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Gut microbiota predict development of postdischarge diabetes mellitus in acute pancreatitis

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ABSTRACT

Background Postdischarge morbidity and mortality is high in acute pancreatitis (AP) and pathophysiological mechanisms remain poorly understood.

Objectives We aim to investigate the composition of gut microbiota and clinical long-term outcomes of prospectively enrolled patients with AP to predict postdischarge complications.

Design In this long-term follow-up study, we analysed clinical and microbiome data of 277 patients from the prospective multicentre Pancreatitis-Microbiome As Predictor of Severity trial. The primary endpoint was the association of the microbial composition with postdischarge mortality, recurrent AP (RAP), progression to chronic pancreatitis, pancreatic exocrine insufficiency, diabetes mellitus (DM) and pancreatic ductal adenocarcinoma.

Results Buccal (n=238) and rectal (n=249) swabs were analysed by 16S rRNA and metagenomics sequencing using Oxford Nanopore Technologies. Median follow-up was 2.8 years. Distance-based redundancy analysis with canonical analysis of principal coordinates showed significant differences for β-diversity (Bray-Curtis) for postdischarge mortality (p=0.04), RAP (p=0.02) and DM (p=0.03). A ridge regression model including 11 differentially abundant species predicted postdischarge DM with an area under the receiving operating characteristic of 94.8% and 86.2% in the matched and entire cohort, respectively. Using this classifier, a positive predictive value of 66.6%, a negative predictive value of 96% and an accuracy of 95% was achieved.

Conclusion Our data indicate that the admission microbiome of patients with AP correlates with postdischarge complications independent from multiple risk factors such as AP severity, smoking or alcohol. Microbiota at admission show excellent capacity to predict postdischarge DM and may thus open new stratification tools for a tailored risk assessment in the future.

Trial registration number NCT04777812.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Acute pancreatitis exhibits a considerable postdischarge mortality and morbidity.
⇒ Gut microbiota at admission are associated with severity of acute pancreatitis.

WHAT THIS STUDY ADDS

⇒ We report significant association of the gut microbiota at admission with postdischarge mortality, recurrent pancreatitis and diabetes mellitus (DM).
⇒ Postdischarge DM is robustly predicted using 11 rectal species with an area under the receiver operating characteristics of 94.8% in a confounder matched cohort.
⇒ 11 differentially abundant species yielded a positive predictive value of 66.6%, a negative predictive value of 96% and an accuracy of 95% for postdischarge DM.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Stratification of follow-up consultation based on microbial patterns might likely help to tailor surveillance protocols and save healthcare costs.
⇒ In-depth understanding of pathogenic bacteria and their metabolites might lead to the development of preventive strategies to reduce postdischarge complications such as DM.

INTRODUCTION

Acute pancreatitis (AP) is a common inflammatory disease that often leads to hospital admissions.[1] While most patients experience mild or moderately severe disease, 10–20% of patients develop severe inflammation, a proportion of them with infected abdominal necrosis, organ failure and increased morbidity and in-hospital mortality.[2] Notably, the clinical management of severe AP has considerably evolved over the last years, thereby reducing

in-hospital mortality rates by 35%, bringing the overall mortality of AP down to 2–3%.[3] In particular, goal-directed pain and fluid resuscitation approaches,[4 5] as well as minimally invasive interventions such as endoscopic ultrasound-guided drainage of infected necrotic collections, have advanced in-hospital management of patients with AP.[6] In the past, clinical and scientific interest was primarily focused on these refined minimally-invasive approaches; however, recent data reveal a significant postdischarge morbidity and mortality of patients with AP that was long underestimated and understudied. A large prospective, multicentre study from Hungary reported an approximately threefold increased post-AP mortality rate compared with the general population.[7] In line with these observations, two population-based cohort studies from Denmark and Sweden reported similarly increased mortality rates within 90 days post-discharge (around 5%) even exceeding the in-hospital mortality rate.[3 8] The available literature suggests that postdischarge mortality is higher in patients with more severe AP, and cardiovascular events as well as AP-related sepsis are common causes of death.[3 7] Furthermore, GI diseases, including malignancies and cancer-related cachexia, are reasons for the increased mortality rate, in particular during the first 2 years after the first episode of AP.[7] While these important clinical studies have prompted follow-up protocols for patients with AP after discharge that are increasingly implemented in recent evidence-based guidelines,[9 10] in particular for patients with moderately severe and severe disease, the exact causes and pathophysiological underpinnings of the increased postdischarge mortality are still poorly understood. It is hypothesised that recurrent AP (RAP), development of chronic pancreatitis (CP), low socioeconomic status and comorbidities considerably contribute to the increased post-discharge mortality. In particular, alcohol-related AP, smoking and male sex are known risk profiles for postdischarge complications.[11] AP-related complications such as the development of diabetes mellitus (DM), pancreatic exocrine insufficiency (PEI), CP and pancreatic ductal adenocarcinoma (PDAC) are likely contributing to the observed postdischarge mortality.[12] Interestingly, a large population-based cohort study showed increased mortality and hospital readmission of post-pancreatitis DM compared with patients with DM type 2,[13] indicating that valid tools for predicting the risk of specific postdischarge complications would assist clinicians to tailor surveillance strategies for patients with AP.[12]

We have recently investigated the orointestinal microbiome of patients with AP at hospital admission in 15 European centres. Our data revealed striking alterations of microbial patterns in patients with AP that are associated with disease severity and other clinical hallmark features such as length of hospital stay and in-hospital mortality.[14] β-diversity significantly differed between patients with revised Atlanta classification (RAC I–III), and differentially abundant species over-represented in severe compared with non-severe AP were short-chain fatty acid (SCFA) producers such as *Parabacteroides distasonis*, *Enteroclo-ster bolteae* and *Lachnospiraceae sp*. Here, we hypothesise that the initial admission microbiome of patients with AP might be associated with postdischarge mortality, RAP, progression to CP, development of PEI, DM and PDAC over a period of 3 years. For the first time, we construct diagnostic classifiers using differentially abundant species from admission microbiomes that predict postdischarge complications, in particular DM, with high accuracy, linking long-term outcomes of patients with AP to gut microbiota.

METHODS

Patient cohort and endpoints

Patients with AP from the Pancreatitis-Microbiome As Predictor of Severity (P-MAPS) I cohort (NCT04777812) were contacted between October 2024 and January 2025.[14] Follow-up data were obtained from patients' digital medical records, outpatient visits and phone contact. Local principal investigators collected and stored data pseudonymised in RedCAP. The study endpoints included postdischarge mortality, RAP, progression to CP, development of PEI, DM and PDAC. The P-MAPS I cohort was recruited within 72 hours after hospital admission in 15 tertiary centres from eight European countries between March 2020 and June 2022.[15] eSwabs (Copan, Brescia, Italy) were frozen at −80°C within 60 min after standardised collection.

Patient and public involvement

It was not possible to involve patients or the public in the design, or conduct, or reporting or dissemination plans of our study.

Microbiome analysis

DNA extraction, library preparation and sequencing protocols were previously described in detail.[14 16] In brief, for DNA isolation, the PureLink microbiome kit (Invitrogen) was used, modified according to International Human Microbiome Standards, followed by a purification step with OneStep PCR inhibitor removal kit (Zymo Research). Buccal and rectal samples were sequenced using the whole 16S rRNA and metagenomic approach with Oxford Nanopore Technologies.[16] Oral species may closely mirror the duodenal microbiome and have the potential to translocate to the pancreas,[17 18] whereas rectal swabs have a higher DNA yield for metagenomics sequencing and may thus allow investigation of systemic effects of microbiome-derived bioactive metabolites.

Statistical analysis

In the P-MAPS I cohort, 77 discrete and 2 continuous confounders with potential impact on microbial composition were prospectively collected.[14] Confounders that occurred at least in 5% of respective endpoints were included in a L1-penalised lasso regression, using the respective endpoint as the dependent variable. Afterwards, a 10-fold cross-validation was performed to determine the optimal lambda value. Variables with non-zero coefficients in the final model were considered relevant and subsequently included in multiple regression or distance-based redundancy analysis (db-RDA) of α-diversity and β-diversity calculations. More details regarding diversity measurements and classifier establishment are described in the online supplemental methods.

RESULTS

Patient cohort

In total, 424 patients were recruited for the P-MAPS trial.[14] From this cohort, 10 patients died within 30 days after recruitment. From 120 patients no follow-up data were available. Additionally, 17 patients had a follow-up period of less than 90 days, did not reach an endpoint and thus were excluded. Out of 277 patients with follow-up data, 181 patients were called by phone, and median follow-up period after initial hospital discharge was 2.8 years (figure 1A,B). 66 (24%) patients had RAP, 16 (6.2%) progressed to CP and 20 (7.2%) died after discharge during the follow-up period. Additionally, 18 (7.3%) developed PEI, 16 (7.2%) were diagnosed with DM and 4 (1.5%) were diagnosed with

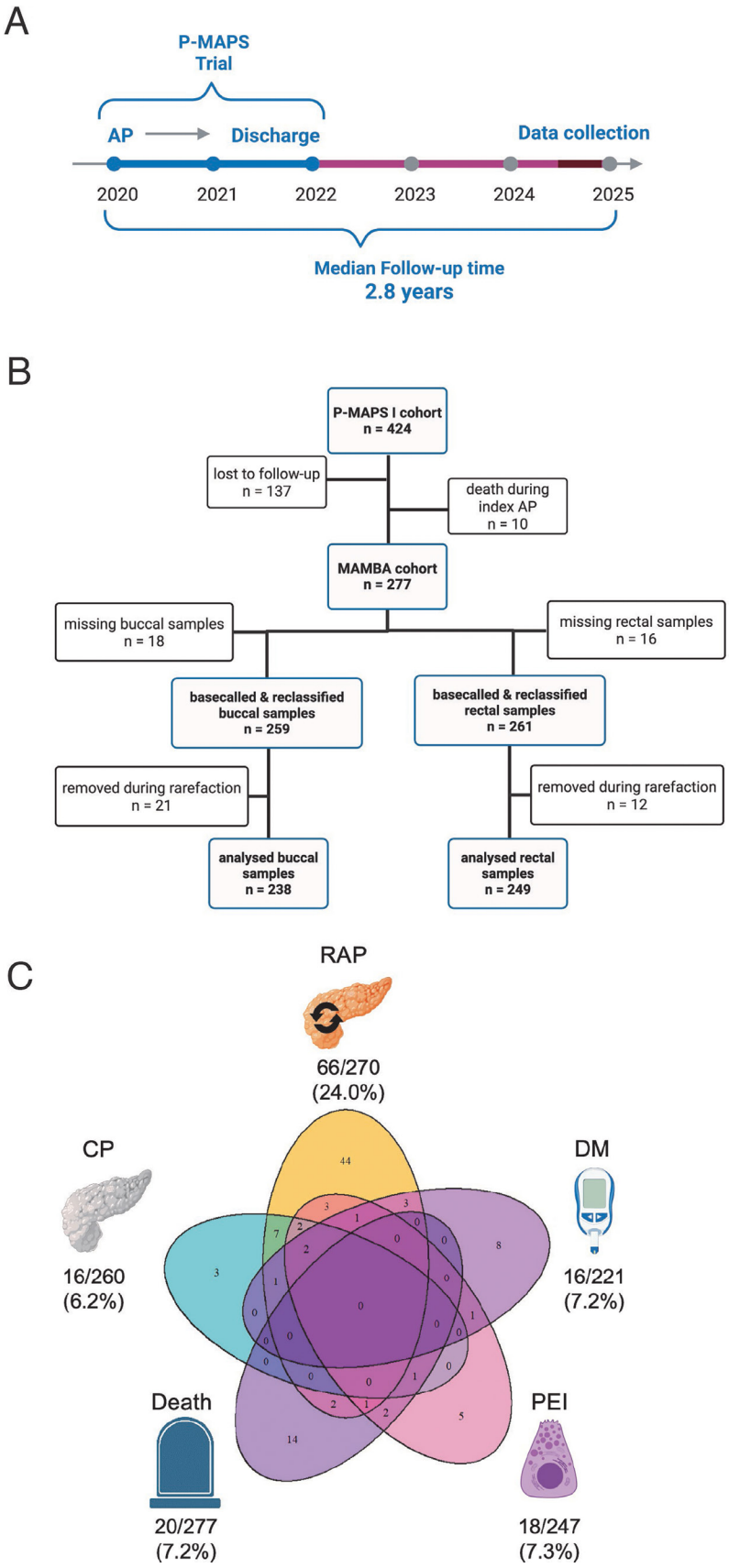

Figure 1 Study overview. (A) Between 2019 and 2021, patients were recruited for the P-MAPS trial. With a median follow-up of 2.8 years, patients were contacted via phone or, in case of unsuccessful calling, data were extracted from digital patient files. (B) STROBE flowchart of the analysed patient cohort. (C) Venn diagram of endpoints. AP, acute pancreatitis; CP, chronic pancreatitis, DM, postdischarge diabetes mellitus, MAMBA, morbidity and mortality by microbiome patterns in acute pancreatitis; PEI, pancreatic exocrine insufficiency, P-MAPS, Pancreatitis-Microbiome As Predictor of Severity; RAP, recurrent acute pancreatitis; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

Table 1 Endpoints associated with index RAC

Endpoints	RAC I	RAC II/III	P value
RAP	47/199 (24%)	19/71 (27%)	0.6
CP	9/194 (4.6%)	7/66 (11%)	0.13
Postdischarge mortality	12/202 (5.9%)	8/75 (11%)	0.2
Postdischarge PEI	8/183 (4.4%)	10/64 (16%)	**0.009****
Postdischarge DM	9/165 (5.5%)	7/56 (13%)	0.13
Postdischarge PDAC	4/196 (2.0%)	0/68 (0%)	0.6

Fisher exact test was performed to reveal possible association between the progression of RAP, CP, postdischarge mortality, development of postdischarge PEI, DM and PDAC.
**p<0.01.
CP, chronic pancreatitis; DM, diabetes mellitus; PDAC, pancreatic ductal adenocarcinoma; PEI, pancreatic exocrine insufficiency; RAC, revised Atlanta classification; RAP, recurrent acute pancreatitis.

PDAC. Among all endpoints, only PEI was associated with severity according to RAC (table 1). PDAC was not further evaluated due to the small number of cases.

Of the 277 patients, 259 buccal and 261 rectal microbial data were re-basecalled and reclassified with an updated library. After normalisation, 238 buccal and 249 rectal samples had sufficient sequencing depth and were thus eligible for further analysis (figure 1B, online supplemental figure S1). Besides RAP and CP, there was no relevant overlap of patients regarding endpoints (figure 1C). Therefore, associations between the orointestinal microbiome and all endpoints were analysed independently.

Association between orointestinal microbiome and postdischarge complications recurrent acute pancreatitis

Out of 238 buccal and 249 rectal samples, five and seven patients had insufficient information regarding RAP, respectively, and were thus excluded for this endpoint. Most patients (49/66) that RAP had one or two recurrent episodes after index AP. 11 patients suffered from three to five further flares and 6 patients were readmitted to hospital more than five times due to RAP. As expected, there is a strong association of RAP with smoking and regular alcohol consumption, and an inverse association of biliary cause of AP, cholestasis and older age (online supplemental tables 1 and 2). Alcohol consumption was determined for stratification of buccal diversity metrics. The evenness was significantly lower in patients with RAP (Shannon: incidence rate ratio (IRR) 0.81, p value 0.015*). No significant differences were obtained for the richness (observed species: IRR 0.97, p value 0.375) and β-diversity metrics (p value 0.264, figure 2A,B, online supplemental tables 3–5). Confounder selection for rectal samples revealed seven confounding parameters (alcohol consumption, alcoholic AP, cholestasis, opiate intake, moderate and heavy smoking, 1–10 cigarettes/day and >10 cigarettes/day, and current antibiotic intake) for which multiple models of α-diversity and β-diversity were stratified for. A lower α-diversity was associated with the occurrence of RAP in rectal samples using observed species (IRR 0.94, p value 0.023*) and the Shannon index (IRR 0.95, p value 0.056, figure 2C, online supplemental tables 6 and 7). Notably, significant differences in β-diversity between RAP and non-RAP were revealed in rectal samples using db-RDA (p value 0.015*, figure 2D, online supplemental table 8).

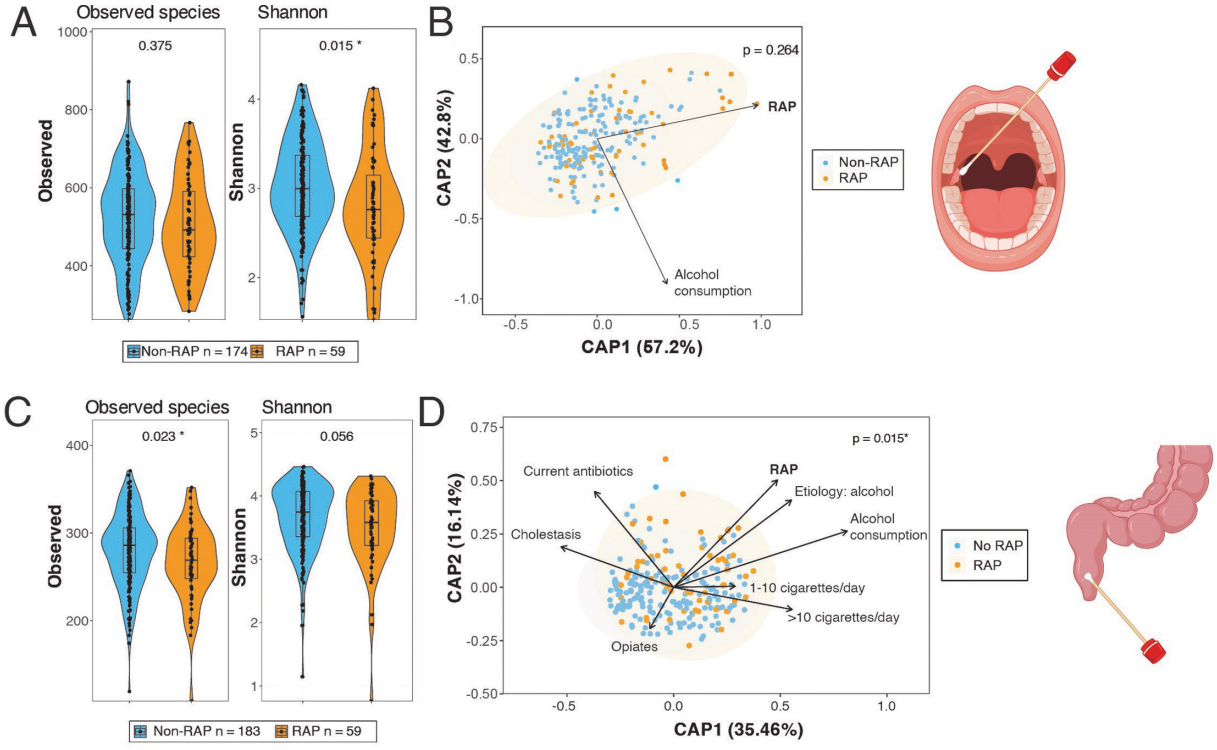

Figure 2 Associations of orointestinal microbiome obtained at the early phase of acute pancreatitis with postdischarge recurrent acute pancreatitis. (A+C) α-diversity was assessed with oral swabs for observed species and Shannon index and was compared between RAP (RAP in orange, non-RAP in light blue). (B+D) db-RDA was ordinated with CAP between patients with RAP and non-RAP. P values for observed species were calculated using multiple negative binomial regression; for Shannon index, either gamma or linear regression was used based on data normality. db-RDA p values were derived from permutational multivariate ANOVA. ANOVA, analysis of variance; CAP, canonical analysis of principal coordinates; db-RDA, distance-based redundancy analysis; RAP, recurrent acute pancreatitis. *p<0.05.

Chronic pancreatitis

Due to unavailable follow-up data, 14 buccal and 17 rectal samples were excluded for α-diversity and β-diversity analysis. 12 out of 16 patients developing CP suffered from prior RAP (figure 1C). Male sex, smoking, current and former alcohol consumption, alcoholic AP and proton-pump inhibitor (PPI) intake were associated with the development of CP (online supplemental tables 9 and 10). Alcoholic AP, no cholestasis, PPI intake and heavy smoking (>10 cigarettes/day) were identified as confounders in both sample sets. Multiple regression models (buccal observed species: IRR 0.99, p value 0.836; buccal Shannon: IRR 0.75, p value 0.074; rectal observed species: IRR 1.04, p value 0.384; rectal Shannon: IRR 1.02, p value 0.638) and db-RDA (buccal p value 0.2, rectal p value 0.593) revealed no significant differences between individuals who developed CP compared with those without CP (online supplemental tables 11–16, online supplemental figures S2A–D).

Postdischarge mortality

In total, 20 patients died within the follow-up period. Half of them died within the first 1.5 years after discharge. The 90-day mortality after discharge was 0.7% (n=2), and the 12-month mortality was 2.9% (n=8, online supplemental figure S3). Various causes of death were reported; however, cancer, cardiovascular diseases, sepsis, infections and respiratory diseases were the leading causes (online supplemental table 17). Older age, higher creatinine values at index admission, neurological, malignant and cardiovascular comorbidities and constipation were associated with postdischarge mortality (online supplemental tables 18 and 19). For buccal samples, confounder selection revealed age, chronic heart insufficiency and non-GI solid malignancies, and all of them were included as covariates for the α-diversity and β-diversity models. Observed species (IRR 0.90, p value 0.076) and Shannon index (IRR 0.88, p value 0.37) were not significantly different between those patients that died after discharge and those that survived (figure 3A, online supplemental tables 20 and 21), but db-RDA revealed significant differences between both groups for postdischarge mortality (p value 0.042*, figure 3B, online supplemental table 22). Age, non-GI solid malignancy, chronic heart insufficiency and intake of antibiotics within a week before sample collection were considered as confounders for rectal samples. After controlling for confounders, both α-diversity (observed species: IRR 1.03, p value 0.399; Shannon: IRR 1.01, p value 0.837) and db-RDA (p value 0.156) did not show any significant differences (figure 3C; online supplemental tables 23–25).

Pancreatic exocrine insufficiency

Criteria used for diagnosis of PEI were decreased stool elastase and symptoms of maldigestion. For 26 buccal and 28 rectal

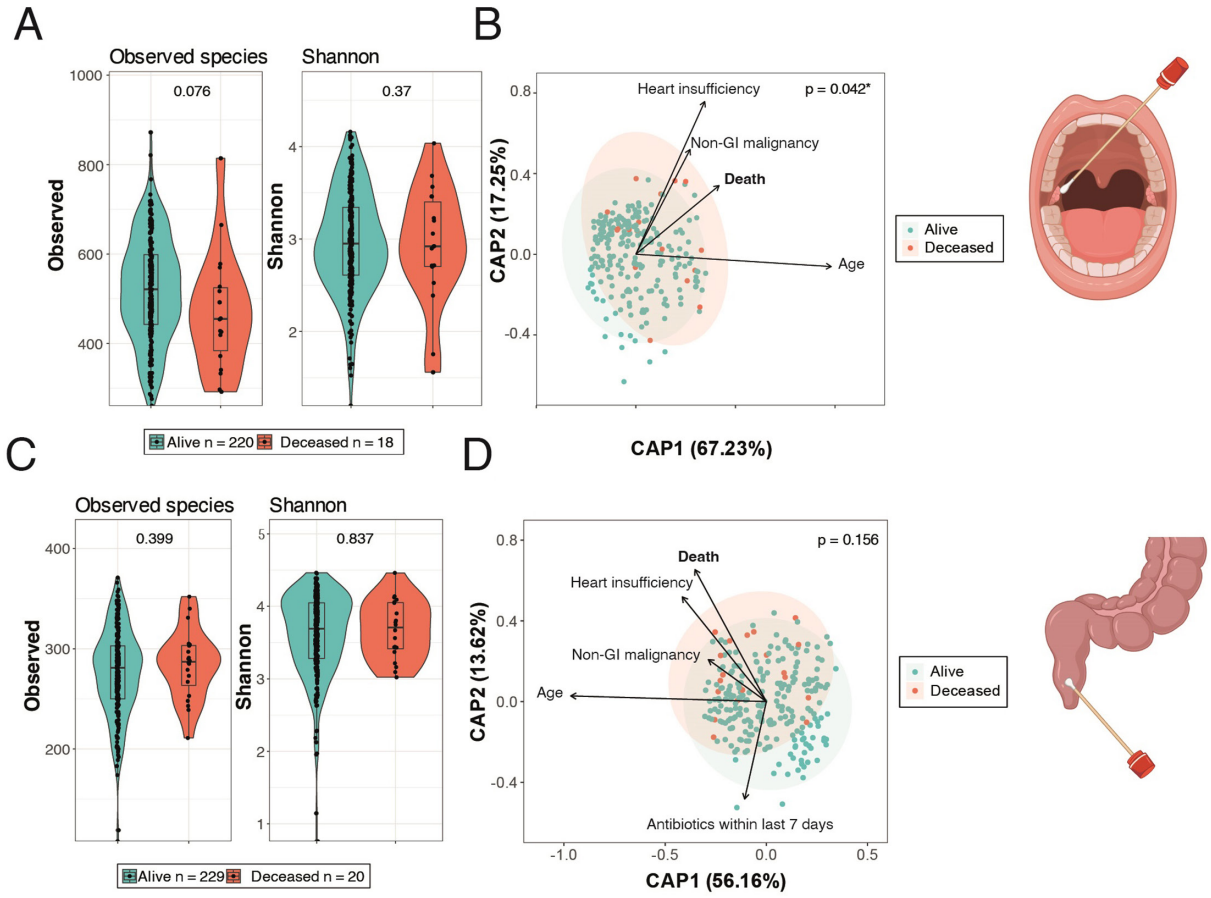

Figure 3 Associations of orointestinal microbiome obtained at the early phase of acute pancreatitis with postdischarge mortality. (A+C) α-diversity was assessed with oral and rectal swabs between patients that died after hospital discharge (violet) and survivors (light green). (B+D) db-RDA between patients that died after hospital discharge and survivors for oral and rectal swabs. P values for observed species were calculated using multiple negative binomial regression; for Shannon index, either gamma or linear regression was used based on data normality. db-RDA p values were derived from permutational multivariate ANOVA. ANOVA, analysis of variance; CAP, canonical analysis of principal coordinates; db-RDA, distance-based redundancy analysis. *p<0.05.

samples, corresponding data for PEI were not available and thus excluded from microbial analysis. Alcohol consumption and non-steroidal anti-inflammatory drug intake were associated with the development of PEI (online supplemental tables 26 and 27). No confounders were identified with L1-penalised lasso regression for buccal and rectal samples, respectively. No significant differences were obtained regarding observed species (buccal: IRR 0.97, p value 0.649; rectal: IRR 1.03, p value 0.432), Shannon index (buccal: IRR 0.92, p value 0.549; rectal Shannon: IRR 1.03, p value 0.525) and Bray-Curtis distances (buccal: p value >0.9; rectal: p value 0.591) in both buccal and rectal samples, respectively (online supplemental figure S4A–D, online supplemental tables 28–33).

Diabetes mellitus

All patients with known DM at index and those without available data for postdischarge DM were excluded. To this end, 47 buccal and 51 rectal samples were not included for analysis. Patients who developed DM after discharge differed from patients without DM regarding cardiovascular diseases at index. For buccal samples, statistical tests for α-diversity and β-diversity were not stratified for confounders as L1-penalised lasso regression did not identify variables with non-zero coefficients and yielded no significant alterations between groups (observed species: IRR 0.93, p value 0.325; Shannon: IRR 0.78, p value 0.135; permutational analysis of variance for β-diversity 0.1; figure 4A,B, online supplemental tables 34–38). Multiple regression models for rectal samples included cardiovascular diseases as covariates and revealed no significant differences in α-diversity for observed species (IRR 0.97, p value 0.61) and Shannon index (IRR 0.93, p value 0.11; online supplemental tables 39 and 40; figure 4C). Remarkably, db-RDA exhibited a significant distance calculation between individuals with and without a development of a postdischarge DM for β-diversity of rectal samples (p value 0.03*, figure 4D, online supplemental table 41).

Microbial classifier for predicting postdischarge complications

Given the significant alterations of the rectal microbiota associated with the occurrence of RAP, the development of postdischarge DM as well as the associations of changes of the buccal microbiota with postdischarge mortality, we set out to further explore the ability of differentially abundant species to predict these long-term complications with regularisation models.

Recurrent acute pancreatitis

To mitigate confounding effects from clinical variables such as alcohol consumption and smoking, patients with RAP were

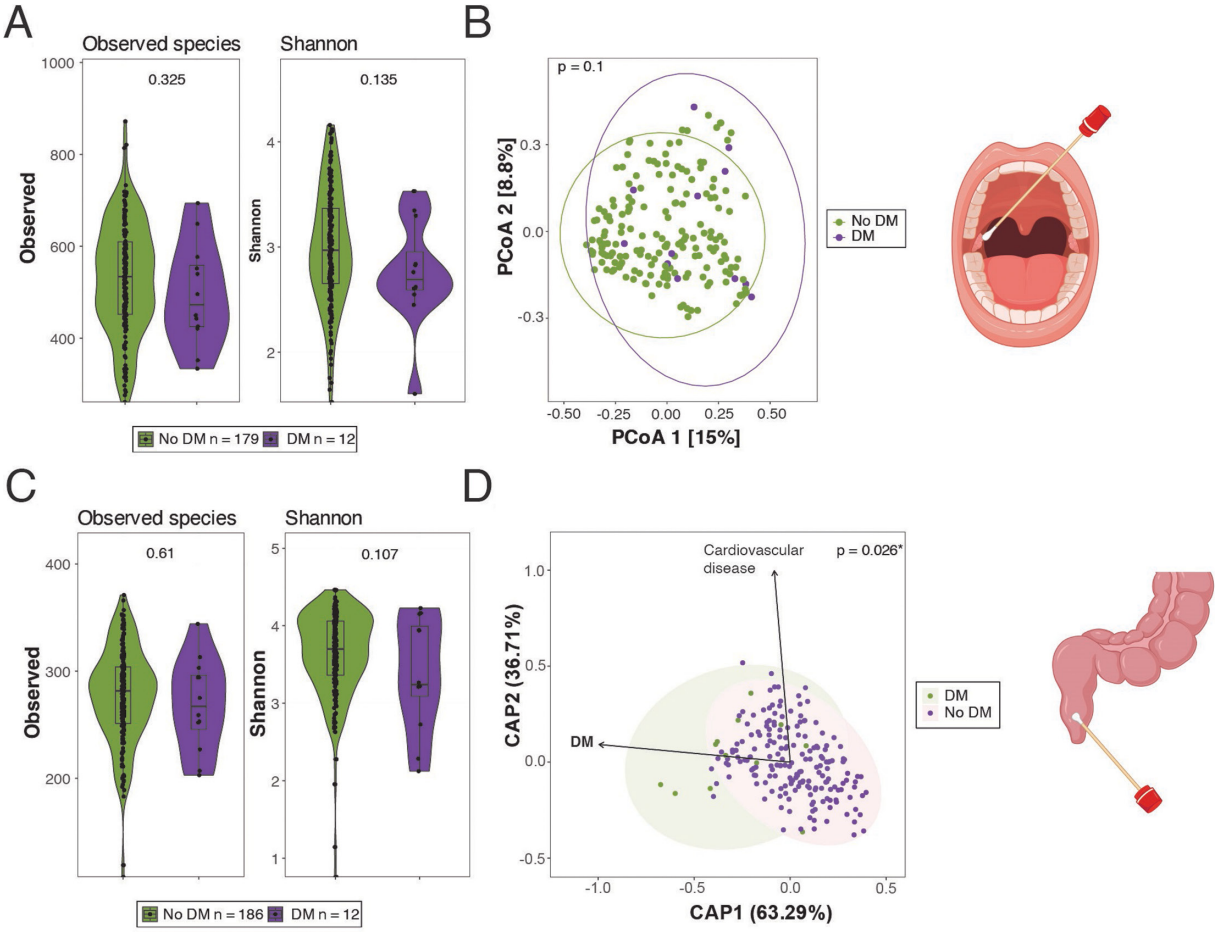

Figure 4 Associations of orointestinal microbiome obtained at the early phase of acute pancreatitis with postdischarge DM. (A+C) α-diversity was assessed with oral and rectal swabs between patients with and without development of a postdischarge DM (no DM in dark green, DM in violet). (B+D) db-RDA for patients with and without the development of postdischarge DM for oral and rectal swabs. P values for observed species were calculated using multiple negative binomial regression; for Shannon index, either gamma or linear regression was used based on data normality. db-RDA p values were derived from permutational multivariate ANOVA. ANOVA, analysis of variance; CAP, canonical analysis of principal coordinates; db-RDA, distance-based redundancy analysis; DM, diabetes mellitus; PCoA, principal coordinate analysis. *p<0.05.

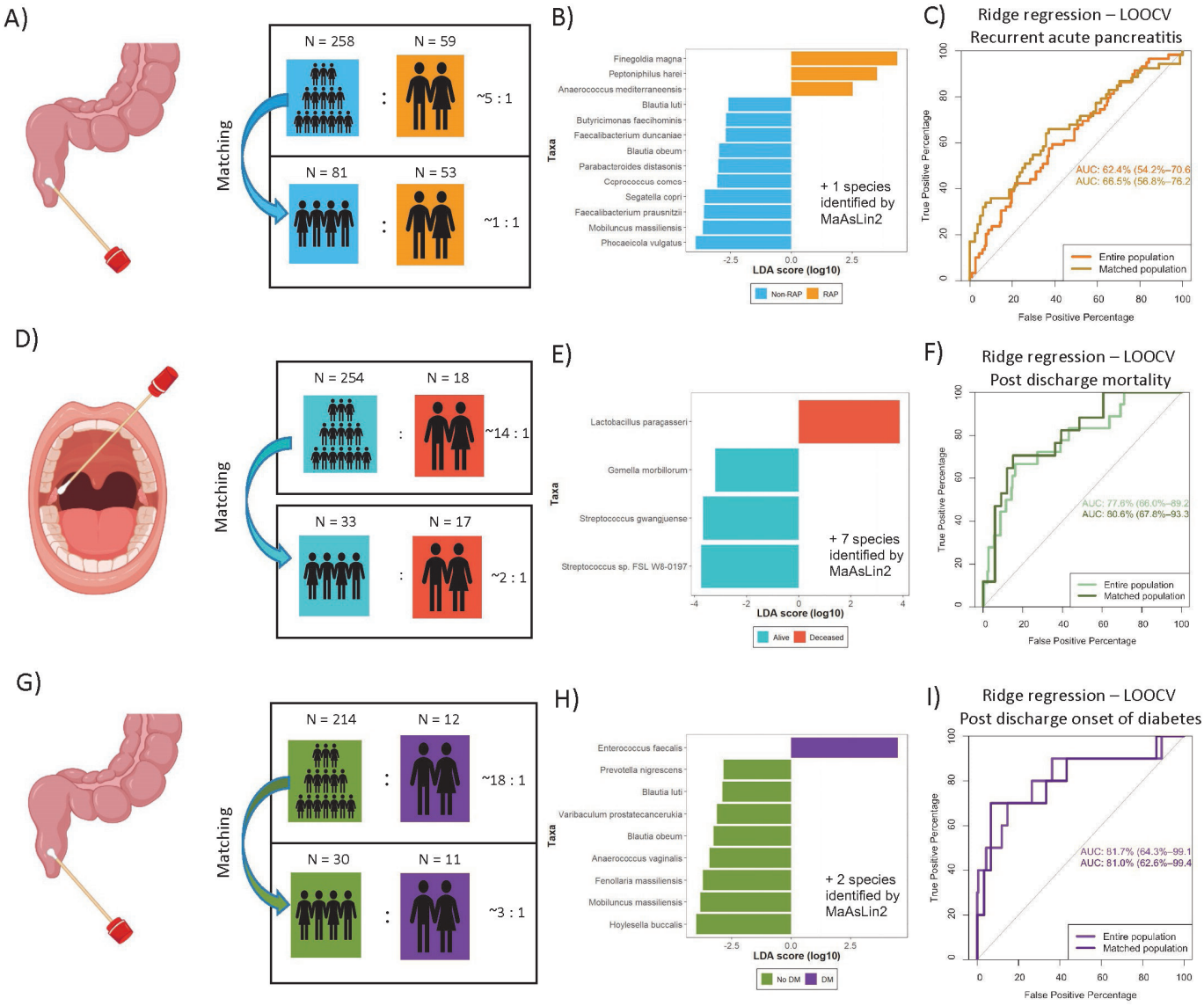

Figure 5 Microbial classifier predicting postdischarge acute pancreatitis related complications. (A) Rectal swabs from patients with RAP (orange) were matched with non-RAP controls (light blue). (B) Differential microbial abundance between groups was assessed using LEfSe. Anaerococcus prevotii was additionally identified by MaAsLin2. (C) An L0-penalised regression model with LOOCV was applied to differentially abundant species in the matched and entire cohorts. (D) Oral swabs from patients who died postdischarge (red) were matched with survivors (light green). (E) Differentially abundant species between matched groups were identified using LEfSe. Terrisporobacter petrolearius, Rummeliibacillus stabekisii, Peptacetobacter hiranonis, Klebsiella electrica, Clostridioides difficile, Exiguobacterium alkaliphilum, Paraclostridium sordellii were additionally identified by MaAsLin2. (F) An L0-penalised regression model with LOOCV was applied to differentially abundant species in the matched and entire cohorts. (G) Rectal swabs from patients with postdischarge DM (violet) and without (non-DM, dark green) were matched. (H) LEfSe was used to identify differentially abundant species. Thomasclavelia ramosa and Bacteroides nordii were additionally identified by MaAsLin2. (I) An L0-penalised regression classifier was constructed using these species for the matched and entire cohorts. AUC, area under the curve; DM, diabetes mellitus; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; LOOCV, leave-one-out cross-validation; MaAsLin2, Microbiome Multivariable Association with Linear Models; RAP, recurrent acute pancreatitis.

matched to non-RAP controls across confounding variables. This yielded a matched cohort of 53 patients with RAP and 81 non-RAP controls for downstream microbial differential abundance analysis (figure 5A, online supplemental table 42). Using Microbiome Multivariable Association with Linear Models (MaAsLin2) and linear discriminant analysis effect size (LEfSe), we identified 14 species exhibiting significant abundance differences (figure 5B, online supplemental figure S5A and table 43). *Finegoldia magna* and *Peptoniphilus harei* were more dominant in the RAP group. Patients without RAP had higher abundances of *Phocaeicola vulgatus* (former *Bacteroides vulgatus*), *Mobiluncus massiliensis* and *Faecalibacterium prausnitzii*. These 14 rectal species were incorporated as predictors in an L0-penalised ridge regression model to classify RAP status. The model demonstrated limited discriminative capacity, achieving an area under the receiver operating characteristics (AUROC) of 66.5% in the matched cohort and 62.4% in the full cohort (figure 5C). A total of 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were significantly inferred using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) with oxidative phosphorylation being more abundant in the non-RAP group, a pathway associated with enhanced

resilience to oxidative stress and maintenance of intestinal barrier integrity (online supplemental table 44).[19 20] Conversely, the valine, leucine and isoleucine degradation pathway linked to inflammation is associated with RAP.[21]

Postdischarge mortality

A matched cohort consisting of 33 survivors and 17 patients that died after discharge was identified in a comprehensive matching including potential confounders using random forest (figure 5D, online supplemental table 45). Differential abundance revealed 11 oral species (figure 5E, online supplemental figure S5B and table 46). *Lactobacillus paragasseri* was more abundant in patients that died after discharge, whereas *Streptococcus sp FSL W8-0197* and *Streptococcus gwangjuense* were dominant in the survivor cohort. The performance of the L0-penalised ridge regression was moderate with 80.6% in the matched and 77.6% in the entire cohort, respectively (figure 5F). In total, 39 KEGG pathways were differentially abundant between the two groups, including pathways related to primary and secondary bile acid biosynthesis, which were associated with postdischarge mortality (online supplemental table 47).

Postdischarge diabetes mellitus

11 patients who developed DM after hospital discharge were matched to 30 patients without postdischarge DM (figure 5G, online supplemental table 48). MaAsLin2 and LEfSe calculations identified 11 species that were differentially abundant between both groups. *Enterococcus faecalis* was more dominant in the DM group, whereas *Hoylesella buccalis* and *M. massiliensis* had the strongest effect size in the non-DM cohort (figure 5H, online supplemental figure S5C and table 49). A ridge regression model including those 11 species was able to predict postdischarge DM with a remarkable AUROC of 94.8% and 86.2% in the matched and entire cohort, respectively (figure 5I). These 11 differentially abundant rectal species yielded a positive predictive value of 66.6%, a negative predictive value of 96% and an accuracy of 95%. Interestingly, the lipopolysaccharide biosynthesis was an inferred pathway among 83 KEGG pathways that was previously associated with diabetes in European women and linked to insulin resistance (online supplemental table 50).[22 23]

DISCUSSION

Despite improved in-hospital management of patients with AP, postdischarge morbidity and mortality remains high, in particular within the first 2 years after the first episode of AP.[7] Increased postdischarged mortality is also known from other conditions such as myocardial infarction or sepsis,[24 25] highlighting the broader relevance of this topic in clinical medicine. Here, we performed a long-term follow-up study of patients from the prospective, multicentre P-MAPS trial including 15 centres in eight European countries. Patients with the first or second episode of AP were enrolled within 72 hours of hospital admission and oral and rectal microbial swabs were collected according to highly standardised operating procedures.[14–16] Here, we investigated both the frequency of postdischarge mortality and morbidity as well as the potential associations with the admission microbiome following reclassification and re-basecalling with an updated microbiota library. Our clinical data confirm earlier studies revealing a relevant postdischarge mortality of 7.2% within the first 3 years after hospital discharge, resulting in a threefold increased mortality rate compared with the initial 30 days mortality rate of 2.4% in the P-MAPS cohort. The 90-day mortality (0.7%) was lower in our cohort than in previously reported data (3.0%).[7] Multiple risk factors have been described for the development of postdischarge complications in large population-based cohort studies.[11] To this end, our study confirms previous data showing that smoking, continued alcohol consumption, older age, male sex, creatinine at index admission, neurological, cardiovascular and malignant comorbidities as well as abdominal surgery have a significant impact on the development of postdischarge mortality and related complications. Surprisingly, the severity of AP according to RAC was only significantly associated with the development of PEI but no other postdischarge complications. For the subsequent microbiome analysis, we carefully accounted for 77 discrete and 2 continuous potential confounders for microbiome diversity. db-RDA ordinated with canonical analysis on the principal coordinates clearly showed the pronounced effect of known confounders for postdischarge mortality and RAP such as alcohol consumption, cardiovascular and malignant comorbidities. Despite accounting for these potential confounders in multivariate models, ß-diversity was significantly different for postdischarge mortality, RAP and development of DM indicating a possible link between the microbial composition and these clinical endpoints.

To further reduce the impact of these potential confounders for subsequent construction of diagnostic classifiers, we defined matched cohorts for postdischarge mortality, RAP and DM. The moderate discriminatory capacity of differentially abundant species for postdischarge mortality (77.6%) and RAP (62.4%) can be explained by the relatively strong impact of these confounding factors on both endpoints. In contrast, only previous cardiovascular diseases had to be accounted for in postdischarge development of DM, highlighting the strong impact of microbial diversity itself on this clinical endpoint. This strong effect was reflected by a very good discriminatory capacity on construction of the diagnostic classifier for the development of postdischarge DM (AUROC: 94.8% for the matched cohort, and 86.2% for the entire cohort). Several heterogeneous meta-analyses evaluated the prevalence of DM after one or more episodes of AP and revealed a wide range between 8% and 54%.[26 27] Notably, earlier literature on the epidemiology of post-AP DM in population-based studies was prone to selection bias leading to wrong assumptions and incorrect risk constellations.[28] In contrast to large population-based studies, the occurrence of postdischarge DM was not significantly associated with AP severity in our cohort, pointing towards an important role of the gut–pancreas axis in regulating β-cell function and autoimmunity independent of the extent of pancreas inflammation. However, there is limited knowledge of the pathophysiology of the development of de novo DM in post-AP. To this end, the gut microbiome might orchestrate a crucial crosstalk between ß-cells and immune cells via antimicrobial peptides.[29] For instance, experimental data in non-obese diabetic (NOD) mice have provided evidence that administration of β-cell produced cathelicidin related antimicrobial peptide (CRAMP) induced regulatory immune cells in pancreatic islets, dampening the incidence of autoimmune diabetes.[30] Intriguingly, CRAMP production by β-cells was controlled by SCFAs produced by the gut microbiota. Furthermore, dysbiosis in newborn NOD mice induced type 1 interferon production by colonic epithelial cells and promoted subsequent development of pancreatic autoimmune response and development of diabetes, highlighting an intricate communication between gut microbiota and pancreatic endocrine function.[31] Notably, CRAMP-expressing probiotics restored colonic homeostasis and interrupted the altered glucose metabolism, preventing autoimmune diabetes in NOD mice.[31] These initial findings provide first experimental evidence that microbial

treatments using probiotics or even metabolites such as SCFA might hold future promise to prevent post-AP DM.

Interestingly, we found *Prevotella nigrescens*, *Blautia luti*, *Varibaculum protatecancerukia*, *Blautia obeum*, *Anaerococcus vaginalis*, *Fenollaria massiliensus*, *M. massiliensis, Thomasclavelia ramosa*, *Bacteroides nordii* and *Hoylesella buccalis* to be more abundant in the rectal microbiome of patients without postdischarge DM. In contrast, *E. faecalis* was more abundant in patients with postdischarge DM. Interestingly, *Blautia* was found to be significantly decreased in patients with new onset DM and restored after adequate antidiabetic medication.[32]

When interpreting our results, several limitations of our study need mentioning. First, the incidence of postdischarge DM was 7.2% and might have been underestimated by the length of follow-up (median 2.8 years) and the lack of standardised glycated haemoglobin measurements. However, previous data had shown that the majority of complications occur during the first 2 years after index admission for AP.[7] Second, faecal elastase was not available for most of the patients and improvement of symptoms on enzyme replacement therapy was not systematically reported, potentially leading to a possible underestimation of PEI in our study population. Moreover, oral and rectal swabs were collected within the first 72 hours after admission, leaving the possibility that the extent of gut dysbiosis was altered during this period. Finally, metabolic prediction using PICRUSt2 yielded intriguing results for several KEGG pathways (eg, lipopolysaccharide biosynthesis), but this approach cannot replace direct measurements of metabolites in blood and stool samples. To this end, the aforementioned limitations will be addressed in the ongoing global P-MAPS II trial (NCT06508502).

In conclusion, we have conducted a long-term follow-up study of patients with AP showing a striking association of admission gut microbiota and subsequent development of postdischarge complications. Differential abundance analysis and construction of a diagnostic classifier yielded a high discriminatory capacity to predict the development of postdischarge DM, thus opening new stratification tools for a tailored risk assessment in the future.

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