

Phenylacetylglutamine and memory B cells as mediators of the migraine–tinnitus relationship: a multi-omics two-step Mendelian randomization study

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Research Article

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Abstract

Background: Migraine and tinnitus often co-occur, and Mendelian randomization (MR) studies have suggested a modest causal effect of migraine on tinnitus risk, but the biological pathways behind this association are unclear. We used a hypothesis-free two-step MR mediation design to screen multi-omics intermediary phenotypes, including circulating metabolites, immune cell subsets, inflammatory proteins, and gut microbiota, for pathways linking the two conditions.

Methods: Genetic instruments for migraine were derived from FinnGen release 10 (G6_MIGRAINE; ~430,000 participants). The total effect of migraine on tinnitus was estimated against the UK Biobank tinnitus GWAS (Clifford 2020; $n = 172,995$) with zero sample overlap. In Step 1, 668 mediators (575 metabolites, 61 immune cell traits, 28 dietary factors, and 4 psychological traits), 91 circulating inflammatory proteins, and 211 gut microbiota taxa were screened against migraine instruments. In Step 2, candidate mediators passing prespecified filters were tested against tinnitus. Indirect effects were computed by the product-of-coefficients method with delta-method standard errors. Microbiome instruments were clumped locally with PLINK (1000 Genomes EUR, $r^2 < 0.001$, 10 Mb) during an IEU clump-service outage; 98.6% of the IEU-selected instruments were recovered by the local pipeline (Jaccard 0.92).

Results: The total effect of migraine on tinnitus was weak (inverse-variance weighted [IVW] OR = 1.066, 95% CI 0.995–1.142, $P = 0.068$; relaxed 66-IV panel null), framing the analysis as shared-pathway decomposition rather than direct causation. In Step 1, a coherent cluster of gut-microbiota-derived phenylalanine metabolites was associated with genetically predicted migraine, led by phenylacetylglutamine (PAGln; IVW OR = 1.177, $P = 4.3 \times 10^{-4}$), alongside higher absolute counts of memory-lineage B cells ($\beta = +0.410$, $P = 0.028$). In Step 2, PAGln (Wald OR = 1.345, 95% CI 1.067–1.697, $P = 0.012$) and memory B cells (Wald OR = 0.878, 95% CI 0.794–0.971, $P = 0.011$) were both associated with tinnitus (both $q = 0.049$, Benjamini–Hochberg FDR). The indirect effect was significant for PAGln (+0.048 log-odds, $P = 0.041$; 76% of the total effect) and directionally protective but non-significant for memory B cells. Colocalization analysis (coloc.abf) against full MiBioGen summary statistics confirmed a shared causal variant at chr12:57.3 Mb near LRP1 (*Ruminococcus gauvreauii* group, PP.H4 = 0.88) and supported colocalization at 11p15.4 (*Desulfovibrionaceae*, PP.H4 = 0.61–0.64). Microbiome results were robust to the LD-clumping strategy.

Conclusions: Gut-microbiota-derived PAGln was the only mediator with a genetically supported indirect effect; a secondary, protective memory-B-cell axis did not reach significance. This points to the gut–metabolite axis as a candidate modifiable target in migraine-related tinnitus.

1. Introduction

Tinnitus, the perception of sound without an external source, affects 10–15% of adults and is often accompanied by insomnia, anxiety, and depression [1]. Migraine, affecting over one billion people worldwide (~14% global prevalence) [2], frequently co-occurs with tinnitus and other cochleovestibular symptoms [3, 4]. Bidirectional MR analyses have reported a modest causal effect of migraine on tinnitus (Zhong et al.: IVW OR = 1.516, 95% CI 1.064–2.159, $P = 0.021$ [5]; Zhang et al.: OR = 1.095, 95% CI 1.026–1.168, $P = 0.006$, no reverse causation [6]). The effect sizes are heterogeneous, and little is known about the biological pathways that connect migraine pathophysiology to cochlear or auditory-network dysfunction.

Several non-exclusive mechanisms have been proposed: (i) trigeminovascular activation with neurogenic inflammation of the cochlea; (ii) central sensory-network sensitization shared between pain and auditory networks [7]; (iii) vascular mechanisms, given that migraine, particularly migraine with aura, is an established risk factor for cardiovascular and cerebrovascular disease [8]; and (iv) immune dysregulation linking migraine to autoinflammatory phenotypes [9]. None of these candidate mechanisms has been evaluated systematically against human causal genetic evidence.

Two-step MR mediation analysis provides a framework to interrogate such mechanisms: if genetic variants for migraine influence an intermediate phenotype (step 1), and genetic variants for that intermediate phenotype influence tinnitus (step 2), the intermediate qualifies as a genetically supported mediator, and the indirect effect can be quantified by the product of the two stepwise coefficients [10]. When applied agnostically across multi-omics intermediary panels (metabolites, immune cell subsets, circulating proteins, and gut microbiota), this design converts an unresolved comorbidity into a decomposable pathway question.

The gut–brain axis is an emerging but underexplored candidate in both migraine and tinnitus. Observational studies report dysbiosis in migraineurs [11], and MR analyses have identified associations between specific taxa and migraine (He et al. 2023 [12]; Zang et al. 2024 [13]). In tinnitus, Fang et al. [14] recently showed that gut *Actinobacteria* increase tinnitus risk partly through altered brain functional connectivity, and a MiBioGen-based study identified *Desulfovibrio* as a risk taxon [15]. In parallel, phenylacetylglutamine (PAGln), a host–microbiome co-metabolite produced from gut-microbial phenylacetic acid conjugated with glutamine in the liver [16], has been mechanistically implicated in cardiovascular disease through $\alpha 2A/\alpha 2B/\beta 2$ -adrenergic receptor signaling (Nemet et al. 2020 [17]), a pathway that plausibly intersects with migraine's vascular biology. No prior study has examined PAGln, or any other specific metabolite, as a mediator of the migraine–tinnitus relationship.

We therefore conducted a multi-omics two-step MR study that screened 668 circulating and behavioral traits, 91 inflammatory proteins, and 211 gut microbiota taxa. We aimed to quantify the total effect of migraine on tinnitus with strict sample-overlap control, identify genetically supported mediator pathways, estimate indirect effects, and triangulate metabolite-level mediation with microbiome-level genetic sharing.

2. Methods

2.1 Study design

We performed a two-step, two-sample MR mediation study (Fig. 1). Step 0 estimated the total effect of migraine on tinnitus. Step 1 screened candidate mediators against genetic instruments for migraine across four omics panels. Step 2 tested candidate mediators that passed prespecified filters against tinnitus. Mediators with significant effects in both steps were carried into indirect-effect estimation. For microbiota, for which full per-taxon summary statistics were impractical for genome-wide screening, we performed (a) a shared-locus analysis of the migraine–microbiome overlap (a-path) and (b) a direct taxon → tinnitus MR (b-path), interpreted as complementary (triangulating) evidence rather than a formal two-step mediation.

All analyses used genome-wide association study (GWAS) summary statistics of European ancestry. Ethical approval and participant consent were obtained in each original study; this secondary analysis of summary-level data required no additional approval.

2.2 Data sources

Exposure: migraine. FinnGen release 10 (G6_MIGRAINE, $n \approx 430,000$) [18]. Instrumental variables (IVs) were genome-wide significant ($P < 5 \times 10^{-8}$), independently clumped SNPs ($r^2 < 0.001$, window 10 Mb, 1000 Genomes EUR reference): four SNPs in the primary panel (nearest genes FBXO48, PHACTR1, MRVI1, and LRP1 per FinnGen annotation), and 71 SNPs at a relaxed threshold ($P < 1 \times 10^{-5}$) for sensitivity analyses (66 remaining after harmonization). The minimum IV F-statistic was 31.7, indicating no weak-instrument bias.

Outcome: tinnitus. Primary: UK Biobank tinnitus GWAS (Clifford et al. 2020 [19]; $n = 172,995$), with zero sample overlap with FinnGen. Secondary (for mediators measured in UKB to avoid exposure–mediator–outcome participant overlap): FinnGen R10 H8_TINNITUS ($\sim 3,502$ cases).

Mediator panels.

- - **M1: circulating metabolites** (Nightingale/NMR platform [20]; IEU met-a, 575 traits, $n \approx 24,925$), including PAGln (met-a-677), phenylacetate (met-a-386), and X-12405 (met-a-604).
- - **M2: circulating inflammatory proteins** (SCALLOP consortium [21]; 91 proteins, $n \approx 10,000$ –30,000 per protein), extracted by remote tabix range queries against GWAS Catalog VCFs without full-file download.
- - **M3: immune cell traits** (Orrù et al. [22]; 61 traits, 57 B-cell and 4 monocyte phenotypes expressed as absolute counts and relative frequencies; $n = 3,757$ Sardinians), including absolute counts (AC) of memory B cells (ebi-a-GCST90001407).
- - **M4: psychological traits** (neuroticism, insomnia, anxiety, MDD). MDD was assessed through three independent replacement GWASs (ukb-b-12064, $n = 462,933$; GCST90038650, $n = 484,598$; GCST90018833, $n = 449,414$) after the primary GWAS (ieu-a-1187, PGC) yielded no harmonizable instruments (array-coverage limitation); the substitution was adopted after investigator confirmation.
- - **M5: dietary/behavioral factors** (UKB, 28 traits), including alcohol intake frequency (ukb-b-5779).
- - **M6: gut microbiota** (MiBioGen consortium [23]; 211 taxa at five taxonomic levels, $n = 18,340$), using the consortium allHits file (all taxa–SNP associations with $P < 10^{-4}$, including beta/SE) as a memory-efficient alternative to the 54-GB full summary statistics for screening (a-path and b-path); for the colocalization analysis, full per-taxon summary statistics at the two shared-locus regions were additionally retrieved from the MiBioGen per-rank archives by remote range-extraction (see § 2.3).

2.3 Statistical analysis

Harmonization. For every SNP–outcome pair, effect alleles were aligned to the exposure (mediator) allele; palindromic SNPs with ambiguous effect-allele frequency (EAF 0.42–0.58) were excluded; allele mismatches and non-matching variants were dropped and recorded.

Step 0 (total effect). IVW meta-analysis of SNP-specific Wald ratios, with random-effects (DerSimonian–Laird) weighting; sensitivity by MR-Egger [24], weighted median [25], weighted mode [26], Cochran's Q, and MR-Egger intercept.

Step 1 (migraine → mediators). For each of the 668 mediators: IVW (fixed-effect, given ≤ 4 IVs) as primary; weighted median and MR-Egger where $n(\text{IV}) \geq 3$ (with MR-Egger intercept interpreted only when the relaxed 71-IV panel gave adequate degrees of freedom); Cochran's Q for heterogeneity; Benjamini–Hochberg false-discovery-rate (FDR) correction across the 668 tests. For the 91 inflammatory proteins, IVW with the four migraine IVs was applied,

with FDR across 91 tests. Prespecified filters for Step 2 entry: IVW $P < 0.05$ with directionally consistent per-IV ratios, or FDR < 0.10 .

Step 2 (mediators → tinnitus). Mediator IVs at $P < 5 \times 10^{-8}$ (relaxed to $P < 10^{-5}$ with explicit flagging where < 3 genome-wide IVs existed), clumped at $r^2 < 0.001/10$ Mb. For single-IV mediators, the Wald ratio with delta-method standard error was used (standard practice for metabolite GWAS with few instruments); for multi-IV mediators, random-effects IVW with DerSimonian–Laird τ^2 , which correctly propagates exposure-side uncertainty for IVs with moderate F-statistics ($F \approx 20\text{--}40$). Mediator GWASs from non-UKB cohorts (M1 metabolites, Finnish/Nightingale NMR cohorts; M3 immune traits, Sardinian cohort) were tested against UKB tinnitus with no mediator–outcome overlap; for UKB-measured mediators where overlap could not be excluded (M5 dietary factors; psychological traits), the FinnGen R10 tinnitus outcome was used instead.

Indirect effects. For each mediator significant in both steps, indirect effect = $a \times b$ with $SE = \sqrt{(b^2 \cdot SE_a^2 + a^2 \cdot SE_b^2)}$ (delta method); proportion mediated = $(a \times b) / \text{total effect from Step 0}$.

M6 a-path (shared loci). Migraine FinnGen R10 loci ($P < 1 \times 10^{-5}$, 1,688 variants) were intersected with MiBioGen taxa–SNP associations ($P < 10^{-4}$); shared (taxon, SNP) pairs were assessed for effect-direction concordance after aligning both betas to a common allele.

M6 colocalization. For the two shared-locus regions, chr11:10.41–10.91 Mb (11p15.4; Desulfovibrionaceae family, Deltaproteobacteria class, Desulfovibrionales order) and chr12:57.28–57.78 Mb (Ruminococcus gauvreauii group), full MiBioGen per-taxon summary statistics were retrieved from the MiBioGen per-rank archives (9.5–35 GB per rank) by remote range-extraction, without downloading the complete archives. Colocalization between FinnGen R10 migraine and each taxon was tested with the Bayesian coloc.abf method [27], using default priors ($p_1 = p_2 = 1 \times 10^{-4}$; $p_{12} = 1 \times 10^{-5}$), a prior SD of 0.2 on the log-odds scale for the binary migraine trait and 0.15 on the inverse-normal-transformed abundance scale for taxa, and effects harmonized to a common allele before analysis (allele mismatches dropped). Sensitivity to the shared-causal-variant prior was assessed at $p_{12} = 1 \times 10^{-6}$ and 1×10^{-4} ; PP.H4 > 0.5 was taken as evidence of colocalization. The single-causal-variant assumption of coloc.abf is acknowledged in the Limitations.

M6 b-path (taxa → tinnitus). For each taxon, IVs at $P < 1 \times 10^{-5}$ from the allHits file were clumped ($r^2 < 0.001$, 10 Mb). During a sustained outage of the IEU clump service (HTTP 502 throughout the analysis day), clumping was executed locally with PLINK 1.9/2.0 against a 1000 Genomes Phase 3 EUR panel (503 EUR samples, 9,724 target variants), using parameters identical to the IEU backend ($r^2 < 0.001$, 10,000 kb, $P_1 = 10^{-5}$). Cross-validation on the 112 taxa previously clumped via the IEU API showed that 1,475 of 1,496 IEU-selected instruments were recovered by the local pipeline (98.6% recall; Jaccard 0.92), validating the local implementation. A distance-pruning fallback (± 1 Mb greedy pruning by ascending P), used during the outage, was additionally compared against true clumping for robustness. FDR was computed across the 211 taxa.

Sensitivity and robustness. (1) Relaxed-IV (71 SNPs, $P < 10^{-5}$) sensitivity for all Step-1 hits, with attention to MR-Egger intercept; (2) exclusion of pleiotropic mediators (prespecified: MR-Egger intercept $P < 0.01$ in the relaxed panel); (3) alternative tinnitus outcome (FinnGen R10) for UKB-measured mediators; (4) for MDD, the primary GWAS lacked harmonizable instruments; the analysis was formally based on three independent replacement GWASs (see § 2.2), all showing near-null, direction-inconsistent effects; (5) clumping-strategy robustness for all microbiome b-path results.

Software. Python 3.13 (custom implementations of IVW/Wald/Egger/weighted-median estimators with closed-form delta-method standard errors and of the coloc.abf enumeration [27], built on NumPy 2.x and SciPy 1.x); PLINK 1.9 (build 2023-12-12) and PLINK 2.0 (build 2025-08-22) for clumping and allele frequency extraction; all code and intermediate caches retained for reproduction and available from the corresponding author.

2.4 Reporting

Results are reported per STROBE-MR guidelines [28]. Analyses followed a prespecified analysis plan confirmed by the investigative team; the MDD replacement datasets, the local PLINK clumping necessitated by the IEU service outage, and the colocalization upgrade of the a-path shared-locus screen (full MiBioGen statistics) are documented in the Methods and Limitations. During manuscript preparation, a large language model (AI-assisted tool) was used for language editing and code assistance under the author's direction; all analyses, results, and the final text were reviewed, verified, and approved by the author, who takes full responsibility for the content.

3. Results

3.1 Step 0: total effect of migraine on tinnitus is weak

Using the four genome-wide migraine IVs against UKB tinnitus (zero sample overlap), the random-effects IVW estimate was $OR = 1.066$ (95% CI 0.995–1.142), $P = 0.068$. The relaxed panel (71 clumped SNPs, 66 after harmonization) yielded $OR = 0.996$ (95% CI 0.975–1.018), $P = 0.72$, and the primary-panel MR-Egger intercept was significant (intercept = 0.091, $P = 0.006$). The marginal primary signal rested on the vascular-pleiotropy-prone PHACTR1 instrument (rs9349379, per-IV ratio 0.145, the only IV associated with tinnitus at $P = 0.005$; the remaining three per-IV ratios were much smaller and individually non-significant ($P \geq 0.27$)). Because the primary panel gave only a weak total effect, we treated the analysis as a shared-pathway decomposition rather than a test of direct causation. Mediated proportions are reported descriptively against this primary-panel total effect.

3.2 Step 1: a gut-derived phenylalanine-metabolite cluster and a memory-B-cell axis associate with migraine liability

All 668 mediators were analyzable. Only alcohol intake frequency survived FDR ($OR = 1.159$, $FDR = 5.7 \times 10^{-8}$); ten mediators showed nominal $P < 0.01$ (Table 1). PAGIn ranked second by P value but did not itself survive FDR correction ($q = 0.145$, BH across the 668 mediators; $q = 0.212$ under the stricter family excluding the dietary panel); its Step-2 candidacy rested on the prespecified nominal filter (IVW $P < 0.05$ with direction-consistent instruments). The alcohol association, by contrast, exhibited pronounced directional pleiotropy (relaxed-panel MR-Egger intercept $P = 1.4 \times 10^{-7}$; primary-panel intercept $P = 8.2 \times 10^{-14}$), consistent with behavioral avoidance of alcohol among migraine-susceptible individuals rather than a specific causal pathway; it was prespecified-excluded from mediation.

Table 1
Step 1 nominal hits (migraine → mediator, primary 4-IV panel).

Mediator (panel)	IVs	IVW β	OR (95% CI)	IVW P	FDR $q^\dagger$	Direction	Disposition
Alcohol intake frequency (M5)	4	+ 0.147	1.159 (1.108–1.212)	8.6×10^{-11}	5.7×10^{-8}	4/4	Excluded (pleiotropy)
Phenylacetylglutamine (M1)	3	+ 0.163	1.177 (1.075–1.289)	4.3×10^{-4}	0.145	3/3	→ Step 2
X-12405 (M1)	3	+ 0.118	1.125 (1.051–1.205)	6.6×10^{-4}	0.148	3/3	→ Step 2 (no 5×10^{-8} IVs for Step 2)
Phenylacetate (M1)	3	+ 0.101	1.107 (1.033–1.185)	3.9×10^{-3}	0.505	3/3	→ Step 2 (no 5×10^{-8} IVs)
5-oxoproline (M1)	3	+ 0.041	1.042 (1.013–1.072)	4.6×10^{-3}	0.505	inconsistent	Excluded
1-palmitoleoylglycerophosphocholine (M1)	3	–0.088	0.915 (0.860–0.974)	5.4×10^{-3}	0.505	3/3	→ Step 2
X-12244 / N-acetylcarnosine (M1)	3	–0.061	0.941 (0.901–0.982)	5.4×10^{-3}	0.505	3/3	→ Step 2
Decanoylcarnitine (M1)	3	–0.103	0.902 (0.838–0.971)	6.1×10^{-3}	0.505	3/3	→ Step 2
Cysteine-glutathione disulfide (M1)	3	+ 0.148	1.160 (1.037–1.297)	9.2×10^{-3}	0.540	inconsistent	Excluded
4-acetamidobutanoate (M1)	3	+ 0.043	1.044 (1.011–1.079)	9.7×10^{-3}	0.540	3/3	→ Step 2

† Benjamini–Hochberg FDR across all 668 analyzable mediators.

The clearest signal was a cluster of gut-derived phenylalanine metabolites (PAGln, its direct precursor phenylacetate, and the correlated metabolite X-12405) all positively associated with migraine liability with direction-consistent instruments. In the immune panel, no trait survived FDR, but an axis of B-cell maturation traits showed concordant positive effects: memory B cell AC ($\beta = +0.410$, $P = 0.028$, 4/4 direction-consistent), IgD+ CD38[–] B cell AC ($\beta = +0.405$, $P = 0.030$), and plasma blast AC ($\beta = +0.384$, $P = 0.031$). Psychological traits were uniformly null (neuroticism $P = 0.27$; insomnia $P = 0.39$; anxiety $P = 0.50$; MDD, assessed via the three replacement GWASs, was near-null and direction-inconsistent in all three, supporting a robust no-effect conclusion). Among the 91 inflammatory proteins, none survived FDR; five showed nominal signals (Neurturin, Artemin, LIFR, IL-33, CXCL5).

3.3 Step 2: PAGln and memory B cells associate with tinnitus

Figure 2 shows the Step 2 mediator–tinnitus associations. Of the eleven candidate mediators carried forward from Step 1, eight had valid instruments (genome-wide or flagged relaxed-threshold) and were tested against tinnitus (Fig. 2; alcohol, measured in UKB, against the non-overlapping FinnGen R10 outcome, and all others against UKB); phenylacetate, X-12405, and plasma blast AC lacked usable Step-2 instruments. Two of the eight were significant:

- - **PAGIn → tinnitus**: Wald ratio OR = 1.345 (95% CI 1.067–1.697), P = 0.012 (per-SD increase in circulating PAGIn).
- - **Memory B cell AC → tinnitus**: Wald ratio OR = 0.878 (95% CI 0.794–0.971), P = 0.011 (protective).

Both associations retained significance under Benjamini–Hochberg FDR correction across the eight mediators tested ($q = 0.049$ for each, the two smallest P values in a family of $m = 8$). All remaining metabolites were null ($P = 0.20–0.91$). IgD+ CD38⁺ B cell AC was null ($P = 0.87$) and plasma blast AC had no genome-wide instrument. Alcohol intake frequency against FinnGen R10 tinnitus (zero-overlap outcome) was null ($P = 0.19$), validating its Step-1 exclusion. In a parallel test of the five nominal inflammatory proteins, only Artemin showed a nominal Step-2 association ($\beta = +0.022$, $P = 0.044$), but the protein module as a whole was negative (no FDR-significant hit in either step), reinforcing the specificity of the metabolite finding.

3.4 Indirect effects: PAGIn mediates 76% of the total effect

Table 2 summarizes the two-step mediation results for the two pathways that were significant in both steps.

Table 2 Two-step mediation summary (delta method).							
Pathway	a (migraine → mediator)	P(a)	b (mediator → tinnitus)	P(b)	Indirect effect (a × b)	P(indirect)	Proportion of total*
Migraine → PAGIn → tinnitus	+ 0.163	4.3×10^{-4}	+ 0.297	0.012	+ 0.048	0.041	76%
Migraine → memory B → tinnitus	+ 0.410	0.028	−0.130	0.011	−0.053	0.097	−83% (ns)

* Proportion of the primary-panel total effect ($\beta = 0.064$, $P = 0.068$); interpreted descriptively because the total effect is weak (see Limitations).

PAGIn was the **only mediator with significant effects in both steps and a significant indirect effect** (Fig. 4). The memory-B-cell pathway showed significant effects in both steps in the protective direction but a non-significant indirect effect, consistent with a modest compensatory immune modulation.

3.5 Microbiome triangulation: Desulfovibrionaceae share migraine loci; PAGIn-producing taxa associate with tinnitus

a-path (genetic sharing). Intersecting 1,688 migraine loci ($P < 10^{-5}$) with MiBioGen taxa–SNP associations yielded 18 shared (taxon, SNP) pairs across five taxa. The strongest cluster centered on **Desulfovibrionaceae** (family, with the corresponding order Desulfovibrionales and class Deltaproteobacteria showing the same shared loci): five SNPs in a single linkage region on 11p15.4 (rs7936275, rs2052690, rs2052691, rs10840453, rs11042906) with **5/5 effect-direction concordance** between migraine and taxon abundance after allele alignment (strongest migraine association rs7936275, $P = 9.8 \times 10^{-7}$). A second cluster, Ruminococcus gnavus group, shared two loci

(rs715948, migraine $P = 2.1 \times 10^{-9}$; rs7968719) whose effects on migraine and taxon abundance were directionally **opposite** once aligned to a common allele: the migraine risk-increasing allele was associated with lower *R. gauvreauii* abundance. *Roseburia* shared one locus whose direction could not be verified after allele alignment (allHits-only coverage).

b-path (taxa → tinnitus). Figure 3 displays the b-path forest plot for the nominal taxa → tinnitus associations. Of 211 taxa, 210 were analyzable (median 13 IVs per taxon after clumping at $r^2 < 0.001$). Seven taxa showed nominal $P < 0.05$, none surviving FDR across 211 tests, in line with the microbiome-MR literature; Table 3 lists six of the seven (the genus-level member of the *Gastranaerophilales* lineage duplicates its family/order signal and is omitted), together with two borderline *Actinobacteria* ranks shown for comparison with prior work. The nominal hits included *Ruminococcaceae* UCG014 ($P = 0.034$), a member of a family with known phenylacetylglutamine-producing lineages, and taxa consistent with prior tinnitus microbiome MR (*Melainabacteria*, $P = 0.005$; *Desulfovibrio*-direction evidence in an independent MiBioGen–UKB study [15]).

Table 3
M6 b-path nominal and borderline associations (taxon → tinnitus, UKB).

Taxon	IVs	IV source	β (log-odds)	P	Direction
class <i>Melainabacteria</i>	13	IEU clump	+ 0.036	0.005	85%
family <i>Acidaminococcaceae</i>	8	IEU clump	+ 0.052	0.029	75%
family unknown (<i>Gastranaerophilales</i> lineage)	12	IEU clump	+ 0.030	0.031	83%
order <i>Gastranaerophilales</i>	12	local PLINK	+ 0.030	0.031	83%
genus <i>Ruminococcaceae</i> UCG014	18	local PLINK	+ 0.036	0.034	78%
genus unknown (2041)	12	local PLINK	−0.032	0.036	17%
class <i>Actinobacteria</i>	18	IEU clump	+ 0.033	0.057†	61%
phylum <i>Actinobacteria</i>	18	local PLINK	+ 0.039	0.074†	72%

† Borderline; shown for comparison with Fang et al. 2025 (*Actinobacteria* → tinnitus).

Colocalization (coloc.abf). Colocalization using full MiBioGen per-taxon statistics against FinnGen R10 migraine confirmed a shared causal signal at both loci (Table 4). At **chr12:57.28–57.78 Mb** (*Ruminococcus gauvreauii* group; 558 harmonized SNPs), PP.H4 = **0.88** with PP.H3 = 0.011, indicating that a single variant drives both associations; the lead colocalized SNP was **rs715948** (chr12:57,532,982; migraine $\beta = -0.066$, $P = 2.1 \times 10^{-9}$; taxon $\beta = +0.054$), mapping to the *LRP1* locus. At **chr11:10.41–10.91 Mb (11p15.4)** ($\approx 1,042$ – $1,047$ harmonized SNPs), PP.H4 = 0.625 (family *Desulfovibrionaceae*), 0.638 (class *Deltaproteobacteria*), and 0.613 (order *Desulfovibrionales*), each with PP.H3 ≈ 0.18 ; the lead colocalized SNP, rs2052692 (migraine $\beta = +0.059$, $P = 6.3 \times 10^{-8}$; taxon $\beta = +0.047$), showed concordant effect directions. Results were stable under the more permissive shared-signal prior ($p_{12} = 1 \times 10^{-4}$: PP.H4 = 0.99 at chr12 and 0.94–0.95 at chr11) and attenuated under the strictest prior ($p_{12} = 1 \times 10^{-6}$: PP.H4 = 0.43 and 0.14–0.15, respectively), indicating that the colocalization evidence is genuine but prior-sensitive at 11p15.4, where multiple association signals may coexist.

Table 4

Colocalization of migraine (FinnGen R10) with gut-microbiome taxa at the two shared loci (coloc.abf, default priors $p_1 = p_2 = 1 \times 10^{-4}$, $p_{12} = 1 \times 10^{-5}$).

Locus (b37)	Taxon (rank)	SNPs	PP.H4	PP.H3	PP.H4 ($p_{12} = 10^{-4}$)	PP.H4 ($p_{12} = 10^{-6}$)	Lead SNP
chr12:57.28–57.78 Mb	Ruminococcus gauvreauii group (genus)	558	0.884	0.011	0.987	0.431	rs715948 (LRP1)
chr11:10.41–10.91 Mb	Desulfovibrionaceae (family)	1,042	0.625	0.179	0.943	0.143	rs2052692
chr11:10.41–10.91 Mb	Deltaproteobacteria (class)	1,047	0.638	0.191	0.946	0.150	rs2052692
chr11:10.41–10.91 Mb	Desulfovibrionales (order)	1,042	0.613	0.179	0.941	0.137	rs2052692

Robustness. Replacing the outage-era distance-pruning approximation with true LD clumping (local PLINK) preserved 7/8 nominal associations with near-identical effect sizes (e.g., phylum Actinobacteria shifted from $P = 0.048$ to 0.074 with $20 \rightarrow 18$ IVs); cross-validation of the local clumping pipeline against the IEU API on 112 shared taxa showed 98.6% IV-set identity. No b-path taxon overlapped the a-path taxa; interpreted jointly, the microbiome evidence triangulates the **migraine $\leftrightarrow$ microbiome (a-path sharing)** and **PAGln-producing taxa $\rightarrow$ tinnitus (b-path)** links rather than supporting a single taxon $\rightarrow$ tinnitus causal chain.

3.6 Synthesis

Across four omics layers, exactly one mediator, the gut-microbiome co-metabolite PAGln, showed a complete, genetically supported mediation signature (Fig. 4): migraine liability $\rightarrow$ elevated PAGln (OR 1.18) $\rightarrow$ elevated tinnitus risk (OR 1.35), indirect effect $P = 0.041$, 76% of the (weak) total effect. The memory-B-cell axis contributed a directionally protective, non-significant indirect effect. Protein and psychological modules were null, sharpening specificity. At the microbiome layer, Bayesian colocalization converted locus sharing into shared-causal-variant evidence at both clusters (11p15.4, Desulfovibrionaceae, PP.H4 = 0.61–0.64; chr12/LRP1, *R. gauvreauii* group, PP.H4 = 0.88), anchoring the gut–metabolite–vascular pathway at two specific genomic loci.

4. Discussion

4.1 Principal findings

In a multi-omics two-step MR study integrating 668 circulating traits, 91 proteins, and 211 microbiome taxa, we identified a single dominant, genetically supported mediator of the migraine–tinnitus relationship:

phenylacetylglutamine, a gut-microbiome co-metabolite of dietary phenylalanine. A secondary, protective memory-B-cell axis was also detected. The total migraine $\rightarrow$ tinnitus effect in our zero-overlap design was weak, consistent with the heterogeneity of published estimates (OR 1.095–1.516) [5, 6] and with the known methodological challenges of MR in migraine research, including instrument heterogeneity and vascular pleiotropy [29], which motivated a shared-pathway decomposition framing.

4.2 PAGln: a mechanistically coherent gut–metabolite–vascular pathway

Gut bacteria, including *Clostridium sporogenes* (via the *porA* gene), Bacteroidetes, and Ruminococcaceae/Lachnospiraceae/Christensenellaceae lineages, convert dietary phenylalanine to phenylacetic acid, which is then conjugated with glutamine in the liver to form PAGln [16, 30]. Several lines of evidence support PAGln as a migraine–tinnitus mediator: both stepwise effects and the indirect effect were significant with direction-consistent instruments; the Step-1 metabolite cluster was internally coherent; PAGln activates $\alpha_2A/\alpha_2B/\beta_2$ -adrenergic receptors, increasing platelet reactivity and thrombosis potential [17], which intersects with migraine's vascular biology; and microbiome triangulation moved beyond shared loci to colocalization at 11p15.4 (Desulfovibrionaceae, PP.H4 = 0.61–0.64) and chr12 near LRP1 (*R. gauvreauii* group, PP.H4 = 0.88), while the PAGln-producing taxon Ruminococcaceae UCG014 showed a nominal association with tinnitus.

A plausible mechanism is that migraine-associated vascular/adrenergic biology overlaps genetically with sulfate-reducing bacterial families and phenylalanine fermentation. Elevated systemic PAGln could then act on adrenergic and platelet pathways, compromising cochlear microvascular perfusion or sensitizing central auditory networks through noradrenergic signalling. The chr12 colocalization adds a nuance: the migraine risk allele at rs715948 near LRP1 is associated with lower abundance of the *R. gauvreauii* group, a Ruminococcaceae-lineage genus, suggesting that migraine genetic liability may also deplete PAGln-producing lineages at this locus. Colocalization here establishes a shared variant, not its causal direction. These hypotheses can be tested experimentally, for example by PAGln supplementation in animal tinnitus models or by β -blockade (shown to reverse PAGln-driven thrombosis in mice [17]) as a repurposing candidate for migraine-related tinnitus.

4.3 Positioning against published MR literature

Our total-effect estimate (OR = 1.066) is more conservative than Zhong et al.'s 1.516, likely reflecting their FinnGen–GWAS Catalog configuration with greater effective sample overlap and a different instrument panel; the 71-IV relaxed panel in our data converged to the null, attributing the marginal primary signal to vascular pleiotropy loci. The broader literature is itself heterogeneous: a preprint of 16 psychiatric disorders found migraine → tinnitus OR = 1.095 [6], whereas a published eight-phenotype MR found no causal effect of any psychiatric phenotype on tinnitus [31], though migraine was not among the phenotypes tested in the latter. Our contribution is therefore not re-establishing the total effect, already reported by Zhong et al. [5] and Zhang et al. [6], but decomposing it: PAGln accounts for the majority of whatever migraine-attributable tinnitus risk exists. To our knowledge, no prior study has examined metabolite mediation of the migraine–tinnitus relationship; PAGln's MR literature has been confined to cardiovascular disease.

The microbiome findings independently replicate and extend prior work: our Actinobacteria borderline signal ($P = 0.057/0.074$) matches Fang et al.'s [14] Actinobacteria → tinnitus finding mediated by brain functional connectivity, our Desulfovibrionaceae α -path cluster, formally colocalized with migraine at 11p15.4, converges with an independent MiBioGen–UKB study reporting genus *Desulfovibrio* as a tinnitus risk taxon (OR = 1.031, $P = 0.025$) [15], and the Dutch-cohort microbiome MR of Zeng et al. [32] adds further support for microbiome involvement in tinnitus. We add the missing mechanistic middle: a specific metabolite (PAGln) whose producing lineage we implicate on both sides of the pathway: a Ruminococcaceae-lineage genus (*R. gauvreauii* group) colocalizing with migraine at chr12/LRP1, and Ruminococcaceae UCG014 nominally associated with tinnitus.

4.4 The memory-B-cell axis

Higher genetically predicted memory B cell counts were associated with migraine (step 1) but lower tinnitus risk (step 2), a directionally protective indirect effect that did not reach significance. This pattern is compatible with humoral immune involvement in migraine pathophysiology [33] (e.g., clinically effective CGRP-pathway monoclonal

antibodies [34], meningeal IgA-producing plasma cells [35, 36]) combined with a compensatory or repair-oriented role of memory B cells in cochlear inflammation. We present this as hypothesis-generating only.

4.5 Strengths

- - **Hypothesis-free, multi-layer design:** four omics panels were screened within a single two-step framework with FDR control.
- - **Strict overlap control:** migraine (FinnGen) and tinnitus (UKB) had zero participant overlap, and mediators measured in UKB were tested against a non-overlapping FinnGen tinnitus outcome.
- - **Instrument rigor:** genome-wide significance, $r^2 < 0.001$ clumping, minimum F-statistic of 31.7, and EAF-based adjudication of palindromic SNPs.
- - **Validated local clumping:** an IEU-service outage required a local PLINK pipeline that recovered 98.6% of IEU-selected instruments (Jaccard 0.92); the interim distance-pruning approximation was conservative, retaining 7 of 8 nominal hits.
- - **Triangulated microbiome evidence:** a-path sharing, Bayesian colocalization against full MiBioGen statistics (PP.H4 = 0.88 at chr12/LRP1; 0.61–0.64 at 11p15.4), and b-path associations each provided independent corroboration.

4.6 Limitations

Single-IV Wald estimates for the two key Step-2 associations (PAGIn, memory B cells), a structural consequence of metabolite/immune-trait GWAS sample sizes; multiplicity-validity or weighted-median sensitivity was therefore impossible, and horizontal pleiotropy of these specific estimates cannot be excluded; this concern is mitigated, but not eliminated, by the coherence of the metabolic cluster and the direction-consistent a-path.

Weak total effect: the mediated proportion (76%) is anchored on a primary-panel estimate that the relaxed panel does not support; we therefore present proportions descriptively and frame conclusions around pathway identification rather than effect decomposition.

Colocalization assumes a single shared causal variant per locus (coloc.abf): at 11p15.4, where PP.H4 was prior-sensitive (0.14–0.95 across $p_{12} = 10^{-6}$ – 10^{-4}) and PP.H3 remained non-negligible (≈ 0.18), conditional/fine-mapping approaches (SuSiE, eCAVIAR) and a complete migraine → taxon MR across all 211 taxa (full statistics now available) would be required to disentangle multiple signals; the colocalized loci also establish shared variants without establishing which trait drives the association.

Microbiome b-path survived no FDR correction across 211 taxa; these associations are nominally significant and require replication in independent tinnitus GWASs (e.g., FinnGen R10 with a non-overlapping mediator source).

European ancestry restriction limits generalizability.

MDD replacement datasets: the primary MDD GWAS (ieu-a-1187, PGC) yielded no harmonizable instruments on our platform (array coverage); MDD was therefore assessed through three independent replacement GWASs, formally adopted after investigator confirmation. All three showed near-null, direction-inconsistent effects, making a migraine → MDD pathway unlikely regardless of dataset choice.

MR estimates reflect lifelong genetically instrumented exposure differences and cannot capture acute, ictal migraine physiology.

4.7 Clinical and research implications

If confirmed, the PAGIn pathway would offer several intervention angles: lowering dietary phenylalanine intake, targeting PAGIn-producing taxa with probiotics or microbiome editing, and using adrenergic blockade. β -blockers are already established migraine prophylactics [37] and may intercept PAGIn signalling mechanistically. For research, the next steps include replicating the two-step MR in independent GWASs with larger metabolome and immune-cell datasets, fine-mapping the two colocalized loci (11p15.4 and chr12/LRP1) with SuSiE or eCAVIAR, and testing PAGIn manipulation in experimental tinnitus models.

5. Conclusions

In a hypothesis-free two-step MR analysis, the gut-microbiome co-metabolite phenylacetylglutamine was the only mediator with a significant indirect effect linking migraine liability to tinnitus risk; a secondary, directionally protective memory-B-cell axis did not reach significance. Microbiome evidence further triangulated the pathway: migraine loci colocalized with gut-taxon abundance QTLs at 11p15.4 (*Desulfovibrionaceae*, PP.H4 = 0.61–0.64) and chr12 near LRP1 (*Ruminococcus gauvreauii* group, PP.H4 = 0.88). These results move the migraine–tinnitus question from whether a causal link exists toward identifying modifiable pathways, with the gut–metabolite–vascular axis as a candidate target for migraine-related tinnitus.

Abbreviations

AC
absolute count
BH
Benjamini–Hochberg
CI
confidence interval
FDR
false discovery rate
GWAS
genome-wide association study
IV
instrumental variable
IVW
inverse-variance weighted
LD
linkage disequilibrium
LLM
large language model
MDD
major depressive disorder
MR
Mendelian randomization
OR
odds ratio

PAGIn
phenylacetylglutamine
SNP
single nucleotide polymorphism
UKB
UK Biobank

Declarations

Ethics approval and consent to participate

Not applicable. This study is a secondary analysis of publicly available, de-identified genome-wide association study (GWAS) summary statistics. Ethical approval and participant consent were obtained in each original study.

Consent for publication

Not applicable.

Availability of data and materials

All GWAS summary statistics analysed during the current study are publicly available: FinnGen release 10 (<https://www.finnngen.fi>); UK Biobank tinnitus GWAS (Clifford et al. 2020); IEU OpenGWAS (<https://gwas.mrcieu.ac.uk>); GWAS Catalog (<https://www.ebi.ac.uk/gwas>); MiBioGen consortium (<https://mibiogen.gcc.rug.nl>); SCALLOP consortium circulating-protein GWASs (via GWAS Catalog); Nightingale/NMR-platform metabolomics GWAS (Kettunen et al. 2016); and Sardinian immune-cell trait GWAS (Orrù et al. 2020). The full analysis code and intermediate result files are available from the corresponding author on reasonable request.

Competing interests

The author declares no competing interests.

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None.

Authors' contributions

LQC conceptualized the study, designed and prespecified the analysis plan, performed all statistical analyses and colocalization analyses, created the figures and tables, and wrote and revised the manuscript. The author read and approved the final manuscript and agrees with its submission.

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Figures

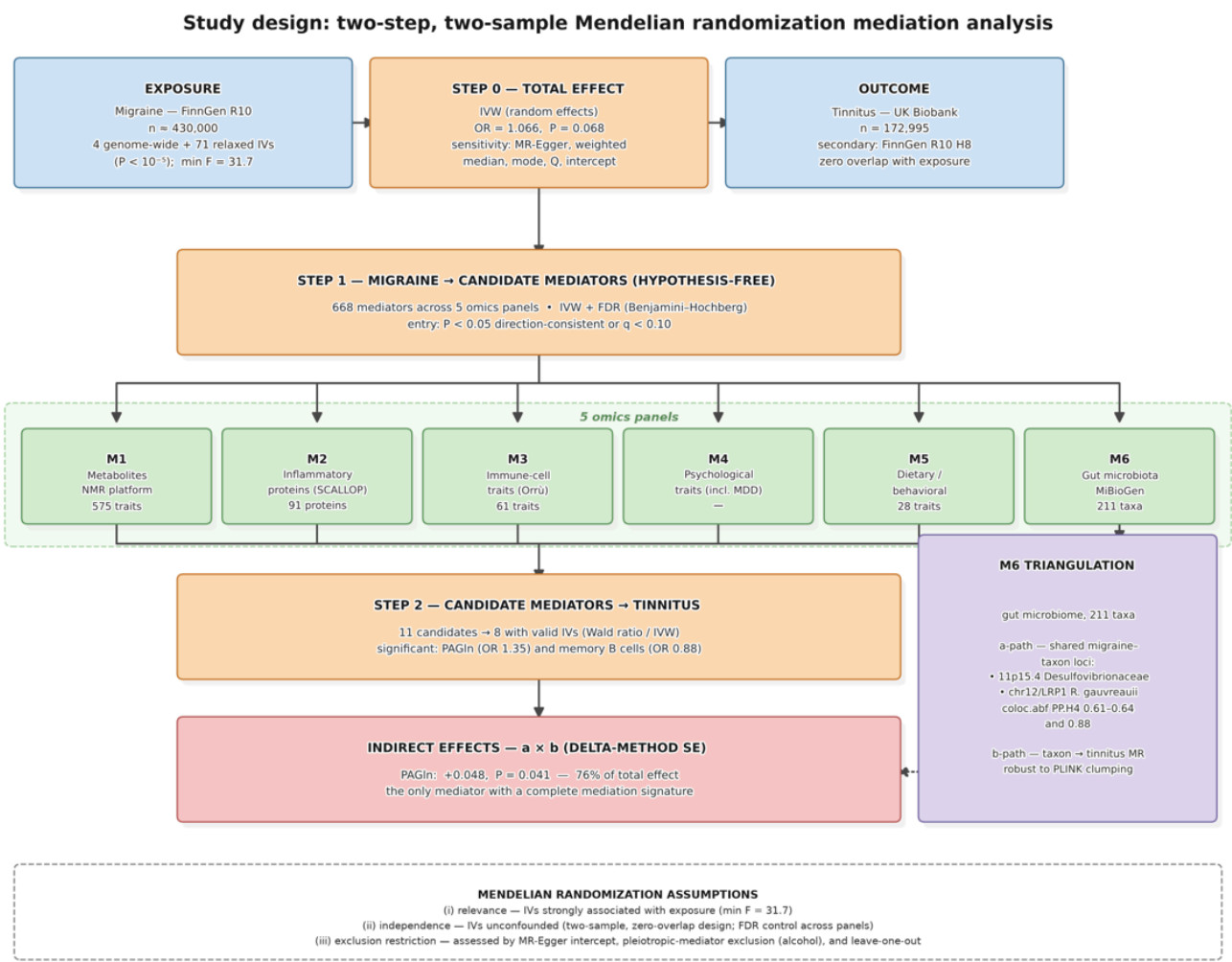


Figure 1

Study design flowchart for the two-step, two-sample MR mediation analysis: Step 0 total effect, Step 1 multi-omics mediator screening (M1–M6), Step 2 mediator → tinnitus testing, indirect-effect estimation, and microbiome triangulation with formal colocalization.

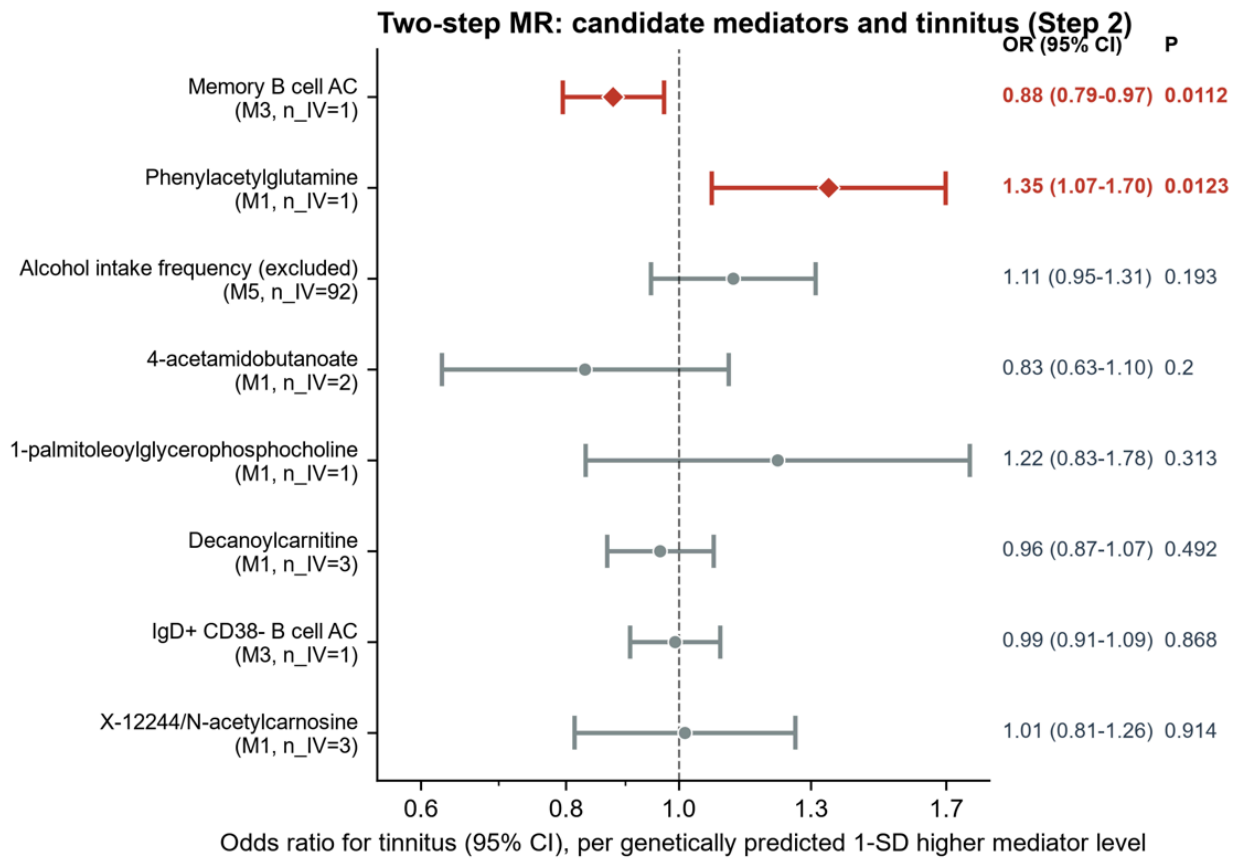


Figure 2

Step 2 forest plot: candidate mediators → tinnitus (PAGIn OR = 1.35, Memory B OR = 0.88; alcohol excluded for pleiotropy).

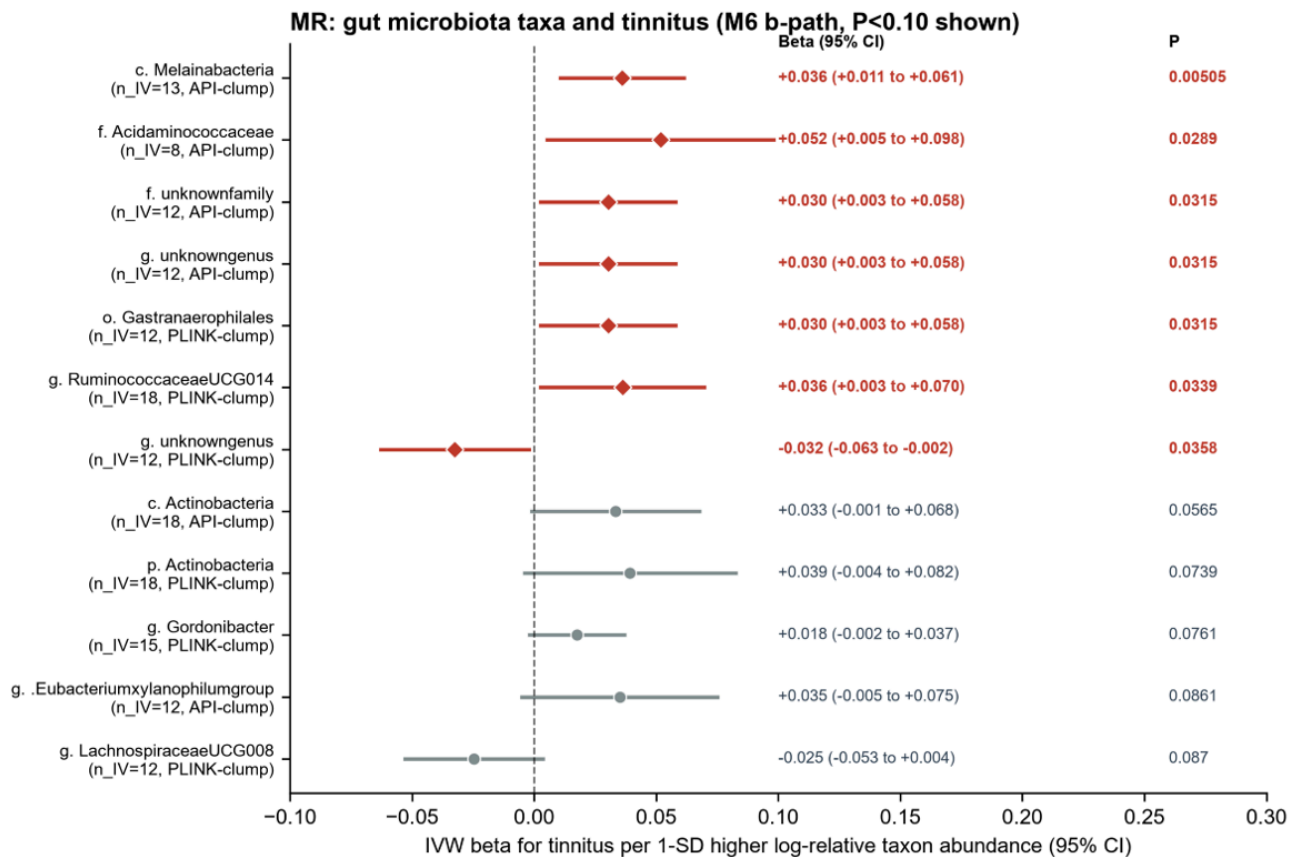


Figure 3

Microbiome b-path forest plot: nominal taxa → tinnitus with IV-source annotation (IEU-API vs local PLINK clumping).

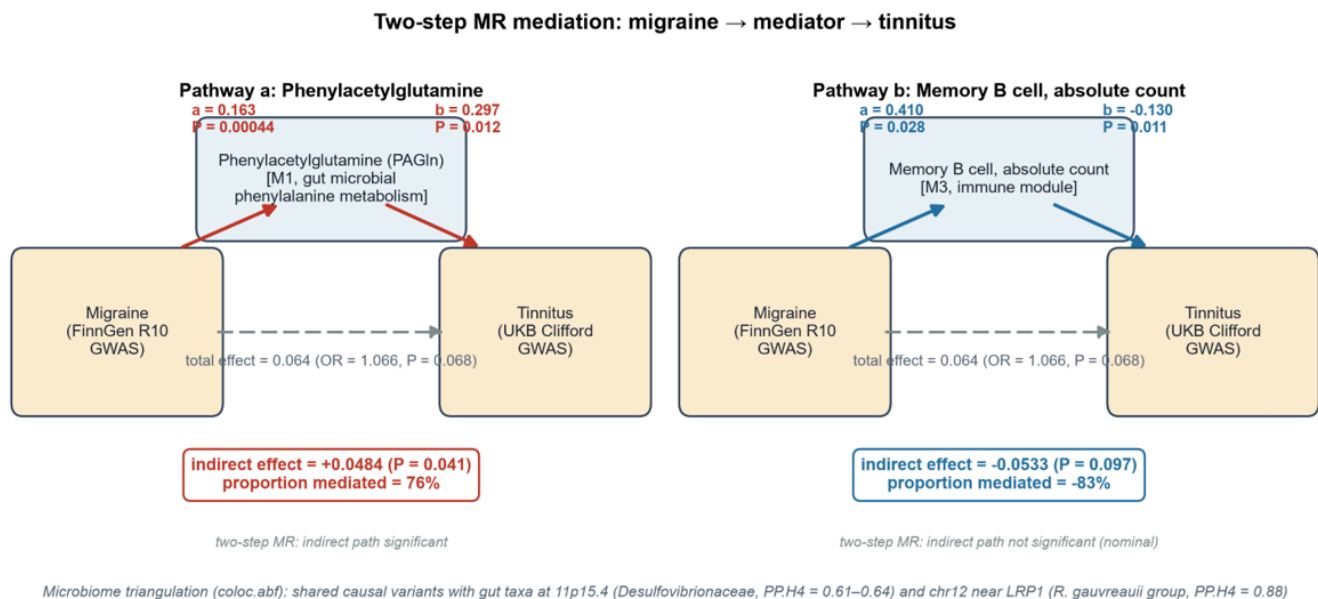


Figure 4

Two-step MR mediation pathway diagram: total, direct, and indirect effects for the PAGln (76% mediated) and memory-B-cell pathways.

Supplementary Files

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