance of *Ruminococcus torques* can be a contributing factor to the incidence of severe respiratory symptoms in COVID-19 patients. The results of the colocalization analysis reveal that the abundance of *Ruminococcus torques* and the expression of RASIP1 in colon tissue share a causal factor and has a high colocalization probability with the occurrence of severe respiratory symptoms, implying that they both play important roles in the development of severe COVID-19.

Myo-inositol has been reported to downregulate the expression of IL-6 levels inhibiting the downstream inflammatory response (28). Furthermore, myo-inositol, as precursor of inositol-phosphate, stimulates surfactant production in lung tissue, and thus could represent a potential preventive strategy for COVID-19 (28, 29). Consistent with this, we provide causal evidence for directionally consistent effects of myo-inositol on severe COVID-19. Bi-directional Mediation analysis results indicates that myo-inositol mediated 13.7% effect of *Howardella* on severe COVID-19, while the mediation effect of myo-inositol via *Butyrivibrio* is 12% for severe COVID-19.

A recent study indicates that gut microbiome of patients with post-acute COVID-19 syndrome are characterized by higher levels of *Ruminococcus gnavus*(30), which has been shown to promote inflammatory responses and impair barrier functions by producing inflammatory polysaccharides(31). We also show that *Ruminococcus gnavus* causally increases the risk of severe COVID-19 using univariate MR analysis. Furthermore, Mediation analysis results reveal that *Ruminococcus gnavus* mediates more than one third effect (36.8%) of N-acetylalanine on severe COVID-19.

Notably, *Ruminiclostridium 6* was previously found to have a strong positive correlation with the levels of ghrelin (32), which exerts immunomodulatory functions in COVID-19 infection, such as the suppressive effects on pro-inflammatory cytokine production including IL-1 β, IL-6 and TNF- α (33). Therefore, it is conceivable that the causal effect of *Ruminiclostridium 6* on severe COVID-19 may result from ghrelin. A recent MR analysis also reveals 2-stearoylglycerophosphocholine and alpha-glutamyltyrosine, as causal metabolites, increase the risk of severe COVID-19 (34). These findings could explain why the causal effect of *Ruminiclostridium 6* on severe COVID-19 is mediated by via 2-stearoylglycerophosphocholine and alpha-glutamyltyrosine (18.0% and 14.5%, respectively) in our mediation analysis.

Ruminococcus torques, also known as *Mediterraneibacter torques*, is an anaerobic and gram-positive intestinal bacteria which belongs to the genus *Mediterraneibacter* in the family *Lachnospiraceae*. According to earlier research, *Ruminococcus torques* is positively associated with intestinal paracellular permeability and gastrointestinal disorders (35, 36). Increased intestinal permeability could cause endotoxemia and activate the inflammatory response, which ultimately raises the risk of various diseases including severe illness in COVID-19 patients (37, 38). Additionally, an increase in the abundance of *Ruminococcus torques* is associated with constipation and diarrhoea in children with autism, and the presence of gastrointestinal symptoms has been demonstrated to be an independent risk factor for severe COVID-19 (39, 40). Therefore, *Ruminococcus torques* could have a potential role in the development of severe respiratory symptoms in COVID-19 patients.

Ras interacting protein 1 (RASIP1) is a vascular-specific GTPase signaling regulator involved in a variety of functions, including the Rho signal transmission pathway. RASIP1 regulates the stability of vascular endothelial connections, which is relevant to vascular barrier function, and mediates the regulation of Rho in intrinsic barrier function through Rap1 (41, 42). RASIP1 depletion reduces the barrier function of vascular endothelial cells induced by Rap1 (43). The disruption of endothelium barrier can result in chronic inflammation, atherosclerosis and vascular leakage, as well as the development and progress of COVID-19 (44). Interestingly, *Ruminococcus torques* and RASIP1 are both associated with cell permeability, and in our investigation, they seem to have a very strong colocalization. Previous database analysis results indicate that rs35866622 decreases the abundance of *Ruminococcus torques* while increases the expression of RASIP1indicating a negative association relationship. As a result, increased abundance of *Ruminococcus torques* coupled with the decreased RASIP1 expression are associated with the disruption of cell barrier and increased permeability, thereby ultimately increase the risk of COVID-19 worsening which is consistent with our MR results.

From the 53 metabolites found to increase the risk of severe COVID-19, we pinpoint the key pathways including ascorbate and aldarate metabolism, beta oxidation of very long chain fatty acids and oxidation of branched chain fatty acids. In these signals, vitamins (ascorbate and aldarate metabolism) have been reported responding to the risk of COVID-19 and its severity. Vitamin C is a potential antiviral agent and may improve immunity. Supplementation with high-dose vitamin C could increase the survival rates of patients with severe COVID-19 by decreasing inflammation and pathogen infectivity and viral yield, improving immune response, alleviating tissue and organ damage(45). Numerous evidences confirm vitamin D insufficiency is associated with greater severity of COVID-19 infection, even the more recent Omicron subvariant of COVID-19(46, 47, 48). Vitamin D administration has been found to be associated with less severe COVID-19 and resulted in a decreased risk of death and admission to intensive care units in patients with COVID-19 (49). In addition, fatty acid metabolism is a crucial event for many viruses to complete their life cycle, and a common consequence of infection by many viruses is to change the nature of lipid metabolism usually from fatty acid oxidation to fatty acid synthesis(50). Fatty acid oxidation is the most powerful pathway to generate energy, and significant impairment in fatty acid oxidation has been reported in patients with post-acute COVID-19 syndrome(51, 52). Our results indicate that the severe COVID-19 causal associated metabolites were significantly enriched in pathways of beta oxidation of very long chain fatty acids and oxidation of branched chain fatty acids. Therefore, fatty acid metabolism offers another promising target to control the COVID-19 infection extent.

It is also worth noting that 5 metabolites (2-tetradecenoyl carnitine, carnitine, cis-4-decenoyl carnitine, decanoylcarnitine and octanoylcarnitine) in the carnitine metabolism pathway were identified to be causal associated with severe COVID-19. Consistent with our findings, a UPLC-MS/MS-based widely targeted metabolomics study also reveals several carnitine family members are significantly reduced in severe COVID-19 patients versus healthy controls subjects and mild COVID-19 patients(53). Carnitine metabolism balance plays an important role in maintaining normal physiological functions through its anti-inflammatory, antioxidative, anti-apoptotic, anti-fibrosis and biomembrane-stabilizing properties(54). Carnitine deficiency occurs in multiple diseases such as sepsis, advanced liver cirrhosis and endocrine disorders(54). Severe COVID-19 patients usually exhibit metabolic disorders and multiple organ dysfunctions, the downregulated carnitine in the severe patients may contribute to impaired organ function. Additionally, alanine, as another important metabolic pathway for COVID-19 severity, is revealed by the pathway enrichment of 4 identified metabolites (aspartylphenylalanine, leucylalanine (X-14189), leucylalanine (X-14304) and N-acetylalanine). A key physiological function of alanine is to transport

pyruvate and glutamate from the muscles to the liver, a process known as the glucose–alanine cycle. Data from patients with different severity grades of COVID-19 show that circulating pyruvate level is the strongest determinants of severe COVID-19(55), and a meta-analysis indicates elevated glutamate is associated with an increased risk of COVID-19 severity(56).

Conclusion

In conclusion, our comprehensive MR analyses identify 13 human gut microbiota and 53 human serum metabolites causal associated with COVID-19 severity. We also find 11 mediated relations on the causal effects of COVID-19 pathology. Our findings reveal the landscape of how gut microbiota and circulating metabolites may affect COVID-19 progression. These causal microbiota and metabolites have the promise to be served as clinical biomarkers for risk stratification and prognostication, and novel basis to unravel the pathophysiological mechanisms of severe COVID-19.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Institute of Clinical Pharmacology, Central South University.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Xi Li, Longbo Zhang and Jian-Quan Luo participated in research design. Han Yan, Longbo Zhang, Jian-Quan Luo, Si Zhao and Wei Zhang participated in the writing of the paper. Han-Xue Huang, Pan Xie, Xin-He Cai and Yun-Dan Qu participated in the performance of the research. Xi Li and Han Yan prepared the figures. All authors reviewed the manuscript.

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Figures

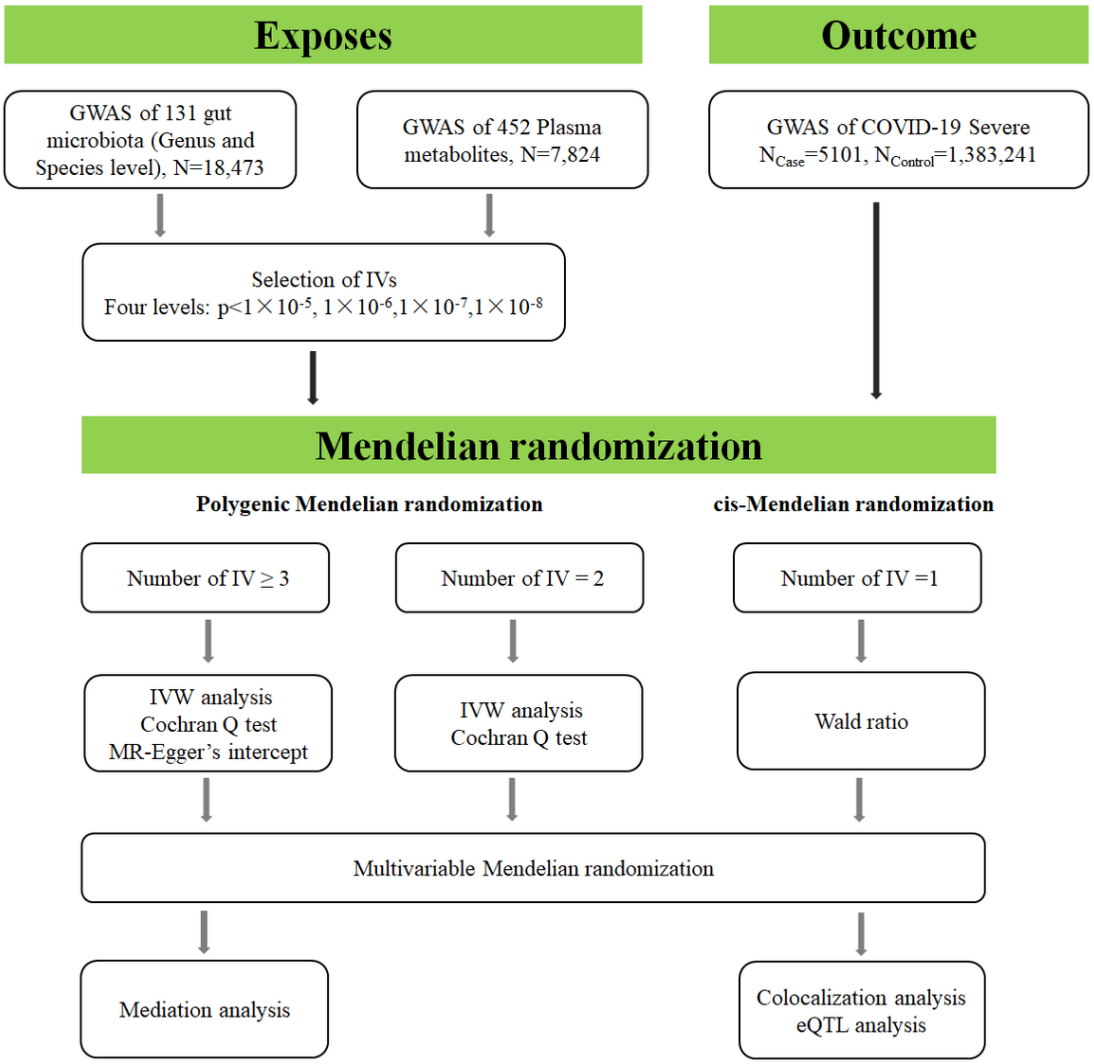

Figure 1

Research flow chart of the study.

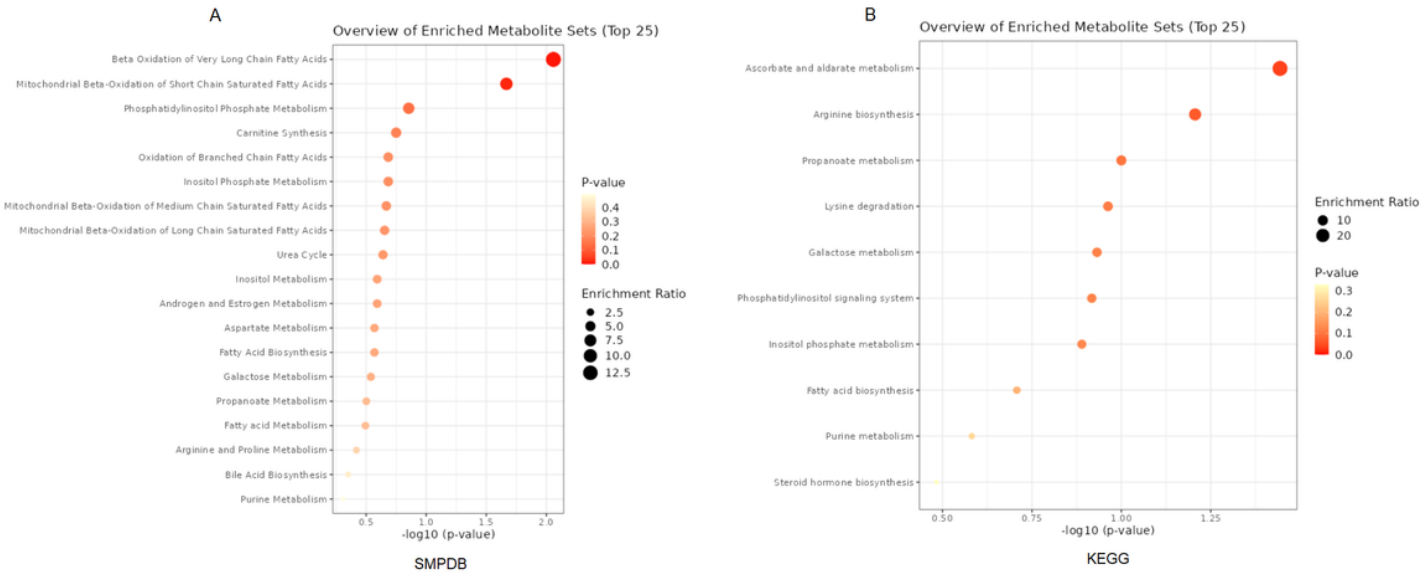

Figure 2

Enrichment analysis results of the causal metabolites of severe COVID-19.

A: Based on SMPDB. B: Based on KEGG database.

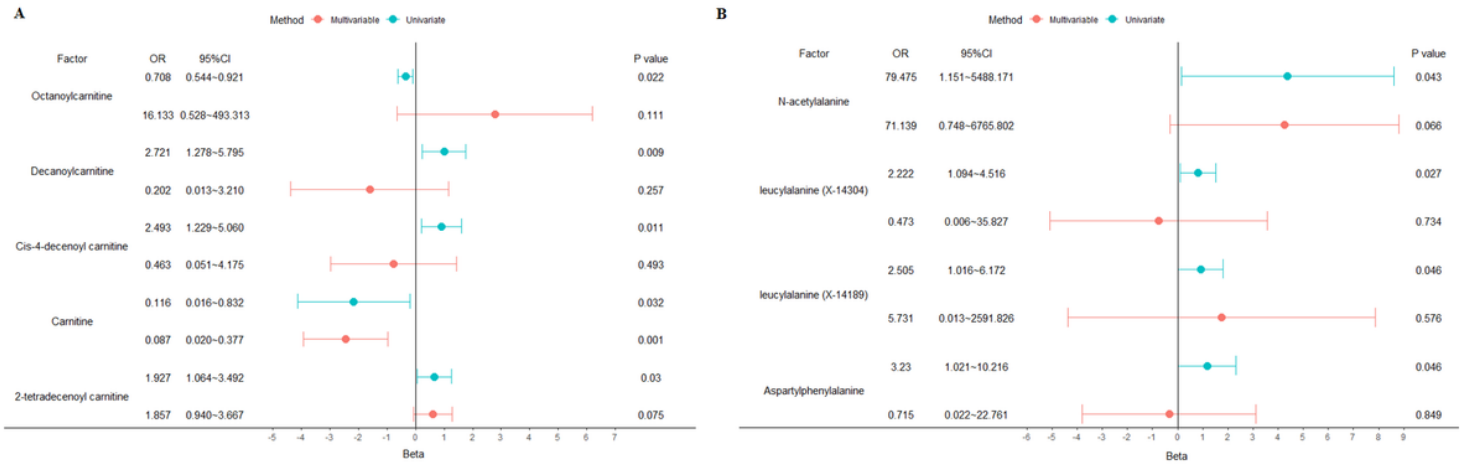

Figure 3

Univariate and multivariate MR analysis results of carnitine and lalanine related metabolites. A: carnitine. B: lalanine.

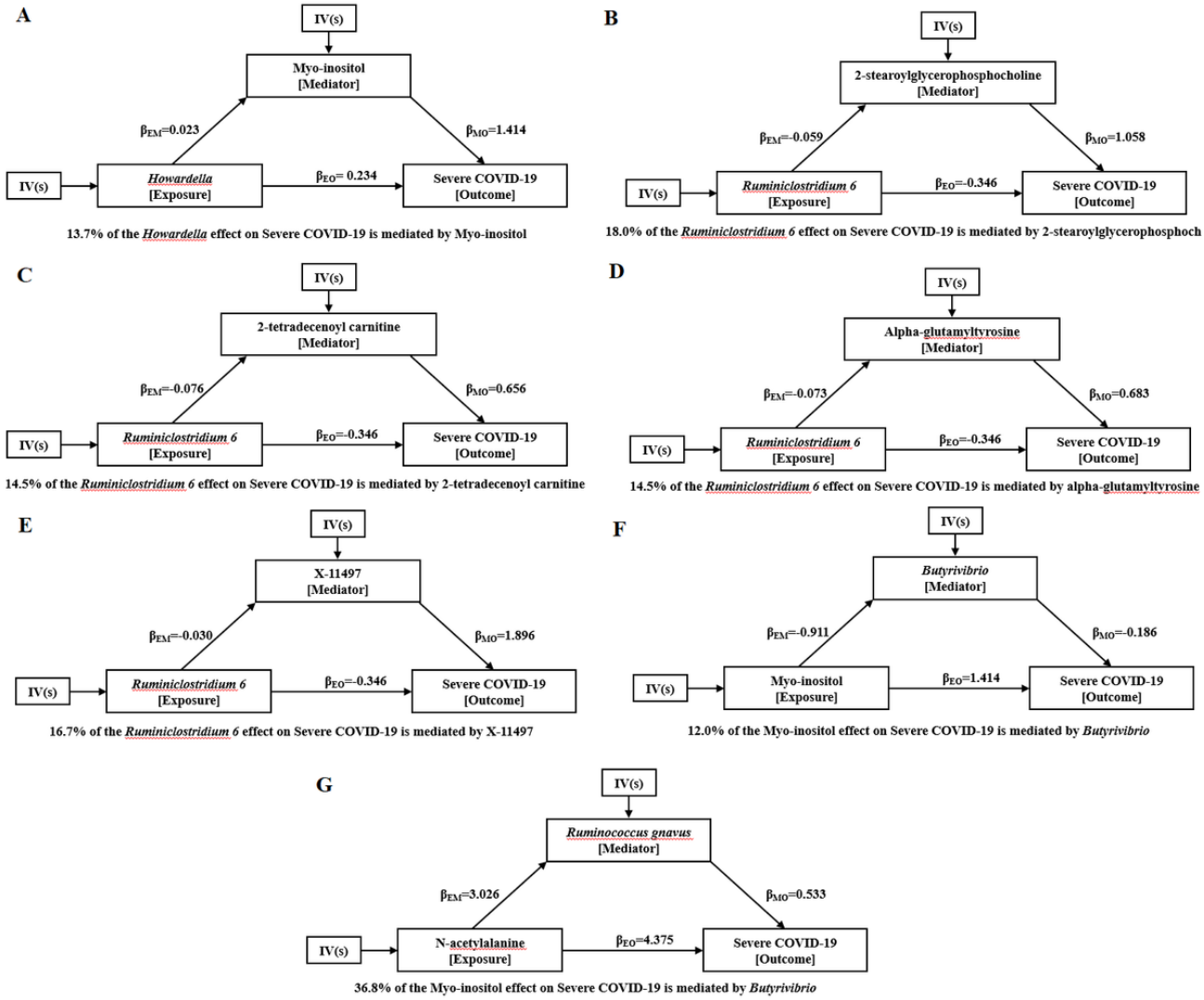

Figure 4

Mediation effect relationship of metabolites and bacteria on severe COVID-19.

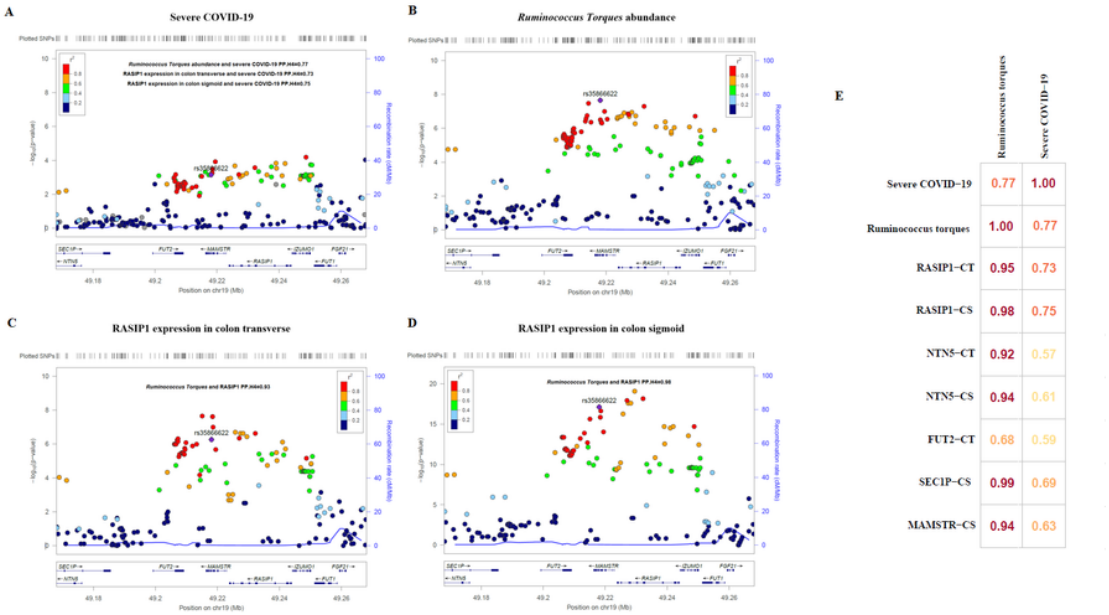

Figure 5

Regional association plots and matrix diagram of colocalization analysis results.